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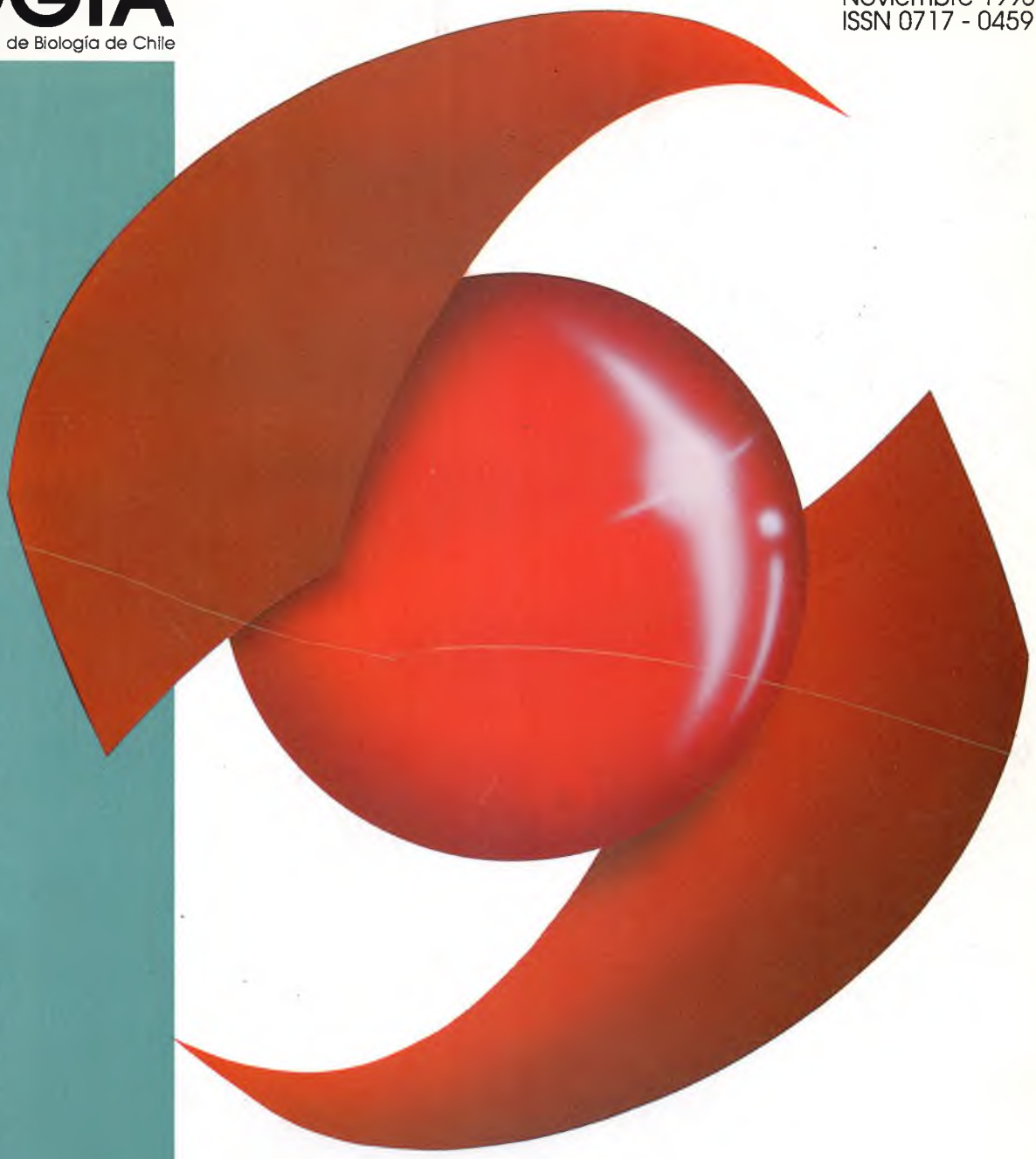
VIII PABMB Congress

The Pan-American Association for Biochemistry and Molecular Biology



Program and Abstracts

XXXII Annual Meeting of the Argentinean Society for Biochemistry and Molecular Biology Research

XIX Annual Meeting of the Society of Biochemistry and Molecular Biology of Chile

November 16/21 1996
PUCON, CHILE

PROGRAM

Saturday 16		Sunday 17	Monday 18	Tuesday 19	Wednesday 20	Thursday 21
	9: 00 10:00	Lecture **Nestor Carrillo**	Lecture **Ramón Latorre**	Lecture **William Whelan**	Lecture **Joseph Martial**	9:00 *to* 11:30 **Symposium** Biochem Educ
		Coffee Break	*Coffee Break*	*Coffee Break*	*Coffee Break*	*Coffee Break*
Congress Registration	10:30 13:00	**Symposia** 1 - 2 - 3 - 4	**Symposia** 8 - 9 - 10	**Symposia** 15 - 16 - 17 - 18	**Symposia** 19 - 20 - 21 - 22	12:00 *to* 12:15 Closing Ceremony 12:15 *to* 13:15 Closing Lecture **L. de Meis**
		Lunch	*Lunch*	*Lunch*	*Lunch*	
	14:30 16:30	Posters 1	Posters 2	**Free Afternoon**	Posters 3	**Chao!**
18:30 *to* 19:30 Opening Ceremony		*Coffee Break*	*Coffee Break*		*Coffee Break*	
19:30 *to* 20:30 Opening Lecture **Susan Taylor**	17:00 19:30	**Symposia** 5 - 6 - 7	**Symposia** 11 - 12 - 13 - 14		**Symposia** 23 - 24 - 25	
	19:45 20:45	Lecture **Peter Dolphin**	Lecture **Georges Dreyfus**		20:15 *to* 21:00 Typical Chilean Songs and Dances	
20:45 *to* 22:30 **Cocktail Party**	21:00	*Dinner*	*Dinner*	*Dinner*	*Dinner* **Party**	

SYMPOSIA SUBJECTS

1. From egg to embryonic nervous system. *Roberto Mayor*
2. Protein-ligand interactions. *Emilio Cardemil*
3. Molecular aspects of Archaea. *Carlos Jerez*
4. Host-parasite relationship in Chagas disease. *Norbel Galanti*
5. Protein kinases involved in cell division. *Jorge Allende*
6. Intracellular protein glycosylation. *Armando Parodi*
7. Cholinergic macromolecules and disease. *Nibaldo Inestrosa*
8. Protein folding. *Octavio Monasterio*
9. Biotechnology. *Pablo Valenzuela*
10. Cell calcium signaling and regulation. *Cecilia Hidalgo*
11. Oxidative stress. *Federico Leighton*
12. Signal transduction. *Héctor N. Torres*
13. Regulation of enzyme activity. *Jorge Babul*
14. Cell adhesion and recognition. *Enrique Brandan*
15. The supramolecular organization of metabolism. *Tito Ureta*
16. Biodegradation of aromatic compounds. *Rafael Vicuña*
17. Yeast biochemistry and genetics. *Antonio Peña*
18. Signal transduction and gene expression. *Loreto Holuigue*
19. Enzyme structure and function. *Juan Carlos Slebe*
20. Mechanisms of viral pathogenesis. *Eugenio Spencer*
21. Hormone receptors. *Ricardo Boland*
22. Cellular biology of neuronal cell differentiation. *Hugo Maccio*
23. Molecular genetics of inherited disorders. *Pilar Carvallo*
24. The private life of ion channels. *Ramón Latorre*
25. Regulation of transcription. *Alberto R Kornblihtt*
26. Biochemical education. *Manuel Krauskopf*

Asociación Panamericana de Sociedades de Bioquímica y Biología Molecular

Associação Pan-Americana das Sociedades de Bioquimica e Biología Molecular

The Pan-American Association for Biochemistry and Molecular Biology

VIII PABMB Congress

In conjunction with

XXXII Annual Meeting of the Argentinean Society for Biochemistry and Molecular Biology Research

XIX Annual Meeting of the Society of Biochemistry and Molecular Biology of Chile

November 16-21, 1996

Gran Hotel Pucón, Pucón, Chile

PROGRAM and ABSTRACTS

Acknowledgments

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American Society for Biochemistry and Molecular Biology
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Welcome

On behalf of the Organizing Committee of the VIII PABMB Congress, I extend to all participants the warmest welcome to this triple event, the Eighth Congress of the Pan-American Association for Biochemistry and Molecular Biology (PABMB), held jointly with the XXXII Annual Meeting of the Argentinean Society for Biochemistry and Molecular Biology Research (Sociedad Argentina de Investigación en Bioquímica y Biología Molecular), and the XIX Annual Meeting of the Society of Biochemistry and Molecular Biology of Chile (Sociedad de Bioquímica y Biología Molecular de Chile). Every four years, the Pan-American community of biochemists, professors, post-doctoral fellows, students and collaborators, get together to present and discuss their research in biochemistry, molecular biology, cell biology, molecular genetics, biophysics, and related fields. Our expectation is to stimulate and strengthen the collaboration and exchange of information on the research conducted by members of our societies, and by the invited and interested researchers.

The topics of the symposia and lectures, as well as many names of the invited speakers, were suggested by the constituent and adherent societies. The program committee gave preference to those subjects proposed by several societies and which allow, at the same time, the participation of scientists of different countries. The program includes 8 plenary lectures, 26 symposia, and 3 poster sessions. This book is the summary of all these presentations, approximately 620 in total. Many topics were left out because of restrictions such as the length of the meeting, together with our prospects of a meeting with participants secluded in a place with ample time for exchange of experiences. These ideas were important in many decisions taken by the organizers. The beauty of Pucón may ran counter to our expectations.

We open to you the doors of our country and science in what will be a week-long celebration with our colleagues and friends from other countries to share with us the excitement for science and scientific research. We are all convinced that Science is important to know more about us, about everything in Nature, and for the future of our countries.

I would like to thank our colleagues that had the enormous responsibility to make every effort to organize each detail of this meeting. The eventual success of the Congress will be their best reward.

¡Bienvenidos!

Jorge Babul
Chairman, Organizing Committee

Program

Saturday 16th

18:30 - 19:30 Opening Ceremony (Salón Plenario)

Jorge Babul
(*President, Sociedad de Bioquímica y Biología Molecular de Chile*)

Marino Martínez-Carrión
(*Chairman, Pan-American Association for Biochemistry and Molecular Biology*)

Kunio Yagi
(*President, International Union of Biochemistry and Molecular Biology*)

Fablán Jaksic
(*Pontificia Universidad Católica de Chile*)
Ecological overview of Chile: landscape and creatures.

19:30 - 20:30 Plenary Opening Lecture (Salón Plenario)

Chair: Marino Martínez-Carrión

Taylor, S.S. (*Univ. California, San Diego*). cAMP-dependent protein kinase: structural insights into structure and function. (*abstract* 6)

Sunday 17th

09:00 - 10:00 Luis F. Leloir Plenary Lecture (Salón *Plenario*)

Chair: Ricardo Boland

Carrillo, N. (*PROMUBIE, Univ. Rosario*). The plant-type ferredoxin-NADP$^+$ reductase family. Something old, something new, something borrowed. (*abstract* 1)

10:30 - 13:00 Symposia 1- 4

Symposium 1 (Salón Casino)

From Egg to Embryonic Nervous System: A Molecular Analysis

Chair: Roberto Mayor and Heiner Westphal

The process of embryonic development is one of the most exciting problems in modern biology. It transforms an egg in an organism with different organs. The most complex organ is the nervous system. Although the nervous systems of insects, amphibians and mammals look very different and are able to perform very different jobs, the molecular mechanisms involved in its early development are quite similar. In this Symposium, the molecular mechanisms that control some aspects of the patterning of the early nervous system during development will be shown and how these mechanisms are conserved between *Drosophila*, *Xenopus* and mice

Modolell, J. (*CSIC, Univ. Autónoma Madrid*) Stepwise generation of positional information in the *Drosophila* imaginal discs. (*abstract* 11)

Mayor, R. (*Univ. Chile*) How is the limit of the neural tissue established in *Xenopus laevis?* (*abstract* 10)

Carrasco, A.E. (*Univ. Buenos Aires*) Regulatory aspects of anterior-posterior axis formation in *Xenopus laevis.* (*abstract* 9)

Westphal, H. (*NIH, Bethesda*) Factors that control neural development in the mouse. (*abstract* 12)

Sunday 17th, continued

Symposium 2 (Salón Plenario)

Protein-Ligand Interactions

Chair: Emilio Cardemil and Jack Preiss

Protein-ligand interactions are the first step in the development of most biochemical phenomena: enzyme catalysis, allosteric interactions, transport and receptor function. In this Symposium several examples of protein-ligand interactions will be presented, and special emphasis will be given to the techniques employed to analyse such interactions. The topics to be discussed will range from the role of particular amino acids involved in the protein-ligand interaction to the development of specific ligands for a particular target protein.

Bhagavan, N.V. (*Univ. Hawaii*) Exploring the structure of human serum albumin ligand binding sites using recombinant DNA techniques. (*abstract* 13)

Preiss, J. (*Michigan State Univ*) Adenosine diphosphate glucose pyrophosphorylase: mode of interaction with allosteric effectors. (*abstract* 17)

Ceccarelli, E. (*Univ. Rosario*) Involvement of the FAD binding domain amino acids on the function and stability of a FNR-like enzyme. (*abstract* 14)

Ferreira, S.T. (*Fed. Univ. Rio de Janeiro*) Hydrostatic pressure as a tool in the investigation of protein-ligand interactions. (*abstract* 15)

Pérez-Montfort, R. (*Univ. Autónoma México*) Using evolution for specific inhibition of triosephosphate isomerase from Trypanosomatidae. (*abstract* 16)

Symposium 3 (Salón Conguillío)

Molecular Adaptations of Archaea and Bacteria to their Environment

Chair: Carlos Jerez and Ricardo Amils

Micro-organisms respond to environmental changes by reorganizing their genetic expression. Several mechanisms employed to adapt to changes in osmolarity, heat shock, nutrient starvation and other stress conditions are well known in the domains Bacteria and Eukarya. Much less is known about adaptations in the domain Archaea. This group includes several extremophiles living under drastic conditions of salinity, temperature, anaerobiosis and pH. The study of the mechanisms employed by these species to adapt to their environment is interesting both from the basic view and possible biotechnological applications. The symposium will focus on the heat shock response and chaperones, phosphate starvation response, genomic organization and signal transduction systems both in Archaea and Bacteria.

Macario, A. (*Univ. Albany*) Heat-shock genes in Archaea. (*abstract* 21)

Jerez, C. (*Univ. Chile*) Response of the crenarchaeon *Sulfolobus acidocaldarius* to heat shock and phosphate starvation. (*abstract* 20)

Amils, R. (*CSIC, Univ. Autónoma Madrid*) Genomics and molecular biology of extreme halophilic archaea (haloarchaea). (*abstract* 18)

de Mendoza, D. (*Univ. Nac. Rosario*) The role of membrane fatty acid synthesis in *Bacillus subtilis* development. (*abstract* 19)

Symposium 4 (Salón Ranco)

Molecular Basis of Host-Parasite Relationship in Chagas Disease

Chair: Norbel Galanti and Bianca Zingales

This symposium will consider three aspects of the study of the protozoan *Trypanosoma cruzi*. 1) its genome organization compared with the mammalian host, which may be important because of the peculiar behavior of chromatin in Trypanosomatids during the cell cycle. 2) the identification of molecules which may be involved in the defense of the parasite against the immune system of the host, or of molecules which may play a role in the mechanism of invasion of the host by the parasite. 3) basic molecular biology techniques will be shown to define two major lineages in *T. cruzi*, which may represent subspecies of the genus.

Galanti, N. (*Univ. Chile*) Histone genes in Trypanosomatids: transcription and translation. (*abstract* 24)

Cazzulo, J.J. (*Fundación Campomar, Buenos Aires*) Structure and possible functions of cruzipain, the major cysteine proteinase from *Trypanosoma cruzi* . (*abstract* 22)

Frasch, A.C.C. (*Fundación Campomar, Buenos Aires*) *Trypanosoma cruzi* molecules involved in the invasion of the mammalian host . (*abstract* 23)

Zingales, B. (*Univ. Sao Paulo*) DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi*. (*abstract* 25)

14:30 - 16:30 Poster Session 1 (Poster Exhibition Room)

Chair: Emilio Cardemil and Octavio Monasterio

Bioenergetics, Biological Catalysis and Enzyme Regulation, Protein Structure, Microbial Biochemistry, Others.

Abstracts 117 - 288

17:00 - 19:30 Symposia 5-7

Symposium 5 (Salón Plenario)

Regulation of Protein Kinases Involved in Cell Division

Chair: Jorge Allende and J. Silvio Gutkind

Protein kinases are deeply involved in signal transduction pathways of messages that trigger cell division. For this reason it is important to study the mechanisms that regulate some of these enzymes. The symposium will examine some properties of several of these kinases in a variety of organisms from lower eukaryotes to mammalian cells. Aspects such as enzyme structure/function, substrate specificity, transcriptional and activity regulation during differentiation and cellular stress, and diverging pathways of signal transduction will be discussed.

Allende, J.E. (*Univ. Chile*) Studies on protein kinases CK1 and CK2. (*abstract* 26)

Pinna, L.A. (*Univ. Padova*) How do protein kinases recognize their targets? The lesson of protein kinase CK2. (*abstract* 29)

Gomes, S.L. (*Univ. Sao Paulo*) The genes encoding PKA subunits are coordinately induced during differentiation in *B. emersonii.* (*abstract* 27)

Téllez-Iñón, M. T. (*INGEBI, Buenos Aires. Univ. Buenos Aires*) Protein kinases and cell division in *Trypanosoma cruzi.* (*abstract* 30)

Gutkind, J.S. (*NIH, Bethesda*) Signaling from the membrane to the nucleus through small GTP-binding proteins acting on divergent MAP kinase cascades. (*abstract* 28)

Symposium 6 (Salón Casino)

Glycobiology: Intracellular Protein Glycosylation, Folding and Kinesis

Chair: Armando Parodi and Carlos Hirschberg

The symposium will deal with several aspects of protein and glycoproteins metabolism in the secretory and endocytic pathways: a) the mechanism of quality control of glycoprotein folding in the lumen of the endoplasmic reticulum (ER) by which only properly folded species migrate from that subcellular site to the Golgi apparatus; b) the transport of energy-rich compounds required for protein modification (ATP, PAPS, sugar nucleotides) from the cytosol to the lumen of the ER and Golgi, and c) the mechanism of vesicles-mediated intracellular transport of proteins and glycoproteins in the above mentioned pathways.

Parodi, A.J. (*Fundación Campomar, Buenos Aires*) The endoplasmic reticulum mechanism of sensing misfolded glycoprotein structures. (*abstract* 34)

Helenius, A. (*Yale Univ.*) Glycoprotein folding in the endoplasmic reticulum: the calnexin/calreticulin cycle. (*abstract* 31)

Hirschberg, C.B. (*Univ. Massachusetts*) The role of nucleotide transporters in posttranslational modifications in the endoplasmic reticulum and Golgi apparatus of mammals and yeast. (*abstract* 32)

Mayorga, L. (*Univ. Cuyo, Washington Univ.*) Vesicular transport from the perspective of endosome fusion. (*abstract* 33)

Symposium 7 (Salón Conguillío)

Cholinergic Macromolecules and Disease

Chair: Nibaldo Inestrosa and Francisco Dajas

Cholinergic macromolecules are involved in the synaptic transmission at the neuromuscular junction and at central cholinergic synapses. The acetylcholine receptor and acetylcholinesterase have been cloned and the 3-D structure is a subject of intense research. The enzyme is capable of interacting with the amyloid-β-peptide and facilitates the formation of amyloid fibrils, key elements in Alzheimer disease. The symposium will review recent advances in both acetylcholinesterase and the acetylcholine receptor with reference to specific diseases including myastenia gravis, congenital muscular dystrophy and Alzheimer disease.

Barrantes, F.J. (*Inst. Inv. Bioquim., Bahía Blanca*) The acetylcholine receptor ligand-gated channel as molecular target of disease and therapeutic agents. (*abstract* 35)

Taylor, P. (*Univ. California, San Diego*) Distinctions between acetylcholinesterase (AChE) and the nicotinic acetylcholine receptor (nAChR) in structure and regulation of gene expression. (*abstract* 38)

Dajas, F. (*Inst. Clemente Estable, Montevideo*) Functions of acetylcholinesterase in the central nervous system demonstrated by enzyme inhibition. (*abstract* 36)

Inestrosa, N.C. (*Pontificia Univ. Católica Chile*) Role of acetylcholinesterase in the formation of amyloid plaques of Alzheimer's brain. (*abstract* 37)

19:45 - 20:45 Plenary Lecture (Salón Plenario)

Chair: Jack Preiss

Dolphin, P.J. (*Dalhousie Univ*). Reverse cholesterol transport in human plasma: measurement and implications for atherosclerosis. (*abstract* 3)

Monday 18th

09:00 - 10:00 Osvaldo Cori Plenary Lecture (Salón Plenario)

Chair: Jorge E. Allende

Latorre, R. (*Univ. Chile, Centro Est. Cient. Santiago*). The molecular origin of voltage dependence in a Ca^{2+}-activated K^+ channel. (*abstract* 5)

10:30 - 13:00 Symposia 8-10

Symposium 8 (Salón Plenario)

Protein Folding

Chair: Octavio Monasterio and Gregorio Weber

The symposium will review the relationship between folding and oligomerization of proteins in order to understand their interactions with nucleic acids. Surface and secondary structures of proteins will be compared with emphasis in the folding homology between evolutionarily distant but related proteins. The folding pathway will be analyzed following 3-D structures of non native intermediates by a novel procedure. The thermodynamic principles and the influence of chaperonins and other factors on the bifurcation of the pathway will be discussed.

Jameson, D.M. (*Univ. Hawaii*) Steady-state and time-resolved fluorescence studies on *E. coli* ribosomal protein L7/L12: wild-type and site directed mutants. (*abstract* 41)

Silva, J.L. (*Univ. Federal Rio de Janeiro*) Energy linkage between protein folding and protein nucleic acid interactions. (*abstract* 42)

Andreu, JM. (*CIB, CSIC. Madrid*) Tubulin secondary structure and surface analysis suggests folding homology with the bacterial cell division protein FtsZ at the GTP binding site. (*abstract* 39)

Ermácora, M.R. (*Univ. Nacional Quilmes*) Equilibrium folding intermediates mapped by a chemical cleavage approach. (*abstract* 40)

Weber, G. (*Univ. Illinois*) Fluorescence, bifurcations, protein folding and the second law of thermodynamics. (*abstract* 43)

Symposium 9 (Salón Casino)

Biotechnology

Chair: Pablo Valenzuela and Isaías Raw

Burzio, L.O. (*Univ. Austral Chile*) The mussel glue proteins: a paradigmatic strategy of biological adhesion. (*abstract* 44)

Raw, I. (*Inst. Butantan, Sao Paulo*) Recovering a traditional institution through biotechnology. (*abstract* 46)

Penhoet, E.E. (*Chiron Corp. California*) Career opportunities for scientists in biotechnology: numerous & varied. (*abstract* 45)

Rutter, W.J. (*Chiron Corp. California*) Biotechnology and health care: themes and schemes. (*abstract* 47)

Symposium 10 (Salón Conguillío)

Membrane Systems Involved in Cell Calcium Signalling and Regulation

Chair: Cecilia Hidalgo and Alcides Rega

Calcium is a universal cellular second messenger. Due to its dual nature as signalling ion as well as a toxic element, cells maintain a very low intracellular resting calcium concentration. Only transient increments in cytoplasmic calcium concentration are allowed and prolonged elevations of cytoplasmic calcium are lethal to cells. The general topic of the symposium deals with the different cellular systems that control the resting calcium concentration in normal or pathological cells, and that modulate calcium release from intracellular compartments.

Rega, A.F. (*Univ. Buenos Aires*) Biochemical events in the calcium activated ATPase of plasma membranes ($PMCa^{2+}$ - ATPase). (*abstract* 52)

Beaugé, L. (*Inst. Mercedes y Martín Ferreyra, Córdoba*) Membrane components are involved in the ATP modulation of the Na^{+}-Ca^{2+} exchanger in the heart. (*abstract* 48)

Canessa, M. (*Univ. Chile*) Abnormalities of Ca^{2+} homeostasis in essential hypertension. (*abstract* 50)

Camacho, P. (*Univ. Virginia*) Calreticulin modulates calcium wave activity in *Xenopus laevis* oocytes. (*abstract* 49)

Hidalgo, C. (*Univ. Chile, Centro Est. Cient. Santiago*) Luminal regulation of calcium release from sarcoplasmic reticulum vesicles. (*abstract* 51)

14:30 - 16:30 Poster Session 2 (Poster Exhibition Room)

Chair: Juan Carlos Slebe and Juan Olate

Membranes, Lipids, Receptor-Hormones and Growth Factors, Signal Transduction, Cell Biology-Molecular Aspects

Abstracts 289 - 457

17:00 - 19:30 Symposia 11-14

Symposium 11 (Salón Conguillío)

Oxidative Stress

Chair: Federico Leighton and Rafael Radi

Oxidative stress, the imbalance among prooxidants and antioxidants in biological systems is considered today a necessary correlate of aerobic metabolism; it represses or modulates activity at the levels of signal transduction, gene expression and other cell functions. Reactive oxygen species induce chemical changes in nuclear and mitochondrial DNA and are involved in ageing, malignant transformation, proliferation and other phenomena. Progress in this area will be reviewed in the symposium.

Boveris, A. (*IQUIFIB, Univ. Buenos Aires*) Steady state concentration of superoxide and H_2O_2 in subcellular compartiments. Detection and values.

Radi, R. (*Univ. de la República, Montevideo*) The redox biochemistry of nitric oxide and peroxynitrite. (*abstract* 56)

Augusto, O. (*Univ. Sao Paulo, Univ. Stadual Campinas*) Possible roles of nitric oxide and peroxynitrite during murine leishmaniasis. (*abstract* 53)

Cadenas, E. (*Univ. Southern California, Los Angeles*) Mitochondrial DNA oxidative damage. Role of outer and inner membrane electron transfer activities. (*abstract* 54)

Leighton, F. (*Pontificia Univ. Católica Chile*) Natural polyphenols and their role as antioxidants in biological systems. (*abstract* 55)

Symposium 12 (Salón Plenario)

Signal Transduction

Chair: Héctor N. Torres and Günther Schulz

Olate, J. (*Univ. Concepción*) Signal transduction properties of a *Xenopus laevis* muscarinic receptor and adenylyl cyclase effector system. (*abstract* 57)

Schultz, G. (*Freie Univ. Berlin*) Selectivity and complexity of receptor-G-protein interaction. (*abstract* 58)

Torres, H. (*INGEBI, Univ. Buenos Aires, Buenos Aires*) Nitric oxide transduction pathway in *Trypanosoma cruzi*. (*abstract* 59)

Monday 18th, continued

Symposium 13 (Salón Casino)

Regulation of Enzyme Activity

Chair: Jorge Babul and Susana Passeron

The symposium will review: 1) regulation of enzymes by ligands, 2) energy-dependent covalent modification of enzymes, and 3) the special case of multicatalytic complexes. We will be concerned with a cascade of interconvertible enzymes, a mutant form of phosphofructokinase which mediates a futile cycle in *E. coli* strains, the phosphorylation of the 20S proteasome, a multicatalytic complex for the degradation of regulatory proteins, and with toxic metabolites that act as enzyme inhibitors in tissues of patients with inherited disorders.

Cárdenas, M.L. (*CNRS- Marseille*) Cascades of interconvertible enzymes as metabolic switches. Application to the glucose-induced switch between glycogen phosphorylase and glycogen synthase in the liver. (*abstract* 60)

Gulxé, V. (*Univ. Chile*) Lack of allosteric regulation of phosphofructokinase and its effects on gluconeogenic metabolism in *Escherichia coli*. (*abstract* 61)

Wajner, M. (*Univ. Fed.Rio Grande do Sul, Porto Alegre*) Inhibition of brain and liver succinate and β-dehydrogenase in methylmalonic acidemia: A model to study the effect of toxic metabolites on the intermediary metabolism. (*abstract* 63)

Passeron, S. (*Univ. Buenos Aires*) Phosphorylation of *Candida albicans* multicatalytic proteinase complex (20 S proteasome) by protein kinase CK. Evidence for a conserved process. (*abstract* 62)

Symposium 14 (Salón Ranco)

Extracellular Matrix-Cell Adhesion and Recognition

Chair: Enrique Brandan and Moisés Selman

The symposium will focus on the current understanding of the interaction between cells and the extracellular matrix, the mechanisms involved in signalling from the extracellular to the intracellular, the participation of extracellular matrix components on the presentation of growth factors to the cell, the interaction between extracellular matrix constituents and the cytoskeleton, the mechanisms involved in regulation of gene expression of matrix components and, finally, the participation of the extracellular matrix in apoptosis.

Brandan, E. (*Pontificia Univ. Católica Chile*) Proteoglycans: Essential extracellular matrix components during skeletal muscle differentiation. (*abstract* 64)

Brentani, R.R. (*Ludwig Inst. Cancer Res., Sao Paulo*) The α5β1 integrin is a hybrid cell surface chondroitin-heparan sulfate proteoglycan. (*abstract* 65)

Carey, D.J. (*Weis Center Res., Danville*) Structure and function of Schwann cell extracellular matrix. (*abstract* 66)

Cancela, E. (*Univ. Algarve - UCTRA, Portugal*) Expression of two vitamin K dependent proteins of the extracellular matrix during early development of the teleost fish Sparus aurata.

Selman, M. (Inst. Nac. Enf. Resp. México). Epithelial apoptosis, fibroblast proliferation and matrix metabolism during chronic lung injury. (*abstract* 67)

19:45 - 20:45 Plenary Lecture (Salón Plenario)

Chair: Antonio Peña

Dreyfus. G. (*Univ. Autónoma México*). The bacterial flagellum: structure, function and genetics. (*abstract* 4)

Tuesday 19th

9:00 - 10:00 Plenary Lecture (Salón Plenario)

Chair: Tito Ureta

Whelan, W.J. (*Univ. Miami*). The biogenesis of glycogen and other storage polysaccharides. (*abstract* 7)

Tuesday 19th, continued

10:30 - 13:00 Symposia 15 - 18

Symposium 15 (Salón Casino)

Metabolic Organization

Chair: Tito Ureta and Athel Cornish-Bowden

The cytoplasm is a highly concentrated anisotropic solution in which enzymes catalyzing reactions of a pathway are close to each other by: a) protein-protein associations as in multienzyme complexes; b) membrane-protein interactions; c) protein associations to the cytoskeleton. This view of the cytoplasm results in the concepts of metabolic compartments and channelling and a new level for metabolic control from reversible binding and/or translocation of enzymes to different sites of the cell. The symposium will review the concepts of cytosol, the cellular location of enzymes, the consequences of enzyme associations for the theory of channelling and flux control and the evolution of compartmentation.

Clegg, J.S. (*Univ. California Davis*) Metabolic organization in the interorganelle cytoplasm of eukaryotic cells. (*abstract* 68)

Preller, A. (*Univ. Chile*) Glycogen synthesis in amphibian oocytes as a model system for the study of metabolic organization. (*abstract* 70)

Cornish-Bowden, A. (*CNRS-Marseille*) Kinetic implications of channelling between consecutive enzymes in metabolic pathways. (*abstract* 69)

Ureta, T. (*Univ. Chile*) Evolutionary thougts about the organization of enzymes *in vivo.* (*abstract* 71)

Symposium 16 (Salón Ranco)

Novel Aspects on the Biodegradation of Natural and Man-made Aromatic Compounds

Chair: Rafael Vicuña and Dietmar Pieper

Aromatic compounds are widely spread in the environment. Some are continually being replenished by organisms, whereas others were biosynthesized in the past and are found now as fossil fuels. In addition, several chlorinated aromatics are released to the biosphere as a result of industrial development. Fungi and bacteria play a major role in the biodegradation of aromatic structures and have evolved highly versatile pathways, aerobic or anaerobic, to cleave rings and remove halogens. Fungi can mineralize lignin, which is an abundant deposit of organic carbon on earth. The symposiumwill discuss recent advances in this field..

Pieper, D.H. (*Nat.Res.Center Biotechnol, Braunschweig*) Microbial degradation of haloaromatic compounds: on the necessity to develop biocatalysts with complete metabolic pathways. (*abstract* 74)

González, B. (*Pontificia Univ. Católica Chile*) Expression of the chlorophenol-degrading ability of *Alcaligenes eutrophus* JMP134 (pPJ4) in a complex system. (*abstract* 73)

Tiedje, J. (*Michigan State Univ*) Diversity in chlororespiration. (*abstract* 75)

Cullen, D. (*Forest Products Lab., Madison*) Recent advances in the molecular genetics of the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* (*abstract* 72)

Symposium 17 (Salón Conguillío)

Metabolic Regulation in Yeasts

Chair: Antonio Peña and Pedro Moradas-Ferreira

Fraenkel, D.G. (*Harvard Univ*) Yeast glycolysis with lowered enzyme levels. (*abstract* 76)

Lagunas, R. (*CSIC Madrid*) Control of the yeast sugar transport. (*abstract* 77)

Moradas-Ferreira, P. (*Univ. Porto*) Expression of *Kluyveromyces marxinaus GAP*1 gene encoding a surface protein associated with cell adhesion. (*abstract* 78)

Peña, A. (*Univ. Autónoma México*) Changes, effects and regulation of cytoplasmic and vacuolar internal pH of yeast. (*abstract* 79)

Tuesday 19th, continued

Symposium 18 (Salón Plenario)

Signal Transduction and Regulation of Gene Expression in Plants

Chair: Loreto Holuigue and Luis Herrera-Estrella

Light, pathogens and wounding are the most important environmental signals that modulate gene expression in plants. Recently, important advances have been reported about the mechanisms of signal perception, intracellular transduction and gene activation. The symposium will focus on the genetic, molecular and biochemical approaches that are currently being used to identify components of the signal transduction pathways and to elucidate events occurring at DNA level that control gene expression.

Bowler, C., (*Stazione Zool, Naples*) Transduction of light signals in plants. (*abstract* 80)

Herrera-Estrella, L. (*Centro Inv. Est. Avanz. México*) Identification of minimal photoresponsive elements and signal transduction to activate them. (*abstract* 81)

de Oliveira, D.E. (*Univ. Federal Rio de Janeiro*) Identification of genes induced in earlier steps of the hypersensitive response in *Arabidopsis thaliana*. (*abstract* 83)

Holuigue, L. (*Pontificia Univ. Católica Chile*) Mechanism of transcriptional activation mediated by salicylic acid in to bacco. (*abstract* 82)

Peña-Cortés, H. (*Inst. Genbio. Forschung, Berlin*) Wound-induced gene expression in plants and signals involved in its regulation. (*abstract* 84)

Wednesday 20th

9:00 - 10:00 Plenary Lecture (Salón Plenario)

Chair: Manuel Krauskopf

Martial, J. (*Univ. Liége*) Molecular mechanism of the interaction between the prolactins and their receptors. (*abstract* 8)

10:30 - 13:00 Symposia 19 - 22

Symposium 19 (Salón Plenario)

Enzyme Structure and Function

Chair: Juan Carlos Slebe and Marino Martínez Carrión

The symposium will present current developments in protein chemistry that contribute to our understanding of enzymes, including genetic, structural, spectroscopic and analytical tools, as well as traditional methods. Features characteristic of the family of allosteric enzymes will be described. New insights to questions such as: Why do cells build oligomeric proteins? How proteins fold? How proteins interact together to perform their biological function? will be presented. Specific aspects of recognition of preproteins for mitochondrial import also will be discussed.

Slebe, J.C. (*Univ. Austral Chile*) Residues at subunit interfaces of mammalian fructose-1,6-bisphosphatase: effects on the regulatory properties. (*abstract* 87)

Wolosiuk, R.A. (*Fundación Campomar, Buenos Aires Univ. Buenos Aires*) The concerted action of thiol/disulfide ex change and noncovalent interactions in the modulation of chloroplast fructose-1,6- bisphosphatase. (*abstract* 88)

Nowak, T. (*Univ. Notre Dame, Indiana*) The modulation of catalysis by the allosteric pyruvate kinase. (*abstract* 86)

Martinez-Carrión, M. (*Univ. Missouri*) Protein structure and function in biological environments: needles in haystacks? (*abstract* 85)

Wednesday 20th, continued

Symposium 20 (Salón Conguillío)

Molecular Mechanisms of Viral Pathogenesis

Chair: Eugenio Spencer and Marshall Horwitz

Animal viruses have developed several strategies to overcome the host response against infection. Some are able to modified the genes coding for the viral surface proteins increasing their hability to infect permissive cells. Other viruses have developed alternative systems that involve the synthesis of genes products that alter the overall protective response of the host, while others may change their replication cycle. Virulence may be defined as a settle between the viral efficient use of the host and host antiviral response. Each of the four viruses considered in the symposium represents an example of one of the above mechanisms that may account for the pathogenesis of the viruses

Patton, J.T. (*NIH, Bethesda*) Role of the viral gene products in rotavirus replication. (*abstract* 91)

Romanowski, V. (*Univ. Nac. La Plata, Univ. Nac. Quilmes*) Basic and applied molecular genetics of arenaviruses. (*abstract* 92)

Lamas, E. (*Inst. Pasteur, París*) Hepatitis c virus (HCV): implications of genetic variability in HCV persistence and associated liver cancer. (*abstract* 90)

Horwitz, M.S. (*Albert Einstein Coll. Med., New York*) Adenovirus e3 immunoregulatory proteins: functions and interactions with cellular proteins. (*abstract* 89)

Symposium 21 (Salón Casino)

Hormone Receptors

Chair: Ricardo Boland and Ralph A. Bradshaw

Peptide and amine hormones interact with target cells with G protein-linked receptors containing seven transmembrane domains. Cell-surface receptors for growth factors have tyrosine kinase activity and are not coupled to G proteins. Classically, the actions of steroids are thought to be exclusively mediated by cytoplasmic/nuclear receptors. The symposium will review the characteristics and G protein interactions of gonad melatonin receptors. Also, advances on the molecular basis of transmembrane (vasopressin) and growth factor receptor activity regulation will be discussed. In addition, a novel non-genomic mechanism of steroid hormone (vitamin D) action involving stimulation of second messenger pathways will be described.

Valladares, L. (*Univ. Chile*) Melatonin and physiological function: characterization of receptors in testis. (*abstract* 96)

Birnbaumer, M. (*Univ. California, Los Angeles*) Phosphorylation of the human V2 vasopressin receptor. (*abstract* 93)

Bradshaw, R.A. (*Univ. California Irvine*) Growth factor mediated-signal transduction in PC12 cells: studies with chimeric receptors and mutant cell lines. (*abstract* 95)

Boland, R. (*Univ. del Sur Bahía Blanca*) Non-genomic signal transduction pathways of 1,25-dihydroxy-Vitamin D_3. (*abstract* 94)

Symposium 22 (Salón Ranco)

Neurobiochemistry: Molecular Cell Biology of Neuronal Cell Differentiation, Growth, Degeneration and Death

Chair: Hugo Maccioni and Ricardo Tapia

The symposium will provide an overview of advances on the cellular and molecular basis of differentiation, growth, degeneration and death of neural cells. The control of the expression of cell surface gangliosides during differentiation, the influence of extracellular signals in controlling apoptosis and the expression of receptor systems for growth factors and their role in the survival of neurons will be examined in neural retinal cells. The effect of drugs that stimulate glutamate release or inhibit glutamate transport on hyperexcitation/neurodegeneration, and the relationship between excitatory aminoacids and brain adaptive changes in response to damage in dopaminergic pathways will be discussed.

Maccioni, H.J.F. (*Univ. Nacional Córdoba*) Compartmentation of ganglioside synthesis in the Golgi complex of neural cells. (*abstract* 100)

Linden, R. (*Univ. Federal Rio de Janeiro*) Mechanisms of apoptosis in the developing retina. (*abstract* 99)

Wednesday 20th, continued

Arruti, C. (*Univ. de la República, Montevideo*) Involvement of FGF-2 in the survival of chick embryo neural retina cells. (*abstract* 97)

Tapia, R. (*Univ. Autónoma México*) Hyperexcitation and neurodegeneration mediated by glutamate receptors in vivo and in cell cultures. (*abstract* 101)

Bustos, G. (*Pontificia Univ. Católica Chile*) Excitatory amino acids and its relationship with adaptative changes in the brain in response to degeneration of central dopamine neurons. (*abstract* 98)

14:30 - 16:30 Poster Session 3 (Poster Exhibition Room)

Chair: Rosalba Lagos and Rafael Vicuña

Molecular Biology, Plant Biochemistry, Biotechnology

Abstracts 458 - 619

17:00 - 19:30 Symposia 23 - 25

Symposium 23 (Salón Conguillío)

Molecular Genetics of Inherited Disorders

Chair: Pilar Carvallo and José M. Cantú

Molecular Genetics has greatly evolved, especially since the Human Genome Project started in 1988. The positional cloning strategy has had a great effect in our knowledge about the localization, structure and function of human genes, especially those related to human disorders. There is now a high number of genes responsible for diverse pathologies in which mutations have been described. The symposium will focus on the molecular bases of different inherited disorders and will cover the analysis of mutations related to different gene functions, as well as mitochondrial DNA mutations in relation to the ageing process and age associated diseases.

Cantú, J.M. (*Univ. Guadalajara, Jalisco*) Molecular diagnosis and mapping in genetical medicine. (*abstract* 102)

Carvallo, P. (*Univ. Chile*) Isolated growth hormone deficiency: from deletions to point mutations. (*abstract* 103)

Vainzof, M. (*Univ. Sao Paulo*) Molecular biology enhancing our understanding and improving the prevention of hereditary myopathies in the brazilian population. (*abstract* 105)

Linnane, A.W. (*Monash Univ., Australia*) Mitochondrial DNA mutation and segregation during the ageing process. (*abstract* 104)

Symposium 24 (Salón Casino)

The Private Life of Ion Channels

Chair: Ramón Latorre and Enrico Stefani

The symposium is concerned about a class of ubiquitous integral membrane proteins denominated ion channels. Because of their immense diversity, we will perforce concentrate in a few aspects that include: the conformational changes a calcium channel undergoes when confronted to a change in transmembrane voltage; the use of toxins in unveiling the structure of calcium channels; we will also discuss the molecular biology of sodium channels involved in sodium reabsorption; finally, we will discuss how ion channels are involved in keeping our intestines in good shape.

Stefani, E. (*Univ. California, Los Angeles*) Charge movement and pore opening in cloned calcium channels: effects of regulatory â subunits. (*abstract* 109)

Wednesday 20th, continued

Cassola, A.C. (*Univ. Sao Paulo*) Peptide toxins designed for blocking high voltage-activated Ca^{2+} channels. (*abstract* 107)

Canessa, C. (*Yale Univ*) The epithelial sodium channel and its role in hypertension. (*abstract* 106)

Sepúlveda, F.V. (*Univ. Chile, Centro Est. Cient. Santiago*) Modulation of swelling-activated Cl^- channels by Cl^-. (*abstract* 108)

Symposium 25 (Salón Plenario)

Regulation of Transcription

Chair: Alberto Kornblihtt and Malcolm J. Low

Progress on the study of molecular mechanisms underlying the function and regulation of transcription has advanced greatly. Our knowledge of the physiology of gene regulation will ultimately require the precise definition of the multimolecular processes built up on a given gene's DNA and RNA, by well known and still unknown factors. The symposium addresses molecular studies on gene expression of the key enzymes of protein synthesis (amino-acyl tRNA synthetases) in a prokaryote, as well as the regulation of vertebrate genes whose products are essential for positioning cells in place (fibronectin), neuroendocrine control (pro-opiomelanocortin) and analgesia (β-endorphin).

Orellana, O. (*Univ. Chile*) Transcriptional analysis of the tyrosyl tRNA synthetase and ribosomal RNA genes from *Thiobacillus ferrooxidans.* (*abstract* 112)

Kornblihtt, A.R. (*INGEBI, Univ. Buenos Aires*) Transcriptional regulation of the fibronectin gene. (*abstract* 110)

Low, M.J. (*Oregon Health Sci. Univ*) Neuroendocrine cell-specific expression and hormonal regulation of the proopiomelanocortin gene in transgenic mice and a novel pituitary melanotroph cell line. (*abstract* 111)

Rubinstein, M. (*INGEBI, Univ. Buenos Aires*) β-endorphin "knock-out" mice produced by targeted mutagenesis in embryonic stem cells. (*abstract* 113)

Thursday 21th

9:00 - 11:30 Symposium 26

Symposium 26 (Salón Plenario)

Biochemical Education: Towards Excellence in Pan-American Ph.D. Programs

Chair: Manuel Krauskopf and Rogerio Meneghini

Krauskopf, M. (*Univ. Austral Chile*) Scientific profiles and capabilities of the faculty involved in Ph.D. programs in Chile. (*abstract* 114)

Lennarz, W.J. (*State Univ. New York, Stony Brook*) Problems and trends in graduate education in the biomedical sci ences in the United States. (*abstract* 115)

Meneghini, R. (*Univ. Sao Paulo*) Collaborative work, a way of improving the scientific production and the quality of the graduate programs in Brazil. (*abstract* 116)

12:00 - 12:15 Closing Ceremony (Salón Plenario)

12:15 - 13:15 Plenary Closing Lecture (Salón Plenario)

Chair: Jorge Babul

de Meis, L. (*Univ. Fed. Rio de Janeiro*). The channel function of the different Ca^{2+}-transport ATPase isoform. (*abstract* 2)

Technical Lectures

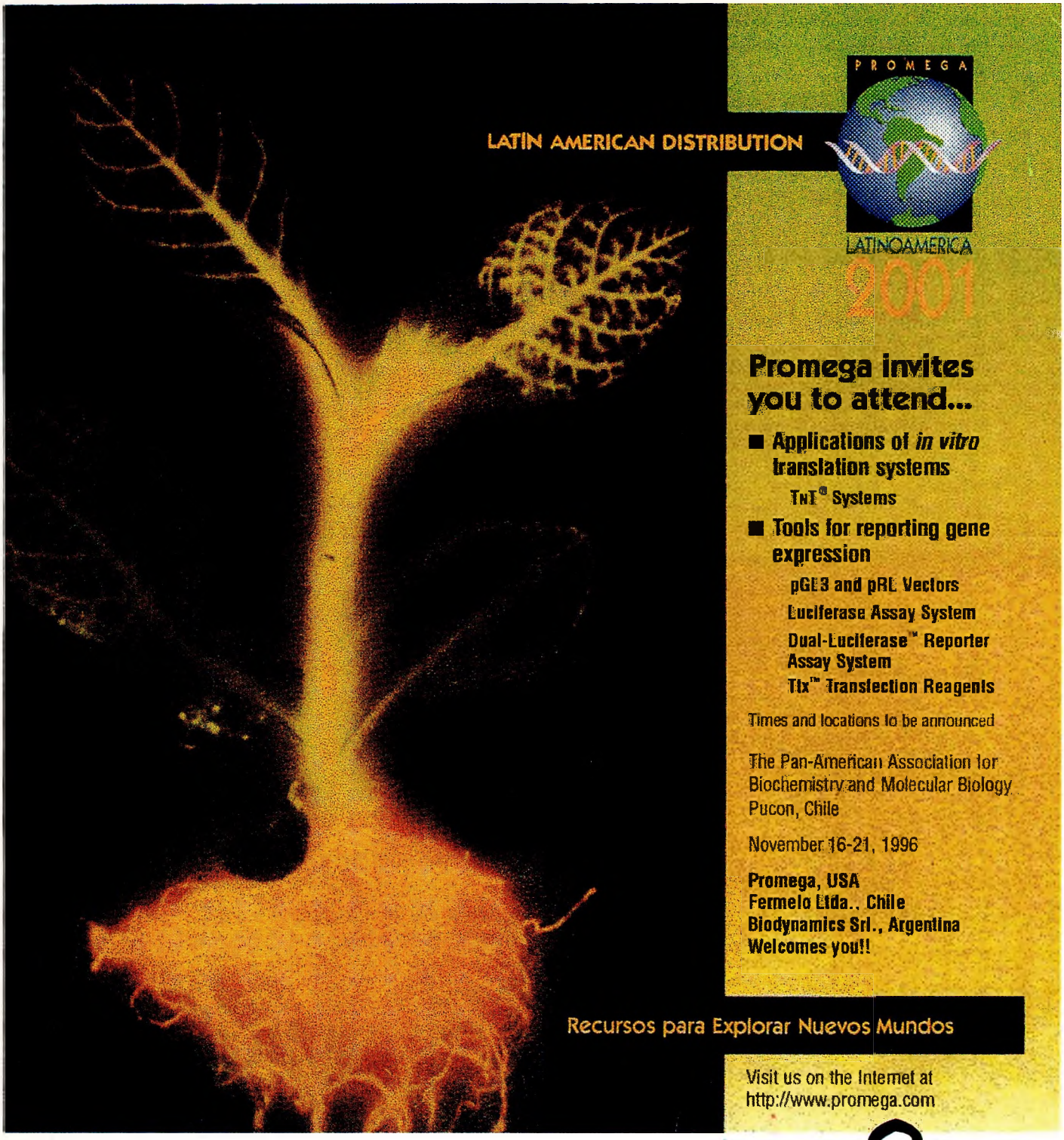
Salón Casino

Monday 18th

14:30 - 15:00	Scott Lesly *(Promega Co.)* **Applications of *in vitro* translation systems**
15:10 - 15:40	Jaime Nunes de Souza *(Life Technologies)* **Advances in RT-PCR and cloning PCR products**
15:50 - 16:20	Claudio Vilariño *(Pharmacia)* **Molecular DNA analysis**

Wednesday 20th

14:30 - 15:00	Scott Lesly *(Promega Co.)* **Applications of *in vitro* translation systems**
15:10 - 15:40	Jaime Nunes de Souza *(Life Technologies)* **Advances in RT-PCR and cloning PCR products**
15:50 - 16:20	Claudio Vilariño *(Pharmacia)* **Molecular DNA analysis**



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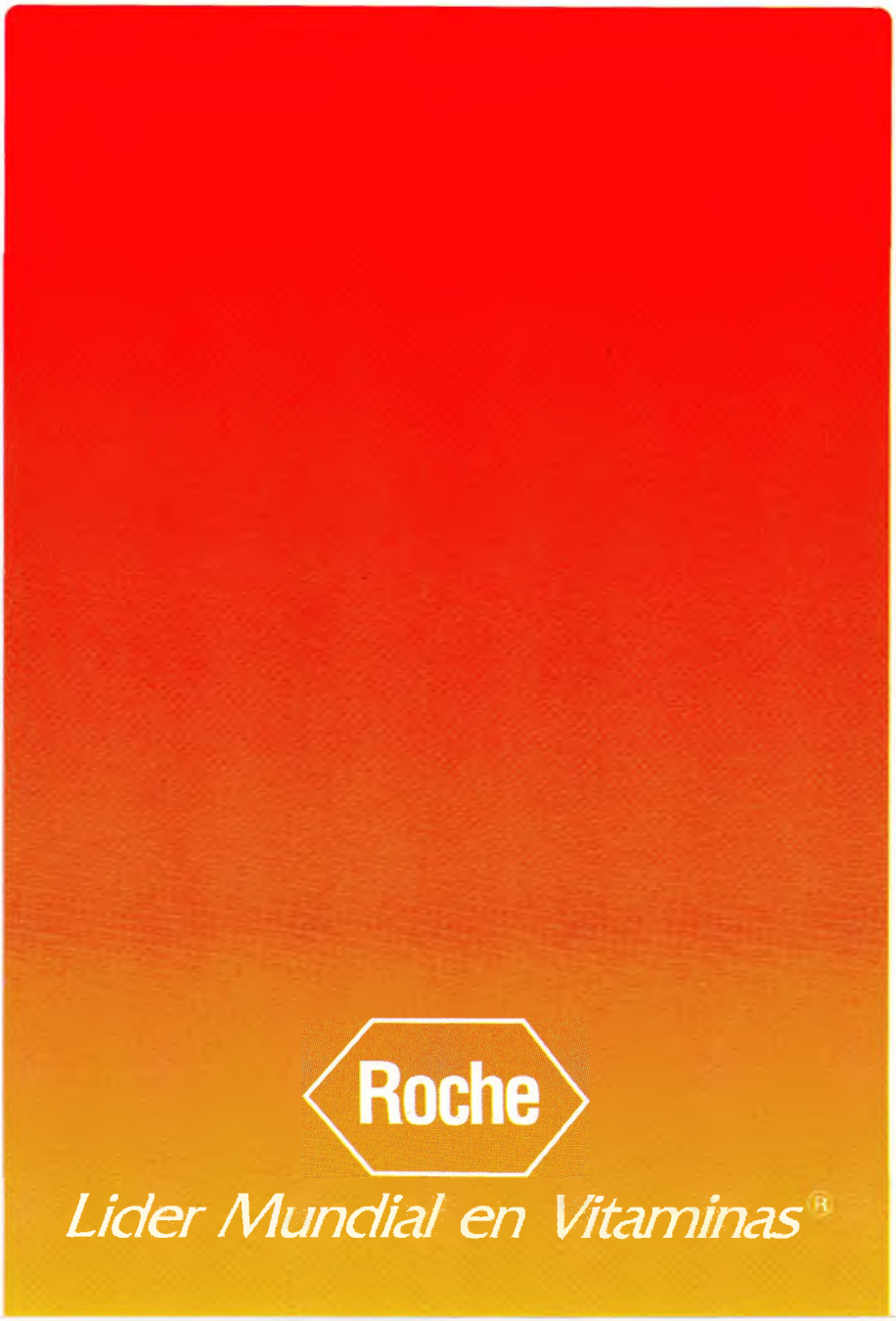

Plenary Lectures

1

THE PLANT-TYPE FERREDOXIN-NADP$^+$ REDUCTASE FAMILY. SOMETHING OLD, SOMETHING NEW SOMETHING BORROWED. Carrillo, N. PROMUBIE, Biochemistry Faculty. University of Rosario, Suipacha 531. 2000 Rosario, ARGENTINA. e-mail: carrill@unrobi.edu.ar.

Ferredoxin-NADP$^+$ reductases (FNR) are FAD-containing enzymes that catalyze electron transfer between NADP(H) and electron carrier proteins (ferre- or flavodoxin). FNR isoforms are found in bacteria, mitochondria and chloroplasts, in which they participate in a variety of redox metabolic pathways. Plant FNRs were isolated forty years ago, and their biochemistry and function thoroughly studied during the 60' and 70'. However, research on these enzymes gained *momentum* in recent years, particularly after determination of the atomic structure of a plant FNR at high resolution, the characterization of cDNA and genomic clones encoding FNRs from plants, vertebrates, algae and bacteria, and the resulting expression of functional reductases in microorganisms like *Escherichia coli*. These achievements opened the route to probe FNR function and structure using site-directed mutagenesis, and allowed establishment of experimental setups to investigate, under defined conditions, the molecular mechanisms underlying biogenesis, intracellular sorting, folding and holoprotein assembly of this ubiquitous flavoenzyme.

An unexpected development of this research was the recognition of the identity between *E. coli* FNR and a known *locus* conferring resistance to oxygen radical toxicity. Since then, the key role played by eucaryotic reductases in detoxification of active oxygen species was established on firm grounds, providing insights into the target(s) and mechanism of FNR scavenging activity. *In toto*, ferredoxin reductases display several outstanding properties, that make them excellent model proteins to address broad biological questions.

2

THE CHANNEL FUNCTION OF THE DIFFERENT Ca^{2+}-TRANSPORT ATPase ISOFORMS. Leopoldo de Meis, Inst.Ciencias Biomedicas, Dept. Bioquimica, Universidade Federal Rio de Janeiro, Rio de Janeiro, Brazil.

The Ca^{2+} transport ATPase from sarcoplasmic reticulum can be reversed and mediates an efflux of Ca^{2+} coupled to ATP synthesis. Recently, it has been shown that efflux of Ca^{2+} can be uncoupled from the synthesis of ATP. The rate of this efflux is increased several folds and the ATPase operates as if it were a Ca^{2+} channel when drugs that interact with the enzyme, such as phenothiazines, local anaesthetics, the fatty acids arachidonic and arachidic acid and heparin or other glycosaminoglycans are added to the medium. The effects of these drugs is antagonized by the ATPase ligands Ca^{2+}, Mg^{2+}, K^+ and the highly specific inhibitor of the Ca^{2+} ATPase thapsigargin.

Heat is released when Ca^{2+} leaks through the ATPase. The ratio between heat released and ATP hydrolyzed (ΔH^{cal}) in intact vesicles, which are able to form a transmembrane Ca^{2+} gradient, is higher than that found in leaky vesicles. The ΔH^{cal} of ATP hydrolysis becomes more negative in presence of drugs that uncouple the Ca^{2+} ATPase and conversely, is less negative whe drugs than increase the coupling of the ATPase, such as dimethyl sulfoxide, are added to the medium.

The channel function of the sarcoplasmic reticulum ATPase provides a new putative pathway by which Ca^{2+} could be released within the cell. The reversal of the Ca^{2+} pump and the effects of uncoupling drugs has also been caracterized for the Ca^{2+} ATPases of human platelets and rat brain.

3

REVERSE CHOLESTEROL TRANSPORT IN HUMAN PLASMA: MEASUREMENT AND IMPLICATIONS FOR ATHEROSCLEROSIS. Dolphin, P. J., *Guérin, M., and *Chapman, M. J. Department of Biochemistry, Dalhousie University, Halifax, Canada. (E-Mail: pdolphin@is.dal.ca) and *INSERM Unité 321, Hôpital de la Pitié, Paris, France.

The incidence & severity of atherosclerosis is positively correlated with the level of plasma Low Density Lipoprotein (LDL) cholesterol & is inversely correlated with plasma High Density Lipoprotein (HDL) levels. The protective role of HDL is thought to result from its ability to remove cholesterol from peripheral tissues. Cell-derived cholesterol in HDL is esterified by plasma lecithin:cholesterol acyltransferase & subsequently moved by the Cholesteryl Ester Transfer Protein (CETP) to plasma Very Low Density Lipoprotein (VLDL) & LDL in exchange for triacyl-glycerols. Subsequent uptake of VLDL remnants & LDL by the liver completes the "Reverse Cholesterol Transport" pathway. Humans with dysfunctional CETP have high plasma HDL & are resistant to atherosclerosis. We developed a new method for the simultaneous measurement of the rate of cholesteryl ester (CE) mass transfer & rate of exchange between HDL, VLDL & LDL catalyzed by CETP in human plasma. This method was utilized to study CE transfer in the plasmas of normolipidemic (N), mildly hypertriglyceridemic (HTG), heterozygous Familial Hypercholesterolemic (FH) & Combined Hyperlipidemic (CH) subjects before & after drug therapy. In N & FH subjects LDL is quantitatively the major acceptor of HDL-CE. VLDL is qualitatively a better acceptor of HDL-CE than LDL & is quantitatively the major acceptor in HTG & CH subjects where there was no net mass transfer of HDL-CE to LDL. HMG Co-A Reductase inhibitors lower LDL levels & HDL-CE transfer in FH subjects but do not normalize the LDL profile by decreasing the relative proportion of the denser, more atherogenic LDL particles. Fenofibrate treatment of CH subjects reduced plasma VLDL & CETP-dependent transfer of CE & also normalized the LDL profile by reducing the proportion of denser LDL particles.

4

THE BACTERIAL FLAGELLUM: STRUCTURE, FUNCTION AND GENETICS. Dreyfus, G[1]. and Camarena, L.[2]. [1]Instituto de Fisiología Celular and [2]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México. México D.F. E-mail: gdreyfus@ifcsun1.ifisiol.unam.mx

Most bacteria move about their environment by means of the motility organelle known as flagellum. This organelle spans the cells envelopes and is composed of a filament, hook and basal body. It responds to chemotactic stimuli through a complex array of signals. The number and disposition of the organelle varies with bacterial species. They can be either randomly distributed (peritricous flagellation), polar or laterally inserted. It is a highly organized structure that is genetically controlled by more than 40 genes in enteric bacteria. Functional homology between almost all the corresponding genes in *E. coli* and *S. typhimurium* has been demonstrated. Whereas this has not been studied in detail for other bacterial species.

We are particularly interested in the photosynthetic bacterium *Rhodobacter sphaeroides* which contains a single medial flagellum with unique kinetic and structural properties. Studies on the structure and genetics of the flagellum of this phototrophic bacterium are revealing interesting differences with other motile bacteria. *Rhodobacter sphaeroides* is a monoflagellated bacterium that rotates its single flagellum in one direction to either swim or tumble. Using transposon mutagenesis we are identifying a group of genes involved in the process of morphogenesis and rotation of the flagellum. Structural genes have also been identified. We are also carrying studies on the structure of the filament i.e. secondary structure of polymeric and monomeric flagellin.

5

THE MOLECULAR ORIGIN OF VOLTAGE DEPENDENCE IN A Ca^{2+}-ACTIVATED K^+ CHANNEL. Latorre, R. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Centro de Estudios Científicos de Santiago, Santiago, Chile.

Excitable membranes play a fundamental role in the generation, transmission and transduction of information in the nervous system. The generation of an action potential, a fast transient change in the membrane potential of the cell, is produced by the increase in permeability of ion-selective channels. Changes in membrane permeability are driven by changes in membrane potential and this is the fundamental property of voltage dependent channels. In 1952 Hodgkin and Huxley (*J. Physiol.* 117, 500-544) proposed that the voltage dependence of the ion permeability is the orientation of charges within the membrane which gate the ion channel open or closed in response to changes in the electric field. Cloning of voltage dependent potassium channels showed that these intrinsic membrane proteins possess a peculiar sequence in the fourth putative transmembrane domain (S4). The sequence consist of four to seven basic residues, arginines and lysines separated by hydrophobic amino acids. This sequence has been shown to be the voltage sensor postulated by Hodgkin and Huxley. Calcium- and voltage-dependent K^+ (K_{Ca}) channels seem to have an intrinsic voltage sensor and to be an evolutionary bridge embracing mixed properties of voltage- and ligand-gated ion channels. In this conference, I will discuss the structural elements involved in the Ca^{2+} and voltage dependent opening of cloned K_{Ca} channels combining molecular-genetic and biophysical methods. I will show that Ca^{2+} appears to be acting by decreasing the energy necessary to displace the S4 segment in the electric field. Supported by FONDECYT, Cátedra Presidencial, and NIH.

6

cAMP-DEPENDENT PROTEIN KINASE: STRUCTURAL INSIGHTS INTO STRUCTURE AND FUNCTION.

Susan S. Taylor
Department of Chemistry & Biochemistry. University of California, San Diego. La Jolla, CA 92093-0654.

cAMP-dependent protein kinase (PKA), one of the simplest members of the protein kinase family serves as a template for the entire family. The crystal structure of the catalytic subunit revealed the general fold of the highly conserved catalytic core. The active site is located in a cleft between two lobes with Thr197, an essential phosphorylation site, located in an activation loop at the edge of the cleft. Crystal structures of PKA with various nucleotides, peptides, and inhibitors reveal a range of open and closed conformations. Kinetic analysis shows that the phosphoryl transfer step is fast (500/s). The rate limiting step is much slower (20/s) and is associated with product release and opening of the cleft. The networking required for peptide binding, nucleotide binding, and the associated conformational changes is extensive.

Both the regulatory (R) subunits and PKI bind to C in a bipartite manner. They share a common inhibitor site that binds at the cleft interface but use different surfaces of C to bind tightly. The complementary docking surfaces on C and RI have been mapped in detail based on the crystal structures of both proteins. The RI structure also defines the two cAMP binding sites and provides a basis for understanding how the cooperative binding of cAMP causes activation of PKA. The inhibitors also contribute to subcellular localization. The R-subunit docks to anchoring proteins while PKI has a nuclear export signal (NES).

(Supported by grants from the NIH and from the American Cancer Society).

7

THE BIOGENESIS OF GLYCOGEN AND OTHER STORAGE POLYSACCHARIDES. W.J. Whelan. J. Lomako and W.M. Lomako, Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, Florida, USA, e-mail: wwhelan@mednet.med.miami.edu

Glycogen synthesis is initiated on an autocatalytic, self-glucosylating primer, glycogenin, which constructs a maltosaccharide chain on its Tyr-194, from which the glycogen molecule is built by glycogen synthase and branching enzyme. A study of this system has led to the discovery (or rediscovery) of a stable intermediate in glycogen synthesis and degradation, which we have named proglycogen (Mr 400 kDa).

The proportion of proglycogen to mature glycogen varies widely from liver to muscle to heart. There is the potential greatly to increase the glycogen content of muscle and heart by causing the conversion of proglycogen into mature glycogen. This would be of importance in exercise physiology.

Glycogenin has been cloned and expressed by Roach and by ourselves. The human gene for glycogenin has been mapped to chromosome 3, band q24. The relation of glycogenin function to the aberration of glycogen metabolism in Type II diabetes is under investigation.

Priming of storage polysaccharide synthesis by an autocatalytic protein may be a general phenomenon. From corn we have isolated, sequenced and expressed another autocatalytic, self-glucosylating protein, amylogenin. Although its properties are similar to glycogenin there is no sequence homology.

Supported by NIH grant DK 37500.

8

BETWEEN THE PROLACTINS AND THEIR RECEPTORS.

Joseph Martial[1], Sandrina Kinet[1], Paul Kelly[2] and Vincent Goffin[1,2]
[1] Laboratoire de Biologie Moléculaire et de Génie Génétique, Université de Liège, B-4000 Sart Tilman, Belgium.
[2] Laboratoire d'Endocrinologie Moléculaire (INSERM U 344), Hôpital Necker, F-75730 Paris, France.

Prolactin (PRL) is a pituitary hormone involved in many different activities mainly related to lactation, reproduction, osmoregulation and immunoregulation. The first step of the mechanism that will lead to expression of PRL bioactivities is the interaction of the hormone with its receptor (PRL-R), called lactogenic receptor which belongs to the superfamily of cytokine receptors.

Our recent work aimed at identifying the aminoacids of the hormone involved in its specific interaction with the lactogenic receptor.

Using a hPRL model built on the basis of the crystallographic coordinates of pig growth hormone, we selected a series of residues belonging to the theoretical binding site1 (helix1, helix 4 and loop 1) and binding site 2 (helix 1 and helix 3) of hPRL. These residues were selectively mutated one by one, the mutants produced in E.coli, purified and assayed in two biological tests based on the Nb2 cells (rat lymphoid cells). Recently, an additional test using the homologous human lactogenic receptor was developed. The results allow us to identify the residues important for the biological activity of the hormone and to propose a complete mechanism for the interaction between the PRL's and their receptors.

Symposia

Symposium 1 - **From Egg to Embryonic Nervous System: A Molecular Analysis**

9

REGULATORY ASPECTS OF ANTERIOR-POSTERIOR AXIS FORMATION IN *XENOPUS laevis*. Carrasco. A. E. Instituto de Biología Celular y Neurociencias, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina. (e-mail: rqcarras@criba.edu.ar)

We have isolated *Xenopus laevis sonic hedgehog (X-shh)* from a cDNA library. It is initially transcribed in the dorsal blastopore lip and later becomes strongly expressed in the axial midline structures (floor plate, notochord and hypochord), the Rathke's pouch, the posterior-ventral region of the visceral arches and ventral regions of the brain, except the infundibulum. A specific antibody found the endogenous protein in the notochord and the cement gland. The latter is devoid of *shh* trancripts, suggesting that the protein has migrated from a different site.

Treatment of *Xenopus laevis* embryos with 10 μM all-trans retinoic acid at the beginning of gastrulation increased the expression of *X-shh* in the posterior notochord and reduced it in the anterior floor plate. In contrast, 7.5 μM of the retinoid antagonist Ro 41-5253 decreased *X-shh* expression in the posterior notochord. These results indicate that endogenous retinoids regulate the spatial expression of *X-shh* in the caudal domain.

Overexpression of chicken *shh* in *Xenopus* embryos produced anterior defects including different degrees of microcephaly, reduction of the cement gland and eyes. The phenotype was associated with a reduction of the expression pattern of *Xotx2* in the forebrain and the retina. This phenotype is opposite to that obtained after overexpression of another member of this multigenic family, *banded hedgehog (bhh)* (Lai, C-J., Ekker, S. C., Beachy, P. A., Moon, R. T. (1995) Development 121: 8, 2349-2360). We conclude that *shh* may be antagonizing *bhh* action and that normal anterior development might require a balance between these antagonic pathways

This research was made possible by grants from the E. E. C. (EU CT 93-0017)

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HOW IS THE LIMIT OF THE NEURAL TISSUE ESTABLISHED IN *Xenopus laevis*?.

Mayor, R . Guerrero, N., Martínez, C., Glavic, A., Gómez-Skarmeta, J.L., Linker, C., Marchant, L. and Cofré, J. Depto de Biología, F. de Ciencias, U. de Chile, Santiago, Chile.

The neural plate is induced in the ectoderm by a signal coming from the dorsal mesoderm, however only a small part of the ectoderm becomes neural tissue, the rest differentiates as epidermis. The limit of the neural plate is established at the junction of these two tissues and in this region the neural crest are produced. Our interest is to understand how the neural crest are induced at the border of the neural plate. Several hypotheses have been proposed but the experimental analysis has been difficult because the absence of any early marker for the neural crest. We have used the expression of a Zinc-finger gene, called *Xslug* to study the induction of the prospective neural crests in *Xenopus*. The *Xslug* gene is expressed in the neural crest much earlier than any morphological diferentiation of this tissue.

By grafting labeled neural plate into lateral epidermis we have shown that neural crest can be induced by an interaction between neural plate and epidermis. Furthermore, neural crest cells come from both tissues. We have proposed a model in which the prospective neural crest are induced by an interaction between a dorsal signal coming from the neural plate and a ventral signal coming from the epidermis. We have explored the role of the notochord as a possible source of positive and negative signals. In order to study the molecular nature of these signals we have overexpressed *Noggin*, showing ectopic expression of *Xslug*. In addition a combination of *Noggin* and FGF is enough to induce *Xslug* in isolated ectoderm. We have blocked the FGF signaling by mutating the FGF receptor, and found an specific inhibition of *Xslug*. Taken together these results suggest that the neural crest are induced by a combination of *Noggin* and FGF. On the other hand, we have explored the role of a new gene that in *Drosophila* is involved in neural patterning. The *Xenopus* homologous of this gene, called Xiro, is expressed in the neural plate and its overexpression is able to change the limits of the neural tissue. This result suggest that the molecular mechanism by which the neural tissue is patterned has been conserved between fly and vertebrate.

Fondecyt 1969910, Conicyt/CSIC, U. de Chile

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STEPWISE GENERATION OF POSITIONAL INFORMATION IN THE *DROSOPHILA* IMAGINAL DISCS. J. Modolell. Centro de Biología Molecular Severo Ochoa, Consejo Superior de Invetigaciones Científicas and Universidad Autónoma de Madrid, 28049 Madrid, Spain.

An outstanding problem of Developmental Biology is the generation of positional information necessary to create species-specific patterns. We use the *Drosophila* wing imaginal discs, the precursors of most of the thorax and wings of this fly, to study the positioning of pattern elements like the cuticular sensory organs (SOs) and the wing veins. Initially, the imaginal discs are subdivided in large compartments defined by cell lineage restrictions. The compartment borders act as organization centers that help the patterning of the whole disc by means of signalling molecules that act at a distance. Somehow this signalling is translated into heterogeneous distributions of transcriptional factors (activators and repressors) in smaller, partially overlaping territories of different shapes (prepattern). Downstream genes are activated by specific combinations of prepattern factors in even smaller domains. I shall show that the prepattern genes of the *Iroquois* complex, which encode putative transcriptional controllers of the homeodomain family, integrate the signalling of the dorso/ventral and anterior/posterior compartment borders to define two smaller territories centered on the prospective wing vein L3. These transcriptional controllers in turn, and in combination with additional activators and repressors, promote expression of SO and vein forming genes in still further restricted domains. Thus, the gradual subdivision of the disc epithelium by the members of this genetic hierarchy creates the positional information essential for patterning.

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FACTORS THAT CONTROL NEURAL DEVELOPMENT IN THE MOUSE

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The central and peripheral nervous systems develop along intricate patterns that are set in the mid-gestation mouse embryo. These patterns are generated by the concerted action of inter- and intracellular signals that we study by a combination of molecular and genetic approaches. We have isolated a number of murine LIM-homeobox genes (Lhx) that play pivotal roles as nuclear transcription factors in neural development. Based on loss-of-function studies in gene-targeted "knockout" mutants, one of the Lhx genes is required for the development of the eye and the anlagen of the hippocampus and the neocortex. Other members of the Lhx family control important aspects of motoneuron development in the spinal chord. We have also studied the involvement of secreted factors in neural development. The Sonic Hedgehog (SHH) gene is instrumental for the formation of ventral fields and for setting the midline along the anterior-posterior axis of the neural tube. Another secreted factor, GDNF (Glial cell line-Derived Neurotrophic Factor), is essential for the development of the enteric nervous system. Thus, each of these factors holds a key position in the complex circuit of neural patterning during mouse development.

Symposium 2 - **Protein-Ligand Interactions**

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EXPLORING THE STRUCTURE OF HUMAN SERUM ALBUMIN LIGAND BINDING SITES USING RECOMBINANT DNA TECHNIQUES. Bhagavan, N.V., Petersen, C.E., Ha, C., Park, D., Harohalli, K., Jameson, D.M., and Mower, H.F. Department of Biochemistry and Biophysics, John A. School of Medicine, University of Hawaii, Honolulu, Hawaii, USA.

The familial dysalbuminemic hyperthyroxinemia (FDH) phenotype results from a natural human serum albumin (HSA) mutant, with histidine instead of arginine at amino acid position 218. This mutation results in an enhanced affinity for thyroxine. Site-directed mutagenesis and a yeast protein expression system were used to synthesize wild type HSA and FDH HSA as well as several other HSA mutants. Studies on the binding of thyroxine to these HSA species using equilibrium dialysis and quenching of tryptophan 214 fluorescence suggest that the FDH mutation affects a single thyroxine binding site located in the 2A subdomain of HSA. Site-directed mutagenesis of HSA and thyroxine analogs were used to obtain information about the mechanism of thyroxine binding to both wild type and FDH HSA. These studies suggest that the guanidine group of arginine at amino acid position 218 in wild type HSA is involved in an unfavorable binding interaction with the amino group of thyroxine, while histidine at amino acid position 218 in FDH HSA in involved in a favorable binding interaction with thyroxine. Neither arginine at amino acid position 222 nor tryptophan at amino acid position 214 appears to favorably influence the binding of thyroxine to wild type HSA.

Currently we are using site-directed mutagenesis to modify the structure of the 1A and 3A subdomains of HSA. The 1A subdomain binds nitric oxide, while the 3A subdomain binds digoxin. Specific modifications of the 1A and 3A subdomains will allow us to determine the structural basis of the HSA/ligand interactions for nitric oxide and digoxin respectively.

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INVOLVEMENT OF THE FAD BINDING DOMAIN AMINO ACIDS ON THE FUNCTION AND STABILITY OF A FNR-LIKE ENZYME. Ceccarelli, E., Ottado, J., Calcaterra, N., Orellano, E., and Carrillo N. PROMUBIE, Biochemistry Faculty, University of Rosario, Suipacha 531. 2000 Rosario, ARGENTINA. email: cecca@unrobi.edu.ar

The noncovalently bound FAD cofactor of plant-type ferredoxin-$NADP^+$ reductases (FNR) displays extensive interaction with two tyrosyl residues (corresponding to positions 89 and 308 in pea FNR), placed on opposite sides of the flavin ring. The contribution made by these residues to the function and stability of the flavoprotein was studied using site-directed mutagenesis. The phenol side chain of the C-terminal Y308 is stacked coplanar to the flavin group. Fluorescence measurements indicate that this interaction plays a significant role in FAD fluorescent quenching by the apoprotein. $NADP^+$ titration experiments suggest that the phenol ring of Y308 may fill the nicotinamide binding pocket in the absence of the nucleotide. The stability of the mutants, judged by thermal and urea-induced denaturation studies, was lowered with respect to the wild-type enzyme, with nonaromatic replacements showing the most dramatic effects. The decrease in thermodynamic stability correlated with declines in catalytic activity and in the accumulation of recombinant FNR forms in *Escherichia coli*. On the other hand, replacement of Y89 resulted in extensive breakdown of the FNR conformation, presumably reflecting a cavity effect. The results suggest that the presence of the electron-rich aromatic side chains adjacent to the isoalloxazine ring is essential for proper docking and stabilization of FAD during FNR biogenesis.

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HYDROSTATIC PRESSURE AS A TOOL IN THE INVESTIGATION OF PROTEIN-LIGAND INTERACTIONS
Ferreira, S T., Felice, F G., Soares, V.C., and Coelho-Sampaio, T. Department of Biochemistry, ICB/CCS, Federal University of Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil

Protein-protein and protein-ligand association reactions are often accompanied by positive standard volume changes, leading to pressure dependence of the association constants. An increase in pressure under isothermal conditions may be used to perturb protein subunit association or ligand binding, and thermodynamic parameters pertaining to these interactions may generally be derived. Fluorescence spectroscopy has been widely used to follow pressure-induced transitions in proteins. Recent examples from our laboratory will be presented on the use of these methodologies to investigate the coupling between ligand-binding and subunit interactions in two systems: 1) tetrameric rabbit muscle pyruvate kinase; and 2) the dimeric calcium-transport ATPase of human erythrocyte plasma membrane. Pyruvate kinase dissociates/unfolds under pressure up to about 3.5 kbar, which is accompanied by inhibition of enzyme activity. Subunit dissociation/unfolding and inhibition of enzyme activity are protected in the presence of the physiological activators K^+ and/or Mg^{2+}, and notably by the substrate, phosphoenolpyruvate. Reversible dissociation of dimeric calcium-ATPase was also observed at pressures up to 2.4 kbar. The physiological regulators of ATPase function, Ca^{2+} and calmodulin, were shown to modulate inter-subunit interactions in the ATPase.

Supported by FINEP, CNPq, CAPES and FAPERJ (Brazil)

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USING EVOLUTION FOR SPECIFIC INHIBITION OF TRIOSEPHOSPHATE ISOMERASE FROM TRYPANOSOMATIDAE. Pérez-Montfort, R., Becker, I., Rojo-Domínguez, A. and Gómez-Puyou, A. Deptos. de Microbiología y Bioenergética, Instituto de Fisiología Celular, Depto. de Medicina Experimental, Facultad de Medicina U.N.A.M., Depto. de Química, U.A.M., México, D.F., México.

Glycolysis is a key metabolic pathway for energy production in *Trypanosoma brucei*, *T. cruzi* and *Leishmania mexicana*. Inhibition of the glycolytic enzyme triosephosphate isomerase (TIM) kills these parasites. TIM is an ubiquitous enzyme with a highly conserved 3-D structure, but also with regions diverging in the amino acid (aa) sequence. The development of compounds for selective inhibition of essential enzymes of infectious agents has generally focused on the active site, a highly conserved region. We hypothesized that by identifying and targeting a non-conserved aa that is important for the function and/or stability of the enzyme from a specific species, selective inhibition could be achieved. Testing our hypothesis with TIM from different species, we showed that Cys 14 is such an aa in the enzyme from *T. brucei*. The reported sequences of other protozoan parasites, including *L. mexicana*, and plants also have Cys in equivalent positions, but mammalian enzymes do not. We could specifically inhibit TIM activity in the former enzymes and in glycosomes of *T. cruzi*. The sequence of the gene encoding for TIM in *T. cruzi* showed an equivalent Cys. This approach could be applied to the rational design of drugs against Trypanosomatidae.

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ADENOSINE DIPHOSPHATE GLUCOSE PYROPHOSPHORYLASE: MODE OF INTERACTION WITH ALLOSTERIC EFFECTORS. Preiss, J., Wu, M., Ballicora, M.A., Sheng, J. and Fu, Y. Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824 U.S.A.

Most ADPglucose pyrophosphorylases (ADPGlc PPases) are allosterically activated by glycolytic intermediates and inhibited by AMP, ADP or inorganic phosphate (Pi). Higher plants, green algae and cyanobacterial ADPGlc PPase's activator is 3-P-glycerate (3PGA) and inhibitor is, Pi. *Escherichia coli* ADPGlc PPase is activated by fructose-1,6-bis-P (FBP) and inhibited by AMP. Allosteric mutants of *E. coli*, *Chlamydomonas reinhardtii* and maize endosperm ADPGlc PPases have been isolated and their effects on glycogen and starch accumulation indicate that allosteric control of ADPGlc PPase is important physiologically, in regulating starch and bacterial glycogen synthesis. Chemical modification and site-directed mutagenesis studies show that the activator site for the *E.coli* enzyme is at Lys39, for *Anabaena* enzyme, at Lys382 and 419, for potato tuber enzyme, at Lys441 on the catalytic subunit and at Lys417 on the regulatory subunit. Thus, the *E. coli* FBP activator site is close to the N-terminal and the cyanobacterial and higher plant ADPGlc PPases 3PGA site closer to the C-terminal. Computer analyses procedures for predicting secondary structure, hydrophobic cluster analysis and PHDsec, show that the cyanobacterial, enteric and plant (both subunits) enzymes are very similar in secondary structure with the substrate and allosteric binding sites predominantly in loop areas of the subunits. Limited proteolysis of the *E. coli* enzyme with proteinase K results in enzyme fully active without activator and fully resistant to AMP inhibition suggesting, that the N-terminal is part of the regulatory domain for both the activator and inhibitor sites. Activator may by binding, prevent the N-terminal region from inhibiting the substrate sites and enable the enzyme to become active. Further studies of the limited proteolyzed *E. coli* enzyme are planned. This research is supported by grants from DOE and USPHS.

Symposium 3 - **Molecular Adaptations of Archaea and Bacteria to their Environment**

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GENOMICS AND MOLECULAR BIOLOGY OF EXTREME HALOPHILIC ARCHAEA (HALOARCHAEA). R. Amils, P. López-García, J.P. Abad, J. Antón, I. Marín, J.L. Sanz, C. Briones and E. Sánchez. Centro de Biología Molecular, CSIC_UAM, Universidad Autónoma de Madrid, Cantoblanco, Madric 28049, Spain. "osantacruz@vmax.cbm.uam.es".

Haloarchaea have atracted the interest of microbiologists due to their unique system to overcome the osmotic pressure created by the extreme salt conditions of the habitats in which they develop by concentrating inside the cell equivalent concentrations of potassium as compatible solute. A great deal of interest has been focused on their genome organization and function at noncanonical conditions during the last decade since they constitute a branch of the domain archaea that is phylogenetically distant from the bacterial and eukaryotic reference systems. We have been studying in our group compara tive genome organization of haloarchaea using pulsed field gel electrophoretic techniques in addition to structural and functional aspects of their translational apparatus. An update of the results will be presented in this communication.

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THE ROLE OF MEMBRANE FATTY ACID SYNTHESIS IN *BACILLUS SUBTILIS* DEVELOPMENT. **de Mendoza, D., Perez, C. and Schujman, G. Programa Multidisciplinario de Biologia Experimental, Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina**

A paradigm for studies on cell differentiation is the primitive developmental process of spore formation in *Bacillus subtilis*. During sporulation, *B. subtilis* is differentiated in two different cell types, the mother cell and the prespore. Sequential activation of sigma factors, whose activities are confined to one cell or the other, leads to differential gene expression in the two cells. We have found that *cis*-unsaturated fatty acids in vitro specifically inhibit a signal transducing protein kinase required for initiation of sporulation. These observations suggest that fatty acids may act as a relevant signal for cellular differentiation. To study the role of membrane lipid synthesis on sporulation, we constructed a *B. subtilis* strain bearing a conditional mutation in genes coding for the acetyl-CoA carboxylase, the first enzyme of fatty acid synthesis. Growing this strain in non-permissive conditions revealed that fatty acids synthesis was necessary for sporulation. To determine at what point sporulation was blocked in the absence of fatty acid synthesis, we used *lacZ* transcriptional fusions to several genes involved in sporulation as well as electron microscopy. The results of these experiments revealed that fatty acids synthesis during the early stages of morphogenesis is a key event for activation of the first forespore-specific sigma factor, sigma F.

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RESPONSE OF THE CRENARCHAEON *Sulfolobus acidocaldarius* TO HEAT SHOCK AND PHOSPHATE STARVATION. Osorio, G. and Jerez, C.A. Depto. de Bioquímica, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

To find out whether the archaea might possess sensory responses homologous to the two-component bacterial signalling systems, we studied the adaptive response of the thermoacidophilic archaeon *Sulfolobus acidocaldarius* BC65 to phosphate starvation. When cells were subjected to phosphate limitation, the levels of synthesis and the degree of phosphorylation of several proteins changed, as detected by two-dimensional non-equilibrium pH gradient electrophoresis of cells labeled *in vivo* with [^{35}S]methionine or $H_3{}^{32}PO_4$. After another growth-restricting treatment, a heat shock (from 70° to 80°C), a general inhibition of protein synthesis was observed. Under phosphate starvation, a 36 kDa protein became phosphorylated without its synthesis being significantly modified, suggesting a probable regulatory role during adaptation of the cell to the change in the external environment. In Southern blot analysis with specific probes from very conserved regions of the *phoR* and *phoB* genes from *Escherichia coli*, we found a positive hybridization with *S. acidocaldarius* chromosomal DNA fragments. This suggested the presence in *S. acidocaldarius* BC65 of genes related to the *E. coli* genes involved in the phosphate starvation response system. This appears to be the first evidence of the possible existence of a two-component sensory system in a microorganism from the archaeal kingdom *Crenarchaeota*.

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HEAT-SHOCK GENES IN ARCHAEA. Macario, A.J.L. and Conway de Macario, E. Wadsworth Center, Division of Molecular Medicine, NYSDOH; and Dept. of Biomedical Sciences, The University at Albany, Albany, New York, USA.

The Hsp70/DnaK heat-shock protein is a molecular chaperone coded by the *hsp70/dnaK* gene and is highly conserved in the phylogenetic domains Bacteria (eubacteria) and Eukarya (eukaryotes). Less is known on the occurrence and degree of sequence conservation of this protein and its gene in the other domain, Archaea. *dnaK* genes have been cloned from the genome of the archaeal species *Methanosarcina mazei* S-6 (1991), *Halobacterium marismortui* (1992), and *Halobacterium cutirubrum* and *Thermoplasma acidophilum* (1994), and sequenced. The encoded proteins are most similar to those from Gram + eubacteria. The only archaeal *dnaK* locus fully sequenced is that of *M. mazei* S-6. Its organization is: 5'-*orf16-grpE-dnaK-dnaJ-orf11-trkA*-3'. These genes are expressed constitutively with monocistronic messages, except for *orf11-trkA* which are cotranscribed. Heat-shock induces *grpE*, *dnaK*, and *dnaJ*, but turns down *orf16* and *orf11-trkA*. Other stressors cause the same effects, although some either induce or turn down *orf11-trkA* depending on the dose. Most notably, the regulatory signals in the promoter regions of these six genes are not of eubacterial but of eukaryal type, *M. mazei* S-6 being a prokaryote notwithstanding. Current research aims at elucidating the transcription initiation mechanism. (Supported in part by a grant from NYSERDA.)

Symposium 4 - **Molecular Basis of Host-Parasite Relationship in Chagas Disease**

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STRUCTURE AND POSSIBLE FUNCTIONS OF CRUZIPAIN, THE MAJOR CYSTEINE PROTEINASE FROM *TRYPANOSOMA CRUZI*. J.J. Cazzulo. Instituto de Investigaciones Bioquímicas "Luis F. Leloir" Fundación Campomar. Buenos Aires, Argentina. jjcazzulo@iris.iib.uba.ar
Cruzipain is the major cysteine proteinase (CP) present in *Trypanosoma cruzi*. Its expression is developmentally regulated, levels in epimastigotes being one to two orders of magnitude higher as compared with the other parasite forms. The enzyme is encoded by a number of tandemly repeated genes (up to 130 in the Tul 2 strain), located in two to four chromosomes. Mature cruzipain consists of a catalytic domain, with high homology to other CPs of the papain family, like cathepsins S and L, and a C-terminal domain (C-T), homologous to that present in most CPs from other Trypanosomatids. The C-T, which can be purified after self-proteolysis, contains post-translational modifications (N-glycosylation, with high-mannose, hybrid monoantennary, or complex biantennary chains; disulfide bridges; and still unknown modifications in seven Thr residues). These modifications, together with the simultaneous expression of different genes with polymorphisms in the C-T, are probably responsible for the heterogeneities in charge and apparent size found in natural enzyme preparations. The C-T is responsible for the immunoreactivity of cruzipain, which is an immunodominant antigen in chronic Chagas disease. Possible functions of cruzipain are (1) protein digestion in the lysosomes; (2) defense of the parasite against the immune system of the host; (3) participation in the differentiation steps of the life cycle; these can be blocked by irreversible CP inhibitors, which are able to inhibit cruzipain inside living parasites. **Supported by SAREC (Sweden) and TDR/WHO.**

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***TRYPANOSOMA CRUZI* MOLECULES INVOLVED IN THE INVASION OF THE MAMMALIAN HOST.** Alberto Carlos C. Frasch, Fundacion Campomar, Buenos Aires, Argentina.
Protozoan parasites make use of different strategies to invade multicellular organisms. The strategy developed by *Trypanosoma cruzi* is essentially the invasion of host cells together with the evasion of the host immune response. A number of parasite epitopes conformed by amino acid repeats in tandem might be involved in distracting the host response against irrelevant molecules. One amino acid repeat epitope, SAPA antigen, is linked to a second domain having trans-sialidase (TS) activity. TS is required by the parasite to invade cells, and antibodies to the enzymatic domain effectively inhibit this activity essential for the parasite survival. Since SAPA is not required for the TS activity of the natural molecule, we proposed that SAPA repeats are required for interacting with the immune system of the host, first delaying and later boosting the antibody response against the catalytic domain of the molecule. Thus, SAPA repeats would have a dual function in preventing and boosting antibody generation at different periods of the infection. A second group of molecules involved in the invasion of cells by *T. cruzi* are glycoproteins of the mucin type. Mucins are the acceptors of the sialic acid transferred by the trans-sialidase. These molecules also contain amino acid repeats in tandem, which are directly related to the function of these molecules. These Thr-rich repeats are the target sites for O-glycosylation, where the acceptors for the sialic acid are placed. Supported by TDR/WHO and SAREC (Sweden)

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HISTONE GENES IN TRYPANOSOMATIDS: TRANSCRIPTION AND TRANSLATION. Galanti, N.; Galindo, M.; Sabaj, V.; Espinoza, I. and Toro, G.C. Depto. Biología Celular y Genética, Facultad de Medicina, U. de Chile, Santiago, Chile.

Trypanosomatids are flagellates that appeared on earth approximately 1.5 billion years ago. Histone genes in Trypanosomatids are of interest considering that these flagellates do not condense their chromatin during mitosis.

In contrast to higher eukaryotes, Trypanosomatids histone genes are in separate chromosomes. Transcripts for histones are polyadenylated; in higher eukaryotes they are not. Sequence similarity of Trypanosomatid core histones with other species is concentrated in the globular region; the amino terminal is highly divergent. Trypanosomatid histones H1 seem to be of low molecular weight, with closest homology to the carboxyl terminal of the higher eukaryote histones H1.

Contrary to these peculiar features of histone genes in Trypanosomads, synthesis of these proteins seem to follow the pattern described in higher eukaryotes. Thus, synthesis of core histones and of most of histone H1 is down-regulated in G1, G2 and M-phase cells, while it is stimulated in S-phase cells.

Trypanosoma histone genes structure and their transcription may constitute interesting targets for a rational approach to the study of these protozoa.

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DNA MARKERS DEFINE TWO MAJOR PHYLOGENETIC LINEAGES OF *TRYPANOSOMA CRUZI*. Zingales, B.[1], Fernandes, O.[2], Macedo, A.M.[3], Campbell, D.A.[4], Souto, R.P.[1] [1]Dep. de Bioquímica, USP, São Paulo, Brazil; [2]Dep. de Bioquímica e Biologia Molecular, FIOCRUZ, Rio de Janeiro, Brazil; [3]Dep de Bioquímica e Imunologia, UFMG, Belo Horizonte, Brazil; [4]Dep. of Microbiology and Immunology, UCLA, Los Angeles, USA.

Parasitic protozoa within the taxon *Trypanosoma cruzi* are considered to be derived from multiple clonal lineages, and show broad genetic diversity as a result of propagation with little or no genetic exchange. We have analyzed a wide sample of *T. cruzi* isolates from vertebrate and invertebrate hosts by PCR amplification of a ribosomal RNA gene sequence, a mini-exon gene sequence and random amplified polymorphic DNA (RAPD). Amplification of the distinct rDNA and mini-exon gene sequences indicated a dimorphism within both of the tandemly-repeated genes: 125-bp or 110-bp products for rDNA and 300-bp or 350-bp products for the mini-exon. Within individual isolates, one of three associations was observed: the 125-bp rDNA product with the 300-bp mini-exon product (defined as group 1), the 110-bp rDNA product with the 350-bp mini-exon product (defined as group 2), and the presence of both rDNA amplification products with the mini-exon group 1 product (group 1/2). The RAPD analysis showed variability between individual isolates, however, tree analysis clearly indicated the presence of two major branches. Interestingly, the rDNA/mini-exon group 2 isolates correlated precisely with one branch of the RAPD-derived tree, group 1 and group 1/2 isolates correlated with the other branch. Our studies show a clear division of *T. cruzi* into two major lineages that present a phylogenetic divergence high enough to define two subspecies. Support: FAPESP and CNPq.

Symposium 5 - **Regulation of Protein Kinases Involved in Cell Division**

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STUDIES ON PROTEIN KINASES CK1 and CK2. J.E. Allende. **Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Santiago, Chile. E-mail: jallende@abello.dic.uchile.cl**

The protein kinase superfamily is now the largest known group of highly related enzyme proteins since more than 400 species of these enzymes are known. All protein kinases are related structurally but the different species can be arranged by sequence comparison into groups that share similar functions. The reversible phosphorylation of proteins catalyzed by protein kinases and phosphatases is the post-translational modification most widely used in eukaryotic cells to modify the biological activity of these macromolecules. Recently the preponderant involvement of several protein kinases in the control of cell division has become evident, making it highly relevant to study the factors that regulate the activity of these kinases. In our laboratory, we are studying two enzymes that are involved in the cell division, protein kinases CK1 and CK2. CK1 is constituted by a subfamily of at least 7 members in mammals and seems to participate in DNA repair. The α species of CK1 from ***Xenopus*** is able to phosphorylate tyrosine residues in synthetic peptides and to autophosphorylate in tyrosine. In ***D. melanogaster***, CK1 α is developmentally regulated and activated by gamma radiation. CK2 has been studied through site-directed mutagenesis of the α and β subunits in order to study the regions involved in its catalytic activity and in subunit interaction. The study of CK2 expression during embryogenesis demonstrates that it is preferentially expressed in the developing neuronal tissue.

Supported by FONDECYT - Chile, The Council for Tobacco Research, the International Centre for Genetic Engineering and Biotechnology.

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THE GENES ENCODING PKA SUBUNITS ARE COORDINATELY INDUCED DURING DIFFERENTIATION IN *B. EMERSONII.* Gomes, S.L., Marques, M.V. and Oliveira, J.C.F., Instituto de Química, Universidade de São Paulo, São Paulo, Brasil e-mail: sulgomes@quim.iq.usp.br

The aquatic fungus *B. emersonii* presents an interesting developmental cycle, which begins with the zoospore, a motile non growing cell, that is triggered to germinate by the presence of nutrients or the addition of cAMP, differentiating into the germling cell. This cell is capable of vegetative growth, which is characterized by intense nuclear proliferation, without cell division, leading to the formation of a multinucleated cell: the sporangium. At any time during growth, shortage of nutrients can induce the sporulation process, which culminates with the intrasporangial formation of the zoospores and their release from the mother cells. A single PKA is present in *B. emersonii*. Its activity is low in vegetative cells, rising sharply during sporulation, reaching maximum levels in the zoospores. After germination PKA activity decreases back to low basal levels. Work from our laboratory has shown that these variations in activity are due to a coordinate transcriptional control of the genes encoding the regulatory (R) and the catalytic (C) subunits. To investigate sequence elements common to both *B. emersonii* R and C gene promoters, which could be involved in the coordinate regulation of these genes, their 5' flanking regions were analysed by gel mobility shift and DNA-footprinting assays. It was determined that different DNA-protein complexes are generated when fragments of the R and C gene promoters are incubated with extracts from cells expressing (sporulating cells) or not expressing (vegetative cells) both subunits. Furthermore, DNase I-footprinting experiments have indicated that a 9-nucleotide sequence element present in both promoters, which was denominated RC box, was protected from DNase I digestion only by non-expressing cell extracts, suggesting a negative control for the induction of R and C genes during sporulation.

Supported by: FAPESP, CNPq and PADCT

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SIGNALING FROM THE MEMBRANE TO THE NUCLEUS THROUGH SMALL GTP-BINDING PROTEINS ACTING ON DIVERGENT MAP KINASE CASCADES. J. Silvio Gutkind. **Laboratory of Cellular Development and Oncology, NIDR, National Institute of Health, Bethesda, MD 20892, USA. E-mail: gutkind@nih.gov**

Critical molecules participating in the transduction of proliferative signals have just begun to be identified. One such an example is the family of extracellular signal-regulated kinases, (ERKs), or MAP kinases (MAPKs). Their function is to convert extracellular stimuli to intracellular signals controlling the expression of genes essential for many cellular processes. The tyrosine-kinase class of growth factor receptors has been shown to regulate MAPKs in a multistep process that involves $p21^{ras}$. However, activation of MAPK by receptors acting through G proteins has been poorly understood. Recently, we have shown that $\beta\gamma$ subunits, not $G\alpha$, act in a Ras-dependent manner to stimulate MAPK, suggesting that signals from G protein-coupled receptors (GPCRs) converge as the level of $p21^{ras}$ with the pathway utilized by receptor-tyrosine kinases. Interestingly, a novel family of kinases structurally related to MAPK, jun N-terminal kinases (JNKs), has been shown phosphorylate ***in vivo*** the N-terminal transactivating domain of the c-Jun protein, thereby increasing its transcriptional activity. We found that only stimulation of GPCRs can led to JNK activation, thus diverging at this level with those signaling routes utilized by tyrosine kinase receptors. This observation led us to hypothesize that JNK and MAPKs might be differentially regulated, and prompted us to investigate the biochemical route controlling JNK. We observed that Ras could weakly activate JNK, however, utilizing a signaling pathway distinct from that regulating MAPks. In Contrast, we found that expression of mutationally activated form of two members of the Rho-family of small GTP-binding proteins, Racl and Cdc42, can initiate an independent kinase cascade leading to JNK activation. Furthermore, we obtained evidence to support that Rac1 and Cdc42 are an integral part of the signaling route linking cell surface receptors to JNK. Our current efforts to elucidate the pathway(s) connecting cell surface receptors to each members of the MAPK-superfamily, and to explore their role in proliferative signaling will be discussed.

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HOW DO PROTEIN KINASES RECOGNIZE THEIR TARGETS? THE LESSON OF PROTEIN KINASE CK2. Lorenzo A. Pinna. Dipartimento di Chimica Biologica dell'Università di Padova, Padova, Italy.

Several features contribute to the substrate selectivity of protein kinases, among which "site specificity", i.e. the ability to recognize definite consensus sequences surrounding the phosphoacceptor aminoacids, appears to play a crucial role especially in the case of Ser/Thr specific kinases. These variably use basic, hydrophobic, prolyl and acidic residues at definite position(s) relative to the phosphoacceptor aminoacid as crucial elements in their consensus sequences. Substantial information about the structural features determining specificity was obtained from studies with protein kinase CK2, a ubiquitous highly pleiotropic enzyme, displaying striking selectivity for very acidic sequences. Mutational studies with CK2 have led to the identification of basic residues that recognize specificity determinants located on the C terminal side of the target aminoacid [Sarno et al JBC 271, 10595-10601 (1996)]. Three basic residues, R191, R195 and, even more, K198, are implicated in the recognition of the determinant at position n+1; K79 and/or R80 contribute to the recognition of the crucial determinant at n+3, together with four consecutive lysines (K74-77) which also play interchangeable roles in the recognition of determinants at positions (+2)/+4/+5. This basic cluster is also implicated in down-regulation of CK2 catalytic subunit by the β-subunit and by polyanionic inhibitors. The relevance of these data to predict the specificity of other kinases will be discussed.

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PROTEIN KINASES AND CELL DIVISION IN *Trypanosoma cruzi*. Téllez-Iñón, Ma. Teresa. INGEBI-CONICET and Facultad de Ciencias Exactas y Naturales, UBA. Buenos Aires- Argentina

Protein kinases are important in the regulation of cellular processess including growth and differentiation.

We have described several protein kinases regulated by second messengers as cyclic-AMP dependent protein kinase, protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase in *Trypanosoma cruzi*. These enzymes are similar to the ones from higher eucaryotes.

TcCaMK is associated with membranes, flagella and is also present as a soluble enzyme. Western blot analysis revealed that the TcCaMK has subunits of 50 and 60 kDa which exhibit autophosphorylating activity. Two different autophosphorylated states differ only in their extent of Ca^{2+}/CaM-dependence. TcCaM K is present in all the forms of the parasite with the highest specific activity found in trypomastigotes. Endogenous substrates are differentially expressed among them.

Our objective is to search for a relationship between signal transduction pathway and cell division. We are studying protein kinases involved in the regulation of cell division of *T.cruzi*. Two genes named tcrk1 and 2 corresponding to cyclin-related kinases (CRKs) with very high identity with CRKs from other trypanosomatids were cloned. The results show that *T. cruzi* possesses protein kinases involved in the regulation of the cell cycle.

Symposium 6 - **Glycobiology: Intracellular Protein Glycosylation, Folding and Kinesis**

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GLYCOPROTEIN FOLDING IN THE ENDOPLASMIC RETICULUM: THE CALNEXIN/CALRETICULIN CYCLE.
Helenius, A., Trombetta, S., Hebert, D., Peterson, J., Chang J.X., Rodan, A., Ora, A., Cannon, K. Chen, W. and Simons, J. Department of Cell Biology, Yale School of Medicine, New Haven, Ct, USA

The ER provides a specialized environment for high capacity production of secretory proteins, glycoproteins, and membrane proteins. Folding is mediated by a large number of resident chaperones and folding enzymes. Some of these also participate in a molecular quality control process that efficiently prevents export and deployment of incompletely folded and misfolded proteins. Our work has shown that folding as well as quality control of glycoproteins is coupled to trimming of the N-linked glycans. Two homologous lectins, the membrane-bound *calnexin* and the soluble *calreticulin*, associate with the partially glucose-trimmed glycans already on the nascent chains. Together with two lumenal enzymes with opposing activities that drive an on-and-off cycle — *glucosidase II* (a soluble heterodimer of 160kDa) and *UDP-Glc:glycoprotein glucosyl transferase* — these lectins promote proper co- and post-translational folding of glycoproteins. Since the system relies on covalent modification of the newly synthesized proteins, and since the chaperones are lectins and do not distinguish between folded and unfolded proteins directly, the mechanism involved is fundamentally different from that employed by classical chaperones in protein folding. Recent studies on the components and the molecular mechanisms of this pathway in mammalian cells and *S. cerevisiae* will be discussed.

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THE ROLE OF NUCLEOTIDE TRANSPORTERS IN POSTTRANSLATIONAL MODIFICATIONS IN THE ENDOPLASMIC RETICULUM AND GOLGI APPARATUS OF MAMMALS AND YEAST.
Hirschberg, C.B., Abeijon, C., Berninsone, P. and Toma, L. Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester, MA 01655, USA, e-mail: Carlos.Hirschberg@ummed.edu

The lumen of the endoplasmic reticulum and Golgi apparatus are the subcellular sites where glycosylation, sulfation and phosphorylation of secretory and membrane-bound proteins, proteoglycans and lipids occur. Nucleotide sugars, nucleotide sulfate and ATP must first be translocated into the lumen before they can serve as substrates in the above reactions. Translocation of these nucleotide derivatives is mediated by highly specific transporters, which are antiporters with the corresponding nucleotide monophosphate, as shown by genetic and biochemical approaches. Studies with mammalian and yeast mutants showed that a defect in a specific translocator results in selective impairment of glycosylation of proteins, lipids and proteoglycans. Several of these transporters have been purified and cloned. Experiments with yeast and mammalian cells demonstrate that these transporters play a regulatory role in the above reactions.

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VESICULAR TRANSPORT FROM THE PERSPECTIVE OF ENDOSOME FUSION.
Mayorga, L., Aballay, A., Berón, W., Colombo, M.I., Barbieri,A., and Stahl, P.D. Inst. Histología y Embriología, Fac. Cs. Médicas, U.N.Cuyo-CONICET, Argentina and Dep. Cell Biology and Physiology, Washington University School of Medicine, USA. E.mail lmayorga@fmed2.uncu.edu.ar.

Protein glycosylation is an ordered process that occurs in several organelles along the secretory pathway. Hence, it requires the sequential transport of proteins among membrane-bound compartments. Macromolecular trafficking among organelles utilizes a complex machinery for the formation, transport, and fusion of vesicles. Several components of this machinery have been identified and a consensus model that fit a large body of observations has been proposed. However, the process is still far from being completely characterized. By using an in vitro assay that reconstitutes fusion among endosomes we have observed that, in addition to the set of factors included in the present model, other proteins are involved in the fusion process. Recent data implicate a zinc-binding protein with a domain similar to the C1 domain of protein kinase C in endosome fusion. Moreover, some results indicate that the role of NSF in endosome fusion may be different from that proposed by the consensus model. The observation that endosomes fuse in the presence of micromolar [Ca^{2+}] suggests that a different annexin-dependent fusion mechanism may be activated by a cytoplasmic calcium increase. Techniques such as rapid freeze and video microscopy in combination with genetic manipulation of factors will provide new insights into the dynamics of vesicle and tubule formation from endosomes and the process of membrane fusion in the endocytic pathway.

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THE ENDOPLASMIC RETICULUM MECHANISM OF SENSING MISFOLDED GLYCOPROTEIN STRUCTURES.
Parodi, A. J. Fundación Campomar, Buenos Aires, Argentina. E-mail: aparodi@iris.iib.uba.ar

Proteins in the secretory pathway adopt their tertiary structures in the lumen of the ER. Species that fail to properly fold are retained in that subcellular location, where they are proteolytically degraded, and are not transported to the Golgi. It has been proposed that the quality control of glycoprotein folding involves glucosylation of misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase (GT). This presentation will deal with a) the molecular basis by which the GT glucosylates misfolded but not properly folded glycoproteins: the enzyme must recognize two elements exclusively exposed in the former conformations, the innermost GlcNAc unit of the acceptor oligosaccharides and patches of hydrophobic amino acids and b) evidence gathered in "in vivo" performed experiments that support the proposed role of the GT: the induction of the synthesis of the GT-encoding mRNA under stress conditions that promote glycoprotein misfolding and the modification of the rate of exit of glycoproteins from the ER upon inhibition of removal of the glucose units added by the GT.

Symposium 7 - **Cholinergic Macromolecules and Disease**

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THE ACETYLCHOLINE RECEPTOR LIGAND-GATED CHANNEL AS MOLECULAR TARGET OF DISEASE AND THERAPEUTIC AGENTS. F. J. Barrantes. Instituto de Investigaciones Bioquímicas de Bahía Blanca, 8000 Bahía Blanca, Argentina.

During the last two decades a convergence of techniques in various disciplines has led to important advances in our understanding of the structure and mechanisms of action of several ion channels and of the multiplicity of functions that they perform. Ion channels underlie a great variety of cellular functions; correspondingly they can be affected by a variety of pathological conditions leading to abnormal structure and/or function, either by mutation or in an acquired form. The nicotinic acetylcholine receptor (AChR), the best studied ligand gated ion channel, is not the exception to this rule, and is known to be the target of inherited and acquired diseases. The exquisite sensitivity of the patch-clamp technique, along with the insights provided by genetic engineering (especially site-directed mutagenesis), have built the foundations and begun to fill out the details of the molecular mechanisms of channel gating, ion permeation and block. More abnormal conditions associated with the AChR are likely to be discovered in the near future, as our understanding of the structural-functional relationships and principles derived from the study of this paradigm ligand-gated ion channel progresses even further. This knowledge will lead, in turn, to the development of new therapeutic strategies, some of which are already being discussed or implemented for the treatment of AChR pathologies.

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FUNCTIONS OF ACETYLCHOLINESTERASE IN THE CENTRAL NERVOUS SYSTEM DEMONSTRATED BY ENZYME INHIBITION. Federico Dajas. Gustavo Costa, Alejandro Ricciardi, Federico A. Dajas Bailador. División Neuroquímica, Instituto de Investigaciones Biológicas Clemente Estable. Avda. Italia 3318, Montevideo, Uruguay. Email:fdajas@iibce.edu.uy.

Functions of acetylcholinesterase (AChE) in the central nervous system (CNS) are not fully understood yet. The exact nature of its cholinolytic actions as well as its non-cholinergic functions are the center of debate.

We have studied the role of AChE in the CNS [striatum (ST), Substantia Nigra (SN) and Locus coeruleus (LC)] utilizing Fasciculin (FAS), a powerful peptidergic AChE inhibitor.

In adult rats, AChE inhibition provoked functional and behavioral changes (circling, catalepsia) which were fully blocked (ST), partially blocked (SN) or not blocked at all (LC) by atropine. Interpretation of these results is difficulted by the fact that AChE inhibition by FAS (and other AChE inhibitors) is accompanied by a simultaneous blockade of nicotinic actylcholine receptors. Important changes in cell aggregability were observed after FAS in developmental nervous system, in retinal cell cultures which show a marked AChE activity resistant to FAS. These changes varied according to the inhibitor that was utilized and the way cells were grown. The effects of AChE in development have also been confirmed in *in vivo* models.There is important experimental evidence to support complex and diverse functions of AChE in the CNS, beyond its cholinolytic activity.

This work has been supported by IPICS (Uppsala, Sweden), SAREC (Sweden), CSIC (Uruguay) and the European Union.

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ROLE OF ACETYLCHOLINESTERASE IN THE FORMATION OF AMYLOID PLAQUES OF ALZHEIMER'S BRAIN. Inestrosa, N.C. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Alzheimer's disease (AD) is characterized by the presence of senile plaques where amyloid-β-peptide (Aβ) is the major component. Brain acetylcholinesterase (AChE) consistently colocalizes with amyloid deposits including the senile plaques. We have investigated whether AChE affects the known spontaneous *in vitro* formation of amyloid fibrils by Aβ homologous synthetic peptides. We report here that AChE directly promotes amyloid fibril formation, as shown by thioflavine-T fluorescence, turbidity, congo red binding and electron microscopic studies. AChE accelerates fibril formation of both wild type Aβ and an analogue (Aβ Val 18→Ala) which alone produces few amyloid-like fibrils. The action of AChE was independent of the subunit array of the enzyme, it was not affected by active site inhibitors and it was partially blocked by peripheral anionic binding site ligands including, propidium, fasciculin and a monoclonal antibody. We have compared the ability to induce amyloid deposits between AChE and others senile plaques-associated proteins, such α_1-antichymotrypsin, apolipoprotein E (apoE), apoE3, apoE4, laminin and fibronectin. Results indicates that AChE seems to be the strongest Aβ-promoting factor among them. Our *in vitro* data suggest that AChE, in addition to its role in cholinergic synapsis may function by accelerating Aβ formation and could play a role during amyloid deposition in AD.

Supported by FONDECYT 1940694 and a Presidential Chair in Science.

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DISTINCTIONS BETWEEN ACETYLCHOLINESTERASE (AChE) AND THE NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) IN STRUCTURE AND REGULATION OF GENE EXPRESSION, Palmer Taylor, Department of Pharmacology, UCSD, La Jolla, CA 92093-0636

AChE and the nAChR are two major functional proteins in cholinergic synapses. Despite their interaction with a common neurotransmitter and similar developmental patterns of gene expression, the structures of the binding sites and the control of gene expression for the two proteins differ substantially. X-ray crystallography shows the AChE active center to be centrosymmetric to the subunit and at the base of narrow gorge, whereas various studies show the nAChR binding site to be at 2 of the 5 subunit interfaces of this pentamer. Expression of mutant and chimeric AChEs and AChRs has delineated amino acid side chains contributing to the specificities of the respective sites. Distinct members of the family of three fingered peptide toxins bind to the target molecules selectively. Fasciculin binds to entry portal of the active center gorge in AChE, yet only partially occludes substrate entry. The α-toxins bind across the two subunit interfaces of the nAChR and are competitive with agonists. Studies of mutant peptide toxins define residues in the toxins, AChE and the nAChR involved in the interactions. Residues at the interface of the fasciculin-AChE complex are also identified in the crystal structure, yet not all of the interfacial residues contribute to the binding energy. Striking differences in control of gene expression are found for AChE and the nAChR. Transcriptional activation of genes encoding the individual subunits governs nAChR expression, while alternative mRNA processing of a single gene and mRNA stabilization control diversity of species and expression of AChE.

Symposium 8 - **Protein Folding**

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TUBULIN SECONDARY STRUCTURE AND SURFACE ANALYSIS SUGGESTS FOLDING HOMOLOGY WITH THE BACTERIAL CELL DIVISION PROTEIN FtsZ AT THE GTP BINDING SITE. de Pereda, J.M., Leynadier, D., Evangelio, J.A., Chacón, P. and Andreu, J.M. C I B, CSIC, Madrid, Spain.

Separate alignments of 75 α-tubulin, 106 β-tubulin, 14 γ-tubulin sequences, and 12 sequences of the bacterial cell division protein FtsZ have been employed to predict their secondary structures. The predicted secondary structure of the αβ-tubulin dimer averages 33% α-helix, 24% β-sheet and 43% loop, which is compatible with CD and FTIR spectroscopy. The predictions have been compared with sites of limited proteolysis by twelve proteases at the surfaces of the αβ-dimer and taxol-induced microtubules. From 24 experimentally determined nicking sites, 18 are at predicted loops or at the extremes of secondary structure elements. Combining this with other biochemical information, a set of surface and distance constraints is proposed for the folding of β-tubulin. The FtsZ sequences are only 11-16% identical to the tubulin sequences. However, the predicted secondary structures show two clearly similar regions, at tubulin positions 95-175 and 305-350, corresponding to FtsZ 65-135 and 255-300 respectively. The first region consists of a predicted loop1-helix-loop2-sheet-loop3-helix-loop4-sheet fold, which contains the inverted phosphate binding motif (KR)GXXXXG (loop1), and the tubulin-FtsZ signature G-box motif (SAG)GGTG(SAT)G (loop3). A simple working model envisages the inverted P-loop1 and the G-box loop3 together at the nucleotide binding site, whilst loops 2 and 4 are at the surface of the protein, in agreement with proteolytic and antigenic accessibility results in tubulin. The model is compatible with studies of tubulin and FtsZ mutants. It is proposed that this region constitutes a common structural and evolutionary nucleus of tubulins and FtsZ which is different from typical GTPases.

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EQUILIBRIUM FOLDING INTERMEDIATES MAPPED BY A CHEMICAL CLEAVAGE APPROACH, Ermácora, M. R., Departamento de Ciencia y Tecnologia, Universidad Nacional de Quilmes, Bernal, Argentina.

Solving the folding problem requires a knowledge of the 3D structure of nonnative states along the folding pathway. However, analysis of nonnative states by NMR spectroscopy has proven difficult. A new chemical cleavage technique was developed to map the backbone fold of partially folded proteins. The method is based on the conformation dependent chemical cleavage of the polypeptide chain by reactive oxygen species generated at a cysteine specific tethered EDTA-Fe reagent [Ermácora et al., *Proc Natl Acad Sci* 1992, 89: 6383-6387; Platis et al., *Biochemistry* 1993, 32: 12761-12767; Ermácora et al., *Biochemistry* 1994, 33: 13625-13641]. The new approach was used to characterize the structure of a nonnative state, SNase1-135, a C-terminal truncated form of staphylococcal nuclease (lacking the last 14 of 149 residues), that acquires a compact state under physiological conditions. The chemical cleavage pattern described for the nuclease fragments shares similarities with that found for the related full length nuclease variants. This indicates a common proximity of sites and common solvent accessibility in the native and nonnative states. Novel cleavage sites are observed at residues that would be buried in the native structure but become exposed by the unfolding of helix 3. Taken together, these data are consistent with a surprisingly native-like conformation for much of SNase1-135. Thus the nonnative state of staphylococcal nuclease seems to adopt a global native-like tertiary fold with the exception of the segment comprising helix 3 and the connecting loop between helix 2 and 3 which is unfolded [Ermácora et al. *Nature Struct Biol* 1996, 3: 59-66] Extensive tertiary interactions in the SNase fragment system were not apparent by NMR criteria. The chemical cleavage analysis of SNase1-135 fragment complements the previous NMR analysis and extends our understanding of the structure of this nonnative state. The manner in which the backbone topology is stabilized in absence of extensive tertiary interactions is now a major problem in the field of protein folding. The chemical cleavage method should provide a useful structural tool for the analysis of these nonnative states, particularly in cases where NMR analysis is impractical.

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STEADY-STATE AND TIME-RESOLVED FLUORESCENCE STUDIES ON *E. COLI* RIBOSOMAL PROTEIN L7/L12: WILD-TYPE AND SITE DIRECTED MUTANTS. Jameson, D.M. Dept. Biochemistry and Biophysics, University of Hawaii, Honolulu, HI 96822, USA.

The essential role of the ribosome in protein biosynthesis has been known for many years and considerable effort and ingenuity has been devoted to isolating and characterizing the individual proteins and RNA species which comprise this complex macromolecule. We now enjoy a wealth of structural and functional information on the ribosome yet a detailed understanding of the coordinated chemical and physical processes involved in protein biosynthesis is still elusive. Such understanding requires, in part, more knowledge of the dynamic aspects of ribosomal components and the conformational alterations which occur during ribosomal function. We have utilized steady-state and time-resolved fluorescence methods to investigate the ribosomal protein L7/L12, which is a 12 kD protein present in four copies in the 50S subunit of *E. coli* ribosomes. Oligonucleotide site-directed mutagenesis was used to introduce cysteine and/or tryptophan residues at specific locations along the peptide chain. in both the C-terminal and N-terminal domains. Various sulfhydryl reactive fluorescence probes were attached to the cysteine residues and used to elucidate dynamic aspects of different regions of the protein as well as to study the dimer/monomer equilibria of several L7/L12 variants. Self-energy transfer between fluorescein probes on adjacent subunits and heterotransfer between tryptophan and AEDANS probes were also utilized to elucidate conformational aspects of L7/L12. The data indicate that the two C-terminal domains have considerable flexibility to move independently of each other and with respect to the dimeric N-terminal domain. The data also indicate that, contrary to the usual depiction of L7/L12, the two C-terminal domains are on average well-separated

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ENERGY LINKAGE BETWEEN PROTEIN FOLDING AND PROTEIN NUCLEIC ACID INTERACTIONS. Silva, J.L. and Foguel, D. Departamento de Bioquimica, Instituto de Ciencias Biomedicas, Universidade Federal do Rio de Janeiro, RJ, 21941-540, Brazil.

Our working hypothesis is that the nucleic acid-protein recognition is coupled to protein folding and oligomerization. In the case of Arc repressor, the stabilization of the native dimer is determined by the specificity of the protein-DNA interaction. This free-energy linkage is absent in a mutant Arc protein (P8L), which binds to operator and to nonspecific DNA sequences with equal, low affinity. The coupling between DNA binding and the conversion of partially folded monomers to native dimer seems to account for the ability to recognize operator DNA. The use of hydrostatic pressure has several advantages because it affects the structure of the protein but not that of the nucleic acid. By cold-denaturing the protein repressor-operator complex at sub-zero temperatures under pressure, we have shown that the entropy increases greatly when Arc repressor binds tightly to its operator sequence but not when it binds loosely to a nonspecific sequence. Increase in entropy enables the protein to distinguish between a specific and a non-specific DNA sequence. We postulate that the formation of a protein-operator DNA complex is followed by an increase in nonpolar interactions and release of solvent, which would explain its entropy-driven character, while solvent would not be displaced in nonspecific complexes. The thermodynamic data derived from pressure, calorimetric and binding studies clearly point in the direction of protein-nucleic acid recognition requiring mutual accommodations in the structures of the protein and the nucleic acid. In conclusion, hydrostatic pressure has permitted to assess intermediate states in the assembly of viruses, multimeric proteins and protein-nucleic acid complexes, addressing questions of macromolecular recognition.

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FLUORESCENCE, BIFURCATIONS, PROTEIN FOLDING AND THE SECOND LAW OF THERMODYNAMICS. **Weber G., School of Chemical sciences, University of Illinois, Urbana IL 61801, USA.**

The cyclic process of absorption and emission of light by a stable fluorophore in dilute solution involves competing radiative and radiationless transitions and the bifurcation that determines their relative proportions occurs at the lowest singlet excited state. From the empirical observations it is clear that, while the second law of thermodynamics is obeyed in the alternative processes of radiative and radiationless decay, the bifurcation ratio does not depend on the magnitude of the entropy increase in the complete cycle. Instead it is determined by probabilities that are quantitatively unrelated to heat exchanges and cannot be expressed as such. It is surmised that similar bifurcations exist in the process of protein folding, and the choice between alternative processes is not solely determined by the differences in free energy at any point in the competing pathways, but also, and most importantly, by the specific probabilities that fix the bifurcation ratio. As a result, the difference in free energy between the final folded forms need not represent their respective proportions at equilibrium. Chaperonins and other determinants of the folding path may be expected to act by influencing the bifurcation ratios that are required for a predominance of physiologically folded molecules. This analysis demonstrates by a specific example that the second law of thermodynamics does not prescribe, *per se*, a unique outcome for natural processes; it only limits them to the extent stated by the Clausius-Duhem inequality, that the entropy must increase in their course towards equilibrium.

Symposium 9 - **Biotechnology**

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THE MUSSEL GLUE PROTEINS: A PARADIGMATIC STRATEGY OF BIOLOGICAL ADHESION. Tatiana Silva, Veronica A. Burzio, Joel Pardo, Ricardo Schroeder, Luis A. Burzio and Luis O. Burzio. Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia Chile.

Mussels have developed adhesive strategies to solid surfaces to survive in the turbulent intertidial zone, consisting of the byssus The most important precursors of the byssal adhesive are proteins known as polyphenolic proteins, containing a variety of hydroxylation such as the modification of Tyr residues to 3,4-dihydroxyPhe or Dopa, as wells as the hydroxylation of Pro, Lys and Arg residues.These proteins consist of array of peptides which are repeated in tamdem several times in the primary structure of the adhesive.

The chemistry surrounding the sclerotization of the byssus is not well understood, but the process seems to be strongly related to the presence of proteins containing Dopa and the oxidation of these residues to o-quinones. *In vitro* experiments suggested that the crosslinking involves the covalent interaction between Dopa and oxidized Dopa derivatives such as α, β-dehydroDopa.

Since the glue protein exhibits strong adhesion to a variety of surfaces (glass, plastics, metals and ceramics), there is a great interest to develop new uses of these adhesives for medical and industrial purposes. Since these adhesives are able to mediate the attachment of proteins to a solid support, a system has been developed to enhance the immobilization of antigens or antibodies for diagnostics purposes using EIA (Supported by BioCollico Ltda).

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CAREER OPPORTUNITIES FOR SCIENTISTS IN BIOTECHNOLOGY: NUMEROUS & VARIED

Edward E. Penhoet, Chiron Corporation, Emeryville, California, USA

Scientists tend to perceive career opportunities narrowly in the field of biotechnology with an understandable focus on research. The reality, however, is much broader than the perception. Scientific training is very useful across the entire spectrum of careers in the field. The successful commercialization of a potentially useful research breakthrough requires scale-up and process development, formulation of the product, animal testing, pilot scale manufacturing, manufacturing, clinical testing, data evaluation, submission of clinical results and manufacturing data to regulatory authorities, preparation of sales and marketing literature, and, finally, marketing and sales of the product -- each and every one of these activities represents a career opportunity for a scientist. The list does not stop here, however. Other areas where a scientific background can be very important include patent law, business development and technology evaluation, public relations, and general management.

My presentation will examine each of these opportunities and provide a closer look at the relationship of scientific training to job success in each category.

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RECOVERING A TRADITIONAL INSTITUTION THROUGH BIOTECHNOLOGY

Isaias Raw. Instituto Butantan, S.Paulo, Brazil

Many countries established, at the turn of the century, biomedical Institutes hoping to produce the immunobiologicals they needed. Most of them cannot cope with new developments and requirements.

Ten years ago a Center of Biotechnology was established in the Institute, and started to redesign the plant and process for the production of hyperimmune sera against animal venom and bacterial toxins, being today one the largest world producers. A new concept of product enclosed plants were introduced and applied to large plants were tetanus, diphtheria and pertussis. New development includes the production of poly and monoclonal anti human lymphocyte sera, recombinant hepatitis B vaccine, rabies produced vero cells and eritropoetin. Experimental acellular pertussis and meningitis C/B vaccines will be tested soon.

This technological chance has brought new resources to the Institute, that has also recovered basic science research, and has created a atmosphere for the integration of the basic science laboratories, development and production.

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BIOTECHNOLOGY AND HEALTH CARE: THEMES AND SCHEMES

William J. Rutter, Chiron Corporation, Emeryville, CA, USA.

Biotechnology can make the greatest impact in health care by focusing on disease control rather than disease management. This involves a comprehensive approach driven by metrics and involving diagnosis and prevention as well as treatment. Coupling of these approaches has advantages both from R&D and marketing perspectives.

Using this concept, technology can make a bigger impact, creating and driving markets and allowing the development of commercially attractive businesses. For example, the development of an exquisitely sensitive and quantitative technology to measure nucleic acid sequences allows the determination of viral load. This has facilitated the development of better therapeutic and prevention strategies for viral diseases such as hepatitis and AIDS.

Prevention will also play a bigger role in future health care stategies. Recombinant DNA technology in the past has focused on producing mimics of infectious agents which form safe and efficacious subunit vaccines. Recently a new safe and potent adjuvant has been developed which increases immunological responses from these vaccines. This will not only expand the markets to a large inmunocompromised population (infants & aged) but also will stimulate the use of these preparations as therapeutic agents.

Symposium 10 - **Membrane Systems Involved in Cell Calcium Signalling and Regulation**

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MEMBRANE COMPONENTS ARE INVOLVED IN THE ATP MODULATION OF THE Na^+-Ca^{2+} EXCHANGER IN THE HEART, Luis Beaugé and Graciela Berberian, Instituto de Investigación Médica "Mercedes y Martín Ferreyra", Casilla de Correo 389, 5000 Córdoba, Argentina.

Cardiac membrane vesicles were prepared by differential centrifugation and loaded with 160 mM NaCl, 0.1 mM EDTA and 20 mM MOPS.Tris (pH 7.4 at 37ºC). Usually there was 38 % i.o., 36 % r.o. and 26 % leaky vesicles. ^{45}Ca uptake was measured in media with high (160 mM) and low (10 mM) Na^+, 20 mM MOPS.Tris (pH 7.4 at 37ºC) 5 mM Mg^{2+} and 0.1 mM EGTA. Low Na^+ had the equivalent to 260 mosmols of KCl, NMG.Cl or Bis.Tris. Propane.Cl (BTP). In the presence of 1 µM Ca^{2+}, 0.5 mM vanadate (V) and 5 mM Mg^{2+}, 1 mM ATP increased two to four fold a Na^+ gradient dependent Ca^{2+} influx; in the absence of gradient a 20-40 % increase was observed. This ATP stimulation had the following characteristics: (I) was seen in NMG and BTP but not in K^+ media; (ii) 1 mM ATP-γ-S also stimulated whereas 2 mM AMP-PCP did not; (iii) was due to an increased apparent affinity for Ca^{2+}; a similar K_m^{Ca} shift (from 1-2 µM to 0.1-0.2 µM) was caused by K^+ with and without ATP; (iv) the $K_{0.5}^{ATP}$ was about 0.5 mM; (v) was not observed in the presence of 20 µM eosine (E) without V but it was reinstated when E and V were together. The vesicles lost all Ca^{2+} when the ionofore A23187 was added. These results suggest that in cardiac sarcolemma phosphorylation of a membrane component is involved in MgATP stimulation of the Na^+-Ca^{2+} exchanger. If that is the case, the responsible enzyme(s) must be also membrane bound.

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CALRETICULIN MODULATES CALCIUM WAVE ACTIVITY IN *XENOPUS LAEVIS* OOCYTES. Camacho, P. Department of Neuroscience, University of Virginia, Charlottesville, VA 22908. Intracellular production of inositol 1, 4, 5 trisphosphate (IP_3) activates a receptor-ion channel (IP_3R) of the endoplasmic reticulum (ER). We use the *Xenopus* oocyte translation-expression system together with new *in vivo* confocal Ca^{2+} imaging techniques as a means to directly visualize the functional contributions of proteins active in intracellular Ca^{2+} signalling *[Science* 260:226-229(1993)]. Our recent work has revealed a novel pathway for the luminal regulation of IP_3-mediated Ca^{2+} release [*Cell* 82: 765-7719 (1995)]. Overexpression of the ER luminal Ca^{2+} binding protein Calreticulin (CRT) in *Xenopus* oocytes results in a sustained elevation of intracellular Ca^{2+} with a concomitant inhibition of repetitive Ca^{2+} waves following IP_3R activation. The peak amplitude of the IP_3-induced Ca^{2+} response, is not increased in CRT overexpressing oocytes. Different domains of CRT bind Ca^{2+} in two distinct ways; the central, P-domain binds with high affinity/low capacity while the carboxyl-terminus, C-domain binds with low affinity/high capacity. This suggests potential roles in regulation and storage, respectively, for the P- and C- domains. Deletion mutagenesis implicated the P-domain (and not the C-domain) in inhibition of oscillatory Ca^{2+} release in overexpression studies. CRT modulation of release may result from an interaction with either the IP_3R (to prolong Ca^{2+} release), or with the Ca^{2+}ATPases (to inhibit store refilling). The longest luminal facing loop of the IP_3R (L3 loop) putatively lines the ion channel pore. This loop contains potential sites of direct regulation by CRT (2 glycosylated asparigines) and sites for indirect regulation of thiol groups by an interaction of CRT with PDI (4 conserved cysteines). Co-expression of the L3 loop with CRT, effectively blocks the sustained elevation in Ca^{2+} release obtained by expression of CRT alone, suggesting that the IP_3R is one of the targets of CRT action. Thus, chaperones such as CRT (and Calnexin) which function in protein folding and maturation, may in addition, dynamically modulate ER resident proteins including those involved in Ca^{2+} signalling. Supported by NIH RO1 GM48451.

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ABNORMALITIES OF Ca^{2+} HOMEOSTASIS IN ESSENTIAL HYPERTENSION. Canessa Mitzy. Departamento de Biologia, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Abnormalities of intracellular Ca^{2+} homeostasis and Na^+/H^+ exchange have been implicated in the pathophysiology of human essential hypertension as well as in animal models of hypertension. To further define the nature of cytosolic Ca^{2+} (Ca_i) abnormalities, we have studied peripheral lymphocytes from normal and hypertensive subjects and Ca^{2+}fluxes in cultured vascular smooth muscle cells from normotensive and hypertensive rats. In both hypertensive cells there is an elevation of Ca_i.

Lymphocyte Ca_i was significantly increased in hypertensives compared to normotensives while the subjects consumed high salt diet. Using maximum likelihood analysis, we found that Ca_i levels were distributed in 3 modes. The first mode and 2 upper modes were separated at Ca_i level of 120 nM. The majority of normotensives (86%) and half of the hypertensives (52%) were <120 nM. Subjects with levels <120 nM had a raise in Ca_i when changed to a low salt diet; hypertensives with levels >120 nM did not change Ca_i but their blood pressure fell significantly with salt restriction.

Hypertensives also had increased Na^+/H^+ exchange activity in comparison to normotensives when both were studied in high salt balance. A positive correlation between Na^+/H^+ exchange and Ca_i was observed only in subjects with levels <120 nM. Thus many hypertensive subjects have increased cytosolic Ca^{2+} but this abnormality is not related to enhanced Na^+/H^+ exchange activity in all subjects. The salt-induced change in Ca_i observed in subjects with levels <120 nM and its link to Na^+/H^+ exchange suggests regulation by factors involved in salt/volume homeostassis. Those subjects with Ca_i >120nM, mostly hypertensives, may have abnormalities in this regulation contributing to blood pressure elevation.

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LUMINAL REGULATION OF CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM VESICLES. Hidalgo, C. and Donoso, P. Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile, y Centro de Estudios Científicos de Santiago, Santiago, Chile.

The sarcoplasmic reticulum (SR) of muscle cells has a central role in regulating the free calcium concentration in the myoplasm, that in turn controls the muscle contraction-relaxation state. In skeletal muscle, transverse tubule depolarization elicits massive calcium release from the SR through the ryanodine receptors/calcium release channels. While cytoplasmic regulation of these channels has been extensively studied, their luminal regulation has been less characterized. Only few studies have described activation of vesicular calcium release by increasing luminal Ca^{2+} concentration; calsequestrin, the main SR luminal protein, has been proposed to mediate this activation. In this work, we studied the effect of luminal pH on calcium release kinetics in SR vesicles isolated from rabbit skeletal muscle. Calcium release from ^{45}-Ca^{2+} loaded SR vesicles, measured by a fast filtration method with a high time resolution (ms), was induced by addition of a solution of 2 mM ATP, pCa 5.0, pH 6.8, to vesicles with well-defined luminal pH and luminal [Ca^{2+}]. We found that decreasing luminal pH from pH 6.8 to pH 5.5 overruled the effect of luminal calcium on release kinetics: after triggering release with ATP, high release rate constants were observed even at very low luminal calcium concentrations. We propose that calsequestrin, through protein conformational changes caused either by Ca^{2+} or by protons, has a role in the luminal control of the calcium release channels.
Supported by Fondecyt grants 1940369 and 1961226.

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BIOCHEMICAL EVENTS IN THE CALCIUM ACTIVATED ATPase OF PLASMA MEMBRANES ($PMCa^{2+}$-ATPase). Rega A.F., Herscher, C.J. and Adamo, H.P.. Instituto de Química y Fisicoquímica Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. rega@qb.ffyb.uba.ar.

The $PMCa^{2+}$-ATPase transforms chemical energy into osmotic work by coupling the chemical reaction of hydrolysis of ATP to the vectorial reaction of translocation of Ca^{2+} from the cytoplasm to the medium surrounding the cell. The hydrolysis of ATP takes place along a series of partial reactions including phosphorylation and dephosphorylation of the enzyme which, in addition, adopts two mayor conformational states, typically E_1 and E_2. The vectorial reaction includes the binding of Ca^{2+} to one side and its dissociation from the other side of the membrane. Valuable information on the characteristics of the partial reactions and the effects of natural ligands of the $PMCa^{2+}$-ATPase comes from studies of pre-steady-state kinetics. We have determined the effects of Ca^{2+} and Mg^{2+} on these reactions and the step of the catalytic cycle at which these cations need to bind to be effective. The effects of Ca^{2+} and Mg^{2+} can be ascribed to changes between E_1 and E_2. Conformational changes following binding of ligands can be revealed by an independent procedure such as changes in intrinsic fluorescence. We have observed that intrinsic fluorescence of the $PMCa^{2+}$-ATPase changes with Ca^{2+} and Mg^{2+} in a way compatible with the role that we assigned to these cations in the catalytic cycle of the enzyme. Characterization of biochemical reactions that take place during the transport reaction is essential for the structural information to be interpreted when available.

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Symposium 11 - **Oxidative Stress**

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POSSIBLE ROLES OF NITRIC OXIDE AND PEROXYNITRITE DURING MURINE LEISHMANIASIS

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Activated macrophages simultaneously synthesize nitric oxide and superoxide anion which can react with each other producing peroxynitrite. Consequently, it has been difficult to assess the precise contribution of each of the formed reactive oxygen- and nitrogen-derived species to the microbicidal activities of macrophages, particularly *in vivo*. To explore this problem, we are examining the formation and potential roles of nitrogen-derived intermediates in *Leishmania amazonensis* murine infection. Our results have demonstrated that peroxynitrite is a potent leishmanicidal agent *in vitro* and that both nitric oxide and peroxynitrite are formed during infection of the susceptible BALB/c mouse strain by *L. amazonensis*. Nitric oxide was detected as the nitrosyl hemoglobin complex by electron paramagnetic resonance analysis of blood drawn from mice at different times of infection, and it was shown to increase with the evolution of the disease. Peroxynitrite formation was inferred from immunoreaction of homogenates obtained from footpad lesions with anti-nitrotyrosine antibody; homogenates from parasites drawn from the lesions were also immunoreactive although to a lesser extent. Analysis of protein homogenates by gel electrophoresis and Western blot suggested that peroxynitrite may degrade proteins *in vivo*, in addition to nitrating them. Formation of both nitric oxide and peroxynitrite was not able to eliminate the parasites at least at late stages of the infection.

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MITOCHONDRIAL DNA OXIDATIVE DAMAGE ROLE OF OUTER AND INNER MEMBRANE ELECTRON TRANSFER ACTIVITIES.

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Oxidation of either biogenic amines at the outer mitochondrial membrane or respiratory chain substrates at the inner mitochondrial membrane results in an increase in the intramitochondrial steady-state concentration of hydrogen peroxide.

The former process entails the activity of monoamine oxidases A/B(EC 1,4.3,4, MAO), flavoenzymes that catalyze the oxidative deamination of biogenic amines, such as dopamine, serotonin, and norepinephrine. We examined whether the H_2O_2 formed during the two-electron oxidation of tyramine [4-(2-aminoethyl)phenol] (a substrate for both monoamine oxidases A/B) may contribute to the intramitochondrial steady-state concentration of hydrogen peroxide ($[H_2O_2]_{ss}$) and, thus, be involved in the oxidative impairment of mitochondrial matrix components. The intramitochondrial ($[H_2O_2]_{ss}$) calculated in terms of glutathione peroxidase activity during the metabolism of tyramine was 48-fold higher ($7.71\pm0.25\times10^{-7}$M) than that obtained during the oxidation of succinate via complex II in the presence of antimycin A ($1.64\pm0.2\times10^{-8}$M). This increased $[H_2O_2]_{ss}$ resulted in oxidative damage to the brain mtDNA in terms of single strand breakage, wich was inhibited by the monoamine oxidase inhibitor tranylcypromine and by catalase.

The latter process, oxidation of mitochondrial respiratory chain substrates, was associated with mtDNA base oxidation in the form of 8-hydroxydeoxyguanosine. EPR analysis indicated the formation of hydroxyl radical, which was proportional to the amount of H_2O_2 accumulated.

In both instances, a site-specific mechanism for the formation of hydroxyl radical and leading to mtDNA base oxidation or strand breakage is proposed. The occurrence of a copper pool in mtDNA suggests the homolytic cleavage of H_2O_2 whith formation of hydroxyl radical. These results suggest that the H_2O_2 generated either during the MAO-catalyzed oxidation of biogenic amines and possibly certain neurotransmitters at the outer mitochondrial membrane or during the oxidation of malate/glutamate or succinate by the electron-transfer chain contributes to the intramitochondrial $[H_2O_2]_{ss}$. A relationship was found between an increased $[H_2O_2]_{ss}$ and oxidative damage to mtDNA. This may have potential implications for senescence, mitochondrial dysfunction, and neurodegenerative processes.

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NATURAL POLYPHENOLS AND THEIR ROLE AS ANTIOXIDANTS IN BIOLOGICAL SYSTEMS.

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Through *in vitro* and *in vivo* observations, it has been possible to establish that in addition to the molecular species traditionally recognized as antioxidants (tocopherols, ascorbate, carotenoids, ubiquinol, glutathion, etc) there are various other compounds active as antioxidants. Among the natural polyphenols abundantly present in plants and in vegetable foods, several are at least as active as tocopherols or much more active. Questions derive from these findings: which compounds account for the total antioxidant activity in food?, which for the total antioxidant capacity of plasma and interstitial fluid?; what are the redox interactions among traditional antioxidants and these newly recognized species?; how can we identify systematically these new compounds?; how can we measure total antioxidant capacity in biological systems?

To evaluate the activity of a putative antioxidant, we can measure the decay of a stable free radical exposed to it; it is also possible to study the modification of the oxidative damage exerted by a source of free radicals on a specific biological structure, or, more recently, we can observe the modification of the response to a specific oxidative stress of transcription factors known to respond to oxidative stress, such as NFκB, or the response of genes that are activated by oxidative stress. In our laboratory, we have applied these strategies to the study of flavonoids.

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THE REDOX BIOCHEMISTRY OF NITRIC OXIDE AND PEROXYNITRITE.

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Nitric oxide (NO, nitrogen monoxide) is an endogenously produced free radical which participates in signal transduction as well as in cytotoxic events. The biological activity of nitric oxide is dictated by its reactions with transition metal-containing centers, sulfhydryl groups, molecular oxygen, organic radicals and superoxide radical. Nitric oxide can form reversible complexes with heme and non-heme iron as well as with copper which can modulate protein activity. Electron transfer from nitric oxide at the nitrosyl-metal complex leads to reduction of the metal center and formation of a liganded nitrosating species. The direct reactions of nitric oxide with thiols are rather slow and yield disulfides and nitrous oxide but in aerobic environments nitric oxide evolves to nitrosylating intermediates which may react with thiols to form S-nitrosothiols or hydrolyze to nitrite. Nitric oxide reacts fast with free radicals. The reaction of nitric oxide with organic radicals can terminate free radical preocesses. On the other hand, the combination of nitric oxide with superoxide leads to the formation of peroxynitrite anion ($ONOO^-$). Peroxynitrite anion and its conjugate acid, peroxynitrous acid, ($pK_a = 6.8$) are strong oxidizing and nitrating species which can isomerize to nitrate or evolve to a vibrationally activated intermediate with reactivity similar to that of hydroxyl radical. The fate and redox biochemistry of nitric oxide in biological systems will greatly depend on its steady-state concentration as well as on the availability of its molecular targets.

Symposium 12 - **Signal Transduction**

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SIGNAL TRANSDUCTION PROPERTIES OF A *XENOPUS LAEVIS* MUSCARINIC RECEPTOR AND ADENYLYL CYCLASE EFFECTOR SYSTEM. Olate,J. Dpto. Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile. jolate@halcon.dpi.udec.cl

Recently, we have cloned two oocyte *Xenopus laevis* genes, one encoding for a muscarinic receptor (xlmR) and the other for an adenylyl cyclase (xlAC). Using transient transfection of COS-7 and HEK-293 cells, we studied the functional properties of the xlmR and xlAC. Expression of the xlmR results in the inhibition of AC activity and activation of the MAPK pathway through a mechanism that involves a *Pertussis* toxin sensitive G protein and Gβγ subunits, indicating that the signal transduction properties of the xlmR are similar to mammalian m2 and m4 muscarinic receptors. The xlAC gene is 4,372 bp long which encodes for a protein of 1,355 aminoacids. The xlAC deduced amino acid sequence exhibits a low degree of sequence identity to mammalian enzymes (19-24%), indicating that the Xenopus isoform represent a new member of this protein family. Expression of the xlAC in COS-7 cells resulted in an increased basal AC activity that was stimulated by forskolin, Gpp(NH)p and aluminium fluoride and was insensitive to Ca^{++}-calmodulin. Since no activation of S49 cyc⁻ AC was obtained with the xlGα subunit, we decided to look for proteins that interact with the the xlGαs in vivo by using the two hybrid system. We have detected 6 positive clones that are currently under analysis. (Proyecto FONDECYT 1940256, Proyecto ECOS)

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NITRIC OXIDE TRANSDUCTION PATHWAY IN *Trypanosoma cruzi*. Torres, H. and Flawiá, M. INGEBI (CONICET and FCEN-UBA), Obligado 2490, Buenos Aires, Argentina.

A NO synthase was purified from epimastigote soluble extracts. The enzyme required NADPH, was activated by Ca^{2+}, calmodulin, tetrahydrobiopterin and FAD, and inhibited by N-methyl-L-arginine. Glutamate and NMDA stimulated *in vivo* conversion of arginine to citrulline by epimastigote cells and enhanced cell motility. These stimulations could be blocked by, MK-801 and ketamine and enhanced by glycine and serine. A Na nitroprusside-activated guanylyl cyclase was detected in cell-free, soluble epimastigote preparations. L-glutamate, NMDA and Na nitroprusside increased, *in vivo*, cyclic GMP levels and also enhanced cell motility. This evidence indicates that in *T. cruzi* epimastigotes, L-glutamate controls cyclic GMP levels and cell motility through a pathway mediated by NO.

Using murine cDNA probes of c-Fos and c-Jun, hybridization signals at low stringency were detected in restriction fragments of *T. cruzi* DNA. Employing polyclonal sera raised against human c-fos and c-jun specific polypeptide bands of about 37 and 62 kDa respectively were observed in western blots of cytosolic or nuclear extracts. Moreover, in electrophoretic mobility shift assays using a TRE probe, three complexes were obtained; the two faster migration complexes were specific. Since treatment of epimastigote cells with NMDA increased the polypeptide recognized by the c-fos antiserum it is suggested that AP-1 expression seemed to be under the control of the nitric oxide pathway. (Supported by CONICET, UBA, WHO, F. Antorchas and ICGEB).

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SELECTIVITY AND COMPLEXITY OF RECEPTOR-G-PROTEIN INTERACTION. Schultz, G. Institut für Pharmakologie, Freie Universität Berlin, Berlin, Germany.

The interaction of heptahelical receptors with heterotrimeric G proteins, on one hand, is highly specific and, on the other hand, is complex as many or most receptors interact with more than one G protein. Intranuclear application of antisense nucleotides annealing to the mRNA of G-protein subunits and electrophysiolocal or fluorometric determination of receptor-regulated functions on the single cell level allowed identification of several specific G_o, G_i and G_q/G_{11} heterotrimers that selectively interact with various receptors. Dual coupling of receptors to signalling pathways involving cAMP and calcium elevations has been shown to involve G_s plus G_q/G_{11} or G_s plus G_i stimulation. A few receptors have been identified that couple to even more G-protein subfamilies, i.e. the thrombin receptor activating G_i, G_q and G_{12}/G_{13} proteins (in human platelet membranes) and the TSH receptor interacting with G proteins of all four subfamilies (in membranes of human thyroid). These data support the existence of a G-protein-mediated signalling network in the plasma membrane.

Symposium 13 - **Regulation of Enzyme Activity**

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CASCADES OF INTERCONVERTIBLE ENZYMES AS METABOLIC SWITCHES. APPLICATION TO THE GLUCOSE-INDUCED SWITCH BETWEEN GLYCOGEN PHOSPHORYLASE AND GLYCOGEN SYNTHASE IN THE LIVER. [1]Cárdenas, M. L., [1]Cornish-Bowden, A. and [2]Goldbeter, A. [1]Laboratoire de Chimie Bactérienne, CNRS, 31 Ch. Joseph-Aiguier, 13402 Marseille Cedex 20, France; email cardenas@ibsm.cnrs-mrs.fr. [2]Service de Chimie Physique, Université Libre de Bruxelles, Campus Plaine, C.P. 231, B-1050 Bruxelles, Belgium.

An essential point in the regulation of metabolism is how to produce a sufficiently sensitive response to a signal, i.e. how a metabolic pathway can respond to an effector with enough sensitivity. Cooperativity and high half-saturation concentrations, although necessary for a sensitive response, appear not to be sufficient for allowing an effector to act as a switch of a metabolic pathway. On the other hand, if the action of the effector is not directly exerted on the target enzyme but through a cascade of interconvertible enzymes, a very highly sensitive response can be obtained if certain kinetic requirements are satisfied. The organization of the cascade in closely intertwined cycles of covalent modification multiplies the potential for control. Each of the modifying enzymes in every cycle can be activated or inhibited and can thereby modulate the flux through the target reaction of the cascades. Phosphorylation-dephosphorylation cascades provide a prototype example; they represent an exquisite mode of cellular regulation ranging from regulation of metabolic pathways to control of the cell cycle. One of the best known examples is the cascade controlling the balance between glycogen synthesis and degradation, in which glucose induces a switch between glycogen phosphorylase and glycogen synthetase, such that a sharp threshold is observed. The origin of this threshold will be discussed. [Supported by TOURNESOL]

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LACK OF ALLOSTERIC REGULATION OF PHOSPHOFRUCTOKINASE AND ITS EFFECTS ON GLUCONEOGENIC METABOLISM IN *Escherichia coli*. Guixé, V., Torres, J.C. and Babul, J. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. vguixe@abello.dic.uchile.cl

The reaction catalyzed by phosphofructokinase (Pfk) represents the key commitment step in glycolysis and it is tightly regulated in a wide variety of organisms. In order to assess the physiological importance of the allosteric properties of Pfk, strains of *E. coli* in which only one type of Pfk is expressed, were studied. Strains carrying Pfk-2*, a mutant form of the wild type Pfk-2 isozyme not inhibited by MgATP, grow 3-4 times slower on gluconeogenic carbon sources compared to strains with either of the wild type isozymes, Pfk-2 and Pfk-1.

The futile cycle between fructose-6-P and fructose-1,6-bisP was assessed by measuring the rate of *in vivo* radioactive labeling and the level of fructose-1,6-bisP produced under gluconeogenic conditions using a trace of [U-^{14}C]glucose. Both, the rate of labeling and the level of fructose-1,6-bisP, were higher in the strain with the mutant enzyme compared to the others strains. Also, strains bearing the mutant enzyme, show elevated intracellular concentrations of fructose-1,6-bisP and produce higher levels of radioactively labeled CO_2 when supplied with [U-^{14}C]glycerol as only carbon source.

These results suggest that the strain bearing Pfk-2* sustains higher rates of futile cycle during gluconeogenesis than the others strains. The occurrence of futile cycle would be at the basis of the slow growth phenotype of this strain in gluconeogenic carbon sources. (Fondecyt 1950297).

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PHOSPHORYLATION OF *Candida albicans* MULTYCATALYTIC PROTEINASE COMPLEX (20 S PROTEASOME) BY PROTEIN KINASE CK2. EVIDENCE FOR A CONSERVED PROCESS Passeron, S. Cátedra de Microbiología, Facultad de Agronomía, Universidad de Buenos Aires and CIBYF (CONICET), Buenos Aires, Argentina. passeron@ifeva.edu.ar

Protein degradation is now emerging as an important mechanism of cellular regulation. The eukaryotic 20 S proteasome (MCP), as the catalytic core of the main soluble proteolytic machinery, is responsible of the degradation of important regulatory proteins. We have purified to apparent homogeneity the MCP from yeast cells of *C. albicans*. The purified particle exhibited canonical characteristics: a large molecular mass (680 kDa) composed by several subunits ranging from 20 to 32 kDa, at least three different substrate specificities and a cylindrical shape consisting of a stack of four rings with a hallow center. Consensus sequences for tyrosyl and seryl/threonyl kinases are found in the amino acid sequence derived from MCP subunit cDNAs. Very recently, it has been reported that two subunits (C8 and C9) of mammalian MCP are in vivo phosphorylated and can be phosphorylated in vitro by protein kinase CK2. We have found that *C. albicans* MCP was efficiently phosphorylated by homologous CK2 in a polylysine dependent manner, on at least two subunits of 32 and 27 kDa with a stoichiometry of 4 mole of Pi per mole of MCP. The Km for MCP was 15 nM which is compatible with its intracellular concentration. Preliminary experiments suggest that the proteasome catalytic function is not significantly affected by these phosphorylations. Heterologous phosphorylation assays using MCPs from croaker muscle and *C. albicans* and protein kinase CK2 from rat liver, *C. albicans* and human recombinant CK2 catalytic α subunit seem to indicate that phosphorylation of MCP by CK2 is a conserved mechanism. The identification of *C. albicans* proteasome phosphorylatable subunits is under study

Supported by grants from UBA, CONICET and ICGEB.

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INHIBITION OF BRAIN AND LIVER SUCCINATE AND β-DEHYDROGENASE IN METHYLMALONIC ACIDEMIA: A MODEL TO STUDY THE EFFECT OF TOXIC METABOLITES ON THE INTERMEDIARY METABOLISM. Wainer, M. Departamento de Bioquímica, UFRGS, Porto Alegre, RS, Brasil.

High amounts of methylmalonic acid accumulates in tissues of patients with methylmalonic acidemia, an inherited metabolic disorder caused by deficiency of methylmalonyl-CoA mutase activity and clinically characterized by neurological dysfunction. In the present study we report our findings on the effect of methylmalonic acid on glucose and ketone bodies utilization in cerebrum of young rats. We observed that the metabolite provokes important inhibitions of succinate dehydrogenase and β-hydroxybutyrate dehydrogenase in brain and liver. As a result, glucose cannot be fully oxidized in brain, leading to a higher lactate release and lower CO_2 production. Besides, brain cannot use properly the alternative substrates ketone bodies since the conversion of β-hydroxybutyrate to acetatoacetate is impaired. All this leads to a deficit of energy and consequently to excitotoxic mechanisms, what is demonstrated by the induction of convulsions by intrastriatal administration of methylmalonic acid and by their prevention by MK-801, a NMDA antagonist. The blockages of succinate conversion to fumarate and acetoacetate to β-hydroxybutyrate in liver may respectively explain serum low levels of glucose and ketone bodies verified in these patients. Therefore, our results may be related to the neurological symptoms commonly found in methylmalonic acidemia.

Symposium 14 - **Extracellular Matrix-Cell Adhesion and Recognition**

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PROTEOGLYCANS: ESSENTIAL EXTRACELLULAR MATRIX COMPONENTS DURING SKELETAL MUSCLE DIFFERENTIATION. Brandan, E. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago, Chile.

The role for the extracellular matrix (ECM) in the regulation of cell differentiation and gene expression seems to be essential in those systems where several cell types are present. Particular ECM components in skeletal muscle differentiation seems to be required. A special interest has been focused on one type of macromolecules found in the ECM, the proteoglycans. These macromolecules are found either associated to the plasma membrane and to the ECM and several functions have been described. In particular they seems to be essential in process such as cell adhesion, cell migration, cell proliferation and maintenance of the differentiate state.

In this presentation we will focus on the biochemical characteristics of several proteoglycans found associated to skeletal muscle tissue, such as decorin, glypican, syndecan-1 and perlecan. We will examine the level of expression of those proteoglycans during skeletal muscle differentiation and how the expression is regulated, and the possible functions of these macromolecules during skeletal muscle differentiation, specially focused to the presentation and/or sequestration of growth factors, strong inhibitors of skletal muscle terminal differentiation.
(Supported by Fondecyt 1960634)

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THE α5β1 INTEGRIN IS A HYBRID CELL SURFACE CHONDROITIN-HEPARAN SULFATE PROTEOGLYCAN. Veiga[1], S.S., Elias[1], M.C.Q.B., Gremsky[1,2], W., Porcionatto[3], M.A., Silva[3], R., Nader[3], H.B. and Brentani[1], R.R. [1] Ludwig Inst. for Cancer Research, S. Paulo. [2] Dept. Cellular Biology, Federal Un. of Parana, Curitiba. [3] Dept. of Biochemistry, Federal Un. of S. Paulo, Sao Paulo, Brazil.
Fibronectins are glycoproteins implicated in a wide variety of cell-adhesive process as platelet sub-endothelial adhesion and aggregation, phagocytosis, cell differentiation and during tumor cell dissemination in metastasis. All cellular properties in which fibronectins have been implicated represent events mediated by cell surface receptors that interact with fibronectins basically through RGD (integrins) and glycosaminoglycans (GAG) binding domains (proteoglycans). Herein, working with human melanoma cell line Mel-85 based in immunoprecipitation reactions from cell extracts that were iodine (^{125}I) cell surface labeled or metabolically radiolabeled by sodium sulfate (^{35}S) we were able to detect α5β1 integrin as the only sulfated integrin compared with other $\alpha_{(s)}\beta1$ heterodimers expressed by these cells. This facultative sulfation was confirmed by fibronectin-affinity chromatography, two dimensional electrophoresis and by chemical reduction producing a comigration of both α5 and β1 integrin chains characteristics of this integrin. Based in deglycosylation procedures as chemical β-elimination, proteinase K digestion and susceptibility to chondroitinase ABC and heparitinase Type I and II of the α5β1 heterodimer we were able to confirm this integrin as a proteoglycan molecule.

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STRUCTURE AND FUNCTION OF SCHWANN CELL EXTRACELLULAR MATRIX. Carey, D.J. Weis Center for Research, Geisinger Clinic, Danville, PA, USA
During the terminal differentiation of peripheral nerves Schwann cells synthesize and assemble a complex extracellular matrix (ECM) that consists of both basement membrane and interstitial matrix components. Studies utilizing primary co-cultures of rat embryo Schwann cells and sensory neurons have shown that Schwann cell contact with assembled ECM is essential for their terminal differentiation, which is characterized by a cessation of proliferation and migration and the elaboration of myelinated and unmyelinated ensheathment of axons. Schwann cells synthesize a number of ECM receptor proteins, including several types of membrane associated heparan sulfate proteoglycans. A novel, peripheral nerve specific ECM protein was purified, based on its ability to bind with high affinity to N-syndecan, a transmembrane proteoglycan. This protein, p200, is secreted by Schwann cells and co-assembles with collagen I and fibronectin into a fibrillar ECM in Schwann cell cultures. p200 consists of a collagen-like domain that is responsible for the assembly of the protein into trimers, plus a non-collagenous domain that confers heparin binding activity. Purified p200 promotes Schwann cell adhesion and spreading. p200 is expressed in peripheral nerves only during the period of Schwann cell differentiation. These findings suggest an important role for p200 and its receptor in peripheral nerve development.

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EPITHELIAL APOPTOSIS, FIBROBLAST PROLIFERATION AND MATRIX METABOLISM DURING CHRONIC LUNG INJURY. Selman, M., Instituto Nacional de Enfermedades Respiratorias, México.
Independent of etiology, chronic lung injury usually evolves to diffuse pulmonary fibrosis which represents and advanced, progressive, and generally lethal lung disorder. The evolution of a chronic, unresolving lung inflammation to a fibrotic process is characterized by abnormal proliferation of mesenchymal cells, specifically fibroblasts and myofibroblasts, and by the accumulation of excessive amounts of matrix proteins, mainly collagens. Three crucial events seem to occur during this pathological response: a persisting fibroproliferative reaction in both interstitial as well as intraalveolar spaces, an increase in apoptotic cell death of alveolar epithelial cells, and a progressive collagen accumulation which irreversibly distorts the lung architecture. Programmed cell death is provoked by factor(s) secreted by fibroblasts, and there is some evidence suggesting that the activity of the factor(s) is dependent upon nitric-oxide-initiated oxidation or nitrosylation by the fibroblasts. In addition, disruption of basement membrane extracellular matrix may enhance epithelial apoptosis. Excessive collagen deposition is the result of an imbalance in the collagen turnover rates characterized by a transient increase in collagen synthesis, with a decrease in collagen degradation. Fibrosis is considered otherwise, the final common pathway of a variety of chronic lung disorders, and in this context, the diagnosis of pulmonary fibrosis implies the recognition of an advanced stage in the evolution of a complex process of abnormal repair.

Symposium 15 - **Metabolic Organization**

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METABOLIC ORGANIZATION IN THE INTERORGANELLE CYTOPLASM OF EUKARYOTIC CELLS. Clegg, J.S. Bodega Marine Laboratory, University of California (Davis), Bodega Bay, California, USA. jsclegg@ucdavis.edu

It has long been known that various metabolic pathways occur ir membrane-limited cytoplasmic compartments. More recently it has been realized that metabolic organization is far more extensive, albeit more subtle, than previously thought. This presentation will consider the *cytosol,* an ambiguous term often assigned to the soluble phase of the cytoplasm. Historically, this extensive compartment was viewed as a concentrated solution containing (chiefly) proteins, various metabolites and inorganic ions. Much of intermediary metabolism was thought to occur there through the random collisions of freely diffusing enzymes, cofactors and intermediary metabolites. That view, possibly still held by some, is now highly suspect and, in my opinion, incorrect. Available evidence indicates strongly that the *cytosol in vivo* is neither a crowded solution nor a site for much metabolism. I will make the case that enzyme-enzyme, and enzyme-cytoskeletal interactions dominate metabolism in the interorganelle cytoplasm. The ultrastructural equivalent of this metabolic organization could be the microtrabecular lattice (MTL) described by Keith Porter using HVEM. Those images, although static snapshots, are more in keeping with the collectiv evidence on cytoplasmic structure than is the "crowded cytosol" I will consider some potentially far-reaching consequences of this *MTL paradigm* to current thought about metabolism, transmembrane modulation and the intracellular transmission of signals.

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KINETIC IMPLICATIONS OF CHANNELLING BETWEEN CONSECUTIVE ENZYMES IN METABOLIC PATHWAYS. Cornish-Bowden, A. and Cárdenas, M. L. Laboratoire de Chimie Bactérienne, Centre National de la Recherche Scientifique, 31 chemin Joseph-Aiguier, 13402 Marseille Cedex 20, France; email athel@ibsm.cnrs-mrs.fr.

Study of the kinetics of metabolic pathways and other multi-enzyme systems during the past two decades has required revision of some long-standing ideas of metabolic regulation. In particular, the idea of a "rate-limiting" enzyme whose activity controls the flux through an entire pathway is misleading, as control of flux is shared by all of the enzymes of a system. This is the fundamental idea of metabolic control analysis. However, it was derived mainly from consideration of multi-enzyme kinetics in terms of independent non-interacting enzymes, whereas simultaneous advances in knowledge of cell architecture have shown that it is also misleading to regard the cytoplasm as a mixture of enzymes in free solution. Among other points that need to be allowed for in any discussion of kinetic behaviour *in vivo* is the increasing evidence that the product of an enzyme can be directly transferred, or "channelled", to the active site of an enzyme that uses it as substrate, with no need for it to pass through free solution. How far does the new knowledge of the complexity of cell structure require a revision of the conclusions from metabolic control analysis? Many quantitative details need to be modified when channelling and other aspects of enzyme-enzyme interaction are taken into account, but the qualitative picture is not greatly changed: it remains fair to think of flux control as shared between the enzymes of a system. Moreover, effects of channelling on the free concentration of a channelled intermediate are much smaller than one might guess.

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GLYCOGEN SYNTHESIS IN AMPHIBIAN OOCYTES AS A MODEL SYSTEM FOR THE STUDY OF METABOLIC ORGANIZATION. Preller, A., Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. (apreller@abello.dic.uchile.cl).

Labelled glucose (microinjected or present in the incubation medium) is readily metabolized by frog oocytes into glycogen, the main product of glucose metabolism in these cells. Glucose incorporation occurs preferentially by an indirect pathway that requires prior degradation of glucose to three carbon compounds, which are then, via gluconeogenesis, converted into the glucosyl units of glycogen. All enzymes of glucose utilization are present in oocytes homogenates.
Recent advances in metabolic organization indicate that enzymes, and hence metabolic pathways, have a discrete cellular location. Enzyme-enzyme plus enzyme-membranes or enzyme-cytoskeletal interactions would support metabolic pathways organization. Glycogen synthesis pathway in frog oocytes seems in accordance with this statement. We have found that, in these cells, glycogen location is particularly restricted to the cell cortex both in animal and vegetal hemispheres, with glycogen granules filling the plasma membrane microvilli. Also, glycogen is abundant in the perinuclear region. Some enzymes of the biosynthetic pathway, i.e. fructose-bisphosphatase colocalize with the polysacharide. Cytoskeleton proteins such as tubulin exhibit the same cortical and perinuclear distribution. Implications of these findings in metabolic architecture will be discused. (Supported by grant 1940583, Fondecyt).

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EVOLUTIONARY THOUGHTS ABOUT THE ORGANIZATION OF ENZYMES *IN VIVO*. Ureta, T. *Departamento de Biología, Facultad de Ciencias, Universidad de Chile, P.O. Box 653, Santiago, Chile.* e-mail: tiureta@abello.dic.uchile.cl

Enzymology has come a long way since enzyme hunters began to characterize catalytic activities with ever more sophisticated tools for assays, purification, mechanistic studies, etc. Following the dictum of Ephraim Racker (*"don't waste clean thoughts on dirty enzymes"*), enzymologists have achieved high-resolution X-ray crystallography of fairly pure crystals and the determination of amino acid sequences, and the ultimate accomplishment: studying enzymes without ever touching the protein, relying instead on the nucleotide sequence of an open reading frame. On the other hand, modern enzymologists are increasingly coming back to the recipe laid out more than fifty years ago by Otto Warburg (*"study enzymes under the most natural conditions of action, in the living cell itself"*), *i.e.*, in the state of utmost impurity!

Using Racker's and Warburg's advice in a recursive manner, modern enzymologists have reached the working hypothesis that metabolic pathways (or parts thereof) are catalyzed by enzymes which may exist as: *1*) functionally independent activities; *2*) multienzyme complexes; *3*) bound to structural proteins of the cytoskeleton; *4*) multifunctional proteins. In different organisms, a given pathway (*e.g.*, fatty acid degradation) may adopt one or several of the arrangements summarized above, thus providing the opportunity to infer evolutionary strategies favoring the diverse alternatives. I will use an evolutive approach to review the organization of enzymes involved in fatty acid synthesis and degradation, urea synthesis and the glycolytic pathway. (Supported by FONDECYT Grant 1940583).

Symposium 16 - **Novel Aspects on the Biodegradation of Natural and Man-made Aromatic Compounds**

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RECENT ADVANCES IN THE MOLECULAR GENETICS OF THE LIGNIN-DEGRADING BASIDIOMYCETE *PHANEROCHAETE CHRYSOSPORIUM*. Cullen, D. Institute Microbial and Biochemical Technology, Forest Products Laboratory, One Gifford Pinchot Dr., Madison, WI 53705 USA. Email: dcullen@facstaff.wisc.edu.

Extracellular enzymes of *P. chrysosporium* implicated in the degradation of lignin and organopollutants include lignin peroxidases (LiPs), manganese peroxidases (MnPs) and glyoxal oxidase (GLOX). Knowledge concerning the structure, genomic organization, and transcriptional regulation of *lips, mnps*, and *glx* has advanced considerably during the past 10 years. Multiple LiP isozymes are encoded by a family of 10 structurally related genes designated *lipA* throught *lipJ*. Three *mnps* have been sequenced, and *glx* is encoded by a single gene. Genetic and physical mapping show close linkage among 8 *lips*, while *lipD* and *lipF* remain unlinked to each other or to any *mnps* or *glx*. Transcriptional regulation of *lips, mnps*, and *glx* has been investigated using RT-PCR techniques. Dramatic modulation of transcript patterns has been observed in response to media composition. These methods have been extended to *P. chrysosporium* in complex substrates including wood chips and soils contaminated with polycyclic aromatic hydrocarbons (PAHs). These recent results will be discussed in some detail.

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EXPRESSION OF THE CHLOROPHENOL-DEGRADING ABILITY OF *Alcaligenes eutrophus* JMP134 (pPJ4) IN A COMPLEX SYSTEM. B. González. Lab. de Microbiología. Facultad de Ciencias Biológicas. P. Universidad Católica de Chile, Chile. bgonzale@genes.bio.puc.cl

Alcaligenes eutrophus JMP134 (pJP4) grows, among other chloroorganics, on 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,6-trichlorophenol (2,4,6-TCP). The efficiency of degradation of these compounds, as well as the survival of strain JMP134 and endogenous microorganisms were assessed in short-term batch or long-term-continuous incubations of this strain with a chloroorganic- and organic matter containing- industrial effluent. After six days of incubation, 2,4-D (400 ppm) or 2,4,6-TCP (40-150 ppm) were extensively degraded (70-90%). Under these conditions, native microorganisms were unable to degrade such level of compounds. Less degradation of 2,4,6-TCP by strain JMP134 was observed at 200-400 ppm of compound, by the presence of native microorganisms, and by the lower organic matter content of an aerobically treated effluent. Degradation of 2,4-D and 2,4,6-TCP was slightly dependent on the amount of starting inocula of strain JMP134. This strain also degrades 2,4-D and 2,4,6-TCP when the effluent was amended with a mixture of these two compounds, or the same mixture plus other chlorophenols. In long-term incubations, strain JMP134 was unable to keep a high, stable population, although high rates of 2,4,6-TCP degradation were observed with as low as 10 cultivable strain JMP134 cells/mL, suggesting the activity of endogenous 2,4,6-TCP degraders. Long-term incubations did also remove significant levels of 2,4-D and a mixture of four chlorophenols, in the presence of higher amounts of strain JMP134.

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MICROBIAL DEGRADATION OF HALOAROMATIC COMPOUNDS: ON THE NECESSITY TO DEVELOP BIOCATALYSTS WITH COMPLETE METABOLIC PATHWAYS

D.H. Pieper. R. Blasco, M. Mallavarapu, M. Prucha, R. Erb, B. Jakobs, B. Gonzales[1], R.-M. Wittich, B. Happe and K.N. Timmis Dept. Environm. Microbiol. GBF - Ntl. Res. Centre for Biotech., Braunschweig, Germany and [1]Dept de Biol. Cellular y Molecular, Pontificia Univ. Catolica de Chile, Santiago, Chile

The majority of microorganisms able to mineralize chloroaromatics initiate metabolism by transformation to chlorocatechols by unspecific peripheral enzymes. A wide variety of chloroaromatic compounds are thereby converted to a group of related metabolic products. However only a small fraction of bacteria able to transform chloroaromatics simultaneously exhibit the metabolic potential for mineralization. Thus, routes other than the chlorocatechol pathway, which have the potential to metabolize these compounds are of considerable relevance in nature.

We will show that both 4-chloro- and 3-chlorocatechol can be transformed into the antibiotic protoanemonin by enzymes of the widespread 3-oxoadipate pathway and that, moreover, protoanemonin formation can occur by distinct metabolic routes. Evidence will be presented that toxicity of chloroaromatics towards chloroaromatic metabolizing organisms can be due to the formation of protoane-monin and that the formation of protoanemonin can account for the poor effectiveness of PCB cometabolizing organisms in natural environments. Additionally, we will show that misrouting of chlorocatechols into the meta-cleavage pathway can create major problems and a self-inhibiting metabolic route for chlorodioxin metabolism will be presented.

Evidently, biocatalysts harbouring effective complete metabolic routes for chloroaromatics are necessary. Gene cassettes harbouring chlorocatechol degradation genes have now been developed. Transfer of this cassette into appropriate hosts result in effective mineralization but also detoxification of any misrouted substrate.

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DIVERSITY IN CHLORORESPIRATION Tiedje, J., Löffler, F., Sanford, R. and Cole, J. Center for Microbial Ecology, Michigan State University, East Lansing, MI, USA.

A number of bacteria have now been shown to grow by chlororespiration, a process in which the reductive dechlorination of organochlorine compounds serves as the sole electron acceptor resulting in growth. This process occurs only under anaerobic conditions and most but not all chlororespirers are obligate anaerobes. In most cases these organisms exhibit considerable substrate specificity for their organochloro electron acceptor, and in some cases they are obligate chlororespirers, i.e. no other electron acceptor is known to support growth. The chlororespirers we have studied comprise 5 different substrate or taxonomic groups: Gram negative *Desulfomonile* strains use 3-chlorobenzoate as electron acceptor, Gram positive *Desulfitobacterium* species use chlorophenols as their electron acceptors, a new group of facultative anaerobic myxobacteria, "*Anaeromyxo dehalogens*" uses 2-chlorophenols as electron acceptors, a new Gram negative delta Proteobacteria uses trichloroacetic acid as its electron acceptor, and a number of diverse strains have been shown to use tetrachloroethene as their electron acceptor. We have partially purified the dechlorinase from *Desulfitobacterium chlororespirans* strain Co23: it is a membrane bound protein with specificity for multiple substituted aromatic compounds. Chlororespirers partition a majority of their electron flow to dechlorination, and all we have studied reduce H_2 concentrations to below 1 ppm, consistent with the theoretical prediction if this redox couple was supporting respiratory energy production. These characteristics help distinguish cometabolic (fortuitous) dechlorination, such as by methanogens, from chlororespiration.

Symposium 17 - **Metabolic Regulation in Yeasts**

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YEAST GLYCOLYSIS WITH LOWERED ENZYME LEVELS. Fraenkel, D.G. Microbiology and Molecular Genetics, Harvard Medical School, Boston MA 02115, USA.
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Functioning of pathways in cells presents well appreciated problems: uncertainties about the pathways themselves; whether the enzymes act independently with the same kinetic characteristics as in the test tube; and aspects of rate limitation. The present work involves a complicated example. The Gcr system of *Saccharomyces cerevisiae* has at least two proteins, Gcr-1p and Gcr-2p, which together make a transcriptional element necessary for high expression of most glycolytic genes. gcr1 (1) and gcr2 (2) mutants are both markedly affected in levels of the several enzymes. As might be expected, gcr1 mutants do not grow on glucose. But gcr2 mutants grow quite well. Data on glycolytic flux, enzyme and metabolite levels, obtained with H. Uemura, will be used to illustrate problems and uses of such analysis.

1) Clifton, D., and Fraenkel, D.G. (1981). J. Biol. Chem. 256, 13074-13078.
2) Uemura, H., and Fraenkel, D.G. (1990). Mol. Cell. Biol. 10, 6389-6396.

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CONTROL OF THE YEAST SUGAR TRANSPORT
Lagunas, R. Instituto de Investigaciones Biomédicas, CSIC. Arturo Duperier, 4. 28029-Madrid, Spain.

Sugar transport is the first rate limiting step of glycolysis in *Saccharomyces cerevisiae* and several strategies are used by this organism to adjust this uptake to the different environmental conditions. One of these strategies is the catabolite inactivation of the sugar transporters that occurs when protein synthesis is impaired upon exhaustion of a nitrogen source in the medium and that results in important physiological effects. This inactivation mainly affects the Vmax, follows first order kinetics and is an energy dependent process stimulated by fermentable substrates. Using polyclonal antibodies against a recombinant maltose transporter protein and appropriate mutants it has been shown that this inactivation is due to a proteolysis that occurs independently of the function of the proteasome and that takes place in the vacuole after internalization by endocytosis.

Actin microfilaments are required in yeast fluid-phase as well in α-factor endocytosis when bound to its receptor. Using mutants defective in β-tubulin, actin and the actin-binding proteins Sac6 and Abp85, as well as nocodazole and cytochalasin D which respectively inhibit formation of microtubules and microfilaments, it has been shown that actin microfilaments are only partially required for endocytosis of the maltose transporter while microtubules are not. The observed quantitative differences between actin network requirements of α-factor receptor and maltose transporter endocytosis raise the question of whether this is due to a different contribution of distinct endocytic pathways to these two processes

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EXPRESSION OF *KLUYVEROMYCES MARXINAUS* GAP1 GENE ENCODING A SURFACE PROTEIN ASSOCIATED WITH CELL ADHESION

Fernandes P., Moreira R.F., Moradas-Ferreira P.: Instituto de Ciências Biomédicas Abel Salazar e Centro de Citologia Experimental, Universidade do Porto, Porto, Portugal

The yeast *K.marxianus* genome contains a glyceraldehyde-3-phosphate gene family of 3 members (1). The coding region of one of the genes (*GAP1*) has a 80% homology with the other two genes, while the homology for the upstream region is not significant. *GAP1* is constitutively expressed either on glucose or ethanol carbon source and encodes a 37KDa glycoprotein which is localised in the cell wall, being accumulated when cells flocculate. This protein does not contain a signal peptide, nevertheless the protein is targeted to the cell surface as indicated by different evidences. A *gap1* mutant strain do not accumulate the protein in the cell wall and no longer forms flocs. Thus GAP1p is involved in cell adhesion and uses an alternative pathway to be targeted to the cell surface.

1. Fernandes P.A., Sena-Esteves M., Moradas-Ferreira P.(1995) "*Characterization of the glyceraldehyde-3-phosphate dehydrogenase gene family from K.marxianus - PCR-SSCP as a tool for the study of multigenic families*" Yeast 11, 735-733

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CHANGES, EFFECTS AND REGULATION OF CYTOPLASMIC AND VACUOLAR INTERNAL pH OF YEAST. Peña, A., Martínez, G.A., Calahorra, M. Instituto de Fisiología Celular, UNAM. Apartado 70-242. 04510 México, D. F., México.

The internal pH of yeast has been measured by following the fluorescence changes of pyranine introduced into the cells by electroporation. The values obtained in resting cells (slightly below 6.0), are increased by incubating the cells at high pH values in the presence of a substrate, or by the addition of monovalent cations plus glucose, following the same selectivity pattern of their transport. These results appear to be due to the function of the plasma membrane H^+-ATPase, which drives the transport of the cations, or can even pump protons at high pH values of the medium in the absence of any cations. If the cells are electroporated and looked at under the miscroscope, pyranine is observed only in the cytoplasm; however, after some time in the presence of glucose, it is concentrated in the vacuole, which allowed to measure the pH of this organelle. The values obtained for the pH of the vacuole are more or less in agreement with those found by other authors; however, after preincubating the cells, it was found that glucose, which can produce an increase of the cytoplasmic pH, on the contrary, produced a small decrease of the vacuolar pH. The addition of monovalent cations after preincubating the cells in the presence of glucose, produced an increase of both the cytoplasmic and the vacuolar pH of the cells. There are some implications of these observations, since it appears that the vacuole may not play such a significant role in the regulation of the internal pH of the cell, at least not as a proton sink. Besides, the changes of the cytoplasmic pH are relevant to the regulation of at least several steps of yeast metabolism which were studied.

Symposium 18 - Signal Transduction and Regulation of Gene Expression in Plants

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TRANSDUCTION OF LIGHT SIGNALS IN PLANTS

Chris Bowler, Stazione Zoologica, Naples, Italy; Gunther Neuhaus, University Freiburg, Germany; Nam-Hai Chua, The Rockefeller University, New York, USA

The phytochromes are the best studied plant photoreceptors, controlling a wide variety of responses at both whole plant and single cell levels. Three signal transduction pathways, dependent on cGMP and/or calcium, are utilized by phytochrome to control the expression of genes required for chloroplast development and anthocyanin biosynthesis. Control of the relative inputs into these different transduction pathways may be a means by which plant cells can adjust their biochemical status, in particular for controlling the levels of photoprotectants (such as anthocyanins) and the relative amounts of the different photosynthetic complexes. If this were the case one would predict the existence of cross-talk mechanisms between the different phytochrome pathways, which would allow the activities of each pathway to be known by the others and which would allow regulatory signals to pass between them. Experiments will be presented that have elucidated some of these negative regulatory mechanisms. In addition to these studies of light-activated processes, we have also performed some experiments to understand how phytochrome can down-regulate other events. For example, the expression of several genes is known to be negatively-regulated by light. We have determined how such genes are regulated by phytochrome in the context of calcium and cGMP and have identified an 11 bp cis-element present within the promoters of such genes that is both necessary and sufficient to mediate light down-regulation.

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Identification of Minimal Photoresponsive Elements and the signal transduction to activate them. Argüello-Astorga, G. and Herrera-Estrella, L. Depto. de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados, I.P.N.-Unidad Irapuato, México.

The work done by several research groups has shown that the promoter region of *cab* and *rbcS* genes contains several Light Responsive Elements (LREs), regarded as functionally redundant. This redundancy of LREs has constituted an obstacle to define the precise role of each of the many protein factors interacting with the control regions of these genes. To overcome this difficulty, it is indispensable to delimit Minimal Photoresponsive Elements (MPEs), which can be defined as minimal native sequences which fused to a heterologous truncated promoter (itself inactive), are able to promote light inducible transcription. By means of a phylogenetic and structural analysis of the *rbcS* upstream sequences, we identified a small region which is "structurally" conserved in *rbcS* genes from dicotyledonous as well as from monocotyledonous plants. A 52 bp synthetic oligonucleotide which spans this conserved region was fused to a CaMV 35S minimal promoter (-46 to +8). This chimaeric promoter was shown to be able to activate the expression of GUS in transgenic tobacco. This expression was found to be light-regulated and specific of photosynthetic tissues. This is, as far as we know, the shortest native *rbcS* sequence that has been experimentally defined as an LRE. This sequence includes the binding sites of two nuclear factors (IBF and GBF), which are also known to interact with sequence elements present in *cab* promoters. Selective mutation of the binding sites for GBF and GA-1 within this MPE, allowed us to establish that have different functions but operate coordinately to activate transcription in light grown plants. Experiments aimed to determine some of the components in the signal transduction pathway of light activation of this MPE will be presented.

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MECHANISM OF TRANSCRIPTIONAL ACTIVATION MEDIATED BY SALICYLIC ACID IN TOBACCO

Ramírez, I., Ojeda, H., Stange, C., Hidalgo, P.and Holuigue L. Departmento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile. P.O. Box 114-D, Santiago, Chile.

Salicylic acid (SA) is an important signal that mediates defense reponses induced by pathogens in plants. Thus, it has been reported that SA is responsible for the transcriptional activation of some defense genes occuring after pathogen infection. The mechanism by which SA signal is transduced from the cytoplasm to the nucleus to activate transcription is still not clear. In order to inquire about this mechanism, we assess the identification of nuclear components of the SA-mediated signal transduction pathway. With this purpose, we have been using defined promoter sequences identified as Salicylic Acid Responsive Elements (SAREs), like the *as-1* element from CaMV 35S promoter. This sequence, that specifically binds to the nuclear factor ASF-1, was previously characterized to respond to SA with an immediate early kinetics. Using *in vitro* DNA-protein binding assays with nuclear extracts from tobacco SA-treated plants or from control water-treated plants, we were able to determine that SA produces an increase in ASF-1 binding activity. Our results indicate that this increase in binding activity is mediated by protein phosphorylation events. Coincidently, an increase in protein kinase activity is detected in nuclear extracts after treatment with SA. This results support the idea that a nuclear protein kinase and the transcriptional factor ASF-1 are components of the SA-mediated signal transduction pathway.

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IDENTIFICATION OF GENES INDUCED IN EARLIER STEPS OF THE HYPERSENSITIVE RESPONSE IN *ARABIDOPSIS THALIANA*

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The hypersensitive response (HR) is a plant defense mechanism active against viruses, fungi and bacteria. It involves the rapid and localized cell death at the site of infection, depriving the pathogen of living host cells and, therefore, limiting its further spreading. Resistance of the host plant is strongly correlated with the expression of HR, although it is not clear *if it* is the cause or the consequence of resistance. Recent data suggest that death of host cells during de HR results from the activation of an intrinsic cell death program. The identification of plant genes implicated in such a program are of considerable interest from the fundamental and applied point of view. The object of this work was to undertake a genetic study of the earliest steps leading to HR. The phytopathosystem chosen was the interactions between *Arabidopsis thaliana* and *Xantomonas campestris* pv. *campestris*, in which the HR had been previously characterized (Lummerzhein et al. (1993). *MPMI*, 6, 532-544). The phytopathogenic bacterium *X. c.* pv. *campestris* is the causal agent of black rot of crucifers, resulting in major crop lost. As many xanthomonads, *X.c.*pv.*campestris* infect also weeds related to the host plants, including *A. thaliana*. This small weed is uniquely suited for molecular cloning experiments, due to its reduced genomic size. The genes induced during the first hour of the incompatible interaction between *A. thaliana* and *X.c.* pv. *campestris*, were analyzed using the differential display approach. Among the several potentially induced cDNA fragments already identified, three of them showed similarities with sequences described in data banks: a leucin aminopeptidase from *A. thaliana*, a calcactrin from *Atriplex numularia*, and a protein kinase related to APK1a and b from *A. thaliana*. The full-length transcripts were identified and their expressions on different stress conditions were evaluated. Their possible role on the HR will be discussed.

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WOUND-INDUCED GENE EXPRESSION IN PLANTS AND SIGNALS INVOLVED IN ITS REGULATION. Peña-Cortés. H. Institut für Genbiologische Forschung, Ihnestr. 63, D-14195 Berlin, Germany.

Chemical and physical signals have been reported to mediate wound-induced proteinase inhibitor II (pin2) gene expression in tomato and potato plants. Among the chemical signals, phytohormones such as abscisic acid (ABA) and jasmonic acid (JA) and the peptide systemin represent the best characterized systems. Furthermore, electrical and hydraulic mechanisms have also been postulated as putative pin2-inducing systemic signals. Most of the chemical agents are able to induce pin2 gene expression without any mechanical wounding. Thus, ABA, JA, and systemin initiate pin2 mRNA accumulation in the directly treated leaves and in the nontreated leaves (systemic) that are located distal to the treated ones. Recently, it was reported that heat treatment and mechanical injury generate electrical signals, which propagate throughout the plant. These signals are capable of inducing pin2 gene expression in the nontreated leaves of wounded plants. Mechanical damage, electric current application and localized burning led to the accumulation of proteinase inhibitors II (Pin2) mRNA in potato and tomato wild type plants in a local and systemic manner. Electric current, similar to mechanical wounding, requires the presence of ABA to induce Pin2 gene expression. Conversely, burning of the leaves activates Pin2 gene expression by triggering directly the biosynthesis of JA by an alternative pathway which is independent of endogenous ABA levels. Increased endogenous levels of JA in transgenic potato plants, obtained by overexpressing the flax allene oxide synthase, does not affect the expression of wound-induced genes. Thus, the increase of JA levels, at least under these conditions, is not sufficient for leading to a constitutive expression of wound responsive genes. Interestingly, the modified levels of JA lead to the formation of damage symptoms in the transgenic plants which cause an accelerated death of the leaves. Involvement of JA in such processes will be discussed.

Symposium 19 - **Enzyme Structure and Function**

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PROTEIN STRUCTURE AND FUNCTION IN BIOLOGICAL ENVIRONMENTS: NEEDLES IN HAYSTACKS? Martinez-Carrion, M., School of Biological Sciences, University of Missouri, Kansas City, 64110 USA.

The message of how a protein folds to acquire its function is contained within its amino acid sequence. *In vitro*, there are many experimental alternatives to investigate the folding process, the conformation of the protein, and the identity and possible roles of the "active" components of the protein. The latter are reasonably assumed that may act in similar ways within the cell. The former is more complicated. From synthesis of the protein until it reaches its final destination, there are often tortuous and mostly unknown paths. Pursuit of those events in intracellular environments where there are thousands of other proteins, some of them at very high concentration presents major challenges. In this work, we describe how analysis of a protein among many others can be investigated and some of the individual steps between synthesis to attainment of final active conformation can be obtained. Emphasis is placed in locating the stages where other macromolecular arrangements, proteins or membranes, assist in harboring and protecting a newborn protein on its way to adulthood inside a cellular compartment. Details of the analysis of conformational intermediates reaching a final structure with enzymatic activity will be presented. A mitochondrial precursor protein and its cystolic homolog are the needles being fetched among the intracellular proteins haystack. (Supported by NIH grants HL-38412 and GM-38341.)

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THE MODULATON OF CATALYSIS BY THE ALLOSTERIC PYRUVATE KINASE. Nowak, T., Larry, J.P. and Mesecar, A.
Dept. of Biochemistry, Univ. of Notre Dame, Notre Dame, IN, USA.

Yeast pyruvate kinase (yPK) plays a key role in glycolysis regulation Fructose-1,6-diphosphate (FDP) is the primary heterotropic activator. The velocity response to phosphoenolpyruvate (PEP), Mn^{2+} and FDP concentration changes were measured to quantitate ligand coupling. Direct binding studies were also performed to measure these effects. The single trp residue 452, located distant from the active site, is conformationally sensitive and allows for quantitation of binding. The coupling effects, given by ΔG are $\Delta G_{Mn\text{-}PEP}$= -2.75 kcal/mol, $\Delta G_{Mn\text{-}FDP}$= -1.6 kcal/mol with $\Delta G_{FDP\text{-}PEP}$= -0.2kcal/mol. The total coupling, $\Delta G_{PEP\text{-}Mn\text{-}FDP}$= 4.3 kcal/mol. It is the divalent metal and not FDP that elicits the key effects. PEP induces cooperativity in Mn^{2+} binding and FDP eliminates cooperativity. The Mn^{2+} and PEP effects on yPK are reciprocal. FDP binding is always cooperative regardless of the yPK complex. Analysis of the trp fluorescence emission spectra of the various complexes demonstrates differences in conformation of the active complexes with and without FDP although both complexes have the same k_{cat}.The use of the slow substrate Br-PEP shows the loss of kinetic cooperativity although cooperative binding (n_H=1.6) still exists suggesting a late step in the kinetic process controls catalysis. These results require a revised model of allosterism with yPK. An analysis of active site changes during activation was performed with NMR studies where the interaction of the activating cation Mn^{2+} with the monovalent activator Tl^+ was quantitated. Tl^+ activates at the same level as K^+. The cations are 7.0Å apart in the yPK-Mn complex and PEP induces a change where the cations are 6.0Å apart. The addition of FDP to the complex makes no additional change in their interaction. The cations are 5.9Å apart in the yPK-Mn-FDP complex. The $1/T_2$ values suggest the cations share a ligand in common, perhaps the phosphate of PEP. Both localized and general conformational changes in yPK occur during activation and catalysis. A differential ligation model is proposed to explain allosterism in yPK.

The research was supported by NIH grant DK17049

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RESIDUES AT SUBUNIT INTERFACES OF MAMMALIAN FRUCTOSE-1,6-BISPHOSPHATASE: EFFECTS ON THE REGULATORY PROPERTIES. Slebe, J.C., Reyes, A., Ludwig, H. and Cárcamo, J.G. Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.

Studies of the mechanism of regulation of fructose-1,6-bisphosphatase (FBPase) by the effectors, and the catalytic mechanism of hydrolysis of fructose-1,6-bisphosphate, have been extended to the structural level by the availability of crystal structures for the pig kidney enzyme and its complexes with AMP and fructose-2,6-bisphosphate (Fru-2,6-P_2), among others. In the native tetrameric enzyme the four subunits are disposed in a D_2 symmetry, in such a way that the structure resembles a dimer of dimers. Each subunit makes contact with two other subunits along two different interfaces: the A (for active) interface, contains the binding site for the substrate and the competitive inhibitor Fru-2,6-P_2, and the R (for regulatory) interface, contains the binding site for the allosteric inhibitor AMP. The two quaternary structures of the enzyme, the R and T forms, differ by a 15° rotation along the R interface, elicited by AMP binding. Selective chemical modification have shown that one Lys and one Arg in the R interface play a critical role in the allosteric transition. Modification of these residues caused the total loss of AMP cooperativity with no effects on AMP affinity. On the other hand, blocking Cys-128 on the A interface, besides perturbing Fru-2,6-P_2 binding, "disconnects" the active site from the allosteric site. This observation suggests that the communication pathway between both sites requires intersubunits contacts. These results link specific interactions between subunits in FBPase to its observed regulatory properties and provides new insights into the conformational equilibrium of the protein. (Supported by grants from FONDECYT 1951215 & 2960060 and grants S-94-10 & S-95-41from DID-UACH).

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THE CONCERTED ACTION OF THIOL/DISULFIDE EXCHANGE AND NONCOVALENT INTERACTIONS IN THE MODULATION OF CHLOROPLAST FRUCTOSE-1,6-BISPHOSPHATASE. Wolosiuk, R.A. Instituto de Investigaciones Bioquímicas [Fundación Campomar, F.C.E.N.-U.B.A., IIBBA-CONICET], Buenos Aires, Argentina.

In illuminated chloroplasts, photochemically reduced ferredoxin converts, via ferredoxin-thioredoxin reductase, the unique cystine of thioredoxin (**trx**) to sulfhydryl groups. Reduced trx returns to the oxidized state by cleaving disulfide bonds of chloroplast fructose-1,6-bisphosphatase (**CFBPase**) and, concurrently, drives the enzyme to an active form. Although all trx contain the conserved amino acid sequence [-W-C-G-P-C-] involved in the reduction of proteins, chloroplast trx-f is the most effective activator of CFBPase. To characterize the underlying mechanism in the interaction between trx and CFBPase, we analyzed the structure-function relationship of interacting proteins.

The concerted action of Ca^{+2} and fructose 1,6-bisphosphate stabilizes CFBPase and stimulates the reductant-mediated activation; protein perturbants weaken the former but further enhance the latter. Moreover, *E.coli* trx becomes indistinguishable from chloroplast trx-f when intramolecular noncovalent interactions of the enzyme are altered with these modulators. Alternatively, variants of *E.coli* trx prepared by site-directed mutagenesis of amino acid residues devoid of redox capacity are more efficient than the wild type counterpart in enhancing the activity of CFBPase.

On this basis, in vitro, and probably in vivo as well, the rate of thiol/disulfide exchange between proteins is modulated by changing not only the conformation of the protein disulfide oxidoreductase(i.e., trx) but also the target enzyme (i.e., CFBPase).

Symposium 20 - **Molecular Mechanisms of Viral Pathogenesis**

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ADENOVIRUS E3 IMMUNOREGULATORY PROTEINS: FUNCTIONS AND INTERACTIONS WITH CELLULAR PROTEINS. Yongan Li, Jian Kang and Marshall S. Horwitz. Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York, USA

Adenoviruses (Ad) encode proteins that are immunoregulatory or affect the mechanism of action of cytokines. Some of these proteins are encoded by early region 3 (E3) genes and include: Ad E3-gp19K, which binds to class I MHC and prevents transport of the complex from the endoplasmic reticulum to the cell surface; Ad E3-14.7K or the heterodimer of Ad E3-10.4K/ 14.5K, which inhibits TNFα cytolysis. We have demonstrated that the Ad E3-14.7K protein can antagonize the antiviral effects of TNFα *in vivo*. In addition, the E3 DNA expressed as transgenes in murine islet cells can prolong allogeneic transplantation and prevent autoimmune diabetes. Because the mechanism of inhibition of TNFα cytolysis by Ad E3-14.7K is unknown, we have used the yeast two hybrid system to isolate cellular proteins (from a HeLa cell library), which interact with Ad E3-14.7K. Four unique cellular proteins named FIPs (14.7K-interacting proteins) were identified. Each of the 4 FIPs colocalizes with Ad E3-14.7K in cultured cells and binds to this E3-viral protein *in vitro*. FIP-1 also binds to multiple phosphorylated cell proteins in a TNF-α dependent interaction. The FIPs do not interact with an Ad E1B protein or its cellular homologue Bcl-2, which also is capable of inhibiting cytolysis by TNFα. FIP-1 is homologous to Ras-related small GTP binding proteins and also contains regions of homology to 2 bacterial metalloproteases. FIP-2 and FIP-3 are homologous and contain leucine zipper domains. FIP mRNAs are expressed ubiquitously in normal human tissues but differ in amounts in various organs. TNFα can increase the amount of FIP-2 mRNA in cell lines. Antisense RNA to each of the 4 FIPs can partially inhibit TNFα cytolysis in a murine cell line. Thus, it appears that Ad E3-14.7K functions as a TNFα inhibitor by interacting with host cell molecules whose functions are being defined.

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HEPATITIS C VIRUS (HCV) : IMPLICATIONS OF GENETIC VARIABILITY IN HCV PERSISTENCE AND ASSOCIATED LIVER CANCER. Eugenia Lamas. Unité d'Expression des Gènes Eucaryotes. Institut Pasteur, & Unité 370 INSERM, Paris, France.

Hepatitis C virus (HCV) is the main aetiological factor of post-transfusional and sporadic non-A/non-B hepatitis. This viral infection is characterized by an extremely high rate (60-80%) of chronic carrier state development, associated with the low-grade, yet persisting, viral multiplication. Among HCV chronically infected patients, the severity of the liver lesion varies markledy, ranging from mild chronic hepatitis to cirrhosis and hepatocellular carcinoma. The pathogenetic mechanisms of HCV infection are poorly known ; in particular the respective importance of viral and host factors is still unclear.

Several studies have suggested the clinical impact of HCV genetic variability. A particular profile of response to interferon of genotype 1b was first suggested from studies in Japan. Thus, univariate analyses, the relative prevalence of type 1b was significantly higher among non-reponders than responders. However, there are obviously several potential biases to be considered before assessing a given genotype as a predictive factor for poor response to treatment. My talk will focus on a brief update of HCV biology and on the potential clinical impact of HCV genetic variability in associated liver cancer.

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ROLE OF THE VIRAL GENE PRODUCTS IN ROTAVIRUS REPLICATION. Patton, J.T.[1], Xiaobo, J.[1], Wentz, M.[2], and Ramig, F.[2] [1]Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD, USA; [2]Department of Virology, Baylor College of Medicine, Houston, TX, USA

Rotaviruses, members of the family *Reoviridae*, are an important cause of acute infantile diarrhea causing nearly one million deaths annually. The rotavirion consists of a core surrounded by an intermediate and outer shell of protein. The viral genome is made up of 11 monocistronic segments of double-stranded (ds)RNA, a number of which encode proteins that affect virulence and host range. To devise methods for altering the virulence of rotaviruses and for developing improved live virus vaccines, we have examined events in the replication and packaging of the rotavirus genome. By analyzing the replication of mutant viral mRNAs in a cell-free template-dependent replication system containing open cores, cis-acting signals in viral mRNAs were identified that promote minus-strand synthesis. Only one signal, that located at the conserved 3'-terminus, was found to be essential for replication. Remarkably, a signal that promotes dsRNA synthesis was also detected at the 5'-terminus. Gel mobility shift assays were subsequently used to determine which of the core proteins, VP1, VP2 and VP3, was responsible for recognition of the mRNA template during RNA replication. The results revealed that VP1, the suspected viral RNA polymerase, while able to specifically recognize the 3'-end of viral mRNA, lacks replicase activity in the absence of other viral proteins.To further define the protein requirement for replication, VP1 and the major core protein VP2 were expressed by recombinant baculoviruses and assayed individually and together for replicase activity. The results demonstrated that alone both VP1 and VP2 lack replicase activity but when combined possess high levels of such activity. These results are consistent with the idea that genome replication and packaging of the dsRNA product into VP2 cores are interdependent events. The information gained from these studies is being used to develop an infectious RNA system for the rotaviruses.

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BASIC AND APPLIED MOLECULAR GENETICS OF ARENAVIRUSES. Romanowski, V[1,2] *[1]: Instituto de Bioquímica y Biología Molecular (IBBM), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata; [2]: Departamento. de Ciencia y Tecnología, CEI, Universidad Nacional de Quilmes, Bernal, Argentina.*

The arenaviruses have a bipartite ssRNA genome with an ambisense coding strategy. The virions are enveloped and package non-equimolar amounts of each genomic RNA species (L, 7kb, and S, 3,4 kb).

The Arenaviridae family comprises fifteen distinct enveloped RNA viruses, some of which have been recognized as etiological agents for serious human diseases. The common feature of these diseases is its restricted geographic distribution and the emerging character of the first outbreaks. The pathogenic arenaviruses of the American Continent cause hemorrhagic fevers. In particular, Junin virus is the etiological agent of Argentine hemorrhagic fever (AHF) and its variants may cause different clinical patterns. More than 30% fatal cases can be reduced to less than 1% by early immune therapy. Therefore, early and rapid diagnosis is extremely important.

Molecular cloning of Junin virus cDNA yielded genomic nucleotide sequence information that was quickly used to develop and validate a RT-PCR based diagnostic assay. No other laboratory methods were able to diagnose the infection due to the low viraemia. An extension of this methodology was used to detect and characterize new arenaviruses in field rodents trapped in Argentina. The list of members of this virus family will probably continue grow due to the availability of simpler technology.

On the other hand, a live attenuated Junin virus vaccine strain has been developed and its S RNA nucleotide sequence has been determined. The nucleotide and amino acid sequence comparisons of the N and GPC genes of the vaccine strain and its genealogically related more virulent strains revealed some changes that are unique to the most attenuated virus. These changes could be provisionally associated with the attenuated phenotype, until a reverse genetic system becomes available for arenavirus research.

This work has been supported by CICBA and CONICET, Argentina.

Symposium 21 - **Hormone Receptors**

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PHOSPHORYLATION OF THE HUMAN V2 VASOPRESSIN RECEPTOR. Innamorati, G., Sadeghi, H. and Birnbaumer, M. Dept. of Anesthesiology and Molecular Biology Institute, UCLA School of Medicine, Los Angeles, CA, USA

The V2 vasopressin receptor (V2R), a member of the family of G-protein coupled receptors, mediates the antidiuretic action of AVP in the kidney. The gene for the receptor is located in the q28-qter segment of the human X chromosome. Mutations of this gene that reduce or abolish receptor activity cause X-linked recessive nephrogenic diabetes insipidus (NDI), one of the diseases characterized by the inability of the kidney to produce concentrated urine. Activation of the receptor by AVP stimulates adenylyl cyclase activity and is followed by receptor desensitization. Studies on the biochemical modifications of the receptor performed in transient transfections experiments revealed a requirement for maturation (probably refolding) for the V2R to become functional. The role of post-translational modifications on receptor expression and function were examined. Because ligand-induced phosphorylation plays an important role in the desensitization of rhodopsin and other receptors of this family, the possible role of phosphorylation in V2R desensitization was examined. The receptor was found to be phosphorylated, and the protein segments required to observe incorporation of radioactive phosphate were identified. The contribution of the carboxyl terminal segment of the receptor to desensitization was assessed by a variety of methods.

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NON-GENOMIC SIGNAL TRANSDUCTION PATHWAYS OF 1.25-DIHYDROXY-VITAMIN D_3. Boland, R., Boland, A.R. de. Depto. de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur. 8000 Bahia Blanca, Argentina .E-mail: rboland@criba.edu.ar.

$1,25(OH)_2$-vitamin D_3 is a pluripotent hormone involved in the regulation of extracellular and intracellular calcium homeostasis, immunomodulation, regulation of cell growth and differentiation, endocrine and reproductive functions and of muscle contraction. Like sex steroids, $1,25(OH)_2D_3$ acts at its target tissues through a nuclear mechanism mediated by a specific receptor. However, various lines of evidence have recently shown that the mode of action of $1,25(OH)_2D_3$ as well as that of the classic steroid hormones, also involve a membrane effect resulting in the activation of second messenger pathways, similarly to peptide hormones, growth factors and neurotransmitters. Studies with cultured muscle cells (myoblasts and myocytes) have provided useful insights into the non-genomic mechanism of action of the vitamin D hormone. It has been shown that $1,25(OH)_2D_3$ rapidly stimulates the influx of extracellular Ca^{2+} through voltage-sensitive Ca^{2+} channels pharmacologically characterized as of the L-type. The hormone sequentially activates phospholipases C and D providing diacylglycerol for PKC activation and inositoltrisphosphate for intracellular Ca^{2-} mobilization. In addition, $1,25(OH)_2D_3$ rapidly stimulates PLA_2 generating arachidonic acid for the eicosanoid pathway. There are data indicating that PKA-dependent phosphorylation events mediate $1,25(OH)_2D_3$-induced Ca^{2+} influx. The PKC pathway also plays a role modulating $1,25(OH)_2D_3$ signal transduction in muscle cells by cross-talk with the PKA system. Various experimental evidences have involved the participation of Gi/Go proteins in $1,25(OH)_2D_3$ coupling to the signalling pathways. The rapidity and specificity by which $1,25(OH)_2D_3$ acts suggest that a plasma membrane-bound receptor is responsible for the initiation of its effects. In agreement with this interpretation, preliminary results obtained by $[^3H]1,25(OH)_2D_3$ binding analysis and photoaffinity labelling experiments with a photolabile $1,25(OH)_2D_3$ analogue are compatible with the presence of a cell surface receptor for the steroid hormone (Supported by CONICET, Volkswagen Stiftung and European Commission research grants).

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GROWTH FACTOR MEDIATED-SIGNAL TRANSDUCTION IN PC12 CELLS: STUDIES WITH CHIMERIC RECEPTORS AND MUTANT CELL LINES.

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PC12 cells, a stable cell line derived from a rat pheochromocytoma, are a well established paradigm from studying growth factor signalling mechanisms. These cells are responsive in various ways to a number of factors, compounds and conditions. The best studied response is the induction of a neuronal morphology, characterized by extensive neurite outgrowth, that is produced by NGF and members of the FGF family. Other factors, such as EGF and IL-6 produce different physiological or biochemical responses indicating the presence of receptors, but do not induce the neuronal-like changes. Interestingly, all four types of factors (NGF, FGF, EGF and IL-6) stimulate intracellular tyrosine phosphorylation events, the profile of which, in several instances, is substantially overlapping. To determine if the phenotypic variations in growth factor responses in these cells results from qualitative or quantitative (or both) differences, we have adopted two experimental approaches: 1) the use of chimeric receptors, stably transfected into whole cells, that allow selective stimulation (without activating in other endogenous receptors); and 2) the utilization of a mutant cell line (subcloned from native cells) with distinctly different response characteristics. The results of these experiments have revealed important features of growth factor responses in these cells, particularly with respect to receptor activation, and have defined a general two-stage mechanism required to induce differentiation. (Supported by NIH research grant AG-09735).

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MELATONIN AND PHYSIOLOGICAL FUNCTION: CHARACTERIZATION OF RECEPTORS IN TESTIS: L. Valladares, R. Pedraza, A.M. Pino. Unidad de Biología de la Reproducción, INTA, Universidad de Chile, Santiago, Chile.

Melatonin is produced rhythmically by the pineal gland. The hormone rhythm is generated by a biological clock in the suprachiasmatic nucleic of the hypothalamus and it is regulated by the daily light-dark cycle. Interaction of melatonin with a specific receptor was postulated to be involved in evoking melatonin action upon the neural-reproductive axis. In addition to its central effects, it has been shown that melatonin exerts direct action upon the gonads by decreasing testosterone production. For many years the identification and characterization of melatonin targets sites remained an elusive goal, frustrating research on receptors and mechanism of action of melatonin. The availability of 2-[^{125}I]-iodomelatonin has led to a large number of reports on potential melatonin receptors. These receptors can exist in two affinity states: in one state the binding site is coupled to G-protein; a second state may be found in which the iodomelatonin binding site and G-protein have dissociated. Our results show the presence of melatonin receptors in cell membranes of rat testis. The affinity of these receptors suggest that they may recognize physiological concentrations of melatonin in the testis. Melatonin is synthesized whithin the gonad and would mediate autocrine responses.

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Symposium 22- Neurobiochemistry: Molecular Cell Biology of Neuronal Cell Differentiation, Growth, Degeneration and Death

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INVOLVEMENT OF FGF-2 IN THE SURVIVAL OF CHICK EMBRYO NEURAL RETINA CELLS. Arruti.C. Laboratorio de Cultivo de Tejidos. Facultad de Ciencias. Facultad de Medicina. Universidad de la República. Montevideo, Uruguay. arruti@bcel.edu.uy

Some growth factors of the FGF family exist in neural retina cells and act as intracrine signals or use membrane receptors. In particular the FGF-2 form is present at early retinal development, and its specific membrane receptors exist before the onset of the neuronal differentiation. To explore the involvement of exogenous FGF-2 in retina cell survival we used dissociated cell cultures. This experimental approach permitted to vary many external parameters (the cell substrate composition and structure, the constituents of the culture medium, the cell density, the spatial relationships between cells, etc.) which allowed the growth and differentiation of different retinal cell types. As an example: several different neural retina cell types, including glial cells, developed in cultures initiated at high or medium cell densities. The administration of exogenous FGF-2 produced significant increases in the number of glial cells, as well as modifications in the stereotypic organization of the cell colonies. Even if the presence of FGF-2 increased the survival in vitro of either the differentiated neurons or their precursors, they never survived as long as the glial cells. A fraction of the glial cells underwent a phenotype change, becoming lens cells. The transdifferentiation events were sensitive to exogenous FGF-2. The differentiated lens phenotype attained by these cells reached terminal stages, as it was found by structural and molecular evaluations. Chromatin degradation indicated that the neuronal loss resulted from apoptosis, as in non-treated cells.

In conclusion: FGF-2 acting through receptor mediated pathways could contribute to the survival of retinal neurons in embryos.

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EXCITATORY AMINO ACIDS AND ITS RELATIONSHIP WITH ADAPTATIVE CHANGES IN THE BRAIN IN RESPONSE TO DEGENERATION OF CENTRAL DOPAMINE NEURONS. Bustos, G. Lab. of Biochemical Pharmacology, Dept. of Cell and Molecular Biology, Catholic University of Chile, Santiago, Chile.

Neurological symptoms associated to the degeneration of nigro-striatal dopamine (DA) neuronal pathways, as it occurs in Parkinson disease, appear very late and when the degenerative process is almost complete. The extensive loss of DA neurons may be compensated by adaptative changes that occur in surviving DA neurons and other neuronal systems existing in basal ganglia.

DA- and glutamate (GLU)-neuronal systems in basal ganglia may reciprocally interact via the mediation of excitatory amino acid (EAA) receptors. These receptors could be crucial to maintain an adequate functional tone during degeneration of DA-neuronal cells. Therefore, we have studied with in vivo microdialysis methodology and molecular biology techniques, the effects of EAA-receptor ligands on DA- and GLU-related neurotransmission in the striatum and substantia nigra of DA-lesioned rats.

The results show that during the course of DA-nigro-striatal pathway degeneration parallel changes occur in the extracellular levels and in the ligand evoked-release of DA, and amino acid transmitters such as GLU, aspartate and γ-aminobutyrate, in the basal ganglia. In addition, changes are observed in the expression of NMDA-NR1 receptor subunit mRNA as detected by in situ hybridization. All these changes depend on the extent of DA-neuronal degeneration.

Therefore, GLU-neuronal systems and EAA-receptor subtypes may actively participate during brain adaptative changes in response to partial degeneration of DA neurons belonging to the nigro-striatal pathway.(Supported by FONDECYT grant 1960329)

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MECHANISMS OF APOPTOSIS IN THE DEVELOPING RETINA. R. Linden, Instituto de Biofisica da UFRJ, Rio de Janeiro, Brasil.

Programmed cell death (PCD) is controlled by multiple, interactive, cell type- and context-dependent mechanisms: During development of the nervous system, many neurons undergo PCD in the form of apoptosis, leading to adult neuronal populations. We are studying the mechanisms of apoptosis in the developing retina, using *in vitro* explants that maintain the histological structure and cellular interactions of intact retinal tissue. Extracellular signals controlling retinal cell death include at least two members of the NGF family of neurotrophins - NT-4 and BDNF. Protective effects of both these neurotrophins can be blocked by a soluble form of the TrkB receptor. The neurotransmitter glutamate can act either as a positive or as a negative modulator of retinal apoptosis. Transduction of apoptosis signals in retinal cells include cAMP-dependent protein kinase selectively in neuroblasts, while kinases sensitive to 2-aminopurine are involved in the control of apoptosis in various retinal cell types. Apoptosis-modulating proteins are expressed differentially within the retinal tissue, depending both on cell type/stage of development and on the stimulus that triggers apoptosis. However, the transcription factor c-Jun appears to be universally associated with apoptosis in developing retinal cells. The subcellular localization of such proteins in apoptotic cells may vary with the cell type and context of programmed cell death. The data indicate that retinal cells in various stages of development undergo apoptosis via distinct mechanisms.

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COMPARTMENTATION OF GANGLIOSIDE SYNTHESIS IN THE GOLGI COMPLEX OF NEURAL CELLS

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Ganglioside expression in the CNS is developmentally regulated in a cell-type specific manner. In neural retina cells, Northern blot and "in situ" hybridization experiments evidenced transcriptional regulation of the expression of genes coding for two key enzymes of the synthesizing pathway, as well as posttranscriptional control of these activities. Metabolic labeling of chick neural retina cells in the presence of drugs affecting intra Golgi transport (Brefeldin A, Monensin) localize GM3, GD3 and GT3 synthesis in the proximal (cis-medial-trans) Golgi, with GT3 synthesis occurring in the trans-most aspect. In vitro labeling of endogenous acceptors from Golgi membranes localize the synthesis of GM3 and GD3 both in proximal and distal (TGN) Golgi compartments. Further glycosylation to complex gangliosides (GD1a) occurs in the distal Golgi, where the participating transferases (GalNAcT GalT2, and SialT4) colocalize and are functionally coupled. Use of GM3 for synthesis of GM2 or for synthesis of GD3 depends on the relative activities of GalNAcT and SialT2 and also on the relative concentration of their cognate donor sugar nucleotides. Results indicate that regulation of ganglioside expression rely on a complex machinery for synthesis in the Golgi complex, in which enzyme activities, compartmental organization and sugar nucleotide availability all constitute potential levels of control. (Supported by The Council for Tobacco Research Grant 4218 and SeCyT-UNC 176)

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HYPEREXCITATION AND NEURODEGENERATION MEDIATED BY GLUTAMATE RECEPTORS IN VIVO AND IN CELL CULTURES. R. Tapia. Instituto de Fisiología Celular, Univ. Nacional Autónoma de México, 04510-México, D.F., México. rtapia@ifcsun1.ifisiol.unam.mx

Overactivation of glutamate receptors results in hyperexcitability and neuronal death. This mechanism of cell damage might play a role in slowly progressing neurodegenerative diseases, but it is not clear whether increased levels of endogenous extracellular glutamate are neurotoxic. Since the clearance of glutamate from the synaptic cleft depends on its high affinity transport, we studied the effect of the transport inhibitors pyrrolidine-dicarboxylate (PDC) and dihydrokainate (DHK) on extracellular glutamate and on neuronal damage, both in vivo and in neuronal cultures. Both PDC and DHK induced a notable elevation of extracellular glutamate in vivo, but only DHK produced neuronal death. In contrast, in neuronal cultures a correlation was found between increased glutamate and damage produced by PDC, while DHK was ineffective. This suggests that the drugs act on different types of transporters and that the access of glutamate to its receptors is not the same in vivo and in cultures. We also tested the effects in vivo of 4-aminopyridine (4-AP), a convulsant drug that enhances glutamate release, and of ruthenium red (RR), an inorganic dye which seems to induce excitotoxicity. The results indicate that glutamate-mediated neurotransmission plays a role in the hyperexcitation produced by both 4-AP and RR, but this does not necessarily results in neuronal death.

Supported by DGAPA, UNAM (projects 200493 and 205095).

Symposium 23 - **Molecular Genetics of Inherited Disorders**

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MOLECULAR DIAGNOSIS AND MAPPING IN GENETICAL MEDICINE.

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The molecular age of genetics is changing the concept of medical genetics to genetical medicine. Terms like anticipation, expressivity and penetrance have now an accurate explanation in many instances. Classical clinical genetics has included new concepts of paramount importance: genomic imprinting, triplet repeat expansion, contiguous gene syndromes, are now of current use among geneticists. The diagnosis of many inherited disorders is becoming possible thanks to basic techniques as the Southern blot, DNA sequencing, and PCR. We have been working on the molecular diagnosis of some Mendelian disorders such as hemophiliae A and B, X-linked muscular dystrophy, cystic fibrosis and Hb abnormalities, and searching for microdeletions causing malformative syndromes. Mapping is a more difficult task, which usually needs collaborative parties to organize themselves in a consortium to search for a single gene requiring substantial efforts and coordination; once the results are achieved they allow protocols for routinary diagnosis. Our experience in this area includes familial Alzheimer disease and congenital generalized hypertrichosis. Nowadays a new gene is identified about every 18 hours. This information overwhelms the storage capabilities of an individual memory, thus the the indispensable help of informatics, in which the future of genetical medicine will rest, besides of course, of the good will of the scientists to preserve the basic principles of medicine.

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ISOLATED GROWTH HORMONE DEFICIENCY: FROM DELETIONS TO POINT MUTATIONS.

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Short stature is a very frequent clinical deficiency. Isolated growth hormone deficiency (IGHD) is one of the better studied causes for short stature. The molecular genetic analysis of this deficiency has revealed different kind of mutations in the GH gene going from its complete deletion to point mutations. Our study involves 30 IGHD children, and some of their families. The patients were clinically analyzed by different stimuli tests and classified. Four of these patients show the deletion of the GH gene. Linkage analysis was performed in some other patients and their families, using markers for chromosome 17, where the GH gene is localized, as well as markers for chromosomes 20 and 7 close to the genes for GHRH and its receptor, respectively .The molecular genetic analysis was performed in the GH gene of 7 patients. In two of them we have found a different point mutation, heterozygous, and concordant with the dominant mode of inheritance. The other patients are being analyzed through their GHRH and its receptor genes in order to find some other causes for IGHD.

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MITOCHONDRIAL DNA MUTATION AND SEGREGATION DURING THE AGEING PROCESS. Linnane A.W., Kovalenko S.A., Harms P.J. Centre for Molecular Biology and Medicine, c/o Monash University, Clayton, 3168 Victoria, Australia

In 1989, we proposed that the occurrence of somatic mutations in mtDNA and their accumulation during the life process would lead to a general tissue bioenergy decline thereby making a substantial contribution to the ageing process and age associated diseases. The essence of our proposal was the following. Random mtDNA mutations occur in somatic tissues and progressively accumulate throughout human life. The particular metabolism of cells of different tissues will influence the mutation rate of the mtDNA. The resultant mutations will not be distributed evenly among cells of a given tissue/organ by reason of their stochastic occurrence in dividing and non-dividing cells, and the random segregation of mitochondrial genomes which occurs in dividing cells. A mtDNA mosaic among tissue cells will thus occur, representing the uneven distribution of normal and various mutant mtDNA molecules in different cells of the tissue. This in turn will result in a bioenergy mosaic, wherein the cells of a given tissue/organ will have a range of bioenergetic capacities. Consequently, above a certain threshold, there will be a progressive decline in the physiological and biochemical performance of individual tissues and organs, contributing to the ageing process. The generality of the proposal has now been widely confirmed. Nonetheless whether the extent of mtDNA mutation is sufficient to account for the bioenergy decline observed to occur in tissues of ageing subjects has been questioned. Solution XL PCR and *in situ* XL and chimera PCR will be reported to demonstrate extensive mtDNA mutation in aged subjects sufficient to account for bioenergy decline. This work opens the way to a study of the molecular genetics and mtDNA-ageing relationship.

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MOLECULAR BIOLOGY ENHANCING OUR UNDERSTANDING AND IMPROVING THE PREVENTION OF HEREDITARY MYOPATHIES IN THE BRAZILIAN POPULATION.

Mayana Zatz, Mariz Vainzof, Eloisa de Sá Moreira, Simone Campiotto, Antonia M. Cerqueira, Maria Rita Passos-Bueno. Depto. de Biologia, Universidade de São Paulo, São Paulo, Brazil **Email:mayazatz@usp.br**

Almost 12000 individuals belonging to families affected by different neuropathies have been ascertained by our group since 1969. Our research has focused mainly on muscular dystrophies (Duchenne/Becker, limb-girdle, facioscapulohumeral and myotonic dystrophy) . Our main goals have been: a) to perform genotype:phenotype correlations in an attempt to enhance our comprehension on the underlying mechanisms causing these conditions; b) to verify if there is genetic heterogeneity for the genes which are progressively being mapped; c) to collaborate in the localization of new genes through linkage analysis.

Main results on genotype:phenotype correlations will be presented for Duchenne /Becker patients focusing on our most important observations. The molecular analysis of myotonic dystrophy Brazilian families , as well as the comparison of different tissues in young as compared to older patients will be shown.

Through linkage analysis we have mapped the sixth form of autosomal recessive LGMD (LGMD2F) at 5q. The proportion of the different AR forms in our population was estimated as: 22.7%LGMD2A, 27.3% LGMD2B, 9.1% LGMD2C, 13.6% LGMD2D, 4.5%LGMD2E, 9.1 %LGMD 2F. Our results also show that there is at least one other gene responsible for this phenotype. We have performed genotype-phenotype correlations for most of the LGMD genes . Of particular interest is the observation of patients who carry the same mutation and show discordant phenotype. Supported by FAPESP, PADCT, CNPq, IAEA.

Symposium 24 - **The Private Life of Ion Channels**

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THE EPITHELIAL SODIUM CHANNEL AND ITS ROLE IN HYPERTENSION. Shimkets, R.* and Canessa C.†. *Department of Genetics and †Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, USA. e-mail: Cecilia_Canessa@Yale.EDU

Sodium reabsorption from the extracellular milieu and from body secretions is fundamental for the maintenance of extracellular volume and of blood pressure. The amiloride-sensitive epithelial Na^+ channel (ENaC) located in the apical membrane of many epithelial tissues is one of the most important mediators of Na^+ reabsorption into the body. Molecular cloning of the cDNAs that encode the channel protein has shed light on the structure of this novel channel. In contrast to voltage-activated Na^+ channels, ENaC belongs to a new and distinct family of ion channels, the protein is a multimeric complex formed by the association of three homologous subunits: α, β and γ. The hallmarks of ENaC are its high selectivity for Na^+ over K^+, small single channel conductance (5 pS), slow kinetics with long opening and closing times (lasting several seconds) and its high affinity for the blocker amiloride (K_i 0.1 μM). Genetic screening of kinreds with Liddle's syndrome (a form of autosomal dominant hereditary severe hypertension) has revealed mutations in the β and the γ subunits that increase activity of the channel. The increased activity of ENaC results in an augmentation of Na^+ reabsorption that leads to volume expansion and hypertension. We will discussed relevant structural features of the channel, its main electrophysiological properties and the implications of mutations found in patients with Liddle's syndrome.

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PEPTIDE TOXINS DESIGNED FOR BLOCKING HIGH VOLTAGE-ACTIVATED Ca^{2+} CHANNELS. Cassola, A.C.. Departamento de Fisiologia e Biofísica, ICB, Universidade de São Paulo, São Paulo, SP, Brasil.

The venom of some invertebrates contains Cys-rich peptides that are selective blockers of high voltage-activated (HVA) Ca^{2+} channels. These peptides are powerful pharmacological tools in the characterization of different HVA Ca^{2+} channel types. So far the most extensively studied peptides are the ω-conotoxins, found in the venom of the genus *Conus* (Mollusca, Gastropoda) and the ω-agatoxins, purified from the venom of the spider of the genus *Agelenopsis* (Arthropoda, Aracnida). ω-Conotoxins are relativelly small peptides, with 6 Cys residues bonded by disulfide bridges in a characteristic Cys-scaffold. Although some ω-conotoxins have overlapping effects on Ca^{2+} channels, the molecules show hypervariability, conserving only the basic Cys-scaffold and Glu5. ω-Agatoxins molecules are larger peptides, variable in size and in primary structure. The overlapping inhibitory effects of these toxins and the competition between structurally different molecules for the binding site, pose the question, still unsolved, of the structure of this site in the Ca^{2+} channels. The current working hypothesis assumes a "macrosite" to which structurally different molecules can bind. Recently we have described the primary structure of a 76 residues peptide isolated from the venom of the spider *Phoneutria nigriventer*, and its inhibitory effect on L- and N-type Ca^{2+} channels. This peptide has blocking effects on Ca^{2+} channels similar to those of ω-agatoxin IIIA. Interestingly the peptides, synthesized by different spider genus are highly homologous only in the 40 residues of the amino terminus, suggesting that this moiety is essential for the peptide binding to the channel site.

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MODULATION OF SWELLING-ACTIVATED Cl^- CHANNELS BY Cl^-. Sepúlveda, F.V., Stutzin, A., Eguiguren, A.L. and Cid, L.P. Facultad de Medicina, Universidad de Chile and Centro de Estudios Científicos de Santiago, Chile.

The movement of Cl^- and intracellularly-accumulated organic osmolytes plays a central role in regulatory volume adjustments. It has been postulated that a single swelling-activated anion channel mediates transmembrane movement of both these solutes. We have studied the electrophysiology of the channels and uncovered a dependence of their activity upon extracellular Cl^- such that replacement by a relatively impermeant anion decreases the activity of the channels. The effect of Cl^- removal does not appear to be due to depletion of intracellular anion and it is postulated to relate to a direct effect on the gating akin to what has been termed "gating by the permeant ion" for a different class of Cl^- channels. If the same channels were to provide the exit pathway for intracellular organic osmolytes released during regulatory volume decrease, removal of extracellular Cl^- should also decrease their flux. This was tested by measuring the swelling-induced release of taurine, a sulphonic amino acid accumulated up to 70 mM in many mammalian cells. Taurine permeability is increased up to 20-fold upon osmotic swelling. The increase in permeability is enhance 2-3-fold by replacement of Cl^- in the extracellular medium an effect that is not dependent upon changes in membrane potential. The use of blockers is also able to discriminate taurine permeability and the Cl^- channels. It is concluded that separate entities with opposing sensitivities to extracellular Cl^- mediate swelling-activated Cl^- and taurine efflux. *Supported by Fondecyt.*

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CHARGE MOVEMENT AND PORE OPENING IN CLONED CALCIUM CHANNELS: EFFECTS OF REGULATORY β SUBUNITS. Stefani E,. Neely A,. Olcese R,. Noceti F,. Baldelli P,. Wei C,. Qin N,. Zhou J and. Birnbaumer L. Department of Anesthesiology, UCLA School of Medicine, Los Angeles CA 90095-1778

Coexpression of the beta subunit (β_{2a}) with the α_1 cardiac (α_{1C}) pore forming subunit increased the speed of ionic current activation and shifted to more negative potentials the conductance-voltage curve. This current potentiation occurred without major changes in the voltage dependence of charge movement or in the number of functional channels as judged from the size of the charge movement. We further evaluated the action of the beta subunit on calcium channels by a combined measurement of ionic currents, gating currents, together with variance analysis of ionic currents. We measured in a patch the number of channels (N), the absolute probability of opening (Po) and the number of effective charges per channel. The latter was also obtained in an independent manner from the limiting slope of the conductance-voltage curve. We found that co-expression of the b_{2a} increased the limiting Po value at very high potentials, without significant changes in the number of effective charges per channel (10-12 e^-). These results indicate that the beta subunits can facilitate channel opening not only by shifting the activation curve to more negative potentials the activation curve, but also by increasing the maximum Po that the channel can reach at very high potentials. This facilitatory action is occurring in the closed to open transitions that are weakly voltage dependent and that carry a relative small fraction of the total charge.

Symposium 25 - **Regulation of Transcription**

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TRANSCRIPTIONAL REGULATION OF THE FIBRONECTIN GENE. C.R. Alonso, C.G. Pesce, S. Werbajh, P. Cramer and A.R. Kornblihtt INGEBI and Dept. Biol. Sci., Facultad de Ciencias Exactas y Naturales, UBA, Obligado 2490, Buenos Aires, 1428, Argentina. E-mail: ark@proteus.dna.uba.ar

We have previously proposed a molecular interaction between the liver factors that bind to the CRE and CCAAT sites of the fibronectin (FN) gene, based in the following evidence: (i) the close spacing of 20 bp between CRE and CCAAT elements is conserved in the FN genes from rat, mouse and humans; (ii) footprinting competitions showed that CRE oligonucleotides are able to detach both liver factors; (iii) CCAAT-binding and transcriptional activity of liver extracts are reduced when the distance between the CRE and CCAAT elements is increased; and (iv) CCAAT-binding is stimulated by addition of a liver extract fraction containing the CRE-binding factor ATF-2 (Kornblihtt et al., ***FASEB J. 10***, 248-257, 1996). We provide now binding and immunochemical evidence that nuclear factor I (CTF/NF-I) and CP1 (NF-Y or CBF) are the only liver factors that bind to the -150 CCAAT element of the FN gene, forming distinct complexes. We show that these factors bind less efficiently to the CCAAT site of a FN promoter in which the -170 CRE has been disrupted by site-directed mutagenesis, and that each element contributes positively to the liver transcriptional activity, assessed *in vitro* with a G-less cassette construct and *in vivo* by transfections of human hepatoma cells with FN-chloramphenicol acetyltransferase constructs (FN-CAT). Furthermore, using a method that combines UV-crosslinking and immunoprecipitation, we show that antibodies specific to ATF-2 are able to specifically precipitate protein-protein-DNA complexes containing NF-I and CP1 (Alonso et al., ***J. Biol. Chem.***, 1996, in press). Transient expression in hepatoma cells of FN-CAT constructs in which either the CRE or the CCAAT box have been disrupted by mutation, have also allowed us to determine the role of this interaction in signal transduction processes. The FN promoter analysis was complemented with studies that assess its role in FN mRNA alternative splicing and in down regulation of FN expression in highly metastatic cancer cells (supported by grants of the University of Buenos Aires, CONICET, Fundación Antorchas and ICGEB).

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NEUROENDOCRINE CELL-SPECIFIC EXPRESSION AND HORMONAL REGULATION OF THE PROOPIO-MELANOCORTIN GENE IN TRANSGENIC MICE AND A NOVEL PITUITARY MELANOTROPH CELL LINE.
Low, M.J.*, Liu, B.*, Chan, E.C.*, Feddern, C.*, Young, J.† and Rubinstein, M.† *Vollum Institute, Oregon Health Sciences University, Portland, Oregon, USA and †Dept. Chimica Biologica, Instituto de Investigaciones en Ingeneria Genetica y Biologia Molecular (CONICET), Buenos Aires, Argentina.
The principal sites of POMC gene expression are pituitary corticotrophs and melanotrophs and a subset of hypothalamic and brainstem neurons. In both the fetal pituitary and hypothalamus, POMC mRNA is one of the earliest markers of terminal cellular differentiation. We have used transgenic mice as an expression system to define the important tissue-specific regulatory regions of the POMC gene. A 27 kb cosmid clone encompassing the POMC gene directed neuronal, pituitary, and testicular expression and recapitulated the early ontogeny of POMC expression in pituitary and the transient transcription in spinal cord. A nested 10 kb genomic clone was no longer correctly expressed in neurons but was expressed in pituitary and responded normally to stress. Reporter transgenes containing only 5' flanking sequences of the POMC promoter directed expression exclusively to the anterior and intermediate lobes of the pituitary. Functional expression in transgenics combined with binding studies using AtT20 corticotroph nuclear protein extracts identified two core sequences upstream of the POMC gene promoter that are possible homeodomain binding sites and are critical for gene expression. The putative cognate transcription factor is abundant in AtT20 cells and a novel POMC-expressing melanotroph cell line produced from a transgenic mouse pituitary tumor, but not in other cell lines or tissues. We conclude that multiple disparate *cis* elements in the POMC gene are required for the complete pattern of POMC expression. Ongoing studies are aimed at precisely identifying the neuronal-specific enhancer(s) and cell-specific transcription factors.
Supported by NIH grants RO1 DK40457 (MJL) and a Fogarty International Research Collaboration Award TW00511 (MJL and MR)

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TRANSCRIPTIONAL ANALYSIS OF THE TYROSYL tRNA SYNTHETASE AND RIBOSOMAL RNA GENES FROM *THIOBACILLUS FERROOXIDANS*. Orellana, O., and Salazar. O. Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Santiago,Chile
Aminoacyl tRNA synthetases are key enzymes that ensure the fidelity of the protein biosynthesis. They catalize the esterification of cognate amino acids to the 3' end of tRNA. The expression of the synthetases is coordinated with the other macromolecules involved in protein biosynthesis
Previously, we have cloned and sequenced the gene (*tyrZ)* encoding for a tyrosyl tRNA synthetase (TyrRZ) from the acidophilic, autotrophic bacterium *Thiobacillus ferrooxidans*. The protein encoded is more than 45% homologous to the predicted protein sequences from *tyrZ* from *Bacillus subtilis* and *Haemophilus influenzae* but no more than 25% homologous to the TyrRS from *Escherichia coli* or *Bacillus stearotermophilus*.
DNA sequence analysis of *tyrZ* from *T. ferrooxidans* revealed that the gene is adjacent to the 5' end of $rrnT_2$, an operon encoding ribosomal RNA genes. A 110 base pares DNA spacer with no predicted *rho* independent transcription terminator separates both genes. Reverse PCR and primer extension analysis revealed that *in vivo* both genes are at least partially co-transcribed, suggesting that coordination of the expression of these two genes might take place.
As was revealed by Northern blot and reverse PCR, *tyrZ* is transcribed at a low level in *T. ferrooxidans*. Primer extension analysis shows that *tyrZ* mRNA contains a long leader segment (> 350 nucleotide).Upon tyrosine starvation of the cells, a 6-7 flod increase of the enzymatyc activity was observed. Under these conditions, *tyrZ shows* a complex pattern of transcription initiation. These data suggest that the expression of *tyrZ* might be controled either at the transcription or translation level.
Supported by FONDECYT, NIH, ICGEB and Universidad de Chile.

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ß-ENDORPHIN "KNOCK-OUT" MICE PRODUCED BY TARGETED MUTAGENESIS IN EMBRYONIC STEM CELLS

*Rubinstein, M. and ¶Low, M. *INGEBI (Universidad de Buenos Aires - CONICET) y Depto. Química Biológica, FCEyN (Universidad de Buenos Aires), Buenos Aires, Argentina and ¶Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences Univeristy, Portland, Oregon, USA

The endogenous opioid peptides are proposed to modulate neuroendocrine circuits, autonomic reflexes, analgesia, memory and learning through binding to a family of G-protein coupled receptors. To determine the contribution of ß-endorphin to the overall opioid response we decided to produce transgenic mice deficient in ß-endorphin by targeted mutagenesis in embryonic stem cells. Because ß-endorphin is encoded by the proopiomelanocortin (POMC) gene that is also important as a precursor for ACTH and melanocyte stimulating hormones we designed a targeting vector that encodes a truncated POMC prohormone selectively deficient in the ß-endorphin peptide sequences. The resulting transgenic mice display no overt developmental of behavioral alterations and have a normally functioning hypothalamic-pituitary-adrenal axis. HPLC analysis followed by RIA showed that mice homozygous for the mutation have undetectable levels of ß-endorphin whereas ACTH and ß-MSH levels were indistinguishable from those of their wild type littermates. Mice deficient in ß-endorphin exhibit normal analgesia in response to morphine, indicating the presence of functional µ-opiate receptors. However, these mice lack the opioid analgesia (naloxone reversible) induced by mild swim stress. Mutant mice also display significantly greater non-opioid analgesia in response to cold water swim stress compared with controls and show paradoxical naloxone-induced analgesia. These changes may reflect compensatory upregulation of alternative pain inhibitory mechanisms.

Symposium 26 - **Biochemical Education: Towards Excellence in Pan-American Ph.D. Programs**

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SCIENTIFIC PROFILES AND CAPABILITIES OF THE FACULTY INVOLVED IN Ph.D. PROGRAMS IN CHILE. Krauskopf, M. and Vera, M.I. Instituto de Bioquímica, Universidad Austral de Chile, Valdivia, Chile.

Opportunities for talented individuals to seek advanced education in Chile have been rather scarce. The Ph.D. programs started during the late sixties and commit the work of only a few universities in the country. The number of individuals that have completed their doctoral studies in all fields of science has been low. About 2 Ph.D.s/year/million inhabitants are currently awarded in Chile. Brazil, which depicts the greatest effort in Latin America awards about 10 Ph.D.s/year/million inhabitants. In USA, the comparable indicator is over 150.

Surely the quantitative situation in the region is in itself a matter for concern. However, it is also of importance to assess the quality of the advanced programs that are offered in science. The recognition, by governments of the region, that science & technology are needed for national competitivity have stimulated the involvement of many universities in graduate programs. To ensure growth with quality in countries that lack the so much needed "culture for evaluation" we have started a study of the scientific capacity of the faculty involved in the Ph.D. programs that exist in Chile. To assess performance scientometric indicators were used.

Supported by Grant C-13029 from Fundación Andes and by Chilgener.

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COLLABORATIVE WORK, A WAY OF IMPROVING THE SCIENTIFIC PRODUCTION AND THE QUALITY OF THE GRADUATE PROGRAMS IN BRAZIL. Meneghini,R. Departamento de Bioquímica, Universidade de São Paulo, São Paulo, Brazil.

In a recent period of ten years the Brazilian scientific production and its international impact have increased considerably. Data from the Institute for Scientific Information (ISI) indicate a 100% increase in international publications from 1981 to 1992 in ISI-indexed journals. Citations rates increased even more than that, despite the fact that investment in science has not increased in this period. This brought about a significant change in the outputs of the graduate courses, one of the indicators being the number of publications per Ph.D. thesis.

A close examination revealed that to a great extent this better performance has occurred as a consequence of an increase in national and international collaborative work. Therefore, stronger interaction was the response to the lack of resources. To which extent this was a sociological reaction or was induced by oriented governmental programs is a matter of investigation which will be discussed.

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PROBLEMS AND TRENDS IN GRADUATE EDUCATION IN THE BIOMEDICAL SCIENCES IN THE UNITED STATES. William J. Lennarz. Department of Biochemistry and Cell Biology and Institute for Cell and Developmental Biology, SUNY at Stony Brook, Stony Brook, New York 11794, USA. Email: wlennarz@life.bio.sunysb.edu.

During the last decade there has been a remarkable increase in our knowledge of the structure and the function of the molecular components of cells. This increase is the result of major improvements in the sensitivity and the rapidity in methods of structural analysis at the cellular, subcellular and molecular level. As a result, research in the biomedical sciences has crossed the lines of traditional disciplines to such an extent that these disciplines are no longer well-defined. For teaching purposes at the graduate student level, courses that separate biochemistry, cell biology and molecular biology into unique disciplines are still taught. There are, however, major problems. First it has not become clear how much more new information can be added to such courses. If the older, "basic" information is subtracted one must assume that it has been thoroughly covered at the undergraduate level. But what does one do if this is not the case? If the issue is ignored we will produce Ph.D.'s that lack fundamentals. One solution is to require more rigorous training of undergraduates. A second problem is that the "lines" between cell biology and biochemistry have become so obscure that teaching these subjects as separate courses without very careful coordination leads to duplication of efforts. At a time when there is pressure to provide the latest information, as well as the fundamentals, such duplication is wasteful of the student's time. Another third area in which training of graduate students must be improved is in computer-based retrieval of information and in graphics. Both of these are not taught in any organized way in most Ph.D. programs. Consequently, there is great variations in the skills of Ph.D. students in these subjects, and much time wasted by them as they learn these skills in a haphazard way.

Poster Session 1

Abstracts 117 - 288

Bioenergetics

Biological Catalysis and Enzyme Regulation

Protein Structure

Microbial Biochemistry

Others

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Ca^{2+}-ATPase DETECTED IN CHICKEN ERYTHROCYTES. Alves-Ferreira, M.; Ferreira-Pereira, A. and Scofano, H.M. Dept° de Bioquímica Médica, ICB-CCS, UFRJ, R.J., Brasil.

The plasma membrane (PM) Ca^{2+}-ATPase exists in several isoforms, whose distribution and regulation are tissue specific. In erythrocytes from mammals, cytosolic Ca^{2+} levels are regulated exclusively by the Ca^{2+}-ATPase. In contrast, mature avian erythrocytes are nucleated and possess a short life time. The mechanism of Ca^{2+} regulation in nucleated erythrocytes is unknown. We demonstrated that nucleated erythrocytes (from chicken) possess a Ca^{2+}-ATPase in their PM. A PM preparation activated by Ca^{2+} ($K_{0.5}$ ~ 0.49μM) and Ca^{2+} plus calmodulin (CaM) ($K_{0.5}$ ~ 0.11μM) was obtained, indicating a Ca^{2+} affinity higher than that of the mammalian enzyme. Chicken's ghosts were not activated by acidic phospholipids, the classical activators of PM Ca^{2+}-ATPases. Purified enzyme had a $K_{0.5}$ ~ 2.42 μM for Ca^{2+} in the absence of CaM, confirming its higher Ca^{2+} affinity in the absence of regulators. In addition, the enzyme was activated by nanomolar levels of CaM. On SDS-page, purified enzyme showed one major band with 150 kDa, at variance with the molecular mass of 140 kDa observed for anucleated erythrocytes enzyme. Furthermore, differences on the modulation by taurine were observed. These results suggest that the Ca^{2+}-ATPase from PM of nucleated erythrocytes may be a novel isoform, similar in overall regulation but distinct in its primary structure, kinetic constants and fine regulation.

Supported by CNPq, CAPES, FINEP and FUJB.

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IDENTIFICATION OF MITOCHONDRIAL SUPEROXIDE DISMUTASE ISOZYMES IN HYPOCOTYLS FROM *VIGNA UNGUICULATA* VITA 3 CULTIVAR AND EFFECT OF A SALT-STRESS ON THESE ISOZYMES. Aragão, M. E. F., Lima, M. G. S., Melo, D. F., Oliveira, L.M.N. and Oliveira, A. C., *Orellano, E. C., Depto. de Bioquímica e Biologia Molecular (UFC), Fortaleza-Ce, Brasil. E-mail vestress@ufc.br, *ego@unrobi.edu.ar.un., Rosario, Argentina.

Salt stress can induce the production of superoxide anion ($O_2^{\cdot-}$), Hydroxyl radicals (OH), and H_2O_2. SOD catalyses the dismutation of $O_2^{\cdot-}$ radicals to H_2O_2 and O_2 and protects cells against activated O specie damages. *Vigna unguiculata* Vita 3 cultivar is known to be more NaCl-tolerant than Vita 5 cultivar. In the present work, the mitochondrial SODs from Vita 3 were analized. Seeds were germinated in paper during 8 days, in the presence or in the absence of 100 mM NaCl. Mitochondria were purified from hypocotyls by differential and density-gradient centrifugation and showed 3 SOD isozymes : two Mn-SOD and one Cu, Zn-SOD. SOD isozymes were individualized by polyacrylamide gel electrophoresis and localized on the gels by the method of the NBT reduction by $O_2^{\cdot-}$ radicals generated photochemically. The identification of differents isozymes was realized by the presence of KCN and H_2O_2 into revelation solution. The isozymes presented different molecular mass as well as different isoelectric points, including the Mn-SOD isozymes. No difference on plant growth was observed when thay germinated in the presence of 100 mM NaCl or control conditions. Nevertheless, the mitochondria from treated plants presented a three time increase on full SOD activity. Considering Vita 3 a tolerant-cultivar, an increase of SOD activities associated to salinity may contribute to inhibit $O_2^{\cdot-}$ accumulation avoiding plant growth reduction.

Supported by CNPq, FUNCAP.

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SUCCINATE STIMULATION OF THE ALTERNATIVE PATHWAY IN *Vigna unguiculata* DURING MALATE OXIDATION. Fernandes de Melo, D., #Orellano, E.G., Lima Jr. A., Torquato, J.P.P., Costa, J.H., Nogueira, F.D.L., Aragão, M.E.F., *Jolivet, Y., *Dizengremel, P. and Silva Lima, M. Dept. Bioquímica e Biologia Molecular/UFC. Brasil. dirmelo@ufc.br #Univ. Rosario/Argentina. *Univ. de Nancy/Frace

The stimulation by succinate of the alternative electron transport pathway in mitochondria from two cultivars of *V. unguiculata* (Vita 3 and Vita 5) with different substrates has been shown in a previous work. The highest stimulation by succinate was found in Vita 5 with malate as substrate. In the present work, the alternative oxidase (AOX) activity, stimulated by succinate, was studied during malate oxidation at pH: 6.5, 7.2 and 7.8, either through polarographic traces or through western blot of total mitochondrial protein, probed with a monoclonal antibody to the AOX of *S. guttatum*. Mitochondria were isolated from hypocotyls of dark grown plants. The O_2 uptake values by the alternative pathway determined in relation to cytocromic pathway, were 70, 48 and 30% in pH: 6.5, 7.2 and 7.8 respectively. The stimulation of AOX by succinate was concentration and pH dependent. The maximal stimulations by succinate were obtained with 8mM (pH 6.5), 24mM (pH 7.2) and 32 mM (pH 7.8). Immunoblots yielded two bands (35 and 70 kD) what agrees to experimental data found in other plant mitochondria. These results suggest that succinate displays a physiological role in the regulation of alternative pathway related to malic enzyme and malate dehydrogenase activities.

Financial support: CNPq, CAPES, FINEP, FUNCAP.

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A NOVEL Ca^{2+}-ATPase FROM SEA CUCUMBER MUSCLE, POTASSIUM DEPENDENT AND INHIBITED BY ATP. Ana Maria Landeira Fernandez and Leopoldo de Meis. Departamento de Bioquímica Médica ICB/CCS, Universidade Federal do Rio de Janeiro, R. J.,Brasil

The Ca^{2+}-ATPase from sarcoplasmic-reticulum vesicles catalyzes the Ca^{2+} translocation from the medium into the vesicles lumem using the energy derived from the hydrolysis of ATP. In muscle cells this enzyme is responsible for the maintenance of low cytosolic Ca^{2+} concentration ($<10^{-7}$ M) thereby controlling muscle contraction and relaxation. In this work it was shown that vesicles derived from the Sea cucumber longitudinal muscle fibres are able to accumulate Ca^{2+} in the presence of ATP. The active Ca^{2+} transport was totally inhibited by nanomolar concentration of thapsigargin, a highly specific inhibitor of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and demostrates a high affinity for Ca^{2+}, with a apparent K_m of 0.4 to 2 μM. Differently from the others well studied SERCA, monovalents cations are required to activate the Ca^{2+} transport in Sea cucumber vesicles. The transport increased four folds in the presence of 100 mM KCl. Others monovalent cations like NaCl and NH_4Cl can also activate the Ca^{2+} transport. The Ca^{2+}-ATPase from rabbit skeletal muscle is also sensitive to these monovalent cations depending on the conditions used, but they are not required to the Ca^{2+} transport. The Ca^{2+}-ATPase found in sarcoplasmic reticulum from Sea cucumber muscle like others transport ATPases, ehibits two K_m's for ATP, one of high affinity (10^{-7} to 10^{-5} M) associated with the binding of ATP to the catalytic site and a second regulatory site of lower affinity (10^{-4} to 5 x 10^{-4}) which modulates the V_{max} of the enzyme. Differently from the others transport ATPases, ATP in the mM range are able to inhibited the Ca^{2+} transport. The physiological importance of this inhibition is still unknown. Over 2 mM of free Mg^{2+} also inhibited the Ca^{2+} transport exhibiting a competitive inhibition with the monovalent cations.This work was support by FINEP, CNPq and CAPES.

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RELATIONSHIP BETWEEN SUBUNIT INTERACTIONS AND ATPase ACTIVITY OF SOLUBLE CHLOROPLAST ATP-SYNTHASE (CF_0F_1). *Mignaco, J.A., #Scofano, H.M., #El-Hage, C.S.B., §Gräber, P., and @Creczynski-Pasa, T.B., *LQFPP/UENF, #Dep. Bioq., ICB/CCS/UFRJ, @CFS/UFSC, Brasil, §Ins. Phys. Chem., Freiburg Universität, Germany.

Chloroplast ATP-synthase (CF_0F_1) was subjected to high hydrostatic pressure as a tool to understand the interactions between its subunits. The soluble enzyme was stable and actively hydrolyzed ATP at atmospheric pressure, while the stepwise increase of hydrostatic pressure to 2.0 kbar induced a stepwise loss of Mg^{2+}-ATPase activity (with $P_{1/2} \cong 0.8$-1.2 kbar). The protein concentration dependence indicated that inactivation was due to protein dissociation. The fluorescence quantum yields and the shift in the center of spectral mass of the extrinsic probe ANS, as well as the pattern of subunit labeling by the sulfhydryl label IAEDANS, indicated that at 0.6 kbar a partially reversible dissociation occurred, while at 2.0 kbar the CF_0F_1 underwent a more drastic and irreversible dissociation. This process results in the loss of Mg^{2+}-ATPase activity. The addition of DTT favored recovery of the original spectral characteristics upon pressure release, and partially preserved the stimulation of ATPase activity induced by sulfite, although it did not preserve the overall ATPase activity. The addition of 30% glycerol partially protected against pressure inactivation of CF_0F_1 and efficiently preserved the stimulation by sulfite; glycerol may stabilize the enzyme by maintaining it self-associated, or by stabilizing the dissociated subunits.

Supported by CNPq, KFA, FINEP, CAPES, FUJB and FAPERJ.

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EFFECT OF DIACYLGLYCEROL ANALOGS IN PLATELETS AND SKELETAL MUSCLE SARCO/ENDOPLASMIC RETICULUM VESICLES: CYCLOSPORINE A ANTAGONISM. Cristiana Monteiro Cardoso, Vivian M. Rumjanek, Leopoldo de Meis. Institudo de Ciências Biomédicas, Departamento de Bioquímica, Universidade Federal do Rio de Janeiro, RJ, Brasil.

Phorbol 1,2-myristate 1,3-acetate (PMA) and 1-oleoyl 2-acetyl rac glycerol (OAG) are diacylglycerol analogs that act on Ca^{2+} signaling events. The immunossupressor Cyclosporine A (CsA) is a potent inhibitor of Ca^{2+} mediated responses. Calcium storage in intracellular compartments is promoted by a membrane bound protein, the Ca^{2+} ATPase. Different sarco/endoplasmic reticulum isoforms (SERCAs) are found in human platelets and skeletal muscle. In the present report, CsA, PMA and OAG ability to modify the reactions of the SERCA isoforms present in platelets dense tubular system and "light" sarcoplasmic reticulum were investigated. PMA and OAG inhibits Ca^{2+} uptake, phosphorylation by P_i, ATP synthesis, and increase Ca^{2+} efflux from vesicles previously loaded with Ca^{2+}. Although ATPase activity was inhibited in platelet vesicles (PV), had no effect on muscle vesicles (SR). CsA had little or no effect on PV and SR but was able to antagonize PMA and OAG effect in both PV and SR. The results indicate a direct interaction of PMA and OAG with the Ca^{2+} ATPase. The different responses to the drugs may be related to differences in the aminoacid sequences of the isoforms present in PV and SR.

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MITOCHONDRIAL SENSITIVITY TO AZT

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Mitochondrial myopathy has been reported to be associated with long-term AZT treatment. However, since this syndrome is also observed in HIV infected patients not receiving treatment , its etiology is controversial. We have studied the possibility of tissue-specific effects regarding mitochondrial sensitivity to AZT. When mitochondria isolated from liver, kidney, cardiac muscle and skeletal muscle were respiring with glutamate as substrate NADH oxidase was inhibited 90% by 15 mM AZT. The activity of NADH dehydrogenase using potassium ferricyanide as the electron acceptor, was not affected by the drug. Inhibition of state 3 respiration was observed using cardiac and skeletal muscle mitochondria with succinate as substrate, and a lower inhibition was observed when FCCP-uncoupled mitochondria were tested. Succinate oxidase activity measured in intact mitochondria was inhibited by AZT while the activity in disrupted mitochondria was not affected. The dicarboxylate carrier activity in muscle mitochondria, in presence of succinate and Pi, was then compared to the activity in liver mitochondria. The results show that only muscle mitochondria was inhibited by AZT. Similarly, when the hydrolytic activity of H^+ -ATPase was tested, inhibition by AZT was observed with cardiac and skeletal muscle but not in liver or kidney mitochondria.The sum of these effects indicates a tissue-specific inhibition, although its precise mechanism requires further investigation.

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PLASMA MEMBRANE H^+-ATPase FROM *Vigna unguiculata* cv. EPACE 10. Torquato, J.P.P., Costa, J.H., Fernandes de Melo, D., Silva Lima, M.,*Orellano, E.G. Dpto. de Bioquimica e Biologia Molecular/UFC. Brasil dirmelo@ufc.br. *Univ. Rosário. Argentina .ego@unrobi.edu.ar

The plasma membrane H^+-ATPase is known to energize this membrane for the transport of ions and/or organic solutes. However, the mechanism of proton-motive-force-driven transport is not completely clear. The present work deals with kinetic studies of plasma membrane H^+-ATPase from *V. unguiculata* cv. EPACE 10, (Km, V_{max}), optimum of pH and temperature, termostability, ion regulation and ATPase inhibitors activity. The microssomal nonpurified fraction was isolated from etiolated 7 day old hypocotyls of plants germinated in water and the homogenate centrifuged at 90.000 x *g*/30 min. The purified fraction was prepared in sucrose density gradient (32,5 and 46,5%). ATPase activity was estimated by Pi liberation, spectrophotometricaly measured and revealed a Michaelis-Menten kinetics (V_{max}=62,5mU/mg protein and Km=169μM) for MgATP complex. Optimum pH for ATPase activity was 6.0 to 6.5 and temperature optimal was in the range of 35 to 40°C. The termostability was measured from 20 to 100° C and showed an increasing inhibition with temperature enhancement (I_{50} = 40° C). Inhibition by 0.2mM vanadate was 70-90% and 0-9% with nitrate and azida both at 0.2mM. The enzyme activity in the presence of Li^+, Na^+, K^+, NH_4^+ and Ca^{2+} (30 to 130mM) revealed that Li^+ and Na^+ stimulated this activity by 100 and 34% respectively. Contrarily, K^+, NH_4^+ and Ca^{2+} inhibited it by 32, 47 and 100% respectively. The ATPase activity was Mg^{2+} dependent, but 50mM free Mg^{2+} induced a negative modulation.

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FENAZAQUIN (ACARICIDE/INSECTICIDE) SPECIFIC BINDING SITES IN NADH:UBIQUINONE OXIDOREDUCTASE AND APPARENTLY THE ATP SYNTHASE STALK.

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Fenazaquin is one of several acaricides insecticides,including rotenone and pyridaben,that are reported to act by inhibiting NADH:Ubiquinone oxidoreductase (Complex I) (EC 1.6.99.3)in the range 1-10 nM. (3H)Fenazaquin is used here at 0.8nM as a radioligand with electron transport particles(ETP) of bovine heart mitochondria to develop a new and rapid filtration assay with 42% specific binding(displaced by 2uM rotenone).
Several inhibitors of enzyme activity of Complex I generally correlates with their effectiveness in displacing the affinity probe (3H)Fenazaquin from its binding site in ETP (r2 =0.96,n=18).The photoactivatable probe,diazirinyl analog of fenazaquin (3H) on incubation at 435 nM with ETP the irradiation at 350 nm , results in photoactivatable,irreversible specific binding which is fully protectable with rotenone.The site of labelling was examined by sequential isolation of the only band of specifically labeled protein which appeared at 22-24 kDa on SDS-PAGE.Fenazaquin(and presumably rotenone as well) therefore has at least two specific binding sites in ETP,the anticipated high-affinity site in NADH:ubiquinone oxidoreductase and apparently a low-affinity site of unknown function,photoaffinity labeled in the stalk region of ATP synthase.

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A UNIFIED DESCRIPTION OF COOPERATIVE BINDING CURVES. Acerenza, L. and Mizraji, E. Sección Biofísica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Cooperativity, the departure from hyperbolicity of the fractional saturation of a receptor at equilibrium (Y =[occupied sites]/[total sites]) for different values of ligand concentration (L), is an essential property of many physiological mechanisms. Here we use as a measure of cooperativity $\kappa = dK(L)/dL$ where $K(L) = (1 - Y)\ L/Y$ = [total free sites]L/ [total occupied sites] is the "global dissociation quotient" (Edsall,J.T. et al. (1954) *J.Am.Chem.Soc.* 76, 3054). Cooperative behaviour appears when $\kappa \neq 0$, i.e., $K(L)$ is a function of L. We have shown, for several equilibrium models of cooperative behaviour (e.g. Monod-Wyman-Changeux and Koshland-Némethy-Filmer), that $K(L)$ can be expressed as the weighted average of the microscopic dissociation constants (K_i) where the weights are the corresponding fractions of occupied sites (X_i), ($K(L) = \sum K_i X_i$). As a consequence, the change in the global dissociation quotient with ligand concentration for a dimer is $\kappa = (K_1 - K_2)dX_1/dL$. We conclude that the quantitative importance of a cooperative behaviour in a dimer depends on two factor: i) the difference of the microscopic dissociation constants of the sites and ii) the change in the fraction of occupied sites with ligand concentration.

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ENZYMATIC CHARACTERIZATION OF THE 40 kDa CARBOXY-TERMINAL CATALYTIC DOMAIN OF HUMAN POLY(ADP-RIBOSE)POLYMERASE. *H. Mendoza-Alvarez, [1]G. deMurcia, and *R. Alvarez-Gonzalez. [1] Ecole Superieure de Biotechnologie de Strasbourg, UPR 9003 du CNRS, Strasburg, France and *Department of Microbiology and Immunology, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699.

The carboxyl-terminal 40-kDa catalytic domain (CD) of human Poly(ADP-ribose)polymerase (PARP) was cloned, expressed, and purified to homogeneity as described elsewhere [J. Biol. Chem. 268, 13454-13461, (1993)]. This peptide catalyzes the synthesis of protein bound poly(ADP- ribose) in the absence of DNA with a 500-fold lower efficiency than native enzyme. An analysis of the polymer size distribution of the ADP-ribose chains synthesized by CD by high resolution-PAGE shows that this peptide catalyzes the initiation, elongation and branching reactions. Kinetic studies indicate that the reaction occurs in a: i) time-dependent; ii) NAD-concentration-dependent; and iii) peptide-concentration dependent manner. Surprisingly, the initial rates of the reaction show second order kinetics as a function of CD concentration from 100 to 600 nM levels indicating that a CD-dimer can also be formed in the absence of DNA. Therefore, the carboxy-terminal domain of PARP must contain amino acid residues that contribute to protein-protein interactions during automodification. However, the binding affinity in the CD-dimer must be lower than the affinity of the DNA-dependent dimerization of PARP promoted by peptides present in the 74 kDa amino-terminal fragment which contains the DNA-binding and automodification domains. CD also utilized [^{32}P] 3'-deoxyNAD as a substrate for the ADP-ribose chain initiation reaction. However, due to the lower efficiency of this reaction in the absence of DNA, we could not fully characterize it.
This project was supported by grant GM45451 from the NIH.

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PURIFICATION AND PARTIAL CHARACTERIZATION OF *Faseolus vulgaris* SEED ARYLAMIDASE ACTIVITY. Abdala, A.P., Freitas Jr., J.O. and Alves, K.B. Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brasil.

Faseolus vulgaris seed's extract showed arylamidase activity upon L-Leu-p-nitroanilide (Leu-NiA) and 2-naphthylamides (NA) of L-Leu, L-Arg, L-Ala and L-Met. It seeds (15,47g) were swollen in water and, after tegument was removed, homogenized with 83 ml 0.02 M sodium phosphate buffer (NaPB) pH 7.0. The crude extract was centrifuged at 6000g/30 min/4°C and the supernadant (66 ml), submitted to a Mono Q column, equilibrated and washed with 0.02 M NaPB, pH 7.0. Elution with a linear gradient of 0.02 to 0.5 M NaPB pH 7.0, yielded one protein peak of activity, upon AA-NA of Leu, Ala, Arg and Met as substrates. The pool of active fractions was lyophilized and gel filtered on a Du Pont GF-450 column, equilibrated and developed with 0.1 M amonium acetate pH 8.3. One protein peak with activity upon L-Leu-NA was eluted. SDS-PAGE (10%) showed only one protein band with reduced samples (dithiothreitol). The enzyme has a molecular weight of 36 kDa and optimum pH 6.5. The enzyme activity, upon Leu-NA, was not affected by 2.0 mM $CaCl_2$ or $MnCl_2$ and inhibited by 0.5 mM $ZnCl_2$ (94%), 0.1 mM $MgCl_2$ (28%), 3.33 mM o-phenanthroline (40%) and 33 µM EDTA (44%). Sulphydryl group reagent as 80 µM p-hydroxymercuribenzoate inhibited 92% of the enzyme activity upon Leu-NiA while 3.3 mM 2-mercaptoethanol or dithioerythritol had no action. K_M values determined were: Ala-NA, 1.64×10^{-4} M; Arg-NA, 2.98×10^{-5} M; Leu-NA, 1.92×10^{-5} M; Met-Na, $8{,}95 \times 10^{-5}$ M and Leu-NiA, 1.23×10^{-4} M. The highest catalytic efficiency (V_{max}/K_M) was obtained with Leu-NA. So this aminopeptidase is a metaloenzyme and is -S-S- group dependent.

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IN VIVO STUDIES OF HEPATOBILIARY EXCRETION OF BILIRUBINS. *Bari, S. E. ; #Mora, M. E. and #Awruch, J.. *Departamento de Química Biológica and #Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

Bilirubin IX α is an intermediate pigment in heme catabolism in mammals. It is an hydrophobic compound and requires glucuronization prior to biliar excretion. Bilirubin UDP glucuronosyl transferase (BRUDPGT) is the enzyme capable of this process.

To study BRUDPGT substrate specificity, we designed a series of novel synthetic biliverdins, potential precursors of intrahepatic bilirubins. Biliverdins with different polarities (two to four propionate residues on the tetrapyrrole backbone) and varying conformations (due to intramolecular covalent bridging) were prepared for this purpose. The possibility of intramolecular polar interactions (hydrogen bonding) was specially taken into account in all compounds assayed.

Hepatic metabolism and biliar excretion of the biliverdins was investigated using femoral vein infused Wistar rats. Bilis was collected and analyzed by chromatographic and spectroscopic methods.

We found that the increasing hydrophilicity of the substrate leads to the excretion of unconjugated bilirubins, even when the substrate was able to establish hydrogen bonding as the natural substrate of the BRUDPGT, bilirubin IX α. When polarity of the substrate decreased by irreversible changes in the conformation, conjugation was not observed either, showing that polarity and solubility are not strictly associated to the enzyme specificity. BRUDPGT activity seems to be related to hydrophobic bilirubins only when solubility is diminished due to intramolecular hydrogen bonding.

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THE YEAST HEXOKINASE STABILITY INCREASES BY INTERACTION WITH LYSO - PHOSPHATIDYL CHOLINE MICELLES. Guerra, R. and Bianconi. M.L., Departamento de Bioquímica Médica, ICB/CCS, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, CEP 21941-590, Brazil

Although a large number of hexokinase isozymes is found in a membrane-associated form, the yeast forms are believed to be only free in the cytosol. The effect of zwitterionic lyso-phosphatydylcholine (LPC) micelles on the yeast hexokinase (HK) stability at 35°C was studied in different mediums. After 200 min incubation at 35°C in a medium containing glucose and $MgCl_2$, less than 15% of remaining HK activity was found. Nevertheless, in similar conditions of incubation, when LPC was added it was found over 85% of HK activity. On the other hand, when no glucose was added to the incubation medium (only buffer, buffer plus $MgCl_2$, or buffer plus MgATP), LPC micelles are not able to protect the enzyme against thermal denaturation. The results suggest that the protein conformational change by glucose binding favors the interaction with the micelles leading to a great stabilization of the enzyme. Binding of HK to the interface cause an increase in the affinity for ATP as observed by a decrease in the Km for ATP, in addition to an increase in the Vmax. These results indicate that the interaction with the interface increase the enzyme efficiency.

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INHIBITION OF CYTOCHROME P-450 19 (AROMATASE) BY A NOVEL GROUP OF NON-STEROIDAL NATURAL COMPOUNDS (SESQUITERPENE LACTONES). Blanco, J., Gil, R., Alvarez, C., Oberti, J., Sosa, V., Patrito, L., Genti, S.and Flury, A. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Argentina.

The inhibition mechanism of aromatase (cytochrome P450-19) by a new non-steroidal group of inhibitors, sesquiterpene lactones (SQL), was investigated using human placental microsomes and partially purified enzyme preparations.

Kinetic experiments showed that inhibition by SQL was competitive with respect to the substrate (testosterone). The inhibition constants were Ki:4μM for 10-epi-8-deoxicumambrin-B (10 epi), Ki:21μM for dehydroleucodin (DHL) and Ki:23μM for ludartin (LDT). Spectral titrations using UV-VIS difference spectroscopy revealed that only 10 epi and DHL act as type II ligands. It was observed that the compounds shifted the Soret maximum of the enzyme-substrate complex from 390nm to 419nm, which are opposite in sign to type I and reflect conversion of the heme iron from a high to a low spin state, that is, displacement of testosterone. The spectral dissociation constants using partially purified enzyme were Ks_{app}:29μM for 10 epi and Ks_{app}:50 μM for DHL.

Our data suggest that the hydroxyl group at C-10 of 10 epi could be the axial ligand to the heme iron in the active site. Additionally, the displacement of the heteroatom to different positions, like C-2 (carbonyl) in DHL and C-3/C-4 (epoxide) in LDT, decreased the interaction with the iron atom. We present the computer generated model of the inhibitors that clearly show the relationship between the oxygenation pattern and the inhibitory activity. These data are in good agreement with the kinetic evidence.

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EVIDENCE OF THE PRESENCE OF THREE MOLECULAR FORMS OF SERINE HYDROXYMETHYLTRANSFERASE IN *Crithidia fasciculata*. Capelluto, D. G. S., Cazzulo, J. J. and Cannata, J. J. B. CIBIERG, Facultad de Medicina (UBA) e Instituto de Investigaciones Bioquímicas, Fundación Campomar. Buenos Aires. Argentina.

We have postulated that serine hidroxymethyltransferase (SHMT), a pyridoxal-5'-phosphate (PLP) dependent enzyme, which catalyzes the interconversion of serine and glycine transferring a one-carbon unit to tetrahydrofolate (THF) to form 5,10-methylenetetrahydrofolate (MTF), is involved in the reductive metabolism of CO_2 in *Tryponosoma cruzi*. Moreover, this reaction is of prime importance because MTF is involved in the synthesis of purines, thymidylate, methionine and formyl-methionyl t-RNA. SHMT activity is found in *T.cruzi* , but is lower than those of *C.fasciculata*. By DEAE-cellulose chromatography we have shown the presence of three different molecular forms of the enzyme in choanomastigotes of *C.fasciculata* that we have called forms I, II and III, whereas apparently only one form is present in epimastigotes of *T.cruzi*. We have purified the three forms by Phenyl Sepharose, Hydroxylapatite, Red Agarose and Blue Sepharose chromatographies and Sephadex G200 gel filtration.The forms I, II and III of *C.fasciculata* SHMTs were purified 400, 100 and 7600 fold respectively with a purity of about 90% on SDS-PAGE. We have studied, so far, the kinetics and molecular characteristics of forms I and III. Both forms presented pH optima of 8.4 and bimodal kinetics for L-serine with two zones with a plateau region between them at about 0.4 mM L-serine; two sets of Km values were calculated: 0.38 mM and 0.21 mM (form I) and 0.15 mM and 0.68 mM (form III) for L-serine concentrations lower and higher than 0.4 mM respectively. The Km values for THF were 0.20 mM for forms I and III; both forms were inhibited by excess of substrate when the fixed L-serine concentration was 0.5 mM, but the inhibition was abolished when the L-serine concentration was fixed at 2 mM. Both forms were highly unstable at -20 °C, but they were more stable at 4 °C particularly in the presence of 3.6 mM L-serine. The molecular masses calculated according to Andrews' method were about 225 and 211 kDa for forms I and III respectively, similar to those of yeast, fungi, plants and mammalian SHMTs . The subunit molecular masses were 54.1 and 50.4 for forms I and III respectively, which suggests that the native enzyme like all eukariotic SHMTs studied, has a tetrameric structure.

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A CATALYTIC MECHANISM FOR ARGINASE. Miller, D., Guzmán, L., Celis, R., Uribe, E., Salas, M., Cerpa, J., Herrera, P., Carvajal, N. Departamento de Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

Arginase is a widespread enzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. Even though the sequence comparison of several arginases shows highly conserved regions, indicative of some common structural features, very little is known about residues involved in the catalytic mechanism of the enzyme. In this connection, some chemical modification with diethyl pyrocarbonate (DEPC) and site directed mutagenesis, have indicated a possible role for a histidine residue in the activation of a metal-bound water molecule for attack on the guanidino carbon of arginine. This report describes chemical modification studies of histidine and carboxyl groups in the enzme from *Genypterus maculatus*. The enzyme is inactivated by DEPC with a second order rate constant of 336 M^{-1} min^{-1}; the inactivation involved a single residue of pK_a 6.8 at pH 7 and 25 °C and was reversed by hydroxylamine. Borate, which is known to bind to the metal ion, protected against the inactivation by DEPC. The enzyme was also inactivated by reaction of a single residue with Woodward reagent K (WRK); the second order rate constant of 29.3 M^{-1} min^{-1} and agmatine and guanidinium chloride protected against the inactivation. From the results obtained and previous information, a "*charge relay*"-type system (aspartic acid-histidine-metal bound water) is suggested to be involved in the catalytic action of arginase.

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POTENTIOMETRIC MICROBIAL BIOSENSORS. CALIBRATION CURVES FOR GLUCOSE AND OTHER SACCHARIDES USING STEADY-STATE AND KINETIC METHODS. **Corton, Eduardo and Locascio, Guillermo. Biological Instrumentation Laboratory, Biological Chemistry Department, Fac. de Ciencias Exactas y Naturales, Pab. 2, Ciudad Universitaria, Buenos Aires(1428).** eduardo@qb.fcen.uba.ar

Biosensors are devices composed of biologically active materials such as tissues, enzymes, antibodies, etc. in close contact with some kind of transducer that convert the chemical or biochemical signal into an electrical one. The present work aims to develop a simple method for measuring total sugars in food products. A system with a special laboratory-built CO_2 sensitive electrode whose signal is recorded through an appropiate pH-meter has been studied. The biological system is constituted by a thin layer of cells of *Saccharomices cerevisiae* immobilised on a filtration membrane adequately placed on the CO_2 electrode. Measurements were made in 0.1 M phosphate buffer, pH 6.0 Detection limit for both saccharides was found to be 20 ppm. Calibration curve (mV vs. concentration) proved in both cases to be linear between 200 and 4000 ppm, correlation coefficients being around 0.98 whether steady-state or kinetic method were employed. Provided a minimum concentration range is dealed with, kinetic method allows to take readings after just 2 to 4 minutes instead of the 10 to 15 minutes required with the steady-state method. Kinetic method repeatibility was also studied. Measurements performed on samples of 196 ppm gave x = 0.7047 mV/min, σ_{n-1} = 0.0275 (n=10). We believe this biosensor could be useful for certain quality control applications in some food industries.

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ANGIOTENSIN I CONVERTING ENZYMES FROM URINE OF NORMAL AND HYPERTENSIVE PATIENTS: ACTION UPON NATURAL AND SYNTHETIC SUBSTRATES AND INHIBITORY STUDIES. Costa, R.H.; Casarini, D.E. and Alves, K.B. Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brasil.

Angiotensin I converting enzyme (ACE) is a peptidyldipeptidase, metal and anion dependent which participates of the renin-angiotensin and kallikrein and kinin systems since it converts angiotensin I (AI) to angiotensin II (AII) and also inactivates bradykinin (BK). The aim of this work was to characterize the three enzymes isolated from urine of each group of patients: a) normal (n=10): P_0N, P_1N and P_2N; b) untreated renovascular hypertensive patients (n=8): P_0SD, P_1SD and P_2SD and c) treated renovascular hypertensive patients (n=10): P_0CD, P_1CD and P_2CD. The characterization was made through the study of the enzymes action upon natural and synthetic substrates and inhibitory constants determination. The K_M values found for all enzymes were in 10^{-3}-10^{-4}M order for HHL and in 10^{-4}-10^{-5}M order for Z-Phe-HL, using fluorimetric assay. The hydrolysis of AI, BK and luteinizing-hormone-releasing-hormone (LH-RH) was determined by HPLC analysis. AI was better hydrolyzed by enzymes P_2 from the three groups, BK by the enzyme P_1N, while LH-RH was better hydrolyzed by P_1CD. The K_i values determined using HHL as substrate were in 10^{-6}-10^{-7}M order for captopril and ramipril and 10^{-3}-10^{-6}M order for enalapril. The enzymes were competitively inhibited by those inhibitors. All enzymes were also recognized by Y_1 antibody, raised against human renal ACE, showing homology to this enzyme.

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CHARACTERIZATION OF THE PROTEASE I OF FISH SKELETAL MUSCLE PURIFIED BY DIFFERENT PATHWAYS. Desplats, P.A., Sangorrín, M.P., Martone, C.B. and Sánchez, J.J. Instituto Investigaciones Biológicas FCEN Universidad Nacional Mar del Plata - CC 1245 - Mar del Plata (7600) Argentina, sangorri@uni-mdp.edu.ar

Proteinase I, an enzyme shown to be able to degrade contractile and cytoskeletal elements of myofibrils, was previously purified to apparent homogeneity in our laboratory. Later, we observed two activity zones after electrophoretic analysis of proteinase I (PIa) preparations. Thus we decide to examine for the existence of differences in the same enzyme or the presence of two enzymes. We introduced changes in the purification approach selecting different chromatographic methods: DEAE-Sephacel, Benzamidine-Sepharose, Superose 12 , pool named PIb.

PIa and PIb showed by zymography similar patterns with two bands of gelatinolytic activity. To determine if both genatinolytic activities correspond to the same enzyme we examine the effect of an endogenous inhibitor previously reported (SAIB'89,res.114) to act on crude preparations of proteinase I. This inhibitor affects both gelatinolytic bands in PIa and PIb samples. Equivalent response was evidenced at *in vitro* reactions using synthetic peptides as substrates. These results supports our assumptions that proteinase I isolated by us presents two different forms.

This work was supported in part by the CONICET, CIC, and UNMDP.

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DIMERCAPROL (BAL) INHIBITS δ-AMINOLEVULINATE DEHYDRATASE (ALA-D) BY CHELATION OF ZINC FROM A SITE THAT STABILIZES SULFHYDRYL GROUPS. T.Emanuelli[1]; F.A. Beber[2] ; J. Wollsmeider[2], J.B.T. Rocha[2] and D.G. Souza[1]. [1]Depto de Bioquímica, I.B.,UFRGS, Porto Alegre, Brazil. [2] Depto de Química, CCNE, UFSM, Santa Maria, Brazil.

ALA-D is a sulfhydryl-containing enzyme that requires zinc for maximum activity. The function of zinc in ALA-D is not clear: several studies point to a direct catalytic function for zinc, whereas other results support the assumption that zinc stabilizes the active structure of the enzyme protein, possibly by protecting its -SH groups from oxidation. In a previous study we observed that BAL, a chelating agent used in heavy metals intoxications, inhibits the activity of renal and hepatic ALA-D from adult mice. We proposed that this inhibition could be attributed to a chelating effect of BAL that would remove zinc ions essential for ALA-D activity. The aim of this work was to investigate the function of zinc ions in the activity of ALA-D from rodents and a possible relationship between this ion and ALA-D inhibiton by BAL. We investigated the effect of EDTA (0-100 μM), dithiothreitol (0-10 mM), BAL (0-1 mM) and $ZnCl_2$ (0-100 μM) on the activity of renal and hepatic ALA-D from adult rodents (rat and mouse). Renal and hepatic ALA-D inhibiton by BAL was completely reversed by 25 μM $ZnCl_2$, indicating that BAL was removing zinc from ALA-D. The inhibition of renal and hepatic ALA-D by EDTA up to 25μM and by BAL up to 1 mM were completely reversed by 10 mM DTT, indicating that at these concentrations BAL and EDTA are removing zinc ions involved in maintaining -SH groups from mice ALA-D in the reduced state. On the other hand, ALA-D inhibiton by 33-100 μM EDTA could not be reversed by 10 mM DTT, indicating that EDTA is capable of removing zinc ions more tightly bound to ALA-D. Probably this site for zinc ions is not involved in ALA-D stabilization in a reduced state but are essential for enzyme activity. Our results are in agreement with other studies (Spencer & Jordan, Biochem. J., 290:279, 1993) of ALA-D from E. coli and point to a difference in the function of the two metal-binding sites in ALA-D (Dent et al., Biochemistry, 29:7822, 1990). Accordingly BAL would inhibit ALA-D by removing zinc bound to the less tightly metal-site. Supported by CNPq and FIPE.

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CHARACTERIZATION OF SOYBEAN SEED ACID PHOSPHATASE HAVING PHOSPHOENOLPYRUVATE PHOSPHATASE ACTIVITY. Ferreira, C.V.*, Cavagis, A. D. M.*, Taga, E.M.** and Aoyama,H.*. *Departamento de Bioquímica, I.B. - UNICAMP - 13.083-970, Campinas, SP and **Departamento de Bioquímica, F.O.B. - USP - 17.043-101, Bauru, SP.

The physiological substrates of plant acid phosphatases are unknown. A detailed study on the kinetic characteristics using potential physiological substrates is not available. The aim of this work is to determine the kinetic properties of one fraction of acid phosphatase (AP1) present in latent soybean seed, which catalyzes the hydrolysis of phosphoenolpyruvate. The enzyme activity was determined by measuring the inorganic phosphate released. AP1 presented high activity at pH 4.0-4.5. The Km values were determined for p-nitrophenylphosphate (pNPP), inorganic pyrophosphate (PPi), tyrosine phosphate (Tyr-P) and phosphoenolpyruvate (PEP) at pH 5.5, for the first three substrates and 4.5 for PEP at 37°C. The following apparent Km values were obtained: pNPP - 0.49, PPi - 0.51, Tyr-P - 1.14 and PEP - 0.23 mM. The acid phosphatase were inhibited by molybdate, glycerol, ethanol, fluoride, and Zn^{2+}. The AP1 fraction was activated by Concanavalin A - ConA (30%) when PEP was utilized as substrate. This activation was also observed when pNPP, PPi and Tyr-P were utilized as substrates, however only after pre-incubation of the enzyme with ConA for 15 min. In contrast to other plant acid phosphatases the soybean seed enzymatic form presented high thermal stability at 60°C. The hydrolysis of PEP by soybean seed acid phosphatase suggests that the enzyme could have an important participation in the plant metabolism, since this substrate is a key intermediate of plant glycolysis.

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LANGERHANS ISLETS CONSTITUTIVE NITRIC OXIDE SINTASE (NOSc) ACTIVITY. Fonovich de Schroeder, T.M., Carattino, M.D., Frontera, M. and Catanzaro, O.L. PROSIVAD (CONICET) Junín 956, (1113) Buenos Aires, Argentina.

Nitric oxide (NO) production has been recently found in several tissues. This molecule plays different roles according to the cells in which it is produced. A constitutive and an inducible NOS were described in Langerhans islets.

We measured total pancreatic and Langerhans islets NOSc activity, in Control and streptozotocin (STZ) diabetic rats, through quantitation of (3H)citrulline formation by (3H)arginine hydrolysis. Calcium dependency of the enzyme was also studied.

While pancreas homogenate Control NOSc activity was linear for a wide range of protein concentrations, Langerhans islets reached maximal activity for the enzyme at 150 ug of proteins. Specific activity in the linear range was 29.25 +/- 5.16 nmol . 10-4 / mg of proteins . 45 minutes of incubation. Free calcium concentration higher than 10-7 M inhibited NOSc activity. STZ diabetes rats exhibited lower Langerhans islets enzyme activity than control ones. Histological studies showed severe damage of Langerhans islets in those rats.

These results are in agreement with an impairment of NOSc dependent insulin secretion, responsible for the observed hyperglycemia in diabetic rats, as the consequence of B cells destruction by STZ.

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KINETIC STUDIES OF BOVINE KIDNEY LOW MOLECULAR WEIGHT ACID PHOSPHATASE-SURFACTANT INTERACTIONS. Granjeiro, J.M.*, Maia, M.G.S.T.*, Taga, E.M.**, Aoyama, H.*, and Volpe,, P.L.O.***. *Departamento de Bioquímica, I.B. and ***Departamento de Físico-Química, I.Q. UNICAMP - 13.083-970, Campinas, SP. **Departamento de Bioquímica, F.O.B. - USP - 17.043-101, Bauru, SP.

The aim of this work is to study the effect of surfactants in the activity of bovine kidney low-molecular-weight acid phosphatase. The following compounds were analyzed: sodium octylsulfate ($^{-}C_8$), decylsulfate ($^{-}C_{10}$), dodecylsulfate ($^{-}C_{12}$), tetradecylsulfate ($^{-}C_{14}$); dodecyltrimethyl ammonium ($^{+}C_{12}$), tetradecyltrimethyl ammonium ($^{+}C_{14}$), and cetiltrimethyl ammonium ($^{+}C_{16}$) bromide. The enzyme activity was determined with p-nitrophenylphosphate (pNPP) as substrate, at pH 5.0 (acetate buffer), pH 6.0 and 7.0 (bis-tris buffer). At pH 5.0, concentrations of 100 and 30 times lesser, for anionic and cationic surfactants, respectively, were required to promote the enzyme inactivation by increasing the chain lenght of the surfactants. In contrast to the cationic surfactants, the concentrations of the anionic compounds (except for $^{-}C_{10}$), giving 50% of enzyme inactivation seemed to be independent of the pH. Except for $^{+}C_{12}$, the inactivation constant for the enzyme activity (at 37 °C) was time-dependent and proportional to surfactant chain length. In contrast to $^{-}C_{12}$ effect, the enzyme activity inactivation by $^{+}C_{14}$ was partially reversible. The presence of phosphate clearly protected the enzyme against inactivation by $^{+}C_{14}$ and $^{-}C_{12}$. In the enzyme-surfactant interaction polar interactions should be also taken into account. The acid phosphatase-surfactant interaction was time- and surfactant chain length-dependent, reversible for $^{+}C_{14}$, irreversible for $^{-}C_{12}$, but prevented by the addition of inorganic phosphate.

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PURIFICATION, PHYSICO-CHEMICAL AND KINETIC CHARACTERIZATION OF A CASTOR BEAN ACID PHOSPHATASE. Granieiro, P.A.*, Ferreira, C.V.*, Granjeiro, J.M.*, Aoyama, H*, and Taga. E.M.**. *Departamento de Bioquímica, I.B.- UNICAMP 13.083-970, Campinas, SP, Brazil and **Departamento de Bioquímica, F.O.B. - USP - 17.043-101, Bauru, SP, Brazil.

In this work we described a purification and some properties of a castor bean acid phosphatase isoenzyme. The enzyme was purified 619-fold, with a specific activity of 71.4 μmol. $min^{-1}.mg^{-1}$, by a procedure involving ammonium sulfate fractionation, SP-Sephadex ion exchange, gel filtration and Concanavalin A-Sepharose chromatographies. The purified glycoprotein showed a relative molecular mass of 68,000 (HPLC GPC 100 column). Polyacrylamide gel electrophoresis showed a single protein band coincident with enzyme activity. The enzyme activity was determined at pH 5.0 with p-nitrophenylphosphate (p-NPP) as substrate, at 37°C, for 10 min. An apparent Km value of 0.66 mM was obtained for p-NPP. An apparent activation energy, determined by Arrhenius plot, was calculated to be 55.2 kJ K^{-1} mol^{-1}. The p-NPP hydrolysis was inhibited by 10 mM Fe^{2+}, Cu^{2+}, and Co^{2+}, 0.1 mM molybdate, and 15 mM fructose. Fluoride (5 mM), pCMB (1 mM) and vanadate (0.1 mM) inhibited 60, 80 and 70% of the enzyme reaction, respectively . The enzyme activity was independent of Ca^{2+}, Mn^{2+}, or Mg^{2+}. In relation to substrates, the enzyme efficiently hydrolyzed β-naphtyl-P, pyrophosphate, tyrosine-P, 6-phospho-gluconic acid, and FMN. Phosphotyrosine proteins and pyrophosphate could be potential substrates for castor bean acid phosphatase;.the enzyme reaction was independent of metals and strongly inhibited by fructose.

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THIOL/DISULFIDE EXCHANGE IN PROTEINS: ROLE OF THE REDUCTANT AND THE TARGET PROTEIN IN THE ACTIVITY OF RAPESEED PROTEIN DISULFIDE ISOMERASE AND *E. coli* THIOREDOXIN. Aleiandro P. Heuck, Santiago Mora-Garcia, and Ricardo A. Wolosiuk, Instituto de Investigaciones Bioquímicas, FCEyN, UBA. Bs. As., Argentina. e-mail: aheuck@iris.iib.uba.ar

Thiol/disulfide exchanges in proteins contribute to the stabilization of the structure, regulate enzyme activity and provide a response to oxidative stress. These reactions are modulated by a family of catalysts named protein disulfide oxidoreductases [PDOR]. To analyze the characteristics of these redox processes, we obtained homogeneous preparations of two PDOR: the protein disulfide isomerase of *Brassica napus* seeds [PDI] and the thioredoxin of *Escherichia coli* [Trx]. Moreover, we prepared as target proteins rapeseed napin and di-fluoresceinthiocarbamylated insulin [di-FTC-ins] by protein purification and chemical derivatization, respectively.

In the presence of dithiothreitol, PDI and Trx catalyze the reduction of both di-FTC-ins and napin. However, only PDI is functional in cleaving disulfide bonds of di-FTC-ins when glutathione is the reductant. PDI drives the reduction of target proteins at slightly acidic (optima pH: 6.5) whereas Trx-mediated reductions are fast at alkaline pH (optima pH: higher than 8.0). At variance with this generalization, the optima pH for the PDI-mediated renaturation of scrambled RNase A with dithiothreitol was higher than 8.0.

Taken together above results are congruent with the absence of a common pattern for disulfide bond reduction of proteins. The reductant, the PDOR and the target protein conditionate the thiol/disulfide exchange reaction.

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***E.coli* PHOSPHOENOLPYRUVATE CARBOXYKINASE: THE EFFECT OF CALCIUM REVISED.** San Martín, C., and Jabalquinto, A.M. Departamento de Ciencias Químicas, Facultad de Química y Biología, Universidad de Santiago de Chile.

E. coli phosphoenolpyruvate carboxykinase (PEPCK) catalyses the first committed step of gluconeogenesis:

$$\text{oxaloacetate} + ATP \xleftarrow{Me^{2+}} \text{phosphoenolpyruvate} + ADP + CO_2$$

Previous studies (Goldie,H and Sanwal,D. (1980) J. Biol. Chem. 255, 1399-1405) suggested that this enzyme is allosterically activated by Ca^{2+}, and this enhancement in activity occurs synergistically with respect to Mg^{2+}. In this work we characterized the kinetic effects of three divalent cations: Mn^{2+}, Mg^{2+}, Ca^{2+}, and Mg^{2+}-Ca^{2+}, Mg^{2+}-Mn^{2+} combinations. Our results indicate a lack of a Ca^{2+} activating effect when studying the enzyme activity through the partial exchange reaction and in carboxylation and decarboxylation directions. On the other hand, attempts to determine the $^{45}Ca^{2+}$ binding to the enzyme, in the absence or in the presence of Mg^{2+}, were unsuccessful. Thus *E. coli* PEPCK behaves as well as all PEPCKs described to date, they have two types of binding sites for metal: one that binds nucleotide metal complex (MnATP, MgATP), and other that binds free metal (Mn^{2+}).

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ACTIVATION OF RAT LIVER ALA-DEHYDRATASE BY TAMOXIFEN - KINETIC STUDIES. Llambías, E.B.C. - Departamento de Quimica Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina - ebcl1ǫ qb.fcen.uba.ar

5-aminolevulinate (ALA)-dehydratase catalyzes the conversion of two molecules of 5-aminolevulinic acid into porphobilinogen (PBG). Tamoxifen is a very widely prescribed non-steroidal anti-oestrogen used in the treatment of breast cancer. Kinetics of activation of ALA-dehydratase activity by tamoxifen are reported. Enzyme activity was determined "in vitro" by H.P.L.C. Km and Vm were determined in the presence of different concentrations of tamoxifen. In general, any two lines in reciprocal plots intercept to the right of the ordinate above the abscissa, not showing a common intersection point. Km and Vm increase with the addition of tamoxifen, decreasing after with higher concentrations of the activator. Vm/Km linearly increases with increasing concentrations of tamoxifen. When phosphate buffer concentrations are changed, in the absence of tamoxifen, Km and Vm are maximum at 0.08 M. Hill coeficients are 1 in any case, Slopes and intercepts do not vary linearly with increasing concentrations of tamoxifen or phosphate. Activity is maximum at pH 6.3, while Km decreases when pH is over 6.8. With results obtained we cannot determine yet if tamoxifen is a mixed type non-essential activator, or if ligand substitution reactions are involved. The physiollogical significance of these results needs to be determined as well.

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HYSTERETIC BEHAVIOUR OF *Trypanosoma cruzi* PEP CARBOXYKINASE. Maugeri, D.A.: Paladini, A (h); Cazzulo J.J. and Cannata, J.J.B. CIBIERG, Facultad de Medicina, Universidad de Buenos Aires; INGEBI, CONICET and Instituto de Investigaciones Bioquímicas, Fundacion Campomar. Buenos Aires, Argentina.

The "aerobic fermentation" of glucose that occurs in *Trypanosoma cruzi* leads to the production and excretion into the medium of succinate and L-alanine that depends on a carboxylation reaction catalyzed by PEP carboxykinase:

P-enolpyruvate + ADP + CO_3H^- $\xrightarrow{Mn^{++}}$ oxalacetate + ATP

The carboxylation reaction followed by a continuous spectrophotometric method by coupling it to the malate dehydrogenase system showed a lag time which was dependent upon the enzyme concentration, the presence of ammonium sulfate and dithiothreitol in the assay mixture and the purity of the enzyme preparation used. Previous dilution and preincubation at 0°C of the enzyme preparation elicited considerable activation abolishing practically the lag period except when PEP, sustrate of the reaction, was present in the diluting buffer. The transients kinetics of the progress curves, analyzed according to Neet and Ainslie (Methods Enzymology 64: 192, 1980) where as expected from a system exhibiting hysteretic behaviour, with a transition from a less active to a more active enzyme form. Reversible association-dissociation of the enzyme, with the different polymeric states having different kinetics properties seemed to be the most likely explanation. This was corroborated by pressure-induced reversible dissociation experiments according to Weber's theory. In fact, the application of hydrostatic pressure up to 0.14 kbar resulted in the activation of the enzyme, but at higher pressure values, progressive inactivation was observed, being practically total at 1.8 kbar. Decompression to atmospheric pressure restored the initial activity. When the same experiment was performed with the pre-diluted enzyme only inactivation at pressures higher than 0.14 kbar was obtained. Pre-diluted enzyme in the presence of PEP mimics the behaviour of the non diluted enzyme. We postulate the existence of an equilibrium between different polymeric states according to a model in which inactive polymeric aggregate dissociate to the active dimer, which in turn can dissociate to inactive monomer:

aggregate $\underset{\text{or dilution}}{\overset{\text{Pressure} < 0.14 \text{ k bar}}{\rightleftharpoons}}$ dimer $\overset{\text{Pressure} > 0.14 \text{ k bar}}{\rightleftharpoons}$ monomer

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EFFECT OF DOCA ON THE CATALYTIC ISOFORMS OF THE NA^+, K^+ ATPASE IN RAT BLOOD VESSELS. Mir, Y., Bravo, I., Schuster, A and Michea, L. F. Faculty of Medicine, University Los Andes, Santiago, Chile.

It has been established a close relationship between mineralocorticoids plasma blood levels and hypertension. The sodium pump (Na^+, K^+ ATPase) regulates the reactivity of blood vessels through the intracellular levels of sodium and therefore calcium concentration. The aim of the present study was to determine if mineralocorticoids has an effect on the Na^+, K^+ ATPase activity in the rat aorta; also to see if there is an specific effect on the catalytic isoforms of the enzyme. The activity of the Na^+, K^+ ATPase was measured by ^{86}Rb uptake in intact aortic rings under different concentrations of ouabain to determine total pump activity, α_1 and α_2 catalytic isoforms activity. The studies were carried out in four groups of rats, control, adrenalectomized (ADX), control plus deoxicorticosterone (DOCA) and ADX plus DOCA. The total pump activity in the ADX group was lower compared to the control group (165.9 ± 54 *vs* $110,3 \pm 28$, $p<0.05$) and when compared with control plus DOCA group (183 ± 27, $p<0.05$). The percentage of catalytic α_2 isoform activity also presented significant differences: control = 24% *vs* ADX = 10% ; ADX *vs* ADX plus DOCA = 34% ; ADX *vs* control plus DOCA = 24 % ($p<0.05$ all cases). These results suggest that the mineralocorticoids have a major effect on the pump activity of the rat aorta mainly through the regulation of the α_2 isoform activity.

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ENZYMATIC HYDROLYSIS ON THERMALLY TREATED SOYBEAN PROTEIN ISOLATES OBTAINED IN THE PRESENCE OF CALCIUM Molina Ortiz S., Scilingo, A., Añón, M.C. Centro de Investigación y Desarrollo en Criotecnología de Alimentos, 47 y 116, La Plata, Bs.As. Argentina.

The purpose of our work was to study the action of non-commercial proteases upon soybean protein isolates with both calcium and combined thermal treatment (M10 y M30). The enzymes used were maclura, hieronymine (LIPROVE, UNLP-Argentina) and cucurbita (Lab. Bioquímica, UCV-Chile). The hydrolysis development was followed by the dosage of tyrosine meq soluble in 19% TCA, carrying out a SDS-PAGE of the precipitates obtained. The hydrolysates were analyzed through reverse phase chromatography (HPLC-RP) and differencial scanning calorimetry (DSC), being their water solubility determined. The tyrosine meq increased with time of reaction in all the cases studied. After 16 h of reaction, a major hydrolysis degree with cucurbita, an intermediate degree with maclura and a minor degree with hieronymine was observed. The hydrolysates HPLC-RP patterns showed a zone at short retention time, corresponding to polar nature hydrolysis products, present in a larger amount in cucurbita and maclura. hydrolysates. Another zone is also noticed at long retention time, indicating the presence of hydrophobic structures. The hydrolysates thermograms of the two samples studied were analyzed, obtaining the denaturation temperatures and enthalpies. The electrophoretic patterns showed both the progressive disappearance of some of the typical bands of the soybean protein isolates and the appearance of hydrolysis products, showing differences among the action of the three proteases. An increase in solubility of the hydrolyzed isolates with regard to those that were not hydrolyzed was observed with the three enzymes tried and in all hydrolysis times.

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RABBIT KIDNEY HOMOGENATE'S AMINOPEPTIDASES. Oliveira, S.M.; Freitas Jr., J.O. and Alves, K.B.-Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brasil.

Aminopeptidases hydrolyze peptide bonds liberating the N-terminal aminoacid of peptides and proteins. They also hydrolyze the amide bound of L-aminoacyl-2-naphthylamides (AA-NA) and L-aminoacyl-p-nitroanilides (AA-NiA). Rabbit kidney homogenate shows four distinct aminopeptidases when submitted to a Mono Q column, equilibrated and washed with 0.02 M phosphate buffer (NaPB) pH 7.0 and eluted with a linear gradient of 0.02 to 0.5 M NaPB pH 7.0, at 4°C. The second and third active protein peaks, eluted at 0.5 and 0.9 mS conductance respectively, were lyophilized and gel filtered on a Superdex 75 column, equilibrated and eluted with 0.02 mM NaPB pH 7.0, yielding each, one active protein peak. SDS-PAGE (10%) of these enzymes show only one protein band, with MW 78 and 125 kDa, respectively. Since the second enzyme has highest catalytic efficiency (V_{max}/K_M) for Arg-NA and the third, for Leu-NA, enzymes properties were determined using the best substrate for each enzyme. Both enzymes have optimum pH 7.0, are parcially inactivated by 1.0 mM ions Zn^{2+} or EDTA and 100% inhibited by 1.0 mM o-phenanthroline. The activity of the 1st enzyme was 500% activated by 0.15 M sodium chloride, 100% inhibited by 0.13 mM p-hydroxymercuribenzoate (p-HOMB) while 6.66 mM 2-mercaptoethanol (2-ME) had no effect. The activity of the 2nd, was 30% inhibited by 0.2 M sodium choride, 40% by 0.05 mM p-OHMB and 630% activated by 1.7 mM 2-ME. Ki values determined for puromycin and Bestatin are in 10^{-4}-10^{-7} M range, showing competitive type inhibitions. The 1st enzyme is a basic and the 2nd, a neutral aminopeptidase. (CNPq)

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EFFECT OF RIBOFLAVIN DEFICIENCY ON SUCCINIC DEHYDROGENASE OF LIVER MITOCHONDRIA OF SUCKLING AND WEANED RATS. **Quintana, M.; Ore R. and Marcelo A. Centro de Investigacion de Bioquimica y Nutricion. Universidad Nacional Mayor de San Marcos, Grau 750, Lima-1, Peru.**

A deficient nutrition in vitamin B2, given to weanling rats decreases the succinic dehydrogenase activity, and produces body weight chabges and skin alterations. We are studying how riboflavin deficiency in pregnant rats affects to their offspring. Rats were organized in 3 groups at the 4th day of gestation, group A: control (100% B2), group B: marginal (50% B2), group E: deficient (1% B2). The succinic dehydrogenase activity was measured in the livers of young rats 0, 7, 14 and 21 days old. The enzyme activity decreased signicantly in rats with the marginal and deficient diets, specially in the last group. Rats of the E group showed hepatomegaly and fatty liver, and the skin alterations were observed as early as when they were 14 days old.

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SENSITIVITY DESIGN OF A MONOCYCLIC CASCADE: THE REVERSIBLE CASE. Ortega, F. and Acerenza, L. Sección Biofísica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

The covalent interconversion of an enzyme or metabolite is a mechanism that can generate highly sensitive responses. The aim of this work is to study, in a monocyclic cascade, what values of the kinetic parameters of the converter enzymes are required to obtain a pre-established sensitivity of response. The sensitivity is quantified by the Control Coefficient that represents the relative change in a substrate with respect to the relative change in a velocity. To achieve our aim we apply a method called Metabolic Control Design (MCD) (Acerenza, L.. 1993. *J. theor. Biol.* **165**, 63-85.). It allows one to calculate the kinetic constants of the converter enzymes that, when embedded in the system, would produce the pre-established values of the Control Coefficients. Here we consider the case where the converter enzymes are of the reversible Michaelis-Menten type. Using the MCD procedure we have calculated the values of the equilibrium constants required to obtain a high Control Coefficient. It can be concluded that the higher the sensitivity that we want the system to show the greater the equilibrium constant required. The Michaelis constants compatible with the Control Coefficient chosen were also calculated.The results show that some saturation of at least one enzyme is necessary, and the extent of the saturation is greater for systems closer to equilibrium.

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***C SACCHAROLITYCUM* β-GLUCOSIDASE CATALYZED REACTION: pH INDEPENDENT ACTIVATION ENTHALPHY.** **O'Reilly,S.. *Guerrero,I., Montenegro,V. and Paris,I. Institutos de Química y de *Estadística, Fac de Cs Bs y Mat, Universidad Católica de Valparaíso, Valparaíso, Chile.**

Thermophiles in general resemble their mesophilic counterparts with respect to their carbon and nitrogen sources, as well as their metabolic pathways. As a consequence, their enzyme patterns do not show drastic adaptative alterations. On the other hand, single enzymes must have changed in stability and kinetic properties to cope with the high thermal stress.

We are interested to estimate the pH independent activation enthalpy of an enzyme of termophilic origin, *Caldocellum saccharolitycum* β-glucosidase, and to compared it with the enzyme of a mesophile, almond β-glucosidase.

pH dependent kinetic parameter V_{max} and V_{max}/K_m in a pH (5.4 - 7.0) and temperature (42 - 54°C) range, the enzyme dissociation constants (pK_E), and pH independent V_{max} and V_{max}/K_m by non linear regresion analysis were estimated. Using pH dependent and independent kinetic parameters the corresponding activation enthalphies were calculated. pH independent activation enthalpy obtained were 18,250 ± 1,284 (V_{max}) and 21,020 ± 1,296 (V_{max}/K_m), respectively. In both cases, pH dependent parameters showed a tendency to lower values. Estimated values obtained with almond β-glucosidase were significatively lower. Higher activation enthalpies would in part explain the characteristic dependence of this thermophilic enzyme with temperature. Our results also allow us to postulate the participation of two dissociable groups of the free enzyme, with pK values of 4.66 and 6.95 at 54°C (pHopt=6.32), which were not significantly affected by temperature changes.

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STUDIES ON PROTEIN KINASE CK1α. **Pulgar, V., Tapia, C., Connelly, C.C. and Allende, J.E. Departamento de Bioquímica, Facultad de Medicina and Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 70086, Santiago 7, Chile.**

Protein kinase CK1 (also known as casein kinase 1) is a ubiquitous enzyme that has been swown to phosphorylate several key substrates (aminoacyl-+ RNA synthetases, SV-40 large T, p53, insulin receptor, etc). Seven isoforms of this enzyme have been found in mammalian species and in yeast, one isoform coded by HRR 25 has been involved in DNA repair. In our laboratory we have cloned the human and ***X. laevis* α** isoform of CK1 and have been able to express the ***X. laevis*** enzyme in ***E. coli*** as a fusion protein with glutathione transferase. The purified recombinant enzyme has similar specificity and kinetic contants as the enzyme isolated from rat liver as demonstrated by its phosphorylation of the model peptide. Recombinant CK1α can phosphorylate tyrosine residues in poly (Glu^{80}:Tyr^{20}) and in the peptide RRREEEYEEEE and autophosphorylates in ser/thr and to a lesser degree in tyr. A polyclonar antibody raised against the peptide GRHCNKFLIDFGLAKKY a sequence present in CK1α recognizes recombinant CK1 and immunoprecipitates a CK1 activity from extracts from ***X. laevis*** oocytes and He La cells. This method is being used to study the possible regulation of CK1 in cells under different growth conditions and different treatments.

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CHARACTERIZATION OF A SPHINGOMYELINASE ACTIVITY (SMase, E.C. 3.1.4.12) IN SEMINIFEROUS TUBULES OF IMMATURE RATS WITH pH OPTIMUN IN 6.6. Raimann, P. E.; Bernard, E.A. and Guma, F.C.R.
Departamento de Bioquímica, IB, UFRGS, Brasil.

Several classes of sphingomyelinases have been described in mammalian tissues. One is lysosomal, has an acid pH optimun, and is widely distributed in mammalian tissues. A second has a neutral pH optimun, is stimulated by Mg^{+2} ions, and is particularly enriched in certain neuronal areas. A third SMase, with a neutral pH optimun and no Mg^{+2} requirement, has been described in rat brain myelin. Bovine seminal plasma and reproductive tissues contains a SMase activity that has a pH optimun in 6.5 and requires Mn^{+2} for activation. In the present work we describe a SMase activity in seminiferous tubules of rats. This enzyme has a pH optimun at 6.6, requires Mn^{+2} for activation and also have been linked to particulate structures. Crude homogenates, citosolic and microsomal fractions of seminiferous tubules of 19 days-old rats displayed sphingomyelin split with the range pH 4.8 - 8.0 and showed highest activities at pH 4.8 and 6.6. When we analysed in greast detail the pH 6.6 enzyme, we determined that: upon addition of 0.2% Triton X-100 the release of phosphorylcholine was time and enzyme concentraction dependent and linear until 1 h and 200 μg protein. The K_M value of the enzyme was 30.8 μM. Mg+2 was inefective in activate microsomal pH 6.6 SMase. In conclusion, rat seminiferous tubules of immature rats, like bovine seminal plasma and reproductive tissue has a SMase with pH optimun in 6.6 that can be classified as a neutral enzyme with an obvious requirement for divalent ions in activation. However, it appears to be distint from the previously described neutral SMase in its activation by Mn^{+2}.

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HEXACHLOROBENZENE-INDUCED ALTERATIONS OF MICROSOMAL MEMBRANE PHOSPHOLIPIDS AND PROTEIN TYROSINE KINASE ACTIVITY. Randi A. [1], Billi S.[2], Kolliker Frers R.[1], San Martin de Viale L.[2] and Kleiman D.L.[1]. Departamentos de Química Biológica, Facultades de [1]Medicina y [2]Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

Hexachlorobenzene (HCB) is an environmental pollutant. It is known that the activity of some membrane bound-enzymes depends on their lipid environment. We have investigated the time-course changes (4,8,11 and 18 days) in protein tyrosin kinase (PTK) activity, phosphotyrosine content and phospholipid (PL) composition of liver microsomal membranes (LMM), in HCB-treated (1g/kg b.w.) female Wistar rats (6 months old). LMM were isolated and a) PTK activity was assayed with Poly Glu-Tyr as exogenous substrate, b) endogenous protein tyrosine phosphorylation was examined by immunoblotting c) membrane phospholipids (PL) were separated by TLC and Pi determined by Fiske Subarrow. Results: after HCB treatment the PL content of LMM increased 77% up to 11 days, returning to 31% at 18 days when compared to control rats. Parallel changes were observed in phosphatydilcholine content. PTK activity decreased 70%, after 4 and 18 days, however it became elevated after 8 days of treatment relative to controls. Inmunoblot assays showed time dependent alterations in phosphotyrosine content of LMM of 53, 85 and 170 Kd. Conclusion: Our results showed that, in a time-course study, LMM underwent changes in PTK activity, phosphotyrosine content and PL composition.

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EVIDENCE THAT THE RESPIRATORY NADH DEHYDROGENASE-2 OF *Escherichia coli* IS A Cu^{2+}-REDUCTASE. Rapisarda, V. A. and Massa, E. M.
Dpto. Bioquímica de la Nutrición del INSIBIO e Instituto de Química Biológica, UNT-CONICET. Chacabuco 461, (4000) Tucumán, Argentina.

Copper is both an essential nutrient and a toxic metal for all living organisms. In previous studies of our laboratory, using *E. coli* as a model system for oxidative stress, it was found that the respiratory chain supports a Cu^{2+}/Cu^{+} redox cycle involved in hydroperoxide toxicity. It was suggested that one locus of copper reduction is the NADH dehydrogenase-2 (NDH-2), which is a FAD-containing single polypeptide. The aim of the present work was to determine whether or not the NDH-2 has NADH:Cu^{2+} oxidoreductase activity. For this purpose, a cholate-solubilized preparation of membranes from an *E. coli* strain overexpressing the NDH-2 was chromatographed on a column of hydroxylapatite, to isolate the dehydrogenase from the bulk of the proteins as described in the literature. The dehydrogenase activity peak in the eluted fractions was coincident with a NADH:Cu^{2+} oxidoreductase activity peak. Both activities were absent from the elution profile when a *ndh*$^{-}$mutant was tested. These findings confirm our proposal that the NADH:Cu^{2+} oxidoreductase activity is associated with the NDH-2 protein. Thus, this protein seems to be a member of the flavoenzyme family with metal ion reductase activity. To our knowledge, this is the first time that a copper reductase is identified.

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EFFECT OF UNDERNUTRITION ON SERINE PALMITOYLTRANSFERASE ACTIVITY IN HYPOTHALAMUS RATS. Rotta,L.N.; da Silva,C.G.; Madke,R.R.; Perry,M.L.S.; Trindade,V.M.T. - Departamento de Bioquímica -IB - UFRGS - Porto Alegre - Brasil

Serine palmitoyltransferase (SPT) (EC 2.3.1.50) is the enzyme that catalyses the first step in the biosynthesis of sphingolipids, through the condensation of serine with palmitoyl CoA in a process that occurs in endoplasmatic reticulum. The undernutrition reduces the glicosphingolipids content in the Central Nervous System. We investigated the optimum conditions for the activity of SPT in hypothalamus rat and avaliated this enzyme activity in normo (diet:25% casein) and undernourished (diet:8% casein) animals. The enzyme source was a microsomal fraction from the hypothalamus rats, obtained as described by Mandon et al.(*Eur.J.Biochem.198*:667-674, 1991). The enzyme activity was measured according Merril et al.(*Methods in Enzimology, 209*:427-431, 1992) using [3-^{14}C]serine as radioactive precursor. We confirmed that the 3-keto-sphinganine is a product of SPT activity through TLC analysis (Williams et al. *Arch.Biochem.Biophys.228*:282-291, 1984). To avaliate the activity of SPT we used the incubation time of 20 min/37^{0}C, 1mM [3-^{14}C]serine, 0.16 mM palmitoyl CoA and 150μg of microsomal protein, with agitation. SPT activity in normal rats increased with development from 21th day of gestation to the 14th postnatal day and in undernourished animals the enzyme activity is not altered. These results suggest that undernutrition may be diminushed the level precursors of sphingolipids biosynthesis in hypothalamus rats in the ages studied. (CNPq, FINEP, PIBIC-CNPq/UFRGS, FAPERGS, PROPESP-UFRGS).

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METABOLITE-MODULATED COMPLEX FORMATION BETWEEN FRUCTOSE-1,6-BISPHOSPHATASE AND ALDOLASE Sáez, D. E. & Slebe, J. C. Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.

It has been proposed that enzymes along a metabolic pathway may transfer metabolites directly from the active site of one enzyme to the active site of another, with no intermediates becoming free, implying a protein-protein interaction. This phenomenon is referred as metabolite channeling and is the subject of a considerable controversy (Ovádi, J. (1991) *J. Theor. Biol.* 151, 1-22). A modified Hummel-Dreyer equilibrium chromatography technique was used to test if fructose-1,6-bisphosphate (FBP) induces the molecular association of fructose-1,6-bisphosphatase (FBPase) and aldolase. In the absence of FBP, a unique elution profile with a trough at the free FBPase elution position is obtained. The appearance of this trough is physical evidence that a weak reversible association between FBPase and aldolase occurs. The association constant for the complex formation between FBPase and aldolase is 0.12 μM^{-1}. In the presence of a limited FBP concentration (2:1 with respect to FBPase), the association constant increased to 0.82 μM^{-1}, while at a higher concentration (10:1) the constant reach a value of 4.9 μM^{-1}. In the last case the elution profile showed a new unresolved peak running ahead of the elution peak of aldolase. With a very high substrate concentration (140:1) no evidence of interaction is observed. Such concentration-dependent behavior suggest that the competition between FBPase and aldolase for the limited amount of FBP tends to make the most efficient use of the shared metabolite. This results make a convincing argument for a metabolite-modulated enzyme-enzyme interaction along the gluconeogenic pathway. (Supported by Fondecyt 2930044,4950006, 951215; DID UACH S-95-41)

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ACTIVATION OF MYOSIN AND SUBFRAGMENT-1 (S1) ATPase BY 2,4-DINITROPHENOL (DNP) AND ITS ANALOGS 2-, 3- AND 4-NITROPHENOL (NP). Salerno, V.P., Dinucci, A.N., Ribeiro, A.S., Mignaco, J. and Sorenson, M.M. Departamento de Bioquímica Médica - ICB - CCS - Universidade Federal do Rio de Janeiro, Brasil.

DNP has been shown to affect myosin and muscle contraction. We confirm these results on myosin, compare DNP with mono-NP's and characterize DNP's effects on catalysis using soluble S1. Assays contained (in mM) 50 Hepes, 3 ATP, 5 Mg or $CaCl_2$, ~80 KCl; or 5 EDTA and 600 KCl, (30°, pH 7). Effects of the nitrophenols depended on the cation. In the presence of Mg^{++}or Ca^{++}, 3-NP and DNP activated the ATPase; 2- and 4-NP had little effect, or inhibited. 3-NP required a lower concentration ($K_{0.5} \cong 2$ *vs* 15 mM), but had ~1/2 the maximal effect of DNP (which activated 2.2 to 4 fold). DNP and 3-NP effects decreased as $CaCl_2$ was lowered to 1 mM. All NP's inhibited K^+EDTA-ATPase activity ($K_{0.5}$ 2-15 mM). These differences may reflect structure as well as protonation, since pKa=4.1 for DNP and 7.2 to 8.4 for the other NP's. In kinetic experiments in the presence of Ca^{++}or Mg^{++}, DNP inhibited S1 ATPase at low [ATP] and activated only with higher [ATP] (>1 μM in Mg^{++}, or >100 μM in Ca^{++}). DNP appears to be a rather specific effector of S1 ATPase in the presence of Ca^{++}or Mg^{++}, where activation is associated with the appearance of non-Michaelis-Menten behavior. DNP may act directly on the catalytic site, since its effects vary with [ATP].

Support: CNPq, FAPERJ, CAPES, FINEP, UFRJ.

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HAEM BIOSYNTHESIS IN CHICK EMBRYO. N.L. Pauza, M.L. Barreiro, M.F. Spinelli, A. Senn, C. Zorzano, Y.E. Sopena de Kracoff, A.M. Ferramola de Sancovich and H.A. Sancovich. Departamento Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA, CONICET, Buenos Aires, Argentina.

Haematopoiesis in birds occurs in the same manner as in mammals. One of the main sites of haem synthesis in the bird embryo is the yolk sac membrane (YSM) a further significant site of haem production is the liver (L). Our aim in this work is to compare kinetic properties of the δ Aminolevulinic acid Dehydratase (ALA-D), Porphobilinogen Deaminase (PBG-D) and Uroporphyrinogen Decarboxylase (URO-D), the cytosolic hepatic enzymes of the haem biosynthetic pathway with those of the YSM. Embryos of 17 day development were used and the L and YSM of 5-8 embryos were pooled and sampled for enzyme activities. Tissue samples were homogenised in 0.154 M KCl and 11,000 *x g* supernatants were used as enzyme sources. ALA-D, PBG-D and URO-D activities were estimated by the standard methods used in our laboratory. The three enzyme activities from both sources were proportional to the amount of protein and to the incubation time. The optimum pH of each enzyme reaction from both tissues was around 6.8. Activation energies from Arrhenius plots show similar values with the enzymes from both sources. L and YSM ALA-D, PBG-D and URO-D present Michaelis-Menten kinetics. The Km and Vmax values for the three YSM enzymes were lower and higher respectively, than those from L (e.g., L ALA-D: Km 0.3 mM and Vmax 69 nmole PBG/mg x h and YSM ALA-D: Km 0.1 mM and Vmax 104 nmole PBG/mg x h). These results are in agreement with the relative significance of haem biosynthesis in both tissues during the chick embryo development. The YSM is the main site of haem biosynthesis and erythropoiesis in the bird embryo.

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PURIFICATION AND CHARACTERIZATION OF A PROTEASE AND ITS INHIBITOR, THEIR ACTIONS ON MYOFIBRIL DEGRADATION. Sangorrín, M. P. and Sánchez, J. J. Inst. Invest. Biol. FCEyN Universidad Nacional de Mar del Plata. CC 1245 Mar del Plata. (7600) Argentina. sangorri@uni-mdp.edu.ar

We have reported the existence of a myofibrillar proteolytic activity (protease M) from mouse skeletal muscle and a proteic inhibitor of this activity. Muscle was homogenized and fractionated in myofibrillar (MF) and sarcoplasmatic (SF) fractions by centrifugation. Protease M was extracted from the MF with 40 % etilenglycol, pH was adjusted to 4.5 and chromatographed in Mono Q and Superose 12. Inhibitory activity was isolated from SF with similar chromatographic steps. Gel filtration chromatography revealed a mol. wts. of 120 kDa for the protease M and 150 kDa for the inhibitor. Protease M was characterized with especific protease inhibitors using azocasein, radiolabelled myofibrils and synthetic peptides as substrates. Our results indicated that this enzyme is a serin-protease, stimuladed by K^{+1}, Mg^{+2}, Mn^{+2} and ATP. The cleaving pattern of myofibrillar proteins (SDS PAGE) produced by protease M showed decreasing intensity in the bands corresponding to myosin, actin and some regulatory proteins. The data presented in this study suggest that protease M is implicated in skeletal muscle myofibrillar degradation "in vivo" and the inhibitor isolated may provide a mechanism for controlling its action. A similar system has been reported by us in fish skeletal muscle.

This work was supported in part by the CONICET,CIC y UNMDP.

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UROPORPHYRINOGEN DECARBOXYLASE FROM NORMAL RAT LIVER. PURIFICATION AND KINETIC STUDIES OF TWO ISOFORMS. Ríos de Molina M. C., Chaufan G., Corvi M., Guidi S. and San Martín de Viale L. C. Fac. de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Argentina.

Uroporphyrinogen decarboxylase (UroD) is the key enzyme in human and experimental Porphyria Cutanea Tarda. We have completely separated and purified two molecular forms of UroD (SAIB 95). Now we inform its characterization and the detection of a new form. From the (S) vs (S)/v plots the values of Km and Vmax for the different substrates were calculated and the results obtained are shown in the following table.

ISOFORM	MW	Uro'gen III		Penta'gen III	
		Km	Vmax/Km	Km	Vmax/Km
	(kDa)	(μM)	(U/μM)	(μM)	(U/μM)
IF-a	45	0.27	5.00	0.55	0.87
IF-b	30	5.00	1.16	0.50	0.62
IF-c	17	n.d.	n.d.	n.d.	n.d.

It can be seen that the efficiency values for IF-a were always higher than those for IF-b. This difference was more noticeable at the Uroporphyrinogen level. With IF-a we proved that exist an inverse relation between the activity and the amount of enzyme. IF-a and IF-b should be the homologous of isoenzymes informed by Mukerji and Pinstone (Int. J. Biochem. 24, 1992: 105) from human erithrocytes. This is the first time that a third UroD isoenzyme could be detected.

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COPROPORPHYRINOGEN OXIDASE FROM HUMAN LIVER INVOLVEMENT OF HISTIDINE AND ARGININE IN THE CATALYTIC PROCESS. Sorianello, E.M., Mazzetti, M.B., and Tomio, J.M. Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales. UBA. Buenos Aires. Argentina.

The exact mechanism for the formation of the vinyl groups in protoporphyrinogen IX from coproporphyrinogen III catalized by the coproporphyrinogen oxidase (CPOX), is still unkwon. The two ordered decarboxylations, most probably occurs at the same catalytic site in the mammalian enzyme.To facilitate the characterization of the CPOX both structural as functional, the protein was purified from human and rat liver.Our previous studies suggested that basic amino acids could be involved in the catalytic process. To further investigate this role, kinetic studies were performed with chemical modifiers of proteins. The effect of pH on CPOX activity gave maxima values at pH between 5.8 to 7.3. Still, the enzyme showed significant activity at pH 5.2, which was lost at pH 4.6. Thus, kinetic studies on pH dependence suggested that a histidine may be important to the reaction. Studies were then performed with diethyl pyrocarbonate (DEP). It was found that inactivation of human liver CPOX was concentration (6-50mM)and preincubation time (2-15min) dependent. Enzyme activity was totally lost with 15mM DEP and 2 min interaction. The time course of inactivation was also pH dependent. When CPOX was treated with DEP at acid pH the effect was more pronounced. Dye-sensitized photooxidations with Rose Bengal and Methylene Blue are being assayed.Total inactivation was observed with both reagents at 0.05% .Treatment of CPOX with arginine-specific reagent like phenyl glyoxal (PG) caused a rapid decline in enzyme activity that it was dependent on reagent concentration (10-50 mM). The activity also decreased as function of preincubation time. A total inactivation was observed within 5 min and 25 mM PG. Protection studies with the substrate are under investigation. These results suggest that inactivation of mammalian liver CPOX by group specific reagents was due to the modification of histidine and arginine residues of the enzyme which could play key roles in the oxidative decarboxilation of coproporphyrinogen III.

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INHIBITION OF CRUZIPAIN BY A PEPTIDE DERIVED FROM THE P41 INVARIANT CHAIN AND BY INHIBITORS OF THE CYSTATIN SUPERFAMILY. [1]Stoka,V., [1]Bevec, T., [1]Pungercic, G., [2]Cazzulo, J.J. and [1]Turk, V. [1]Dept of Biochemistry and Molecular Biology, J. Stefan Institute, Ljubljana, Slovenia. [2]Inst. de Inv. Bioquimicas - Fundacion Campomar, Buenos Aires, Argentina.

Cruzipain is the major protease from the protozoan parasite *Trypanosoma cruzi*, belonging to the papain superfamily with a specificity which resembles those of cathepsins L and B (1). The invariant chain (Ii) is a transmembrane glycoprotein associated with the major histocompatibility complex (MHC) class II molecules during early stages of their intracellular transport. The alternatively spliced fragment of Ii which is found only in p41 and not in the major, p31 form of Ii (2), exhibits strong inhibitory activity against cathepsin L (3). In this work we studied the kinetics of inhibition of cruzipain by the Ii fragment in comparison with the endogenous inhibitors of the cystatin superfamily (4). The inhibitors were fast acting (kass = 3.4-79 x 10^6 $M^{-1}s^{-1}$) and tight binding (Ki = 14-72 pM) inhibitors of cruzipain. In both cases, the type of inhibition was shown to be competitive. The inhibitory fragment showed no sequence homology (2) with the inhibitors of the cystatin superfamily (4) and might represent a new superfamily which probably undergoes a different mechanism of inhibition. Cruzipain was inhibited by the Ii fragment in the same extent as cathepsin L whereas related enzymes were weakly inhibited or not at all (3), thus indicating that the active site geometry of cruzipain resembles cathepsin L. (1) Cazzulo, J.J. et al. (1990) Biochim. Biophys. Acta 1037, 186-191. (2) Strubin, M. et al. (1986) EMBO J. 5, 3483-3488. (3) Bevec, T. et al. (1996) J. Exp. Med. 183, 1331-1338. (4) Stoka, V. et al. (1995) FEBS Lett. 370, 101-104.

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ACETYL.CoA HYDROLASE ACTIVITY AND FUNCTION IN *ASCARIS suum* MITHOCONDRIA. Zadila Suárez de Mata[1], Becky de Bruyn[2], and Howard J. Saz[2]. Departamento de Biología Celular, Universidad Simon Bolivar[1], Caracas, Venezuela and Department of Biological Sciences, University of Notre Dame, USA[2].

Incubation of *Ascaris* mitochondria with malate results in the accumulation of acetate, propionate, pyruvate and succinate. Acetate may be formed by either the transfer of CoA from acetyl.CoA to other carboxylic acids or the hydrolysis of acetyl.CoA to acetate and free CoASH. An acetyl.Coa hydrolase activity --specific for acetyl.Coa and propionyl.Coa-- has been partially purified from *Ascaris* mitochondrial soluble fraction. This hydrolase activity has been obtained free from acyl.CoA transferase activities and cannot be accounted for on the basis of a thiolase. Gel filtration indicates an apparent molecular weight of 232,000 for *Ascaris* acetyl.CoA hydrolase activity. Since *Ascaris* is an intestinal parasite which metabolizes primarily anaerobically and accumulates a large number of volatile fatty acids which are formed as the CoA derivatives, the hydrolase would be expected to function in the regeneration of free CoA.

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CALCIUM-DEFICIENT DIET MODIFIES THE KINETIC PARAMETERS OF RAT LIVER MICROSOMAL ACYL CO A SINTHETASE. Marra, C.A., Tacconi de Alaniz M.J.Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP),CONICET-UNLP Facultad de Ciencias Médicas, calles 60 y 120, 1900- La Plata. Argentina. E-Mail: mtacconi@isis.unlp.edu.ar

Previous works have demonstrated that hepatic microsomal acyl CoA- synthetase was stimulated by chronic calcium deficient diet. in the present study we provide evidence that various fatty acids of different carbon length and insaturation, depress the biosynthesis of palmitoyl CoA following different behaviors in control or calcium deprived liver microsomes. In addition, we studied the inhibition evoked by stearic, α-linolenic or arachidonic acids on the biosynthesis of palmitoyl CoA in microsomal suspensions either from control or hypocalcemic rats. In control microsomes stearic acid produced a pure competitive effect, while the others followed a mixed-type inhibition. The competitive effect of stearic acid was not observed in calcium-deprived microsomes. Meanwhile, mixed type inhibition produced by α-linolenic or arachidonic acid diminished in deprived microsomes by means of an increase of the non-competitive component (α Ki). These changes observed in apparent kinetic constants (Km, Vm, Ki, and α Ki) determined by Lineweaver-Burks and Dixon plots, were attributed to important alterations in the physicochemical properties of the endoplasmic reticulum membranes induced by the calcium-deficient diet.

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DELTA AMINOLEVULIC DEHYDRATASE ACTIVITY IN ALBINO *B.GLABRATA* EXPOSED TO LEAD. Mozzarelli, M.N., Giancarlo H., Nahabedian D., Verrengia N. and Wider E. Depto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

Delta-aminolevulic dehydratase (ALA-D) is one of the enzymes involved in heme synthesis. It has been widely recommended as a biomarker of lead exposure in humans and more recently in various aquatic organisms. The purpose of the present work was to investigate ALA-D activity and the relationship between this enzyme and tissue lead levels in albino *B.glabrata*, a freshwater gastropod, exposed to different levels of the metal, employing acute bioassays (t=96 hs) At the highest concentration (2.2 mg Pb/L) 25% of mortality was obtained. Therefore, sublethal levels were chosen: 0.5, 0.1 and 0.025 mg Pb/L. The first of those levels corresponds to the maximum permissible concentration of lead to be thrown in industrial effluents discharged to natural water courses, according to the Argentinian law of hazardous wastes. At this level very high degree of inhibition were observed in all the tissues analysed: 87% ; 82%; 85% and 86% for pulmonary, midgut gland, gonadal and cephalopedal respectively. At the lowest level of lead treatment, the inhibition remained still high *(70%) in midgut gland, decreasing approximately to 50% in all other* tissues studied. Additional *"in vitro"* studies have demonstrated that *ALA-D inhibition, in treated organisms, was partially reversed but the addition of cystein plus Zn, in all tissues tested. The values of enzyme activity positively correlated with the levels of Pb accumulated within* the different tissues for the organisms, since the highest bioaccumulation was observed in midgut gland. According to these results ALA-D activity has shown to be a very useful and sensitive biomarker to asses the levels of Pb exposure in albino *B.glabrata* organisms.

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MELANIN BIOSYNTHESIS IN *BIOMPHALARIA GLABRATA* Verrengia, N., Gonzalez Della Valle, M., Giancarlo, H., Mozzarelli, N., Nahabedian, D. & Wider, E., Depto. de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

Albino and wild type *Biomphalaria glabrata* organisms may be distinguished within the population, and the differences are due to the content, composition and distribution of melanin. Although melanin is thought to serve in photoprotection, it is also considered that might play other functions, some of which are still unknown.

The biosynthesis of melanin involves several steps, but both types of pigment are synthesized from a common precursor, tyrosine, which produces dihidroxiphenyl alanine (L-DOPA) and then dopaquinone, by the action of tyrosinase. Dopaquinone may undergo a series of redox reactions, leading to the formation of the different pigments.

The purpose of the present work was to investigate and to characterise the tyrosinase activity in different tissues of albino and wild type *B. glabrata*, employing L-DOPA as substrate. Previous histochemical studies had demonstrated the present of melanin pigments in the pulmonary, cephalopedal, gonadal and midgut gland regions from albino and wild type organisms. However, the enzyme activity could only be detected in the pulmonary region. Optimal pH values were 6.3 and 6.8 while specific activity were 0.216 ±0.058 and 0.189 ±0.034 (μmol/mg prot) for wild type and albino respectively. In both cases, the enzyme shown nearly the same activity after 24 hr of storage at -14 °C. The kinetic studies done on albino and wild type organisms shown Km values of 8.11×10^{-3}M and 7.25×10^{-3}M. According to these results, melanin biosynthesis would occur in the lung tissue, and then the final product, or other intermediates, would be distributed towards the rest of the tissues.

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ANGIOTENSIN II RECEPTORS ARE FUNCTIONALLY COUPLED TO PROTEIN TYROSINE DEPHOSPHORILATION IN FETAL (E20) KIDNEY MEMBRANES. Alvarez, S.E., Fuentes L.B., and Ciuffo, G.M. Facultad de Quimica, Bioquimica y Farmacia Universidad Nacional de San Luis, San Luis, Argentina.

Angiotensin II (Ang II) exerts various effects on its target tissues through binding to membrane receptors, which have been classified into two pharmacologically distinct subtypes, AT1 and AT2. AT1 receptors belong to the superfamily of G-protein-coupled receptors with seven hydrophobic domains and are functionally coupled to multiple classic second-messenger pahtways. The AT2 subtypes are much less characterized. Transient expression of high levels of AT2 receptors during development strongly suggests a developmental role for this subtipe. In the present study we investigated the modulation of tyrosine phosphorilation by AT2 receptors in rat fetal (E20) kidney membranes. Fetal (E20) kidney membranes were prepared following standard techniques. Phosphorilation was performed on freshly prepared membranes, after stimulation with the effectors for 10 min, in the presence of ATP-Mg^{2+} for 5 min. The reaction was stopped by dilution and membranes were collected by centrifugation. Samples were separated by SDS-PAGE and analyzed by inmunoblotting with antiphosphotyrosine antibodies. Previous studies showed a prevalence of AT2 receptors on fetal tissues and fetal kidneys. Ang II seems to mediate a significant reduction in Tyr phosphorilation of several proteins, in agreement with previous reports for different cell lines. Similar effect was observed in fetal membranes. Tyr phosphorilation has been most often associated with cell proliferation and differentiation and the present observations suggests a possible transduction mechanism for Ang II AT2 receptors at a crucial developmental stage.

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HEPARIN-BINDING LECTIN FROM OVINE PLACENTA. STRUCTURAL CHARACTERIZATION. Ambrosio,A., Iglesias,M.M and Wolfenstein-Todel,C. Instituto de Química y Fisicoquímica Biológicas (IQUIFIB) (UBA-CONICET) Fac. de Farmacia y Bioquímica, Buenos Aires, Argentina.

Many biological roles have been proposed for heparin, so that the study of the heparin-binding proteins is of interest. We have previously reported (SAIB 1995) the isolation of an heparin-binding lectin from ovine placental cotyledones by affinity chromatography on a column of Sepharose-heparin. This lectin showed hemagglutinating activity with rabbit erythrocytes, which was inhibited by heparin and related compounds like λ and κ carrageenan, dextran sulfate, fucoidan and polygalacturonic acid.

By size exclusion chromatography on a Superose 12 HR column a single peak was obtained, corresponding to a molecular weight above 300,000. When this fraction was submitted to SDS-PAGE it dissociated into three bands of 20, 17 and 14.5 kDa respectively.

Reverse phase HPLC on a C4 column allowed the isolation of three peaks, correlating to each of the SDS-PAGE bands, and only the peak corresponding to the 14.5 kDa band recovered the hemagglutinating activity.

This fraction was digested with trypsin. The resulting peptides were separated by HPLC on a C18 column and selected peptides were sequenced. Its amino acid sequence showed complete identity with histone H4.

These results suggest that histone H4 forms a high molecular weight complex with other two proteins, which strongly interacts with heparin. It is possible that the regulatory action of histones is also influenced by its interaction with nuclear proteoglycans.

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ELECTROPHORETIC STUDY OF SOYBEAN PROTEIN HYDROLYSATES. *Scilingo, A., Molina Ortiz, S.,* Añón, M.C. Centro de Investigación y Desarrollo en Criotecnología de Alimentos, 47 y 116, La Plata, Buenos Aires, Argentina.

The purpose of this work was to study the action of non commercial proteases upon soybean protein isolates M10 (1.23mg/g protein, heated for 5 min at 80°C) and M30 (9.73mg/g protein) by means of native and denaturing electrophoresis (with SDS, with or without β-ME) in both one and two dimensions. The enzymes used were maclura, hieronymine (LIPROVE, UNLP-Argentina) and cucurbita (Lab. Bioquímica, UCV-Chile). Tyrosine meq in the soluble fraction of the hydrolysates precipitated in 19% TCA were dosed by the Lowry method. 5-15% and 4-7.5% polyacrylamide gradients were used for the denaturing and native electrophoresis, and the samples obtained from whole hydrolysates and from 19% TCA precipitates were loaded in the gels. In all the cases studied, an increase in the soluble tyrosine meq/min was observed, showing differences among the three enzymes at long time of reaction. These results were corroborated by the electrophoretic patterns, where disappearance of high molecular weight aggregates and of α' y α-7S subunits and decrease of AB-11S and β-7S subunits, typical of soybean proteins, were observed depending on the hydrolysis time. Otherwise, appearance of lower molecular weight peptides, characterized by the bidimensional electrophoresis, were also observed in the hydrolysates. The results obtained allow us to conclude that there are important differences in the action of the three enzymes on the same isolate and in the action of the same enzyme on the two protein isolates used as substrate, thus letting us deduce that they act through different hydrolysis mechanisms.

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STRUCTURE AND FUNCTION STUDIES WITH THE α SUBUNIT OF PROTEIN KINASE CK2. **Antonelli, M., Korn, I., Cosmelli, D., Romero, F., Jacob, G., Connelly, C.C. and Allende, J.E. Departamento de Bioquímica, Facultad de Medicina and Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 70086, Santiago 7, Chile.**

Protein kinase CK2 (also known as casein kinase 2) is ubiquitous in eukaryotes and is composed of catalytic (α, α') and regulatory (β) subunits that form a heterotetramer ($\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, $\alpha'_2\beta_2$). CK2α is a proto-oncogene and is clearly involved in the control of cell division. The α and α' subunits are highly analogous but are coded by different genes. The properties of the recombinant α'subunit from zebrafish (*D. rerio*) has been compared to the α subunit from *X. laevis*. Although both subunits have very similar properties, several interesting differences have been observed. The α' subunit is more resistant to inhibition by polyuridylic acid and to heat inativation than α. On the other hand α' is more sensitive to salt inhibition than α. The most interesting propertiy is that at physiological concentrations of salt the α' activity to phosphorylate casein is stimulated 20 fold by the β subunit, while α is stimulated only 5-8 fold. Site-directed mutagenesis has been used to modify the α subunit to prepare $\alpha F^{50}G^{51}$. This double mutant in the region that interacts with the nucleotide triphosphate, has significantly altered kinetic properties that reduce its catalytic efficiency. Mutations in aspartic 156 and a series of truncation of the carboxyl end have been constructed to study the region of α involved in β interaction.

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CHARACTERIZATION OF BLOOD CLOTTING RELATED PROTEINS IN CAIMAN PLASMA. [1] Araujo, M.S., [1] Tiaen, M.A., [1] Nunes, V., [2]Andreotti, R., [3] Shimamoto, K., [3] Ura, N. and [1]Sampaio, C.A.M. [1] Department of Biochemistry, Escola Paulista de Medicina, UNIFESP, S. Paulo, SP; [2]EMBRAPA, Corumbá, MS, Brazil; [3] 2nd Department of Internal Medicine, Sapporo Medical University, Sapporo, Japan.

The interest on reptilian blood biochemistry lies on its evolutionary importance and on the correlation between snake venom and the human blood coagulation process. In a previous work, we have shown that *Caiman crocodilus yacare* plasma contains a kininogen, that inhibits cysteine endopeptidases and releases a kinin-like peptide by trypsin hydrolysis, detected by bioassay and recognized by BK-specific antibody. This communication describes a serine endopeptidase inhibitor and a Factor X-like protein in the *C.c.yacare* plasma. The purification was performed by the precipitation of the caiman plasma with 1 M $BaCl_2$, in 0.1 M Tris buffer pH 8.0, 1 mM EDTA, 1 mM benzamidine, 50 mg/L polybrene, 0.03 M NaCl for 30 min, at 4 °C. Mostly of Factor X-like activity, measured by the hydrolysis of the specific peptide substrate (S-2765), was present in the precipitate. The supernatant was submitted to a chromatography in a DEAE-Sephadex column in the same buffer and increasing NaCl concentrations. The following activities were separated: a pro kallikrein activator (0.03 M); a kininogen (0.1 M); an enzyme that hydrolyses N-acetyl-Phe-Arg-p-nitroanilide (0.2 M); a trypsin inhibitor not retained by the DEAE- but purified by SP-Sephadex.

(CAPES, CNPq, FAPESP, FINEP and PADCT).

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ISOLATION AND CHARACTERIZATION OF SURFACE PROTEINS OF *Bifidobacterium bifidum* (CIDCA 5310). Bibiloni, R.[1]; Bosch, M.[2]; Pérez, P.[3]; De Antoni, G.[4] and Añón, C.[3] (CIDCA). La Plata, Argentina.

Bacteria of the genus *Bifidobacterium* are normal inhabitants of the human colon that exert beneficial effects on their hosts' health. Their ability to interact with the host intestine wall is related to their surface structure. *Bifidobacterium bifidum* (CIDCA 5310) which has been isolated from infant faeces has been studied. To characterize surface proteins, both extraction and dialysis conditions have been optimized. Solubitity assays were performed with extracted proteins in different conditions. Protein analysis included molecular exclusion chromatography, SDS-PAGE and DSC. Differences in protein cell profile before and after the extraction were studied with SDS-PAGE after short lisis protocol. The ratio ml solvent/mg cell was an important factor in the extraction yield. Ratios over 1 ml solvent/15 mg cell did not give better results. An optimal yield of 1.33 mg protein/gr cell after 60 min extraction at 37 °C has been obtained. Solubility assays pointed out that proteins were insoluble between pH 3 and 9, and in presence of β-mercaptoethanol, but their solubility rised up to 40% in SDS concentrations higher than 0.1%. DSC gave an enthalpy value of 1.32 mJ/mg confirming their suspected denaturalization. Chaotropic agent treatment and abrupt change in ionic strenght during dialysis process could lead to protein denaturation and low solubility values. Protein denaturatón was confirmed with DSC, where the thermogram also showed high content of hydrophobic amino acids. Secuential dialysis against NaCl of different concentrations was the best way to obtain not aggregated proteins.

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ISOLATION AND PARTIAL CHARACTERIZATION OF AN UNUSUAL LIPOPROTEIN FROM *Triatoma infestans* HEMOLYMPH. Finarelli, G.S, Rimoldi, O.J. and Brenner, R.R. Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET-UNLP, Facultad de Ciencias Médicas, calles 60 y 120, 1900-La Plata, Argentina.

All animals have lipoproteins which transport lipids through aqueous media such as blood or hemolymph. In all insects examined up to date, the same type of lipoprotein, lipophorin, has been found. It consists of two apolipoproteins of 240 kDa and 80 kDa, and contains 40 to 50% lipids. In some insects, lipophorin can take up even more lipids when it associates with a third apolipoprotein, Apo-Lp III, of approximately 20 kDa, which is normally found free in hemolymph.

In this work, we isolated, purified and examined chemical and immunological characteristics of a hemolymphatic lipoprotein in *T. infestans* of 20 kDa and 1.19 g/ml density. This lipoprotein comprises only one protein component of 16 kDa molecular weight. The amino acid composition and N-terminal sequence of this small apoprotein have no similarity to any apolipoprotein isolated from other insect species. This lipoprotein contains about 6% lipid with a large amount of hydrocarbons, whereas diacylglycerol, triacylglycerol, free fatty acid and polar lipid levels are low. The fractions obtained by hemolymph density gradient ultracentrifugation were subjected to Western blot analysis run against anti-16 kDa protein serum, showing that this small apoprotein is not associated to any other hemolymph lipoprotein. A conclusion to be drawn is that this apoprotein does not act as an apo-Lp III. Then, the full characterization of this lipoprotein should be investigated to shed light on its function.

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FLEXIBILITY OF ß-LACTAMASES. Bunster, M. Martinez, J.Canales,M.and Cid H.Lab.Biofisica Molecular. Depto.Biologia Molecular.Facultad de Ciencias Biologicas. Universidad de Concepcion, Concepcion,Chile.(Proy DI.94-3158-1)

Class A ß-lactamases are enzymes that hydrolyse the ß-lactam ring of a penicillins and cephallosporins. They have globular shape, and two discontinuous domains are clearly defined;one of them formed by helices and the other by five antiparallel ß-sheets and three helices. The knowledge of the 3D structure of some of these enzymes has allowed partially to explain the broad spectrum activity detected for many ß-lactamases. We have used for all the studies, ß-lactamase 1 from *Bacillus cereus* 569/H. We have built the model using structural homology methods and studied the conformational properties of the enzyme relative to the rates of catalytic reaction for different N-acyl subtituted substrates.We have performed equilibrium denaturation-renaturation experiments in presence of urea; the process has been followed by electrophoretic and spectrophotometric techniques. It was possible to detect inter-mediates.We have detected also a change in the spectroscopic properties of the enzyme when it was exposed to an electric field.This change was followed by kinetic measurements.The effect of an electric field produce a similar effect to that found when the enzyme is used for the catalysis of substrats type A like oxacillin, a biphasic kinetic. This effect was eliminated by crosslinking. Molecular dynamic studies of the flexibility are in progress.

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MODIFICATION OF THE HEMOLYTIC ACTIVITY OF STICHOLYSIN BY FREE RADICALS, Campos, A.M., Lissi, E.A., Lanio, M., Alvarez, C., Pazos, F. Depto. Quimica, Facultad de Química y Biología, Universidad de Santiago de Chile y Facultad de Biología, Universidad de La Habana.

Sticholysin is 20 kD protein with hemolytic activity obtained from a marine anemone. This activity is strongly enhanced by increasing the solution ionic strength. In the present work are reported the changes elicited in the protein behaviour by its incubation in the presence of a free radical source.
Incubation of sticholysin in the presence of 2,2'-azobis(2-amidinopropane) decreases its hemolytic activity in parallel to changes in the tryptophan moieties, as sensed by their fluorescence. These changes are almost independent of the ionic strength of the medium, suggesting that the exposure of the reactive centers is not substantially modified by the presence of salt. The time profiles of the hemolytic activity of the protein after its incubation with the free radical source are discussed in terms of the mechanism of the cytolysin inactivation.
Acknowledgments: This work was supported by FONDECYT (194-1058 and 296-0056) and Fundación Andes.

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DESIGN OF A NOVEL INHIBITOR OF CLASS A β-LACTAMASES BY A COMPUTATIONAL METHOD. Campos. M., González, H., Bocaz, G., Vásquez, O., Paredes, R., Parra C., Zúñiga, F. and Moreno, J. Departamento de Polímeros, Facultad de Ciencias Químicas, Universidad de Concepción, Concepción, Chile.

A new inhibitor bromopenem (6 β-bromo-1-dioxi-penem) for class A β-lactamases has been designed by computational methods. The structure was analysed using the Tripos 5.2 Force Field from the Alchemy III software and compared with the one of the BRL 42715.

The inhibitor is a penem derivative analogous of the substrate and covalently bound to the reactive serine 70.

Bromopenem has some structural features of the following inhibitors: 6 β-bromopenicillanic acid, sulbactam and BRL 42715. Its stability energy is 44.74 (Kcal/mol), mostly lower to the other inhibitors studied.
The interaction between bromopenem and the crystal structure of *E. coli* TEM-1 β-lactamase at 1.8 Å is also given, by using Tripos 5.2 Force Field and RasMol program.

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MOLECULAR MODELING OF MANGANESE PEROXIDASE FROM *Ceriporiopsis subvermispora* Strain 13-1.
Canales M *, Vicuña, R., Lobos, S.
* Biophysics Laboratory, Faculty of Biologial Sciences, University of Concepcion. Concepcion. Department of Celular Biology, Faculty of Sciences, Pontifica Catholic University. Santiago. Chile.
Ceriporiopsis subvermispora is a white-red fungus which produces several isozymes of manganese peroxidase. One of them, is a new manganese peroxidase (Mnp13) which has been isolated, partially characterized and gene-sequenced by Dr. Vicuña and col. Mnp13 sequence shows about 70% homology with the manganese peroxidase from P. crysosporium (Mnp) including sequeces for the characteristic binding sites from Hem, Mn^{2+} and Ca^{2+} ions, this lasts are also presents in Lignin peroxidases,although experimental evidence for the presence of Ca^{2+} ions it is not yet available for the Mnp13 enzyme. Based on the 1.8 Å crystal structure of Mnp we have modeled by homology the three dimensional structure of Mnp13, using the following programs: Insight II and Homology from Biosym Inc., Bragi from the GBF mbH and Amber 4.0. The final model confirm the overall conservation of the structure, the insertion of the sequence TSTG between residues S230 and K231 of Mnp being the main difference between both enzymes. The larger loop corresponding to this zone was modeled from the loop data bank. The local optimization of this zone did not distort the original conformation, since the larger loop followed the direction of the preceding ß-sheet. A local molecular mechanics optimization performed in the region corresponding to Ca^{2+} and Mn^{2+} binding site in Mnp, demonstrated Ca^{2+} and Mn^{2+} ions demonstrated that the stereochemistry and the geometry of binding are conserved in the Mnp13 structure . This study give new evidence supporting the presence of Ca^{2+} ions in Mnp13.

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OVEREXPRESSION OF PIG KIDNEY FRUCTOSE-1,6-BISPHOSPHATASE AS RECOMBINANT AND FUSION PROTEINS. Cárcamo. J.G., Yañez, A., Pinto, R., León, O., Reyes, A.M., and Slebe, J.C. Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.
The catalytic activity of fructose-1,6-bisphosphatase (FBPase) is inhibited in a cooperative form by the allosteric binding of AMP. Chemical modification and X-ray diffraction have indicated a number of amino acid residues that could participate in the mechanism of AMP inhibition, both in the AMP affinity as well as in the cooperative phenomenon. In order to investigate the importance of these residues in structure-function relationships of the FBPase by site-specific mutagenesis, it was necessary to clone and express the pig kidney gene in *E. coli*. This has now been achieved in the strain *BL21 (DE3)*. For expression we used the vector *pET22b(+)*, under control of the T7 RNA polymerase-*lac*UV5 promoter system. The purification of the enzyme was carried out in three steps, which led to FBPase purified to near homogeneity. The use of phosphocellulose instead of CM-sephadex permitted to improve the yield; it was possible to obtain about 5 mg of soluble active protein per liter of culture. The kinetic properties of the recombinant enzymes are essentially identical to those of the native pig kidney enzyme. Furthermore, an alternative expression and purification method is presented, using the vector *pET15b*. This system allows us to obtain large amounts of pure FBPase, as a fusion protein (polyHis-FBPase), after just two purification steps. The overexpression of FBPase in an heterologous system is an important advance towards site-directed mutagenesis studies of the pig kidney fructose-1,6-bisphosphatase. (Supported by FONDECYT 2960060, 1951215; DID-UACH S-95-41).

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BJcuL: A C-TYPE GALACTOSIDE-BINDING LECTIN FROM *BOTHROPS JARARACUSSU* VENOM. Carvalho. D.D., Marangoni S., Oliveira B. and Novello, J.C. Depto Bioquimica UNICAMP-IB-Campinas-SP, BRASIL.
Lactose-inhibitable lectins have been purified from a variety of snake venoms, such as *Bothrops atrox* (Gartner et al, FEBS Letters 117:13, 1980), *Bothrops godmani* (Lomonte et al, Toxicon, 28:75, 1990) and *Bothrops jararaca* (Ozeki et al, Arch.Biochem.Biophys., 308:306, 1994). The interest in those lectins are because their intermediate properties between those of the two animal lectin families: they have a subunit molecular mass of about 14 kDa, are soluble and specific for sugars containing β-galactoside, but require Ca^{2+} for hemmaglutination (Hirabayashi et al, 266:2320, 1991). A galactoside-binding lectin was purified from venom of the snake *Bothrops jararacussu* (BJcuL) by affinity chromatography on immobilized D-galactose. Purified BJcuL showed a single band of about 28 kDa and 14 kDa on SDS-PAGE under nonreducing and reducing conditions, respectively, suggesting that it is a dissulfide-linked homodimer composed of 14 kDa subunits. Maximum yield of lectin per 100 mg of venom was 0,9 mg. Trypsined erythrocytes from pig and cow were agglutinated by BJcuL, with end points of approximately 0.5 µg/ml and 2.0 µg/ml, respectively. On the other hand, erythrocytes from duck and horse did not agglutinate in the presence of this lectin (66 µg/ml). The hemagglutinating activity against pig erythrocytes (with a concentration of BJcuL adjusted for 4,14 µg/ml) was inhibited specifically in the presence of 0.8 mM lactose, 3.2 mM galactose, 3.2 mM raffinose, 50 mM glucose or 0,5 mM EDTA. D-galactosamine, D-glucosamine and mannose had little or no effect on the hemagglutination activity of BJcuL. Financial Support: CAPES, FAEP-UNICAMP, CNPq.

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A STUDY OF AMARANTH ALBUMIN-2 PROTEIN AGGREGATES. Martínez E. N., Castellani O. F., and Añón M. C.. Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA-CONICET-UNLP), 47 y 116, La Plata (1900), Argentina.

Amaranth proteins, of high nutritional value, are worth being included in food formulation. Their structural characterization constitutes the first step for future technological developments. In previous studies, we have found that among amaranth protein fractions, albumin-2 has particular physicochemical properties, different from those of albumin-1, although it shares some structural characteristics with storage proteins (specially globulin).

We analyze this fraction by means of differential scanning calorimetry (DSC), polyacrylamide gel electrophoresis, molecular sieving chromatography and zonal ultracentrifugation. The results show that albumin-2 fraction is integrated by molecules of approximately 300 kDa and aggregates larger than 500 kDa. Treatment with 20 mM β ME during or after extraction, produce a decrease in the amount of aggregates suggesting that disulfide bonds contribute to their stabilization. Albumin-2 preparations with or without aggregates have similar thermal stabilities and denaturation enthalpies which may indicate similarities between molecular unities and aggregate conformations. β–ME at concentrations higher than 20 mM produce a partial unfolding of albumin-2 proteins that is evidenced by a decrease of its thermal stability and denaturation enthalpy. In this case, β ME would be acting as a monohydric alcohol destabilizing the proteins by weakening hydrophobic interactions. Analysis of the partially unfolded proteins show that in the aggregate peptide, dimers of 52 and 56 kDa, equal to those that integrate globulins, interact with monomers of 56 kDa, which are only present in albumin-2 fraction. The capacity of albumin-2 fraction of forming aggregates may be due to the presence of this monomeric peptide in such a location of the molecular structure as to allow intermolecular disulfide bonds be formed.

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INFLUENCE OF pH AND IONIC STRENGTH ON THE CONFORMATION OF AMARANTH PROTEINS. Castellani, O. F., Martínez, E. N. and Añón, M.C. Centro de Investigación y Desarrollo en Criotecnología de Alimentos(CIDCA-CONICET-UNLP) 47 y 116, La Plata (1900), Argentina.

The knowledge of protein conformational changes produced by modifications of the medium can be used to establish adequate conditions of processing before being incorporated in food. As amaranth proteins are valuable food ingredients, we carried out the study of amaranth albumin-2 protein fraction modifications when pH and ionic strength were changed. With this purpose, proteins were analyzed by differential scanning calorimetry, gel electrophoresis, zonal ultracentrifugation and molecular exclusion chromatography.

Calorimetric studies at μ=0.54 and at pHs ranging from 6 to 8.5 showed thermograms with only one endothermic transition which maximum denaturation temperature (T_d) and denaturation enthalpy (ΔH) maintained constant. At pHs higher than 8.5 and lower than 6, T_d and ΔH values decreased, indicating a partial unfolding of proteins. These results correlate with those from chromatographic analysis that show a higher elution volume for the proteins at pH 11. At pHs higher than 12 and lower than 3.5 no transition was observed, suggesting that proteins were completely denatured. In alcaline medium, thermograms with two endothermic transitions were observed, indicating the presence of protein species with different thermal stability. Ultracentrifugation and electrophoretic analysis performed at pH 11 showed that molecular aggregates and free molecules were still present, it might be that at this alcáline pH in which they are partially unfolded, aggregates and free molecules have different thermal stabilities. Calorimetric analysis of proteins at pH 6.5 and different ionic strengths showed that at low μ values (0-0.01), proteins with two different thermal stabilities were present, and as μ values increased, the lower thermal stability proteins increased their T_d till μ = 0.5, in which case a single endotherm was shown. This behaviour may be due to a differential influence of μ upon different proteins. At μ higher than 0.5 an increase of T_d was observed probably due to a lyotropic effect of NaCl. From these results, conditions in which albumin-2 proteins are in different conformations can be established.

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PURIFICATION AND STRUCTURAL CHARACTERIZATION OF A FATTY ACID-BINDING PROTEIN FROM THE ARMADILLO LIVER

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The fatty acid-binding protein (FABP) from the armadillo liver was purified to homogeneity by a procedure involving gel filtration and two anion exchange chromatography runs. The purified protein proved to have a pI between 5.0 to 5.2 and migrated by sodium dodecyl sulfate-gel electrophoresis as a single entity of approximately 14 kDa. The armadillo FABP cross-reacted with antiserum against rat liver FABP but not against rat intestinal FABP. The same as other members of the family, it has a blocked N-terminus.

Amino acid sequencing of peptides obtained by in-gel tryptic digestion shows that the armadillo, despite being one of the less evolved mammals, has an L-FABP belonging to the same type as that of the highly evolved mammals.

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SEARCHING FOR SEQUENCE SIMILARITY TO β-AMYLOID PEPTIDE. Contreras.C., Canales, M., & Inestrosa, N.C. *. Lab. de Biofísica, Fac. Ciencias. Biológicas, Universidad. de Concepción. Chile.

* Depto. Biología Celular y Molecular. Fac. Ciencias. Biológicas, P. Universidad. Católica de Chile.

The main component of brain amyloid in Alzheimer´s disease is a peptide (39-43 residues) called β-Amyloid (Aβ), a proteolytic product of a membrane precursor protein. Amyloid fibrils X-ray diffraction studies indicate a stacking of antiparallel β-sheet conformation. CD and NMR studies suggest α-helix component for N-terminal residues 1-28 of Aβ. The N-terminal could adopt a monomeric α-helix (soluble) or an oligomeric β-sheet (insoluble) conformation (CD & NMR) depending on the pH. This conformational change could limit the rate of amyloid plaque formation. Secondary structure prediction methods suggest a β-strand conformation for the C-terminal domain. Since no atomic data exists for the complete Aβ sequence, we look for homology sequences. We did an extensive homology search using the Smith and Waterman algorithm implemented in BLITZ that yields better results for local similarity. After that, PIMA was used to do multiple sequence alignment. The results showed low homology score and was necessary to study each case further. We found a Triosephosphate Isomerase segment with seconday structure elements in accordance with the former experimental data. We report here the structural implications of this study.

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MODELING OF THE COLLAGENIC TAIL OF ASYMMETRIC ACETYLCHOLINESTERASE. Deprez, P.N. & Inestrosa, N.C. Depto. de Biología Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica, Santiago, Chile.

The asymmetric form of acetylcholinesterase (AChE) consist of three catalytic tetramers attached to a triple helical collagenic tail. The specific localization of the enzyme at the neuromuscular junction is due to specific interactions of the tail with extracellular matrix (ECM) molecules, in particular with proteoglycans. A sequence analysis show that the N-terminal proline-rich domain would form principaly no secondary structure. This domain contains cysteine residues responsible for the linking to the catalytic tetramers and for the stabilization of the collagenic domain. This region contains two heparin-binding consensus sequences (HBCS), probably involved in the interaction with glycosaminoglycans (GAGs). Both the N- and the C-terminal domains do not present any homology with previously known proteins, but the last may belong to the α/β family. This domain contains a cysteine-rich region, probably responsible of covalent interactions with other ECM proteins, as seen with other structurally related collagens. A model of the collagenic region was built using the 1clg PDB structure as template. The structure was submitted to molecular dynamics followed by energy minimization (EM), with a tethering force to glycines ϕ and ψ torsion angles. The model show two high density regions of basic residues that form two broad basic belts around the triple helix, and a less dense basic region between the belts. The putative heparin-binding sites were submitted to EM considering a layer of water molecules. This model will allow us to study the docking of the collagenic tail of AChE with GAGs and other ECM molecules.

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CHARACTERISATION OF MOLECULAR CHAPERONES FROM PURPLE BACTERIA Dionisi, H., Checa, S., Ferreyra, R., López, F., Risso, C. and Viale, A. PROMUBIE (CONICET), Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Univ. Nac. de Rosario, Rosario, Argentina.

Molecular chaperones constitute families of proteins which assist cellular polypeptide folding and translocation, and the resurrection of functional protein structures after denaturation. We cloned previously the GroE-type of chaperones from the purple bacterium *Chromatium vinosum*, and report here their role in the acquisition and maintenance of the functional status of enzymes such as mitochondrial rhodanese and Rubisco. Rhodanese is very sensitive to thermal denaturation, which leads to its rapid inactivation and aggregation. *C. vinosum* GroEL alone did not exert any protection against thermal inactivation, but prevented aggregation when present in the medium. Moreover, rhodanese enzymatic activity could be recovered in the latter conditions, providing that GroES and ATP were subsequently added to the medium.

We characterised *in vivo* the role of DnaK and GroE molecular chaperone systems as putative assistants of Rubisco folding, by using the corresponding *Escherichia coli* mutants. The levels of Rubisco expressed in these cells were estimated by both Rubisco carboxylase activity measurements and immunoblot analyses. *E. coli* strains having reduced cellular levels of DnaK and/or GroE systems possessed severe impairments in generating correctly assembled, functional Rubiscos. These results suggest that DnaK and GroE chaperone play possible synergistic roles in the generation of functional Rubiscos oligomers in different organisms. Our results also suggest that the impairment resides mainly in the generation of structured subunits, rather than in their assembly process.

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PRIMARY STRUCTURE OF THE BASIC FATTY ACID-BINDING PROTEIN FROM THE LIVER OF CATFISH *RHAMDIA SAPO*. Di Pietro, S.M., Dell' Angelica, E.C. and Santomé, J.A. Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina.

The basic fatty acid-binding protein (b-FABP) of *Rhamdia sapo* liver was purified as previously described (Di Pietro et al, SAIB-1994). For a complete amino acid sequencing, the purified protein was digested with endoproteases Lys-C, Glu-C and chymotrypsin and cleaved with CNBr. The resulting peptides were fractionated by reverse-phase HPLC and directly sequenced by Edman degradation except for the blocked N-terminus. This peptide had to be unblocked with N-acylaminoacyl-peptide hidrolase from rabbit muscle before sequencing. The protein conteins 125 amino acid residues accounting for a total molecular mass of 13757 Daltons. Sequence comparison shows that catfish b-FABP is far more closely related to chicken liver-FABP than to mammalian liver-FABP. This fact supports the proposal that these two protein belong to different branches, stemming from a gene duplication.

This work was supported by grants of the Universidad de Buenos Aires and CONICET.

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PYRIDOXAL PHOSPHATE BOUND TO Lys^{288} OF *Escherichia coli* PHOSPHOENOLPYRUVATE CARBOXYKINASE IS A SENSITIVE PROBE OF SUBSTRATE-INDUCED CONFORMATIONAL CHANGES. [1]Encinas, M.V., [2]Cardemil, E., and [3]Goldie, H. [1]Departamento de Ciencias Químicas, Facultad de Química y Biología, Universidad de Santiago, Santiago, Chile, and [2]University of Saskatchewan, Canada.

The reaction catalysed by *Escherichia coli* phosphoenolpyruvate (PEP) carboxykinase (ATP + PEP $\Leftrightarrow$ oxaloacetate + ADP + CO_2) is an important regulatory step in gluconeogenesis. This enzyme is a polypeptide of 540 residues, and is composed of two domains that rotate 20° in the enzyme-ATP^{δ}-Mg-oxalate complex (Tari et al. *Nature Struc. Biol. 3*, 355-363, 1996). In this work, *E. coli* PEP carboxykinase has been specifically labeled in Lys^{288} with pyridoxal phosphate (PLP), reduced with $NaBH_4$, and the pyridoxyl group employed as a probe to sense the protein conformational changes induced upon substrate or metal binding. The binding of Mn^{2+} decreases the fluorescence intensity of the probe, meanwhile the addition of ATPMg produces a marked enhancement of the emission. These results evidence that the binding of nucleotide or metal ion produce notable and distinct conformational changes in the protein. Fluorescence spectroscopy studies on the pyridoxyl-enzyme adduct show that the region where the probe is bound is rather accessible to the solvent. However, the presence of ATPMg produces conformational changes that hinder the probe in the protein matrix. Additionally, this work evidences the usefulness of the pyridoxyl moiety to sense microenvironment changes.

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EXPRESSION OF HUMAN GLUTAMATE DECARBOXYLASE 65 AS A SOLUBLE FUSION PROTEIN IN E. COLI. Papouchado, M.[1], Valdez, S.[1], Poskus, E.[1], and Ermácora, M.[2,3]. [1]Cátedra de Inmunología-IDEHU, UBA-CONICET, [2]IQUIFIB, CONICET and [3]Universidad Nacional de Quilmes, Bernal, Argentina.

Glutamate decarboxylase (GAD) 65-directed autoantibodies can be found in most patients with insulin-dependent diabetes mellitus (IDDM) years before they develop diabetes symptoms. Thus, large amounts of GAD are required to study the humoral and cellular anti GAD response in IDDM, and its potential as tolerogen in the prevention of IDDM.

For these purposes, several constructions for the expression of the protein were designed: GAD was expressed as the whole intact molecule or as fusion proteins with signals that directs the target protein to the peripiasm (pelBGAD and BLAXGAD) or with proteins that increases its solubility (TrxGAD).

Western blotting analysis showed two bands in total cell lysates: one with the Mr of GAD or fusion protein and one of 45 kDa. No GAD was directed to the periplasmic space. TrxGAD was the only protein produced in large quantities as a soluble protein. The rest of the proteins remained as inclusion bodies.

TrxGAD was affinity purified with a yield of 9 mg of protein/ l of E.coli culture and a purity of 90%. The specific enzimatic activity of Trx-GAD (determined measuring the formation of $^{14}CO_2$ from L-[U-^{14}C]glutamic acid) was 2 U/mg (1U= 1 µmol of product/min at 37°C). Immunoreactivity of TrxGAD was studied in 23 sera from patients with IDDM which immunoprecipitate ^{35}S-MetGAD produced in vitro by a rabbit retyculocite system (GAD index range: 0.12-1.19, cutoff for positivity: 0.09). An excess of purified TrxGAD completely blocked the binding of anti GAD to the ^{35}S- tracer (GAD index range: -0.02-0.05). A displacement curve assayed in one diabetic patient serum showed that 20 picograms of the protein blocked 50% of the binding of anti GAD to ^{35}S-GAD.

It is concluded that recombinant human GAD enzimatically and immunologically active can be prepared in large scale from E.coli.

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EFFECTS OF HYDROSTATIC PRESSURE ON RABBIT MUSCLE PYRUVATE KINASE. Felice, F. G., Soares, V. C. and Ferreira, S. T. Department of Biochemistry, ICB/CCS, Federal University of Rio de Janeiro, RJ 21941-950, Brasil.

Rabbit muscle pyruvate kinase (PK) interconverts ADP and phosphoenolpyruvate (PEP) into ATP and pyruvate, and requires Mg^{2+} and K^+ as activators. PK is a tetramer of 60 KDa subunits. Although the kinetic mechanism of PK is well known, studies on how interactions between subunits affect catalysis or the affinity of the enzyme for substrates and effectors are still lacking. In this study, effects of hydrostatic pressure on PK stability were investigated by following enzyme activity and conformational changes revealed by fluorescence spectroscopy. Enzyme activity decreased as pressure was increased and was totally abolished at 3.5 kbars. Subunit dissociation/unfolding and inhibition of enzyme activity were protected in the presence of the physiological activators K^+ and/or Mg^{2+}, and notably by the substrate, PEP. We also noted a protective effect of dithiothreitol (DTT) on the enzyme under pressure, suggesting that intra- or intersubunit disulfide bonds may be formed upon pressurization. Recovery of enzyme activity after pressurization was also affected by DTT. While in the presence of DTT 100% of enzyme activity was recovered within 3 hours after a pressure cycle of 10 min at 2.1 kbars, a control sample treated in the absence of DTT showed only 50% remaining activity. Inhibition of PK by hydrostatic pressure depended on enzyme concentration, indicating that inhibition was coupled to the equilibrium of subunit association/dissociation. These results show that hydrostatic pressure is a valuable tool to study the influence of oligomeric structure on PK function.

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STRUCTURAL STUDIES OF A MESOPHILIC MILK-CLOTTING PROTEASE

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This work describes further characterization of a milk-clotting protease from Mucor bacilliformis, whose biological behaviour could make it a good substitute for bovine chymosin in cheese industry. Mucor bacilliformis is a mesophilic Mucor strain and the protease instability when submitted to heat treatment would make its utilization important for the manufacture of some types of cheese.

The molecular weight, amino acid composition and sequence of several proteolytic fragments have been reported (SAIB 94, 95).

Results from electrofocusing indicated a pI of 5.2. This value agrees with those obtained from other Mucor proteases. The extinction coefficient resulted ε= 1.61 mg^{-1} ml cm^{-1} at 278 nm. Measurements show that the enzyme has the high content of β-sheet characteristic of this protein family.

After cyanogen bromide digestion and SDS-PAGE electrophoresis, the fragments obtained were submitted to "in gel" digestion. The resulting peptide mixtures were resolved by RP-HPLC and then the peptides were microsequenced. Peptides were localized in the primary structure by homology studies. Carboxypeptidase treatment of the native protein and the cyanogen bromide fragments are being performed.

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MOLECULAR AND THERMODINAMIC ANALYSIS IN HEMOGLOBINS OF *CLARIAS sp.* AND *LIOPHIS miliaris.*, Galdames, M. I.. Matsuura, M. A. and Focesi Jr., A. Departamento de Bioquímica, IB, UNICAMP, Campinas, SP, Brasil.

The functional oxygenation and oxidation properties of tetrameric hemoglobin were analyzed for *Clarias sp.*, an air breathing fish, and dimeric hemoglobin of *Liophis miliaris*, a water snake, relating to the molecular and thermodynamic of the ligand binding, the Bohr effect and cooperativity. The oxygenation properties of *Clarias sp.* hemoglobin showed high oxygen affinity, P_{50} of 4.5 torr, absence of Bohr effect, cooperativity n = 1.5, in the stripped form, and an evident heterotropic allosteric effect for ATP, with Kd $3x10^4$ M^{-1}, at pH 7.6. The redox potentials showed a low intrinsic free energy $\Delta G^{o'}$ of 2.0 Kcal mol^{-1}, lower in relation to trout I and human hemoglobin. The T→R transition with pK close to pH 7.5, was independent of the Bohr and organic phosphate effects, this suggesting a histidine ionization associated with this allosteric process. The oxidation-reduction of *Liophis miliaris* dimeric hemoglobin in the stripped form showed conformational tertiary structure and absence of the quaternary transition T→R. The tetramerization, cooperativity and transition ocurr only in the presence of ATP, with $\Delta G^{o'}$ 0.4 Kcal mol^{-1} and electrostatic interaction pH dependent. These hemoglobins properties may be reflect the molecular characteristics of the ancestral primitive vertebrates which high oxygen affinity, low intrinsic free energy with a the specifics substitutions at the $\alpha_1\beta_2$ contact.

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TWO ACETYL XYLAN ESTERASES FROM *Penicillium purpurogenum*: PURIFICATION, PROPERTIES AND STRUCTURE. J. Eyzaguirre[1], L. Egaña,[1] R. Gutiérrez[1], V. Caputo[1], A. Peirano[1], W. Pangborn[1], M. Erman[2], B. Burkhart[2], W. L. Duax[2] and D. Ghosh[2]. [1]Laboratorio de Bioquímica, Pontificia Universidad Católica de Chile, Santiago and [2]Hauptman-Woodward Medical Research Institute, Buffalo, NY.

P. purpurogenum secretes several xylan hydrolyzing enzymes. The acetyl xylan esterases (AXE) have been studied. The amount of AXE produced is related to the degree of acetylation of the carbon source. AXE was concentrated and fractionated by gel filtration. Two peaks of activity (AXE I and AXE II) were obtained. Both enzymes were purified to homogeneity by chromatography and chromatofocusing. AXE I: MW 48,000; pI 7.5; optimal activity at pH 5.3 and 50°C. AXE II: MW 23,000; pI 7.8; optimal activity at pH 6.0 and 60°C. Both enzymes are active towards several acetylated substrates. Antisera against AXE I and II do no cross-react. The amino terminal sequences do not show similarities. These results suggest that AXE I and AXE II are the products of different genes. AXE II has been crystallized and the crystals diffract synchrotron X-rays to better than 0.80Å resolution. The space group is $P2_12_12_1$ and cell dimensions are a=34.9Å, b=61.0Å, c=72.5Å. A diffraction data set of 0.90Å resolution at liquid nitrogen temperature has been collected at the Cornell High Energy Synchrotron Service. We are using Dr. Herbert Hauptman's Shake-n-Bake method to elucidate the three-dimensional structure. Work supported by: FONDECYT (1930673), DIUC, UNIDO (91/065), NSF and NIH (DK26546).

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STEADY-STATE AND TIME-RESOLVED FLUORESCENCE STUDIES ON SINGLE-TRYPTOPHAN MUTANTS OF FRUCTOSE 6-PHOSPHATE, 2-KINASE:FRUCTOSE 2,6-BISPHOSPHATASE. Helms M.K.[1], Uyeda, K.[2] and Jameson, D.M.[1] [1]Dept. of Biochemistry and Biophysics, University of Hawaii, Honolulu, HI, 96822, USA. [2]Biochemistry Dept., VA Medical Center and University of Texas Southwestern Medical Center, Dallas, TX, 75216, USA

The bifunctional enzyme, Fru 6-P, 2-kinase:Fru 2,6 bisphosphatase, catalyzes the synthesis and degradation of Fru 2,6-P_2, the most potent activator of phosphofructokinase. Different mammalian tissues contain specific isozymes of this bifunctional enzyme having variable ratios of kinase/phosphatase activities. The rat testis enzyme contains four tryptophan residues - W15 near the N-terminus, W64 in the kinase domain and W299 and W320 in the phosphatase domain. Using site-directed mutagenesis, the four single tryptophan variants and two double tryptophan variants (W15,W64 and W299,W320) were constructed. The relative fluorescence yields of the single tryptophan variants add up to approximately 1.5 times the yield of the wild-type enzyme suggesting that energy transfer occurs between some tryptophan residues in the wild-type enzyme. Time-resolved and steady-state polarization studies on all these variants revealed extensive energy transfer between W299 and W320 and a lesser extent of transfer between W15 and W64. The binding of either Fru-6-P or Fru-2,6-P_2 results in an increase in the polarization of the W15 variant. Dynamic polarization data demonstrated that this increase results from a reduction in the local mobility of the tryptophan residue.

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GALECTIN-3 IN OVINE PLACENTA. Iglesias,M.M.†, Ambrosio,A.†, Castagna,L.* and Wolfenstein-Todel,C.†. † IQUIFIB, Fac. de Farmacia y Bioquímica, UBA, Buenos Aires, and *CIQUIBIC, Fac. de Cs. Químicas, UNC, Córdoba, Argentina.

Galectin-3 (previously named CBP35, Mac-2, L-29 and IgE-binding protein) is a member of a newly designated family of β-galactoside-binding animal lectins. It has been suggested that this lectin plays a role in cell adhesion, inflammation, cell growth, neoplastic transformation, etc.

Galectin-3 was obtained from ovine placental cotyledons, together with galectin-1, by lactose extraction of the tissue, followed by affinity chromatography on a lactose-agarose column. Both galectins were separated by reverse phase HPLC on a C4 column or by FPLC on a Superose 12 HR column. The purified galectin-3 migrates as a single band on SDS-PAGE showing an apparent molecular weight of 29,000. Size exclusion chromatography showed that it behaves like a monomer at physiological conditions. Both galectins-1 and 3 reacted with a rabbit antiserum raised against a chicken liver galectin-1.

The purified galectin-3 was digested with trypsin and the peptides were isolated by reverse phase HPLC on a narrow bore C18 column. Selected peptides were applied to a polybrene-coated glass filter and sequenced in an Applied Biosystems Model 477 Automatic Sequencer. The partial sequences obtained showed homology with mouse 3T3 fibroblast, dog kidney and human breast carcinoma galectins-3.

This is the first time that galectin-3 is detected in placenta. Its presence supports the idea of its involvement in the modulation of diverse biological phenomena such as differentiation and development.

Supported by UBA-CONICET.

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Abstract withdrawn

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FUNCTIONAL RELEVANCE OF BASIC AMINO ACID RESIDUES IN PHOSPHOENOLPYRUVATE CARBOXYKINASE FROM *Saccharomyces cerevisiae*. Krautwurst, H., Chávez, R. and Cardemil, E. Departamento de Ciencias Químicas, Facultad de Química y Biología, Universidad de Santiago, Santiago, Chile.

Phosphoenolpyruvate (PEP) carboxykinase from *Saccharomyces cerevisiae* catalyses the following reaction in the presence of manganese ions:

$$\text{Oxaloacetate} + \text{ATP} \Leftrightarrow \text{PEP} + CO_2 + \text{ADP}$$

which constitutes an important regulatory step in the biosynthesis of glucose from C_3 and C_4 precursors. The enzyme is composed of four subunits of 61 kDa each, and shows high sequence identity with the corresponding enzymes from bacteria and plants. Limited proteolysis and chemical modification experiments have identified Arg^{76} and Lys^{290} as potential residues involved in substrate binding. These residues, as well as Lys^{256}, a residue belonging to the consensus Gly-X-X-X-X-Gly-Lys-Ser/Thr phosphoryl-binding sequence seen in many nucleotide-binding proteins, have been subjected to site-directed mutagenesis. We have prepared Arg/Gln and Lys/Gln mutants, and have found no change in the V_{max}/K_{mADP} for the Arg76Gln mutant, while the corresponding values for the Lys290Gln and Lys256Gln mutants are reduced to 10% and 0.25% the normal value. These results indicate that Arg^{76} and Lys^{290} are of no catalytic relevance, while suggest that Lys^{256} is an essential residue. Furthermore, these results agree with the location of the equivalent Arg and Lys residues in the crystalline structure of the homologous PEP carboxykinase from *Escherichia coli*. Further experiments are in progress to analise more precisely the function of Lys^{256}. Supported by DICYT-USACH and FONDECYT 1941073.

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UNFOLDING AND REFOLDING OF HORSERADISH PEROXIDASE: INTRINSIC FLUORESCENCE AND CD STUDIES. [1]Lasagna, M.D. [1]Brunet, J.E. and [2]Jameson, D.M., 1 Instituto de Química, Facultad de Ciencias Básicas y Matemáticas, Universidad Católica de Valparaíso, Valparaíso, Chile. 2 Departament of Biochemistry and Biophysics, University of Hawaii at Manoa. Honolulu ,USA.

The guanidinium chloride denaturation and renaturation of Horseradish peroxidase (HRP), a 40.000 D hemoprotein, and its apoenzyme (apoHRP) have been studied using intrinsic fluorescence methodologies and CD measurements. The denaturation curve of HRP monitored by steady-state and time resolved fluorescence parameters and CD measurements in the backbone, aromatic and heme regions, seems to be a typical two-state process, while apoHRP present a three-state process. The denaturation curve of apoHRP indicates the presence of a partially unfolded intermediate. This intermediate was also caracterized by CD measurements in the backbone (222 nm) and aromatic (280 nm) regions and present the characteristic of a Molten Globule state. The unfolding process of apoHRP is completely reversible while the HRP unfolding is irreversible. The refolding path of HRP is very similar to the unfolding/refolding path of apoHRP, indicating that the heme group is not critical in the refolding pathway of HRP.

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***DATURA INNOXIA* LECTIN: ISOLATION AND CHARACTERIZATION. COMPARISON WITH *DATURA STRAMONIUM* LECTIN.** Levy-Benshimol, A. and Melito, C. Instituto de Biología Experimental Universidad Central de Venezuela.

A lectin from *D. innoxia* seeds (DIL) was purified by affinity chromatography on ovomucoid-Sepharose 4B. It contains four polypeptides of 121, 63.4, 58.9 and 54.5 kDa. *Reports indicate that D. strammonium* lectin (DSA) has three polypeptides of 68, 60 and 49 kDa. DIL is highly glycosilated (42% w/w), pentoses accounting for 17% of total carbohydrates. In DSA, carbohydrate accounts for 37% (arabinose and galactose only). Hyp is the most abundant amino acid in DIL, followed by Ser, Glx, Arg, Asx, Gly and Pro. In DSA, Hyp, Ser, Cys and Gly predominate. DIL agglutinates erythrocytes from a wide spectrum of species. Minimal DIL concentration for agglutination of goose erythrocytes is 0.03 µg/ml. Best inhibitor of DIL haemagglutination is chitin. Thyroglobulin and ovomucoid, containing complex N-glycans with D-Galß(1→4)D-GlcNac (N-acetyllactosamine) units at the non--reducing end are also strong inhibitors. Among simple sugars and their derivatives, N,N',N''-Triacetylchitotrioside is the best inhibitor. N-Acetyllactosamine has been reported as a good inhibitor for DSA. DIL agglutinates both mononuclear and polymorphonuclear human cells but is not mitogenic. It decreases by more than 50% the mitogenic response of human lymphocytes, stimulated either by PHA or anti-αCD3 antibody. In contrast, DSA is a strong mitogen and does not antagonize lymphocyte proliferation.

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PHOSPHORYLATION OF TAU PROTEIN BY EMBRYONIC MAP2 KINASE. López, L.A., IHEM, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina.

We recently reported a new protein-kinase, Embryonic MAP2 kinase (EMAP2 kinase), that is able to phosphorylate and change the affinity of MAP2 to microtubule (JCB 270, 12511-12517). This fact allows to restore the transport mediated by kinesin and cytoplasmic dynein on microtubules.

It is speculated that EMAP2 kinase regulates the organelle transport in neurons by phosphorylation of several MAPs.

In this work is tested the ability of EMAP2 kinase to phosphorylate tau proteins and the affinity of this phosphorylated species to microtubules. Bovine and embryonic chicken brain tau were used as substrate. EMAP2 kinase was obtained from a crude MAPs preparation of embryonic chicken brain and isolated by sucrose gradient centrifugation. It was observed that EMAP2 kinase is able to phosphorylate the five tau polypeptides of both bovine and chicken brain preparation at a phosphorylation ratio of 3 ± 0.2 mol^{32}P/ mol tau. The microtubule affinity of phosphorylated tau was the same that unphosphorylated tau. The microtubule transport could be regulated by tau phosphorylation.

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BLOCKAGE OF CYSTEINE-128 MAY OBLITERATE THE COMMUNICATION BETWEEN THE ACTIVE AND ALLOSTERIC SITE ON FRUCTOSE-1,6-BISPHOSPHATASE. Ludwig, H.C., Reyes, A.M. and Slebe, J.C. Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.

Fructose-1,6-bisphosphatase, which catalyzes the hydrolysis of fructose-1,6-bisphosphate (Fru-1,6-P_2, K_M = 2.4 μM) to fructose-6-phosphate plus inorganic phosphate, is inhibited allosterically by AMP. The binding of substrate to the active site potentiates AMP inhibition, which indicates that there is communication between catalytic and allosteric site on the native enzyme. We analyze here the effect of chemical modification of Cys-128, a residue which is located on an intersubunit interface and close to the active site, on the interplay between the active and AMP allosteric site.

We used formycin-5'-monophosphate (FMP) as a fluorescent analog of AMP. FMP inhibits pig kidney enzyme with high affinity and positive cooperativity (I_{50}=23.3 μM, n_H=2.6) as AMP does. Since binding of FMP to the enzyme enhances its fluorescence emission, we used this effect to sense binding of the probe to the allosteric site. FMP binds to the enzyme in the absence of substrate (FMP_{50}= 10.9 μM, n_H=2.4), and Fru-1,6-P_2 promotes FMP binding. Upon limited treatment with *N*-ethylmaleimide (NEM), under conditions that just block Cys-128 (Reyes *et al.* (1993). *J. Prot. Chem. 12*, 159-168), the enzyme retains catalytic activity (K_M=5.0 μM) and propensity to FMP binding (FMP_{50}=16 μM, n_H=2.3). However, Fru-1,6-P_2 no longer promotes FMP binding on the NEM-modified enzyme. These results indicate that Cys-128 may be important for the connection between the catalytic and allosteric site. (Supported by grants from: FONDECYT 1951215 and DID-UACH, S-94-10 and S-95-41).

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RIBOFLAVIN PHOTOREACTIVITY TO THE EYE LENS PROTEINS. M.Mancini, M.Diaz, M.I.Becker, A. de Ioannes and E. Silva. Laboratorio de Química Biológica, Facultad de Química, Pontificia Universidad Católica de Chile. post1@lascar.puc.cl

The effect of visible ligth on photosensitizing processes has been postulated as one of the causes of aging and the formation of eye cataracts. Riboflavin, an endogenous component of this organ, plays a very important role in these processes. Consequently, monoclonal antibodies to the hapten tryptophan-riboflavin, generated by irradiation of a solution of bovine serum albumin in the presence of riboflavin, are able to react with the soluble proteins of the eye lens. This reaction is more important in the old rat lens as comparated to the young ones, and a maximum value is obtained with the soluble protein fraction from a human cataractous lens (M.Diaz et al .Photochem. Photobiol., 63, 762-767, (1996)). The different ocular lens proteins fractions have been isolated and subjeted to visible ligth either as polychromatic or monochromatic (450 nm) radiation in both aerobic and anaerobic conditions. In all cases a photoconversion of the tryptophan residues present in these proteins is observed and the extent of this modification correlates very well with the degree of exposure of these aminoacids in the proteins structure. The aggregation processes that are produced simultaneously to the modification of the tryptophan residues allow us to explain the observed behaviour with aging and cataractogenesis .

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THE DENATURATION OF TRYPSINOGEN AT pH 7,0

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The denaturation of purified bovine trypsinogen, induced by guanidine HCl at pH 7,0 and 25°C, was followed by size-exclusion (SE - HPLC) and difference spectroscopy (DE) at 293 nm. The SE-HPLC data clearly show a three-state transition, with a first transition between 1,0 and 2,0 M, and a second transition between 2,0 and 3,0 M GdnHCl. Based on the elution volumes, the native protein shows a Stokes radius of 18,3 Angstron while the denatured protein shows (at 4,0 M denaturant) a Stokes radius of 28,6 angstron. The intermediate species has a Stokes radius (at 2,0 M denaturant) of 23,1 Angstron. The DE data shows a major transition between 1,0 and 2,0 M but is almost transparent to the second transition. Data with circular dichroism and kinetic of unfolding / refolding of the protein is now being collected in order to describe better these conformational transitions.

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DISSOCIATION AND UNFOLDING OF β-LACTOGLOBULIN A INDUCED BY HYDROSTATIC PRESSURE. Mesquita, V.L.V., Botelho, M.M. and Ferreira, S.T. Departamento de Bioquímica Médica, ICB/CSS, U.F.R.J., RJ 21941-950, Brasil.

β-lactoglobulin (β-LG) is a homodimer of 18 KDa subunits found in milk of mammalian species. We have investigated the unfolding and refolding of β-LG induced by high hydrostatic pressure, pH and temperature through the use of fluorescence spectroscopy, with the purpose of characterizing possible folding intermediates. Application of pressure ranging from 1 to 3500 atm promoted a fast (< 10 min.) and significant (10-12 nm) red-shift in the intrinsic fluorescence emission of β-LG, indicating dissociation/unfolding of the protein. Re-folding upon pressure release occurred with considerable hysteresis, indicating loss of free energy of folding and/or association upon application of pressure. Low temperatures (3 °C) favored pressure-induced dissociation and unfolding, suggesting that stabilization of β-LG structure is driven by entropy. Alkaline pHs also promoted protein conformational changes favoring unfolding. Interestingly, the red-shift of emission spectra induced by pressure was not affected by a 10-fold change in concentration of β-LG, indicating anomalous behavior in pressure-induced dissociation of this protein. In order to investigate the incomplete renaturation after decompression, gel-filtration FPLC analysis was carried out and formation of high MW aggregates was observed. Such aggregates were analyzed by SDS-PAGE and non-denaturating electrophoresis, which showed that formation of aggregates might be caused by disulfide cross-linking. Studies are under way to further characterize the unfolding of β-LG by pressure.

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A COMPARISON OF THE HETEROGENEITY OF SPERM HISTONES IN TWO SPECIES OF SEA URCHINS: PARECHINUS ANGULOSUS AND TETRAPYGUS NIGER. Morín, V., Reyes, E., Puchi, M., Schwager, S*. and Brandt, W*. Universidad de Concepción, Chile, University of Cape Town*, South Africa.

To compare the heterogeneity of the major histone classes present in sperms of the sea urchin Tetrapygus niger and Parechinus angulosus, we have isolated the whole histone complement from sperm nucleus. The major types of sperm-specific histones were purified by chromatography in Biogel P-60 and subjected to aminoacid analysis. Each histone class was further analyzed by HPLC and by electrophoresis in polyacrilamide gels under different denaturing conditions. The similarities between each histone type were established according to all the parameters mentioned above and by their immunological cross-reactivity against Tetrapygus niger sperm histone antibodies. The results obtained may be summarized as follow:

1.- Both sea urchins contain a single type of histone H1. Based on their aminoacid composition, T niger H1 appears to be more acidic than P angulosus H1.

2.- Histone H2A is represented by a single protein that shows similar composition in both species.

3.- Three subtypes of H2B were found in T niger which differ in their electrophoretic migration with the three subtypes of H2B of P angulosus. These histones subtypes are due to allelic variations since they differ in individual T niger specimens.

4.- One major and one minor form of H3 and three subtypes of H4 were found in T niger as compared to one H3 and two H4 in P angulosus. Interestingly, it was observed little immunological cross-reactivity.

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INFLUENCE OF THE C-TERMINAL REGION ON THE GTPase ACTIVITY IN TUBULIN. Nova, E., Rodríguez, P. and Monasterio, O. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Tubulin is a heterodimer composed of α- and β-tubulin, that assembles reversibly to form microtubules. Tubulin has two guanosine nucleotide binding sites, the nonexchangeable site and the exchangeable site (E-site) which is located at the N-terminal region of the β-tubulin. GTP at the E-site is hydrolyzed during the polymerization reaction and this activity plays a central role in the dynamic instability of microtubules. The heterodimer presents a low GTPase activity which is stimulated by Ca^{2+} through a conformational change. In order to determine the relationship between the calcium binding site, located at the C-terminal region of tubulin, and the GTPase activity at the E-site, the effect of pH on the initial velocity of this activity was studied in tubulin and tubulin S (tubulin without the C-terminal peptides). The removal of the C-terminal peptides stimulates over one order of magnitud the GTPase activity and calcium looses its stimulatory effect. Tubulin S shows a maximal activity at pH 7.3 with pKs of 5.8 and 8.4. Tubulin shows two maxima at pH 6.1 and 7.5, with a minimum between them, at pH 6.8. The behavior of the GTPase activity of tubulin with respect to pH was interpreted as a pH dependent inhibitory effect produced by the C-terminal region over the GTPase activity at the E-site of tubulin. We conclude that the C-terminal region is regulating the GTPase activity of tubulin.

This work was supported by Grant FONDECYT 1950556.

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ANALYSIS OF VESPID VENOMS BY CAPILLARY ELECTROPHORESIS Karina Pañak[1], Sergio Giorgieri[1], Osvaldo Cascone[2] y Luis Díaz[1]. Cátedras de [1]Química Analítica Instrumental y de [2]Microbiología Industrial y Biotecnología. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires (U.B.A.).Junín 956 (1113) Buenos Aires. Argentina. Oscar Ruiz[1,3]. [3]Instituto Tecnológico de Chascomús. (INTECH) cc: 164 (7130), Chascomús. Pcia de Buenos Aires. Argentina.

Most allergies to insect venoms are caused by bee, vespid and fire ant stings. The venom composition of vespid, including hornets, polistine wasp and yellow jackets is very similar but the diferences in polypeptides composition have not been described yet. *Polybia scutellaris* subspec. *rioplatensis* (common name: camoati) is a polistine wasp from South America (mainly Argentina and Uruguay). The lack of anaphylactic activity of *P.scutellaris* venom is very curious. Although it is very common in South America and people are repeatedly stung, no hypersensitivity phenomena able to be confirmed by studies of the patients sera have been reported. The venom was obtained by carefully squeezing sacs dissected from frozen insects and lyophilized. The 10 kDa cutt-off ultrafiltrated was chromatographed on a HPLC system with a Vydac 218TP54 (25 cm length) column and monitored at 220 nm. Capillary zone electrophoresis (CZE) was performed on the venoms of *Dolichovespula maculata, Polistes (annularis, exclamans, fuscatus and metricus), Vespa crabro, Vespula (germanica, maculifrons, vulgaris, pensylvanica, squamosa and flavopilosa)* and bee. An uncoated capillary column of 37 cm total length, 50 µm ID was used, 100 mM Phosphate pH 2.50 as the running buffer and on line UV-detection at 214 nm. The rapid separations by CZE revealed differences between venoms. The major peptid from the camoati venom was sequenced and its structure was not found in data banks.

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PHOSPHORYLATION OF *Candida albicans* 20 S PROTEASOME BY THE HOMOLOGOUS PROTEIN KINASE CK2. Pardo, P., Fernández Murray, P., Walz, K., and Passeron, S. Cátedra de Microbiología, Facultad de Agronomía, Universidad de Buenos Aires and CIBYF (CONICET), Buenos Aires, Argentina.

The 20 S proteasome is an essential protease found in all eukaryotic organisms and is responsible for highly specific degradation of most cytosolic proteins. Early reports have described the in vitro and in vivo phosphorylation of some proteasome subunits from several sources. Very recently it has been demonstrated that rat liver 20 S proteasome is in vitro phosphorylated by the homologous protein kinase CK2 at the same sites as it is in vivo phosphorylated. We here present evidence showing that purified 20 S proteasome from *Candida albicans* is in vitro phosphorylated at two subunits (32 and 25 kDa) by the homologous purified protein kinase CK2. The phosphorylation reaction is absolutely dependent of polylysine reaching a total incorporation of more than 4 mol of phosphate per mol of proteasome, as assessed by scintillation counting of the excised SDS-PAGE solved ^{32}P-phosphorylated bands. The 32 kDa band contained 2.5 times more phosphate incorporated than the 25 kDa band at all the times assayed. Preliminary experiments suggest that the proteasome catalytic function is not significantly affected by these phosphorylations. Heterologous phosphorylation assays using 20 S proteasome from croaker muscle and *C. albicans* and protein kinase CK2 from rat liver, *C. albicans* and human recombinant catalytic α subunit indicate that phosphorylation of 20S proteasome by CK2 could be a conserved regulatory mechanism. The identification of *C. albicans* proteasome phosphorylatable subunits is under study.

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THERMAL STABILITY OF MYOFIBRILLAR PROTEINS FROM SMOOTH AND STRIATED MUSCLES OF SCALLOP (*Chlamys tehuelchus*).A DIFFERENTIAL SCANNING CALORIMETRIC STUDY (DSC). Paredi, M.E.[a], Tomas, M.C.[b], Añon, M.C.[b] and Crupkin,M.[a]. [a]Centro de Investigaciones de Tecnologia Pesquera, Mar del Plata, Argentina. [b]Centro de Investigaciones y Desarrollo en Criotecnologia de Alimentos, La Plata, Argentina.

The thermal stability of myofibrillar proteins from invertebrates could be related to the presence of paramyosin.The smooth muscle of the scallop (*Chlamys tehuelchus*) has a greater content of paramyosin than the striated muscle. The purpose of this work was to investigate the possible influence of both the paramyosin content and the chemical environment on the thermal stability of the myofibrillar proteins of the scallop's smooth and striated muscles. DSC thermograms of striated and smooth whole muscles of scallops show two endothermic transitions with Tmax of 53.2 and 79.0°C and 52.7 and 78.0°C respectively. Myofibrils and actomyosin isolated and purified from both types of muscle showed thermograms similar to those of the respective whole muscles, with a slight displacement towards lower temperatures.These results show that for both types of muscle, myosin and paramyosin contribute mainly to the first transition and actin to the second. Since the Tmax of the first transition of smooth muscle is similar to the corresponding Tmax of the striated muscle, a greater content of paramyosin would not be directly reponsible for the thermal stability. A decrease in the Tmax in both transition and in the total denaturation enthalpies with the increase of the ionic strength, was observed in both types of muscles. The most affected zone was the correponding to the first transition of the smooth muscle. Similar results were obtained with high pH values.

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EXPRESSION OF C-TERMINAL TRUNCATED VARIANTS OF iFABP AS A MODEL FOR FOLDING STUDIES, Peisajovich, S. G. Clérico, E. M., Ghiringhelli, P. D., and Ermácora, M. R., Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Argentina.

The characterization of the three-dimensional structure and thermodynamic properties of folding intermediates is crucial to understand the mechanism of protein folding and to unravel the folding code that mediates the spontaneous formation of the native state. However, folding intermediates and partially folded nonnative states are difficult to isolate and populate. We define as nonnative states all states that differ from the native and from the theoretically defined random-coil, including equilibrium folding intermediates and kinetically trapped species. We believe that all these forms are different reading of the folding code and therefore potentially useful to undecode its logic. It is of interest to find conditions or means to stabilize nonnative states. At this moment a limited number of protein models fulfill these conditions. Most of these systems are based on solvent changes (denaturant, pH, etc.) that destabilize preferentially the native state over the partially folded state. A few of them are the result of eliminating cofactors or ligands. A more rational approach is to introduce mutations and deletions on the polypeptide chain that can be expected to produce specific alteration in the folding reaction. C~ or N-terminal truncation is particularly interesting because specific interactions can be eliminated without residue replacement and therefore they do not introduce an extra variable in the system. The best studied case is C-terminal truncated staphylococcal nuclease which produces a stable partially folded state at equilibrium under physiological conditions. We designed and produced a series of variants of iFABP, an all-beta, 14 kDa protein, that may be a new model system for the study of nonnative states. In this series, gradual removal of specific tertiary interactions is achieved by the deletion of three residues from the C-terminus. The engineered variants are: (wt)iFABP$_{1\text{-}131}$, (W6F)iFABP$_{1\text{-}131}$; (W82F)iFABP$_{1\text{-}131}$; (W6F)iFABP$_{1\text{-}128}$; (W82F)iFABP$_{1\text{-}128}$; (wt)iFABP$_{1\text{-}128}$. The systematic exchange of tryptophan residues provides a simplified context for the spectroscopic characterization of the truncated variants. The rationale of the design and a preliminary account of the effect of the truncation on the expression and folding properties of iFABP is presented.

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BOTHROPSTOXIN-II (Asp-49) : AMINO ACID SEQUENCE AND BIOLOGICAL ACTIVITIES: Pereira, M. F., Novello, J. C., Landucci, E. T., Zago, E. B., Haun, M. and Marangoni, S. Departamento de Bioquímica, IB, UNICAMP, Campinas (SP), BRASIL.

Bothropstoxin-II (BthTX-II) from *B.jararacussu* snake venom showing residual phospholipase activity, is a protein with only one polipeptide chain with calculated molecular mass of 13.976 venom.The primary structure of BthTX-II was determined from automatic amino acid sequence of the peptides obtained after Staphylococcus aureus strain V_8 and clostripain digestion of reduced and carboxilated toxin. This toxin displays the amino acid residues which are involved in the Ca^{++} loop, essential for the phospholipase activity, but showed residual phospholipase activity. In the snakes bites, the more commom symptom is a formation of local oedema. The time course of the oedema induced by BthTX-II in foot pad of rats,was fast in the beginning, remaining maximum between 30-60 minutes and decreasing after this time. The pretreatment of the animals with cyproheptadine abolished the edematogenic response of BthTX-II and is indicative that the oedema *in vivo* is caused by mast cells degranulation. Many authors have reported oedema induced by phospholipase A_2 in rats, suggesting that the first phospholipase action in the oedema is the release of amines of mast cells. Inflammation activity of PLA_2 can be attributed to a direct but not an enzymatic effect. These results confirms previous evidences of independent sites for the expression of the enzymatic and pharmacological activities in these molecules. BthTX-II is a Asp-49 PLA_2 miotoxin and have residual catalytic activity, which suggests a new way of classifying this proteins family. Citotoxicity with hamster chinese fibroblasts (V-79 cell line) are in progress. (Financial Support: FAPESP, CNPq, FAEP.)

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STRUCTURAL ANALYSIS OF ACID pH SOYBEAN PROTEIN GELS. Puppo, M.C. and Añón, M.C. Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA-CONICET-UNLP). 47 Y 116. 1900 La Plata. ARGENTINA. Soybean proteins may produce gels conformed by a viscoelastic tridimensional matrix capable of holding water and other substancies, by thermal treatment. The nature of such gel and the behavior of the majority fractions of soybean proteins (11S and 7S) depends on the pH, the protein concentration, type of salts present and their concentration, as well as on the time and heating temperature. Gels from soybean protein isolate dispersions were prepared by heating at 90°C for 30 min at different pHs (2.50-3.50, 8), protein concentration (10 and 14% p/p) and NaCl concentration (0.1-2 M). The Water Holding Capacity (WHC) of the gels was determined by weighing difference, their hardness estimated by a compression up to an 80% deformation in an Instron press, and their viscoelasticity was calculated by dynamic G', G" and tan δ measures in an oscillatory Haake. Assays of gel solubility in distilled water (DW), and in a buffer with denaturing agents, urea, and SDS (BSU) and SDS-PAGE of the soluble fractions were made. Gel hardness increased as the pH and the protein concentration increased. Acid gels with a high water holding capacity presented a low hardness, while pH 8 gels with an approximately 100% WHC presented a higher hardness, increased by the protein concentration; pH 3.25 gels showed a greater elasticity than the pH 8 gels independently from the oscillatory frequency. The results obtained from the hardness, solubility in BSU, and electrophoresis assays of acid gels, may indicate the existence of non-covalent bonds predominance, and the absence of disulfide bonds at pHs lower than the pI. The glycinine AB subunit, present in the DW soluble extracts of acid gels would suggest that the 11S fraction participates to a lesser degree in the gel structure maintenance than the 7S fraction. The acid pH gels presented physical and structural properties different from those of the alcaline pH, a characteristic to be taken into account in the formulation of different acid nature food.

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INHIBITORY EFFECTS OF α_2-MACROGLOBULIN ON PROTEINASES OF EPIMASTIGOTES OF *TRYPANOSOMA CRUZI*. Ramos, A., Remedi, M.S.*, Sanchez, C., Chiabrando, G., and Vides, M. A. Dpto de Bioq. Clínica, Fac. Cs. Químicas, (UNC). C.C 61 - 5016 Córdoba - Argentina. E-Mail: gustavo@fisquim.uncor.edu. * Cat. de Qca. Biológica, Fac. Cs. Médicas (UNC).

Human α_2-Macroglobulin (α_2-M) is a plasma proteinase-inhibiting glycoprotein of broad spectrum, which acts by a molecular trapping mechanism and a rapid depuration from circulation through receptor-mediated endocytosis in hepatocites.

The fact that α_2-M has been reported to inhibit several proteinases of bacteria, fungii and viruses, encouraged us to study the possible interactions between α_2-M and proteolitic enzymes of the parasitic protozoa *Trypanosoma cruzi (Tc)*.

Mixtures containing 15.0 ug of purified α_2-M and crude extracts of Tc epimastigotes from 14-old day cultures ($3x10^6$ to $24x10^6$ cells) were incubated for 24 h at 37°C. Then, mixtures were analyzed by polyacrylamide gel electrophoresis (PAGE) on non denaturing, denaturing (SDS-PAGE), and reducing (reduced SDS-PAGE) conditions. The interaction between α_2-M and proteinases of Tc extracts became evident as judged by: a) formation of "fast" α_2-M on non denaturing PAGE; b) presence of α_2-M-proteinase complexes (Mr>360.000) on SDS-PAGE, and c) appearence of bands corresponding to Mr ≈ 85,000-100,000 on reduced SDS-PAGE, owing to proteolitic fragmentation of the "bait" region in α_2-M subunits (Mr 185,000)

Proteinase activity of the extracts was further investigated by SDS-PAGE containing gelatin in mixtures incubated as described above; showing bands of Mr ≈46,000-53,000 which were greatly diminished in the presence of α_2-M. Furthermore, the time course interaction between α_2-M (15.0 ug), and proteolitic enzymes of Tc extracts ($6x10^6$ cells) was evaluated by measurements of the remanent activity on azocasein in vitro. The results showed percentages of inhibition ranging from 75.7 % to 85.2 % at 1 h and 24 h of incubation of the mixtures at 37°C, respectively.

The results presented herein are consistent with the wide inhibition spectrum of α_2-M. Ocurrence of interactions between α_2-M and proteinases of *Tc*-epimastigotes could be important in the knowledge of its role in Chagas' disease.

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LECTIN BINDING PATTERN OF STALLION SPERM PROTEINS. Retamal, C[1,3]. Thiebaut, P.[1], Alves, E.W.[1,2]. LQFPP, CBB, Universidade Estadual do Norte Fluminense[1], Depto. de Bioquimica., ICB, UFRJ[2], RJ. Brasil / Facultad de Medicina, U. de Chile[3], Stgo, Chile.

Spermatozoa acquire their motility and fertilizing ability during transit through epididymis. The objective of our work is to characterize sperm proteins/glycoproteins from the different epididymal regions, likely involved in these process. Proteins removed from the different epididymal regions were analysed by SDS-PAGE. Proteins from ejaculated sperm membranes were purified by solubilization in Nonidet P-40 and separated in SDS gel, then the bands of interest (14,18,20 kDa) were cut, sonicated and passed through a Sephadex G-25 mini-column. The sugar residues were detected using PAS and FITC-lectin probes.Our results shown that the electrophoretic patterns of sperm glycoproteins from the various epididymal regions and ejaculate were remarkably different.Some lectins (UEA, WGA, LPA) gave a stronger reaction in mature spermatozoa, while others (RCA, WFH, PNA) stained better immature sperm cells. The distribution and density of mannose-, galactose-, Nacetyl glucosamine-, fucose- and sialic acid-containing surface glycoproteins vary depending on the sperm membrane domains and the epididymal source. The purified glycoproteins bands contained N acetyl-glucosamine or sialic acid and fucose residues. The ratio of sugar/protein of these glycoproteins increased with the MW. We are getting the internal aminoacid sequence in order to correlate structure/function of these proteins, since the NH_2-terminal was blocked.Our data suggest a glycoprotein surface remodelling process during the sperm maturation.(Supported by FENORTE, CNPq, VITAE/ANDES FOUNDATIONS)

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INTERMEDIATES IN THE UNFOLDING PATHWAY OF PIG KIDNEY FRUCTOSE-1,6-BISPHOSPHATASE. Reyes, A.M., Ludwig, H.C., Rodríguez, P. and Slebe, J.C. Instituto de Bioquímica, Universidad Austral de Chile, Valdivia, Chile.

Vertebrate fructose-1,6-bisphosphatases are homotetrameric proteins, whose activity (fructose-1,6-bisphosphate → fructose-6-phosphate + Pi) is allosterically regulated by AMP. In the native tetramer each subunit contacts two other subunits through different interfaces; one of these interfaces forms the active site, while the other is located adjacent to the AMP binding site. We studied the unfolding of the enzyme induced by guanidine-hydrochloride (Gdn-HCl) under equilibrium conditions, in order to get information about the stability of the monomer-monomer interactions.

Unfolding of the protein was followed by enzyme activity and by using different physicochemical parameters (protein intrinsic fluorescence, anisotropy and emission intensity of covalently-bound extrinsic fluorescent probes, binding of 8-aniline-1-naphthalenesulphonic acid (ANS), size-exclusion HPLC). On the basis of non-coincident transition curves we found that enzyme unfolding is a multistep process. The enzyme inactivation at about 0.4 M Gdn-HCl precedes protein dissociation. At 1-1.5 M Gdn-HCl the enzyme begins to dissociate first to a dimer and then forms aggregates of high molecular weight; these aggregates bind ANS. No compact monomeric intermediate was detected. Further increase in Gdn-HCl concentration beyond 1.5 M leads to complete unfolding of the protein to unstructured monomers. Then, a feasible mechanism for unfolding is: native protein → inactive tetramer → dimer → aggregates → unfolded chains (Supported by DID-UACH, S-94-10 and S-95-41, and by FONDECYT 1951215).

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ISOLATION AND PARTIAL CHARACTERIZATION OF TWO PROTEINASES FROM *Bothrops brazili* SNAKE VENOM. ESCOBAR, E.J., RODRIGUEZ, E.F., LIMAN J.C., PANTIGOSO, C.A. and YARLEQUE, A.

Laboratorio de Biología Molecular, Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos, Lima, Perú.

A non specific proteinase and a thrombin like enzyme were isolated from the venom of *Bothrops brazili* "jergón shushupe" using a column on Sephadex G-100 gel filtration at pH 6,0 with 0.05M ammonium acetate buffer as a first step. Proteolytic enzyme (caseinolytic activity) was obtained as a homogeneous band on PAGE-SDS after two chromatographical steps on CM-Sephadex C-50 at pH 6,0 and 7,0 respectively. Besides, the purification of thrombin like enzyme was completed through DEAE-Sephadex A-50 ion exchange column at pH 6,0.

The proteolytic enzyme showed a rapid inactivation to heating treatment over 37 °C and its molecular weight was 26,3 Kd under reductor and non reductor conditions. The enzyme was inhibited by 0,05mM EDTA indicating that it is metalloproteinase. Electrophoretical assays with bovine fibrinogen 5mg/ml solution in 0,1M Tris-HCl buffer at 7,4 achieved that the enzyme attack A-α chain very strongly whilst B-β and γ were not affected. Biological assays on mice (18-25 g) showed that this protein is a potent hemorrhagine.

On the other hand, the thrombin like enzyme exhibit the following characteristic: it is a thermolabile protein because of 50% of inicial activity was reduced by heating treatment (40-50°C), its molecular weight was 54 Kd by PAGE-SDS. The addition of Ca^{2+} ions (1-10mM), PMSF serine proteinase inhibitor (1mM) y 2 mercaptoethanol (10mM) produced inhibition of its amidolytic activity while Mg^{2+} ions not affected to this enzyme.

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USE OF HOMOGENEOUS ELECTRONIC ENERGY TRANSFER TO OBSERVE DISSOCIATION AND SUBUNIT EXCHANGE IN THE αβ TUBULIN DIMER. Rodriguez P.H.[1], Monasterio O.[1] and Weber G.[2] Depto. de Biologia, Universidad de Chile, Santiago, Chile [1] and School of Chemical Sciences, University of Illinois, Urbana, Illinois, USA[2].

We studied the subunit dissociation and exchange of the αβ dimer of tubulin by observations of its intrinsic fluorescence and that of conjugates with fluorescein isothiocynate (FITC) with 1-1.3 labels per mol of dimer, which contained only traces (<1%) of free dye. Energy transfer between FITC fluorophores was demonstrated by the increase in polarization observed on excitation at the red edge of the FITC spectrum. A dissociation constant of 1 μM, in agreement with previous literature values, was calculated. The low polarization at high dilution, ≈0.09 is inconsistent with rigid particle separation and is attributed to the additional conformational drift of the separated subunits. The changes on dilution show two processes with half times of 3 and 60 minutes, respectively assigned to dissociation and conformational drift. Subunit exchange is demonstrated by the increase in polarization on mixing with a large excess of unlabelled protein at either low or high dissociation. The exchange was independent of protein concentration showing that the dissociation association cycle is determined by the rate of dissociation of dimers, k_-, and from its temperature dependence k_- is assigned an energy of activation of 20 kcal mol^{-1}. At 2 kbar the subunit exchange is tenfold faster than at atmospheric pressure. On application of pressure the fluorescence spectrum of unlabelled tubulin shows a progressive red shift that at 2.4 kbar reaches ≈800 cm^{-1}. The concentration independence shows that this red shift is a first order effect unrelated to dissociation. The pressure effects on dilute FITC-tubulin shows that unlike previously studied homodimers, pressure increases association, corresponding roughly to ΔV of 10 ml mol^{-1}. Work supported by FONDECYT, grants 1950556, 2950001 and DTI, S-29 (Chile) and NIH grant GM11223 to GW (USA)

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PROPERTIES OF THE TYROSYL tRNA SYNTHETASE (TyrRZ) FROM *THIOBACILLUS FERROOXIDANS*. Salazar, J.C., Zúñiga, R., Salazar, O. and Orellana, O. Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile. Santiago, Chile.

Aminoacyl tRNA synthetases (aaRS) catalize the esterification of the amino acid to the cognate tRNA. This is a crucial step that ensures the fidelity of the protein biosynthesis. The amino acylation of tRNAs is a two steps process as is shown in the following sequence of reactions:

aa + ATP ----------> aa-AMP + PP_i

aa-AMP + tRNA ----------> aa-tRNA + AMP

Bacterial tyrosyl tRNA synthetases are well known. The enzyme from *Bacillus stearothermophilus* has been crystalized and the terciary structure of the amino terminal portion determined by X ray crystallography. We have cloned and sequenced the gene (*tyrZ*) encoding for TyrRZ from *T. ferrooxidans*. The amino acid sequence of the protein product is highly homologous (more than 45% identity) to the predicted TyrRZ from *B. subtilis* and *H. influenzae*. Only 20-25% identity was observed when it is compared to *E.* coli or *B.* subtilis TyrRS. Computer assisted modelling of the terciary structure of TyrRZ from *T. ferrooxidans* predicts the presence of the classical Rossmann fold, present in all class I amino acyl tRNA synthetases.

T. ferrooxidans tyrZ was subcloned in an expression vector and the protein overproduced as a fusion product in *E. coli*. TyrRZ was purified to near homogenity from *E. coli* extracts by affinity chromatography and gel filtration After digestion with thrombin, the enzyme was active and stable at -20°C. The enzyme recognizes *E. coli* $tRNA^{tyr}$ as substrate for amino acylation *in vitro* as well as *in vivo*. These data led as to think that TyrRZ from *T. ferrooxidans* is a representative model for the analysis of the interaction of the tRNA with the enzyme. Site directed mutagenesis of this enzyme to asses the tRNA recognition is currently in progress and will be discussed

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INUSUAL NUCLEAR SPERM SPECIFIC BASIC PROTEINS IN *Tagelus dombeii* (Bivalve, Tellinaceae), Sánchez, L.D., Rojas, N., Núñez, M.A., Lizárraga B. and Chiva, M. (*). Centro de Investigación de Bioquímica y Nutrición. UNMSM. Lima, Perú, (*) Unidad de Química Macromolecular, UPC, España.

The electrophoretic pattern of SBO's from bivalve ***Tagelus dombeii,*** shows different proteins bands that migrate in three main zones of gel: the electrophoretic zone of histones, an intermediate region of the gel and of higher electrophoretic mobility, these are six SBP. Considering that nuclear sperm basic proteins (SBP, protamines) from bivalve have been examined in forty species and all of them pressented similar characteritics in the number of components, electrophoretic mobilities and aminoacid compositions, this distribution of ***Tagelus*** is a particulary interesting, has not been described in other bivalves; studies in electronic microscopy shows an sperm apparentely modified.

Afterwards we will discuss our observations under the general frame of SBP's in other bivalves.

Proyecto de Investigación Conjunta ICI, CONCYTEC, Proyecto FEDU 6010201.

UNMSM: Universidad Nacional Mayor de San Marcos.

UPC: Universidad Politécnica de Cataluyna.

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SPECTROSCOPIC STUDIES OF MITHOCONDRIAL MALATE DEHYDROGENASE FROM PORCINE HEART. Sanchez, S.,[*] Brunet, J.E. and [&] Hazlett, T., [*] Instituto de Química, Facultad de Ciencias Básicas y Matemáticas, Universidad Católica de Valparaíso, Valparaíso, Chile., & Departament of Physics, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.

Mitochondrial Malate Dehydrogenase (mMDH) is a 68 Kda dimeric protein which catalyzes the interconversion of oxalate to malate in the Krebs cycle using NADH as a cofactor. The mMDH monomer-dimer equilibrium, and the influence of ligands on this process are not well understood, even though a variety of methodologies have been brought to bare in this issue. The reported dissociation constants seem to be correlated with the specific activity of the sample. With the high activity sample showing the tightest association. To further investigate this correlation and the solution dynamics of mMDH, we have used both time-resolved and steady-state spectroscopic techniques.

Our preliminary studies show the presence of a sensible amount of higher-order oligomers that could be responsible for the contrasting literature results. Our data also indicate a tight inespecific association between mMDH and fluoresceine, a complexity which was not taken into account in earlier works.

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MODIFICATION OF THIOREDOXIN SPECIFICITY FOR TARGET PROTEINS: SITE-DIRECTED MUTAGENESIS OF *Escherichia coli* THIOREDOXIN

Santiago Mora-García*, José María Delfino* and Ricardo A. Wolosiuk* *Instituto de Investigaciones Bioquímicas, Facultad de Ciencias Exactas y Naturales / *Instituto de Química y Fisicoquímica Biológicas, Facultad de Farmacia y Bioquímica-Universidad de Buenos Aires-Argentina. ✉smgarcia@iris.iib.uba.ar

Thioredoxins (Trx) share a conserved active centre (WCGPCK) involved in thiol/disulfide exchange reactions. Nonetheless, they show significant differences in their reactivity towards target proteins. To unravel determinants that make chloroplastic Trx-f a proficient activator of chloroplast Fructose-1,6-bisphosphatase (CFBPase), charge distribution and conformational factors were analyzed by site-directed mutagenesis on *Escherichia coli* Trx. The introduction of positive charges exposed on the active site surface (E30K, L94K and E30K/L94K) did not mostly alter structural parameters, but greatly enhanced the activation of CFBPase by mutant proteins. On the other hand, replacement of strictly conserved residues preceding the active site in *E.coli* Trx with those peculiar to Trx-f (positions 27-29) caused a sharp modification in the environment of nearby chromophores (as indicated by fluorometric and circular dicroism measurements) and on the overall protein stability. These results correlate with a decreased reactivity with CFBPase. Nature and density of charges around the redox site thus seem to be major determinants of affinity for target proteins. In contrast, conformational shifts ensueing the replacement of conserved structural residues point out to a conformational compensatory mechanism at work in Trx-f.

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PROTEIN KINASE CK2 OF *C. albicans* CONTAINS TWO DISTINCT REGULATORY β AND β' SUBUNITS.

K. Walz, P. Pardo, and S Passeron. Cátedra de Microbiología, Facultad de Agronomía, UBA and CIByF-CONICET

Protein kinase CK2 of has been purified to near homogeneity from yeast cells of the pathogenic fungus *Candida albicans* by a procedure which involves chromatography on DEAE-cellulose, phosphocellulose, Q-Sepharose and heparin-agarose. The purified enzyme has the characteristic properties of animal and yeast protein kinases CK2, i.e. it utilizes ATP as well as GTP as phosphate donors, phosphorylates serine and threonine residues on casein, is inhibited by low concentrations of heparin and is stimulated by NaCl and polycationic compounds such as polylysine, spermine and spermidine. The native form has a molecular mass of 159 kDa. SDS-PAGE analysis indicated that it is composed by four polypeptides (44, 39, 37 and 36 kDa). The 39 and 37 kDa polypeptides were identified as distinct catalytic subunits α and α' by active gel phosphorylation assays and immunological recognition. The 44 and 36 kDa polypeptides, were identified as β' and β subunits by Western blot analysis and autophosphorylation assays. Thermal inactivation showed that the *C. albicans* enzyme drops to negligible values after 4 min of preincubation at 45°C, as reported for the *S. cerevisiae* CK2.

All these data suggest that *C. albicans* protein kinase CK2 has an αα'ββ' heterotetrameric constitution similar to that found in *S. cerevisiae.*

Supported by grants from UBA, CONICET and ICGEB.

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ISOLATION OF RAT BRAIN PROTEINS THAT BIND TO THE LOW MOLECULAR WEIGHT COMPONENT (LMWP) OF α-LATROTOXIN.

Woll, P., Haak, H. and Lizarraga, B. Centro de Investigación de Bioquímica y Nutrición, Facultad de Medicina, Universidad Nacional Mayor de San Marcos, Lima, Perú.

α-Latrotoxin is a well characterized universal stimulator of synaptic vesicle exocytosis and neurotransmitter release that has been extensively employed in physiological studies of neurosecretion. Recently, it was shown that highly purifed toxin preparations contain two components: polypeptides of 1401 (LTX) and 70 (low molecular weight protein, LMWP) aminoacid residues. This complex binds to neurexin, a presynaptic membrane protein; but, the molecular mechanisms of this facilitated exocytosis is still unknown.

To study LMWP role in synaptic vesicle exocytosis, a synthetic peptide corresponding to the C-terminal fragment of LMWP was bound to CH-Sepharose to form an affinity column. Detergent treated rat brain homogenate was applied to this column, washed and eluted with a sodium chloride step gradient, resulting in the purification of two proteins between 40 and 65 kDa.

Proyecto FEDU 6010503

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***pts* GENES IN *Bacillus sphaericus*.**

Aleiandro Alice and C.Sanchez-Rivas. Facultad de Ciencias Exactas y Naturales.Depto de Química Biológica. Universidad de Buenos Aires, Argentina.

Bacillus sphaericus is an enthomopathogenic bacteria specie which presented several metabolic deficiencies such as a poor capacity to grow in hyperosmotic media and to use carbohydrates as carbon source, difficulting its survival and making expensive its industrial cultivation. Several enzymes of the glycolytic pathway being detected, this lead us to suspect that the deficiency would be at a previous step: the sugar transport itself. In Gram positive bacteria sugars are transported by specific phosphoryl-transport coupled to a general system of phosphoryl donors: the phosphoenolpyruvate-phosphotranferase system (PTS). *ptsI* and *ptsH* genes responsible of the PTS system, were generally linked and highly conserved among species. *ptsI* or *ptsH* mutant strains from *B.subtilis* failed to use fructose, glucose and sucrose as was *Bacillus sphaericus* specie. In order to determine which of the transporter genes was missing two approaches were assayed: screening of the conserved genes and introduction of cloned *pts* genes from *B.subtilis* into *B.sphaericus*.. The first approach was performed using several bacilli as controls and *B.sphaericus* strains with different enthomopathogenic activity. Southern blot against *ptsI-ptsH* from *B.subtilis*, and PCR analysis with oligonucleotide primers obtained from highly conserved *ptsH* and *ptsI* gene sequences, failed to detect the presence of any of them in *B.sphaericus* strains, revealing that this specie is the first described gram positive bacterium of low G+C content devoid of them. Introduction by electroporation of the single *ptsI* gene from *B.subtilis* was not enough to restore the sugar utilisation capacity in this bacterium. The introduction of several *pts* genes was in progress.

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CHARACTERIZATION OF A SPONTANEOUS SLOW MILK-COAGULATING MUTANT ISOLATED FROM *LACTOBACILLUS HELVETICUS* CRL 1062. Hébert E.M., Auad L., Raya R.R., Ruíz Holgado A.P. de and Savoy de Giori G.. Centro de Referencia para Lactobacilos (CERELA), Tucumán, Argentina.

The *Lactobacillus helveticus* proteolytic system plays a major role during cheese manufacture. The concerted action of a proteinase and a number of peptidases of the starter are responsible for the production of small peptides and aminoacids which contribute to flavour development and flavour intensity during cheese ripening. The aim of the present study was to evaluate a differential medium for fast and slow milk-coagulating isolates for selecting proteinase negative variants (Prt-) of *L. helveticus* CRL 1062. A *L. helveticus* isolate is considered fast if it coagulates nonfat milk (NFM) within 16 h at 37°C from a 2% inoculum. Slow variants are associated with a defective metabolism of lactose, casein hidrolysis, or both.

A spontaneous slow variant of CRL 1062, named S1, was isolated from a casein-yeast extract-glucose agar medium (CYG), as described by Morelli and col. and modified in our laboratory. The growth rates in NFM of CRL 1062 and S1 were 0.26 and 0.04, respectively. S1 had a "fast" phenotype in NFM supplemented with yeast extract or casaminoacids, but not in NFM supplemented with glucose. Thus, the S1 phenotypes were Lac+ Prt-. However, S1 and CRL 1062 cells, grew in MRSLac, had similar ß-galactosidase and proteinase activities, as determined on the ONPG and N-succinyl-ala-ala-pro-phe-p-nitroanilide substrates, respectively. Further studies, carried out in chemical defined medium (CDM), showed that the S1 slow growth rate could be reverted by the addition of bases and vitamins. The single omission technique, applied to each component in order to determine the true nutritional requirements, showed that sustained growth of the mutant strain was only possible in minimal media supplemented with guanine.

Our data suggest that to devise a nonfat milk minimal medium suitable to differentiate fast and slow milk-coagulating variants of lactobacilli further research is still needed and that addition of guanine (plus adenine) must be considered.

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CHARACTERISATION OF FATTY ACID TRANSPORT IN *Streptomyces coelicolor* A(3)2. Banchio C. and Gramajo H. PROMUBIE (CONICET), Dto. de Microbiologia, Fac. de Cs. Bioquimicas y Farmaceuticas, Univ.Nac. de Rosario, Rosario, Argentina.

Degradation of complex oils and lipids by *Streptomyces* species has been long known. However, there is no background on how free fatty acid are taken up into the cell and then used as a carbon source.

In previous studies we demonstrated that *Streptomyces coelicolor* A(3)2, the best genetically characterised *Streptomyces*, could grow in different chain length fatty acids as a sole carbon source (C4-C18:1). Here we present the characterisation of the fatty acid transport system in this bacterium. Kinetics studies of fatty acid transport (^{14}C-palmitic acid) were performed in *S. coelicolor* grown in MM medium with oleic acid as a sole carbon source and we demostrated that a carrier mechanism facilitates the entry of fatty acids into the citoplasm. These studies also showed that palmitic acid enter via an active unidirectional mechanism.The specificity of this transport system was evaluated by competition assays with fatty acids of different chain length. Long chain fatty acids (C10-C18:1) share the same transport system, but short chain fatty acids seems to penetrate by passive diffusion.

Interestingly, glucose stimulates fatty acids utilisation. In order to characterise further this effect we perform transport studies in cells grown with or without glucose. We observed that two kinetics parameters had changed in cells grown in glucose: an increase the V_{max} and a decrease of the K_m

At the moment we are performing biochemistry and genetics studies with mutants obtained by NTG treatment that have lost the glucose effect.

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Anti-HMW properties of Tn*1000* and effect on plasmid stability in *E.coli*. M.BELLANI[1], C. NUDEL[2] and C.SANCHEZ-RIVAS[1]. [1]Facultad de Ciencias Exactas y Naturales, Microbiología y [2] Facultad de Farmacia y Bioquímica, Biotecnología, Universidad de Buenos Aires, Buenos Aires, Argentina.

In order to investigate the stabilisation properties of Tn*1000* insertions in ColE1 recombinants plasmids, the generation of high molecular weight aberrant replication structures (**HMW**) in *rec*BCD*sbc*B mutants which also resulted in plasmid instability, was studied in pUC plasmids. Into these *E.coli* genetic context, a shift of plasmid replication from the theta mode to a rolling circle mode cannot be reverted due to concomitant lack of ExoV and ExoI activity. We showed that the presence of the transposon in a pU18 plasmid substantially reduces HMW formation. However plasmid stability was not increased, probably due to the site of the transposon insertion which was only some base pairs appart from the plasmid replication origin. In order to control this location and to restrict the probable transposon sequences responsible for these properties, the site-specific-recombination system from Tn*1000* (*res* and resolvase) was cloned into the multi-cloning site of pUC. The resulting recombinant plasmid has proven to reduce significantly HMW formation as well as plasmid instability.

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IMPROVEMENT OF CATABOLIC ABILITIES TOWARD CHLOROPHENOLIC COMPOUNDS IN BACTERIA. [1]Céspedes,R., [1]Varela,C., [2]Jakobs,B., [2]Pieper,D., and [1]González,B. [1]Microbiology Unit. Fac. Ciencias Biológicas. P Universidad Católica de Chile, Chile. [2]Biodegradation Group, Gessellschaft für Biotechnologische Forschung mbH, Braunschweig, Germany.

Acinetobacter junii 5ga, a guaiacol (o-methoxyphenol) degrader is unable to metabolize chloroguaiacolic pollutants, because toxic intermediates like chlorosubstituted catechols and protoanemonin (González *et al*, Appl.Env.Microbiol, 59:3424,1993; Blasco *et al*, J.Biol.Chem. 270:29229, 1995) are accumulated. On the other hand, plasmid encoded catabolic operons *tfd*CDEF and *tcb*CDEF from strains *Alcaligenes eutrophus* JMP134 (pJP4) and *Pseudomonas* sp. (p51), respectively, allow them to degrade chlorocatechols. The long-term purpose of this work is to improve the catabolic ability toward chlorinated guaiacols in *A. junii* 5ga, through the introduction of a chlorocatechol-degrading operon. In the present report, the construction of DNA cassettes containing the *tfd*CDEF or *tcb*CDEF operons in mini-Tn5 delivery system vectors is described. Such system allowed the stable chromosomal insertion of both DNA cassettes. The adequate expression of the enzymes from these cassettes was followed by growing *Pseudomonas putida* KT2442 on 3-chlorobenzoate. The presence of these catabolic genes prevented the accumulation of 3-chlorocatechol observed in the wild type strain.

Supported by FONDECYT 1950075 and 1960262, and the collaborative grant 95005 from FUNDACION ANDES/CONICYT and BMBF-FZK/Karlsruhe. R. Céspedes is a FUNDACION ANDES Ph.D. fellow.

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CHARACTERIZATION OF MUTATIONS WHICH AFFECT PRODUCTION OF MICROCIN J25

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A general search was initiated to identify chromosomal mutations in *E. coli* that reduce production of the peptide antibiotic microcin J25 (MccJ25), which is encoded by the low-copy-number plasmid pTUC100. To avoid abnormal regulatory effects due to titration of regulatory proteins by the excess copies of DNA-regulatory elements resident on multicopy plasmids, we started by cloning the microcin genes in the low-copy-number vector pMM6176, generating plasmid pMJ100. The *E. coli* strain RYC1000 transformed with pMJ100 was mutagenized with transposon Tn*5*. Two chromosomal insertions that caused decreased MccJ25 production were isolated. These strains were designated MJ120 and MJ40. MJ120 is auxotrophic for cysteine. Mapping experiments localized the insertion to the 26- to 30-min region of the *E. coli* genetic map. This raises the interesting posibility that *cysB* (28 min) is the gene affected by the insertion. It could well be that *cysB*, which encodes a transcriptional activator protein involved in cysteine biosynthesis, is also required for efficient MccJ25 production. In strain MJ40, the Tn*5* insertion mapped to the 93- to 95-min region of the *E. coli* genetic map. To locate precisely the position of the inserts, we are currently trying to clone in vivo both mutations.

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IDENTIFICATION OF THE *R. salmoninarum* GLUCOKINASE GENE.

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R. salmoninarum, the etiological agent of BKD is able to enter different cell types both *in vivo* and *in vitro*. To identify the gene(s) involved in the invasion process, we screened a *R. salmoninarum* genomic library, constructed in *E. coli* HB101, searching for clones able to enter the embrionic salmon cell line CHSE-214.The sequence analysis of the 2282 bp insert corresponding to one of the selected clones showed two possible ORFs. Unidirectional deletion of the insert suggested that the ORF located to the 5'end was important for the invasive character of the recombinant bacteria. By comparison with sequences on data bases, we found a 43% residue identity between this ORF and the glucokinase (glk) gene of *S. coelicolor*. The expression of this gene was assessed by Northern blot analysis and RT-PCR. To determine if this ORF corresponds to the glk gene of *R. salmoninarum*, we performed genetic complementation of an *E. coli* mutant lacking glucokinase activity. The mutant strain transformed with the plasmid pPMV189 displayed a 100 fold increase in Glk activity. The insert does not contain the glk promoter since the mutant transformed with the plasmid carriyng the insert in opposite orientation showed no increase of Glk activity above the basal level. We cloned the coding region in the expression vector pGEX-4T2 to overproduce a GST fusion protein. The overexpression of the fusion protein was checked both by PAGE-SDS and measurement of the glucokinase activity. Although the identification of the *R. salmoninarum* glk gene was confirmed, its involvement in the invasion process seams unlikely considering that no metabolic enzyme has been involved in the invasion mechanism of any bacteria. **FONDECYT 2950080 , 1951195 & DID F-96-02**

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SALMONELLA TYPHI GENES INVOLVED IN ANAEROBIC RESPIRATION ARE REQUIRED FOR BACTERIAL INVASION OF Hep-2 CELLS.

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By using MudJ (Kan, *lac*)-directed operon fusion technology, mutants of *Salmonella typhi* whose expression is induced under anaerobic growth conditions were isolated. Characterization with respect to their phenotypes and regulatory properties revealed that two mutants were unable to grow anaerobically in minimal medium containing glycerol and sodium nitrate, suggesting that they were defective in nitrate reductase activity. The anaerobic induction ratio in rich medium did not further increase in response to nitrate. Strains carrying an additional mutation in *oxrA* were constructed, showing a lower level of ß-galactosidase expression both aerobically and anaerobically, however the ratios of anaerobic induction remained unaltered. One of these mutants corresponded to *mod*(*chlD*)::MudJ while the other corresponded either to *moa* (*chlA*) or *mob* (*chlB*)::MudJ. A third fusion which was unable to use either nitrate or fumarate as terminal electron acceptors appeared to occur within a regulatory locus; its anaerobic induction ratio was significantly reduced by the *oxrA* mutation. All mutants showed a reduced ability to enter to and proliferate within HEp-2 epithelial cells, suggesting a role for genes involved in anaerobic metabolic pathways in *S. typhi* invasiveness.

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CHANGES IN DNA TOPOLOGY IN *Bacillus subtilis* TOWARDS THE COLD-SHOCK RESPONSE.

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Plasmid DNA isolated from cells of *Bacillus subtilis* growing at 20°C is significatively more negatively supercoiled than the equivalent DNA aislated from cells growing at 37°C (Grau et. al., Mol. Microbiol.11, 933-941). To investigate the mechanism by wich the decrease in growth temperature increase the DNA supercoil, cultures of *B. subtilis* were shifted from 37°C to 20°C in the presence of rifampicin or chloramphenicol.The results of these experiments revealed that a full increase of DNA supercoil at low growth temperatures requied transcription and translation.

To determine whether the temperature-dependent change of DNA supercoil was related with stress mechanisms, we quantified DNA topological changes in well defined mutants blocked in either Sigma B, a general stress transcription factor, or CspB, the major cold-shock protein of *B. subtilis*. These experiments indicate that changes in DNA supercoil were not related with the activity of proteins involved in stress adaptative response.

Since hydrolysis of ATP is the driving-force behind the introduction of negative supercoils by DNA gyrase, we varied the ATP/ADP ratios using protonophores and uncoplers. These experiments showed that DNA supercoil in *B. subtilis* at differents growth temperatures depends, in part, of the phosphorylation potential.

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COMPARISON OF SECRETED PHOSPHATASE ACTIVITY BETWEEN TRYPANOSOMATIDS PARASITES OF PLANTS AND OF HOUSE FLIES. Dutra, P.M.L., Rodrigues, C.O., Lopes, A.H.C.S., Grillo, L.A.M. and Meyer-Fernandes, J.R.*. Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Brasil. *Departamento de Bioquímica Médica, UFRJ, Brasil.

Some flagellate protozoan of the family Trypanosomatidae are known to be pathogenic for several economically important plants. Phosphatase activity has been characterized in some members of the family Trypanosomatidae, such as *Trypanosoma* and *Leishmania*, which are pathogenic for humans and other mammals. In this work, we have investigated the presence of secreted phosphatase in two species of *Phytomonas* (*P. serpens* and *P. françai*) and in *Herpetomonas mcgheei*, which are capable of infecting plants, and in *Herpetomonas muscarum muscarum*, which is strictly a parasite of house flies. *P. serpens*, *P. françai* and *H. m. muscarum* have showed a very defined secreted phosphatase activity. On the other hand, *H. mcgheei* have not showed any detectable secreted phosphatase activity. Sodium tartrate is known to inhibit secreted phosphatase of *Leishmania* species. The secreted phosphatase activity of *P. françai*, *P. serpens* and *H. m. muscarum* was significantly sensitive to sodium tartrate. When intact cells or membrane enriched fractions of *P. serpens* and *H. m. muscarum* were treated with sodium tartrate, a decrease of phosphatase activity was observed, although *P. françai* and *H. mcgheei* were not sensitive to sodium tartrate treatment.

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LOCATION OF THE GLUCOSYL 1-PHOSPHATE TRANSFERASE DOMAIN IN THE *XANTHOMONAS CAMPESTRIS GUMD* GENE PRODUCT.

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Xanthomonas campestris produces an acidic exopolysaccharide, xanthan, composed of polymerized pentasaccharide repeating units. Previous studies demonstrated that the repeating units are produced by sequential transfer of glucosyl 1-P, glucose, mannose, glucuronic acid, and mannose residues from UDP-glucose, GDP-mannose, and UDP-glucuronic acid to a polyisoprenyl phosphate carrier. The 16 kb *xps* or *gum* gene cluster encodes the transferases required for repeating unit assembly. We are studying the *gumD* gene, encoding a 484 aminoacid protein. GumD mutant produces in vivo no detectable amounts of xanthan, and in vitro showed no ability to synthesize any glycolipid intermediate (Katzen, F. et al., this congress). The GumD protein is homologous to the 476 aa RfbP protein of *Salmonella enterica*, responsible for the the transfer of galactosyl 1-P from GDP-galactose to undecaprenol phosphate. The C-terminal half of GumD is also homologous to the 226 aminoacid ExoY protein of *Rhizobium meliloti*, which is thought to transfer a galactosyl 1-P residue to polyprenol phosphate, the first reaction of succinoglycan synthesis. Results from subcloning and complementation analysis, as well as in vitro synthesis of the glycolipids intermediates, indicate that the interaction with polyisoprenyl phosphate and the glucosyl 1-P transferase activity reside in the C-terminal half of GumD (from aa 290 to 484).

This work was supported partly by grant Ex-240 from UBA.

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LYSOSOMAL GLYCEROPHOSPHOCHOLINE PHOSPHO-DIESTERASE IN *TETRAHYMENA*. Florin-Christensen, M.[1], Guberman, A.[1], Paratcha, G.[2], Florin-Christensen, J.[1] [1]INEUCI, CONICET-UBA, and Cátedra de Biología Celular, Ciudad Universitaria, Pab. II, 4° Piso, 1428-Buenos Aires, Argentina.

Lysosomal degradation of phospholipids in eukaryotes is known to proceed through three main pathways: a) attack by phospholipase C to generate diacylglycerol and phosphocholine, which are then, in turn, hydrolyzed by lipase and phosphatase activities, respectively; b) initial deacylation by phospholipase A_1, attack by phospholipase C of the lysophospholipid and, again, further degradation of the products by lipase and phosphatase activities; and c) complete deacylation of the phospholipids to yield glycerophosphocholine. So far, no lysosomal enzyme has been shown to break down this latter product, which accumulates in preparations of lysosomes of mammalian origin. The ciliate *Tetrahymena thermophila* releases various hydrolases of lysosomal origin to the extracellular culture medium, including phospholipase C and phospholipase A1 and acid phosphatase (AP). We have found that, by contrast to the mammalian lysosomes, *Tetrahymena* possesses strong glycerophosphocholine phosphodiesterase (GPC-PD) activity Further, upon chromatography on Sephadex G-75, DEAE cellulose and decyl-Sepharose 4B, the activity coelutes with AP activity. GPC-PD is also inhibited by AP inhibitors, suggesting that these two activities may reside on the same protein. With these results, *Tetrahymena* becomes the first eukaryote in which lysosomal GPC-PD activity is demonstrated.

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MECHANISM OF ACTION OF KETOCONAZOLE ON *TRYPANOSOMA CRUZI* EPIMASTIGOTES. Florin-Christensen, M.[1], Serrago, P.[1], Mirkin, G.[2], Lammel, E.[2], Isola, E.[2], Florin-Christensen, J.[1] [1]INEUCI, Ciudad Universitaria, Pab.II, 4° Piso, 1428-Buenos Aires and [2]Depto. de Microbiología, Facultad de Medicina, Paraguay 2155, Piso 13, 1113-Buenos Aires, Argentina.

Azole antimycotics, like ketoconazole, inhibit cytochrome P450-dependent demethylation of lanosterol and other methylated sterols. This results in two different effects: (1) depletion of mature demethylated sterol forms and (2) accumulation of methylated precursors. The growth inhibitory effects of these drugs could result from (1), (2) or a combination of both. To decide between these possibilities in the case of *T. cruzi* epimastigotes, we took the following approach: If depletion of sterols mediates the toxicity of ketoconazole, then, exogenous supply of mature normal sterols should relieve its growth inhibitory effects. On the other hand, if the accumulation of methylated precursors is the crucial mechanism, then, lipids from ketoconazole-treated cells should be inhibitory *per se*. *T.cruzi* epimastigotes were grown in LIT-S medium, in the presence of 0, 1 or 10 µg/ml ketoconazole with or without various concentrations of lipids extracted from *T.cruzi* cells, either treated or untreated with 1 µg/ml ketoconazole. Growth was measured turbidimetrically for 9 days. We observed that normal *T.cruzi* lipids (up to 0.5 mg of lipid/ml) did not relieve ketoconazole toxicity. On the other hand, lipids extracted from ketoconazole-treated cells (down to 0.05 mg of lipid/ml) completely prevented growth, while normal lipids in the same concentrations were without effect. Our results strongly indicate that, in *T.cruzi* at least, accumulation of toxic sterol precursors appears to be the main event underlying ketoconazole toxicity. *(Supported by CONICET)*

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Mg^{2+} AS AN EXTRACELLULAR SIGNAL: ENVIRONMENTAL REGULATION OF SALMONELLA VIRULENCE. García Véscovi, E., Soncini, F.C, and Groisman, E.A. Facultad de Ciencias Bioquímicas y Farmacéuticas, Univesidad Nacional de Rosario, Rosario, Argentina, and Washington University School of Medicine, St. Louis, MO, USA.

The PhoP/PhoQ two-component system controls essential pathogenic properties of the facultative intracellular parasite *Salmonella typhimurium* including intramacrophage survival, resistance to host defense antimicrobial peptides and to acid pH, invasion of epithelial cells, the formation of spacious vacuoles, and the presentation of antigens by phagocytic cells. While the expression of these virulence determinants is modulated by PhoP, the physiological signal that governs the regulatory system has remained unknown.

Ions are traditionally thought to act as second messengers or as cofactors in signal transduction cascades. However, while searching for genes regulated by the PhoP/PhoQ regulatory system we recovered *mgtA* and *mgtCB*, encoding two high affinity transporters whose expression is increased in low Mg^{2+} media. To determine whether Mg^{2+} is the signal modulating the whole PhoP/PhoQ system, we evaluated the gene expression pattern of the PhoP-activated genes. Growth in physiological concentrations of divalent cations repressed transcription of PhoP-activated genes and rendered wild-type *Salmonella* phenotypically $PhoP^-$ when assayed for susceptibility to antimicrobial peptides. Mg^{2+} changed the conformation of PhoQ through interaction with its periplasmic domain identifying PhoQ as the first Mg^{2+} sensor. Furthermore, a point mutation in the sensing domain of PhoQ altered the set point for Mg^{2+} and rendered *Salmonella* avirulent. This result establishes that a finely tuned response to environmental Mg^{2+} is essential for the pathogenic properties of *Salmonella*.

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BACTERICIDAL ACTION OF STREPTOMYCIN. Diniello, G., Algranati, I.D. and Goldemberg, S.H. Instituto de Investigaciones Bioquímicas Fundación Campomar, Buenos Aires, Argentina.

The bactericidal action of streptomycin has not yet been completely understood. The antibiotic induces a marked increase of misreading and premature termination of translation and cells synthesize aberrant proteins; some of these are incorporated into the cell membrane and lead to permeabilization, with further uptake of the drug and subsequent complete blockage of protein synthesis.

We have shown that the effect of streptomycin both on protein synthesis and lethality of the antibiotic depends on normal intracellular levels of polyamines in bacteria.

In order to determine a possible differential site of streptomycin action, polyamine-depleted and supplemented bacteria were labelled with ^{35}S-methionine, with or without the antibiotic. Analysis of the different subcellular fractions indicated that putrescine-supplemented bacteria after streptomycin treatment incorporated 70% in the periplasm and 350% in the detergent-insoluble fraction (consisting mostly of abnormal peptides), compared to the culture without antibiotic, while polyamine-depleted cells showed 150% and 100% labelling, respectively.

The results suggest that the differential misreading and proteolysis elicited by streptomycin could explain the dramatically higher lethality observed in the presence of polyamines.

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Identification of *Lactobacillus delbrueckii* subspecies by soluble cell protein SDS-PAGE

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The study of soluble cell protein has been applied to bacterial identification..

By comparison of protein fingerprints in SDS-PAGE, genera and species have been differentiated among lactic-acid bacteria. However this methodology was not used for studying species *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus delbrueckii* subsp. *lactis*.

The aim of this work is to determine if this method can be used to differenciate strains of the subspecies *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus delbrueckii* subsp. *lactis*.

Five strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and six strains of *Lactobacillus delbrueckii* subsp. *lactis*, isolated in our laboratory from raw milk, industrial starters and comercial yogurt were used. Bacteria were grown in MRS and harversted in stationary phase. Cell free extracts were obtained by treatment with lysozime 10 mg/ml and SDS. Samples were analysed by gradient (10-18%) and continuous (12.8%) SDS-PAGE.

All strains have shown characteristic bands of *Lactobacillus delbrueckii*. *Lactobacillus delbrueckii* subsp. *bulgaricus* strains showed 16 coincident bands and 10 different bands when compared with *Lactobacillus delbrueckii* subsp. *lactis*. Differences between subspecies have been found in the MW and in the intensity of some of the bands. This method allows to differenciate strains of the same species. By computerized numerical analysis of electropherograms, the strains were grouped in two clusters with a simple matching coefficient of 75-80%.

The results attained demonstrated that this methodology can be used to distinguish strains of different subspecies.

[1] CONICET [2] CIC

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PURIFICATION TO HOMOGENEITY AND PARTIAL CHARACTERIZATION OF A NEURAMINIDASE OF *TRITRICHOMONAS FOETUS*. Marcelo E. Guerin, Alberto C. C. Frasch and Daniel O. Sánchez. Instituto de Investigaciones Bioquímicas "Fundación Campomar" Buenos Aires, Argentina.

Neuraminidase cleaves terminal sialic acid residues from glycoproteins, glycolipids and oligosaccharides. This enzyme is considered a virulence factor in several microorganisms. We have isolated a neuraminidase from the protozoan parasite *Tritrichomonas foetus*, the causative agent of bovine trichomoniasis. The enzyme was purified more than 7500-fold to apparent homogeneity through chromatography and size fractionation. A molecular weight of 160000 was estimated by gel filtration. The purified protein migrated as a single band of 80000 in a silver-stained denaturing polyacrylamide gels, suggesting that the enzyme is a dimer consisting of two subunits of similar size. This neuraminidase exhibited a broad pH optimum between 5.0 to 6.5 in sodium acetate and sodium phosphate buffers using MU-NeuAc as substrate. The apparent K_m for the hydrolisis of MU-NeuAc was 0.16 mM. The enzyme showed no metal requirements and was fully active in the presence of EDTA. The N-terminus of the purified enzyme will be sequenced and primers will be design to isolate the corresponding gene.

We acknowledge the financial support from TDR-WHO, Universidad de Buenos Aires and CONICET.

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CHARACTERIZATION OF THE BACTERIOCIN PsVP-10.
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Bacteriocins are a heterogenous group of proteins of varying molecular mass and biochemical properties that exhibit bactericidal activity against strains and species closely related to the producer culture.

In this study we surveged the production of a bacteriocin-like substance by a strain of *Pseudomonas* sp. isolated from well-water. The new bacteriocin, PsVP-10, was purified from a culture fluid of *Pseudomonas* sp. mainly by extraction with $CHCl_3$ and cation-exchange chromatography. The purity of the sample was confirmed by RP-chromatography. Aminoacid analysis of the purified bacteriocin revealed the absence of alanine, which may be due to the presence of modified alanines. Since it is known that *Pseudomonas* synthetize siderofores, we analysed the production of the bacteriocin in a culture medium in the presence of Fe^{3+}. The results indicated that PsVP-10 was produced in the presence of this ion. We have also demostrated that the bacteriocin is encoded by chromosomal DNA. Cured strains from the plasmids were able to produce the bacteriocin.

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OSMOTIC REGULATION OF CYCLIC β(1-2)GLUCAN SYNTHESIS. Nora Iñón de Iannino and Rodolfo A Ugalde. Instituto de Investigaciones Bioquimicas. CONICET. Buenos Aires. Argentina.

Brucella, *Rhizobium* and *Agrobacterium* form and secreted cyclic β (1-2)glucans. In *Rhizobium* and *Agrobacterium* this synthesis is osmoregulated. In *Brucella* spp. the synthesis of cyclic β (1-2)glucan is not affected by high osmolarity. Bacteria respond to changes in medium osmolarity by varying the concentration of specific solutes in order to maintain constant turgor pressure. In *Rhizobium* and *Agrobacterium* the cytoplasmic concentration of K^+ and glutamate are increased in response to increment of the osmolarity of the growth media. *In vitro* studies revealed that *Brucella* cyclic β(1-2)synthetase is insensitive to 0.5 molar of KCl or 0.5 molar of K-glutamate, two solutes that strongly inhibited the *in vitro* activity of *Rhizobium* and *Agrobacterium* cyclic β(1-2)glucan-synthetase. Recombinant *Agrobacterium* and *Rhizobium* β (1-2)glucan mutants expressing *Brucella* cyclic β(1-2) glucan-synthetase were not inhibited by increasing the osmolarity of the medium. We proposed that the effect of osmolarity on cyclic β(1-2)glucan synthesis is due to a direct inhibition of the enzymatic activity of the β(1-2)glucan-synthetase by the solutes accumulated by the bacteria when the osmolarity of the media was raised.

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***Xanthomonas campestris gum* mutants: xanthan biosynthesis and pathogenesis.** Katzen, F., Ielmini, V., Ferreiro D., Oddo, C., Becker, A., Pühler A., and Ielpi, L. I. I. B. Fundación Campomar, Universidad de Buenos Aires, Buenos Aires. Argentina and Lehrstuhl für Genetik, Fakultät für Biologie, Universität Bielefeld, Bielefeld, Germany.

Xanthomonas campestris pv. *campestris* is a gram-negative bacterium which is phytopathogenic for cruciferous plants. It produces an extracellular acidic polysaccharide termed xanthan.

The biosynthetic pathway of the xanthan can be divided into three parts: (i) the conversion of simple sugars to nucleotidyl derivatives precursors, (ii) the assembly and the decoration of pentasaccharide subunits attached to an endogenous polyprenol phosphate carrier, and (iii) the polymerization of pentasaccharide repeat units and their secretion.

Several chromosomal regions described as *xpsIII*, *xpsIV*, *xpsVI* and a 35.3 kb gene cluster are related to the first step of the xanthan biosynthesis, while the rest of the biosynthetic pathway functions are proposed, although not definitively proved, to be encoded by the *gum* region. This region spans 16 kb in the *X. campestris* genome and can be divided into 12 open reading frames (*gumB* to *gumM*).

In this work we analyzed several *X. campestris* mutants obtained by gene insertion and plasmid integration within different genes of the *gum* region. The results showed that sugar, pyruvyl and acetyl transferases are encoded by the *gum* region. We also studied whether xanthan gum is required for plant virulence. Results obtained with *gumI)* mutant showed that xanthan is involved but not essential for pathogenesis.

This work was supported partly by grant Ex-240 from U.B.A.

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CRYOPRESERVATION OF *BIFIDOBACTERIUM*: EFFECT OF SUGARS ON VIABILITY, METABOLIC CAPABILITY AND BILE TOLERANCE.
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Bifidobacterium are non-spore forming, anaerobic, Gram+ bacteria that exert benefical effects on their hosts, so they are included in probiotic products. The maintenance of properties of the strains such as growth rate, acid production and bile tolerance, is very important. An adequate manteinance of these properties is ensured by a correct preservation of the microbial strains.

In this study, we evaluated the cryoprotective effect of sucrose and trehalose on *Bifidobacterium* strains: CIDCA 531 and CIDCA 5317. The strains grown to early stationary phase, were frozen in an ethanol bath (-80°C) with and without the sugars at final concentration of 0.3M. We evaluated metabolic capacities (kinetics of growth and acidification) and bile tolerance (plating in solid media with bilis at 0.5%) of both strains frozen with and without both cryoprotectors, before and after 10 days at -80°C.

The results showed that:

a) Both sugars protected effectively metabolic properties and viability of strain 531. Strain 5317 showed better viability in trehalose than sucrose, but these were both smaller than the control without freezing. This strain was unable to grow after freezing.

b) Sucrose and trehalose have different ability for recovery of strains in solid media with bile. Sucrose was better for strain 531 -resistant to 1.0% bile-, and trehalose for strain 5317 -sensitive to 1.0% bile-.

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EXPRESSION IN *Escherichia coli* OF THE GENETIC DETERMINANTS FOR THE PRODUCTION AND IMMUNITY OF MICROCIN E492 FROM *Klebsiella pneumoniae*. Wilkens, M., Villanueva, J.E., and Lagos, R. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile.

Microcin E492 is a polypeptide antibiotic of Mr 6000 that is produced and excreted by *K. peumoniae* RYC492 and is active on strains of *E. coli* and other bacteria from the family *Enterobacteriaceae*. The mechanism of action is through membrane depolarization induced by the formation of pores in the bacterial membrane, and it is the only microcin described so far that is mainly produced in the exponential phase of growth.

The genetic determinants for microcin synthesis and immunity were cloned in E. coli VCS257 into the cosmid vector pHC79 starting from total DNA from *K. pneumoniae* RYC492. The microcin E492 expressed in *E. coli* presented several identical properties than that of produced by *K. pneumoniae*, among them the same molecular weight and the ability to form ionic channels in planar phospholipid bilayers. Microcin E492 expression was mainly in the exponential phase of growth, declining in the stationary phase. The immunity was also produced in the exponential phase of growth and was found to disappear in the stationary phase. The expression pattern of the immunity was also studied using a *rpoS* defective host, and was independent on the expression of σ^s.

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MICROBIAL DEGRADATION OF TRICHLOROPHENOLS. Matus,V[1], Valenzuela,J[1], Zenteno,P.[1], Padilla,L[1], Bumann,U[1,2] and B.González[1]. [1]Lab. de Microbiología. Facultad de Ciencias Biológicas. P. Universidad Católica de Chile. Chile. [2]Technische Universität Berlin. Institut für Verfahrenstechniks, Berlin, Germany.

Trichlorophenols are priority pollutants. In this work, the microbial ability to degrade 2,4,5- and 2,4,6-trichlorophenol (245-TCP and 246TCP) was assessed in several biological systems. Up to 150 ppm of 245TCP were extensively mineralized (measured as $^{14}CO_2$ evolution) in a soil not previously exposed to chloroorganics. On the other hand, no degradation of 245TCP was observed in a chloroorganic containing industrial effluent and in sediment or water column samples from a river exposed to chloroorganic pollution. On the contrary, the closely related compound 246TCP was removed in a soil and an industrial effluent after a short adaptation period. Isolation of strains degrading 245TCP as sole carbon source has been not possible. However, 246TCP degrading strains were obtained after enrichment of water column and sediment river samples. These strains are unable to degrade other trichlorophenols (245-, 236 and 235-TCP). On the other hand, a well-known chloroorganic degrader, *Alcaligenes eutrophus* JMP134, was found to grow on 246TCP and, after adaptation in chemostats, to mineralize 245TCP. The latter suggests that the microbial degradation of 246TCP and 245TCP may proceed through similar pathways.

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IDENTIFICATION, SEQUENCING AND FUNCTIONAL CHARACTERIZATION OF THE GENE CODING FOR PHOSPHO-ADENYL SULFOTRANSFERASE IN BACILLUS SUBTILIS. María C. Mansilla; de Mendoza, Diego. PROMUBIE. UNIVERSIDAD NACIONAL DE ROSARIO. ARGENTINA. Suipacha 531 (2000) Rosario.

Transposon Tn*917* mutagenesis of *Bacillus subtilis* BD170 followed by selection for lipoic acid auxotrophs led to the isolation of strain BD2620, which contained a single Tn*917* insertion in an open reading frame whose deduced amino acid sequence was 31% identical to that of *Escherichia coli* and *Salmonella typhimurium cysH* gene product. The *B. subtilis cysH* gene encodes a phospho-adenyl sulfotransferase, with a deduced molecular mass of 27 kDa. The insertional site was near the beginning of the open reading frame which was located at 139° of the *B. subtilis* chromosome, downstream the *pyrE* gene. The growth of the mutant strain in lipoic acid shows that *B. subtilis* can use this coenzyme as a source of sulfur for cysteine biosynthesis. Expression of *lacZ* fused to the *B. subtilis cysH* promoter was repressed by cysteine and sulfide and induced by sulfur limitation, indicating that *cysH* is controlled at the level of transcription.

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DETERGENT-BASED AQUEOUS TWO-PHASE SYSTEMS FOR *Acinetobacter calcoaceticus* LIPASE PURIFICATION. Bompensieri, S., Miranda, M.V. Cascone, O. and Nudel C.B.
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The use of bacterial lipases in the chemical and food industries is highly dependent on the development of simple and easy to scale-up purification strategies.
Due to the high enzyme hydrophobicity and its affinity for interfaces, detergents are potentially suitable for its selective extraction as well as to preserve its activity.
In this work we compare two detergents (Triton X-114 and a mixture of Triton X-100 and X-45) for lipase purification from an ***Acinetobacter calcoaceticus*** culture broth.
Direct extraction of lipase from the culture broth was achieved at pH 7.6 in both cases: while lipase partitioned almost exclusively to the detergent bottom phase (K between 0.007 and 0.020), total protein concentration was approximately the same in both phases. Yields were in the range of 80-85% with purification factors of 11-14 in a single step or up to 40 in multistep processes.
Results demonstrated the feasibility of detergent-based aqueous two-phase systems for lipase industrial purification from culture broths.

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EFFECT OF DRUGS UPON THE TRYPANOTHIONE AND GLUTATHIONE CONTENT IN DIFFERENT STRAINS AND FORMS OF *TRYPANOSOMA CRUZI*. Maya, J.D°., Coloma, L°., Repetto, Y°. and Morello° A. Departamento de Farmacología, Instituto de la Salud CES. Medellín, Colombia y Departamento de Bioquímica. Facultad de Medicina. Universidad de Chile. Chile.

The most commonly used drugs to treat Chagas Disease are nifurtimox and benznidazole. They act through free radical and electrophilic metabolites formation. Most of the oxidative stress defenses are low or absent in *T. cruzi*. However, glutathione (GSH), glutathionil-spermidine (GSH-SP) and tripanothione ($T(SH_2)$ may play an important role against free radicals. The free thiol concentrations (GSH, GSH-SP and $T(SH)_2$) in the epi, trypo and amastigote forms in different strains of the parasite were studied. Also, the effect of nifurtimox and benznidazole upon the free thiol status in *T. cruzi* was determinated in order to explain their toxicity. The clone Brenner free thiol concentrations in epi and trypomastigotes were 1196,3◆73,5 and 892,9◆76,8 nmol/g fresh weight respectively. For the LQ strain, they were 977◆90,7 and 999,5◆48 nmol/g fresh weight respectively. Clone Dm28c contained 612,0◆24,2 and 661,0◆24,2 nmol/g fresh weight respectly. The most abundant thiol was tripanothione (62-72%). When the epi or trypomastigotes forms of the parasite were treated with 20 uM nifurtimox or 100 uM benznidazole, the concentrations of all three reduced thiol decreased strongly. The amount of tripanothione was the most affected, decreasing over 80%. In summary, we concluded that free thiol concentration vary among the forms and strains of *T. cruzi*. Nifurtimox and benznidazole may produce their toxic effect by lowering the reduced thiols in *T. cruzi*, and further drug metabolites conjugation with macromoleculas. Supported by SIDA-Sweden and Fondecyt 1961095/1996.

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CHOLINE TRANSPORT AND ITS RELATIONSHIP WITH THE Pi CONCENTRATION AND OSMOTIC STRESS IN *Pseudomonas aeruginosa*. Pallotti, C., Lucchesi, G.I., Salvano, M.A., Lisa, A.T. y Domenech, C.E. Dpto. Biología Molecular, FCEFQN, Universidad Nacional de Río Cuarto, Cba. Argentina.

We have previously shown that *P. aeruginosa* grown in a medium with choline synthesizes an active transport system formed by, at least, two components: one of high affinity (Km=3μM) and another of low affinity (Km=400μM). Other authors have shown that the inorganic phosphate (Pi) transport in cells grown in a low Pi medium is mediated also by two components, one of high affinity (Km~1μM) and another of low affinity (Km~10μM). Since the synthesis of some enzymes dependent on the Pho regulon may be also induced by the presence of choline in the culture medium, we hypothesized that the Pi concentration might also be controlling the expression of an additional choline transport. Therefore, we selected culture media containing high (39mM) or low (0,25mM) Pi, with choline or succinate as the carbon source. This work shows that *P. aeruginosa* grown in the presence of choline in a low-Pi medium displays the same choline uptake as that detected in a high Pi medium. The characterization of both systems, by the utilization of spheroplasts and periplasmic extracts, indicated that the high-affinity component was dependent on choline-binding proteins. The low-affinity component could be detected in intact spheroplasts. An additional component for the choline uptake (Km=25μM) was detected in bacteria which were grown in the absence of choline in a low Pi medium with succinate and ammonium chloride as the carbon and nitrogen sources. This system may be also differentiated by its insensitivity to osmotic shock produced by the presence of high concentrations of NaCl or sucrose. These findings demonstrate an analogy to the low affinity Pi transport system which is resistant to osmotic shock.

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CRUZIPAIN: MEMBRANE ISOFORMS AND THEIR EXPRESSION IN DIFFERENT STAGES OF *TRYPANOSOMA CRUZI* . F. Parussini, V. G. Duschak and J.J. Cazzulo. Instituto de Investigaciones Bioquímicas "Luis F. Leloir" Fundación Campomar. Buenos Aires, Argentina. jjcazzulo@iris.iib.uba.ar

Cruzipain is the major cysteine proteinase (CP) present in *Trypanosoma cruzi*. Its expression is developmentally regulated, levels in epimastigotes being one to two orders of magnitude higher as compared with the other parasite forms. Natural cruzipain is produced as a mixture of isoforms, probably due to the simultaneous expression of several genes. We have recently found evidence confirming the presence of a membrane isoform, which partitions in the detergent phase upon extraction with Triton X-114 and phase separation, and is liberated by treatment with phospholipase C from *Bacillus cereus*, thus suggesting that it is glycophosphatidyl inositol (GPI)-anchored to the membrane. When the epimastigotes were disrupted by grinding with silicon carbide and the homogenate was fractionated by differential centrifugation, the putative membrane form was enriched in the microsomal fraction, which is known to consist mostly of plasma membrane fragments. Triton X-114 extracts of epimastigote, amastigote and trypomastigote forms of the RA strain of the parasite presented this amphiphilic isoform, at different levels and with different electrophoretic mobility. Purification of total cruzipain isoforms from the three parasite stages was attained by affinity chromatography on cystatin-Sepharose. Electrophoretic analysis of the purified material obtained confirmed the differential expression of cruzipain isoforms along the parasite's life cycle. **Supported by SAREC (Sweden) and TDR/WHO.**

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EFFECTS IN *BACILLUS SUBTILIS* OF A CONDITIONAL LETHAL MUTATION IN THE ACETYL CoA CARBOXYLASE, THE FIRST ENZYME OF THE FATTY ACID SYNTHESIS. Perez, Carlos; Marini, Patricia; de Mendoza, Diego. PROMUBIE. UNIVERSIDAD NACIONAL DE ROSARIO. ARGENTINA. [Suipacha 531 (2000), fax:54-41-253058, email: CPEREZ@agatha.edu.ar]

A *Bacillus subtilis* strain was constructed in which the *accBC* operon coding for the biotin carboxylase (BC) and biotin carboxyl carrier protein (BCCP), subunits of acetyl CoA carboxylase (ACC) was placed under control of the isopropyl-ß-D thiogalactoside (IPTG) inducible *spac* promoter. This strain was dependent upon the presence of IPTG for growth, lipid synthesis and expression of the BCCP subunit, indicating that *accBC* is an essential operon in this organism. The mutant could grow in medium containig branched fatty acids and the fatty acid composition of cellular lipids depends of the fatty acid used to support growth. In sporulation media this strain increased in mass in the presence or absence of IPTG, but only sporulated in the presence of IPTG. This finding illustrates the importance of fatty acid synthesis in cellular differentiation.

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CELL WALL FROM *MUCOR ROUXII*: ULTRASTRUCTURE AND PROTEIN COMPOSITION. Pereyra, E., Moreno,S, Rico, H., Mormeneo, S. and Sentandreu, R. Dpto. Química Biológica, Fac. Ciencias Exactas y Naturales, UBA, Argentina, Dpto. Microbiología, Fac. Farmacia, Universidad de Valencia, España.

Mucor rouxii spores grow isodiametrically in the presence of cAMP analogs and polarized growth is prevented. Cellular volume of rounded cells is increased when cells are cultured for several hours; under these conditions we observed that the cell walls were fragile and showed abnormal characteristics under a light microscope.

We have studied the ultrastructure of the cell walls of growing spores by TEM and have observed that the cells treated with cAMP analogs have increased several times their wall thickness, and show alteration in the depostion of its components.

There are many data supporting the idea that the temporal and spatial regulation of the synthesis and assembly of wall polymers are critical for the properties conferred to the walls. It has been suggested that proteins within the cell wall serve as specific recognition molecules that join the different cell wall components. We have begun the study of the cell wall proteins of spores grown aerobically, anaerobically and with cAMP analogs in an attempt to know the differences between the culture conditions. Sequential treatment of the cell walls with chaotropic agents, quitinase and quitosanase were performed. SDS-PAGE electrophoresis developed with silver stain and Western-blot stained with Concanavalin A-peroxidase or biotin streptavidin-peroxidase showed preliminary differences that deserve further study.

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TRANS ACTIVATION OF *Escherichia coli ato* GENES BY A *Bacillus megaterium* REGULATOR. Pettinari, M.Julia , Vázquez, Gustavo J. and Méndez, Beatriz S. Departamento de Quimica Biologica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

A *B.megaterium* genomic library in *E.coli* HB101 using vector pHV33 was screened for β– ketothiolase activity, and a positive clone was found.

When the insert contained in this clone was sequenced, using both automatic and manual sequencing methods , its translation product was found to have a remarkable homology with many activator and sensor proteins, among them the activator and sensor proteins of the *ato* operon of *E.coli*. This operon codes for the synthesis of the enzymes of short chain fatty acid utilization; thiolase II and the acetil CoA:acetoacetate CoA transferase.

To further analyze the effect of the products of the cloned region, the transferase activity was measured. Both enzymes were constitutively expressed in *E.coli*, while no activity was detected in the same conditions in a strain without the insert containing plasmid.

This is the first report of *B. megaterium* proteins which are capable of activating an *E.coli* operon.

This work was supported by grants from UBA and CONICET

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DETECTION OF RESERVE POLYMERS SYNTHESIS GENES IN NATURAL BACTERIAL POPULATIONS. López, Nancy I. [1], Pettinari, M. Julia [2] and Méndez, Beatriz S.[2] Departamento de Ciencias Biológicas[1] y Departamento de Química Biológica[2] . Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Buenos Aires. Argentina.

Accumulation of intracellular storage polymers such as polyhydroxyalkanoic acids (PHA)s or polyphosphate (poly P) has been considered a biological advantage used by bacteria in a changing environment. We have previously seen that the capacity to accumulate PHAs endows bacteria with enhanced survival capabilities. However, the polymer could not be detected in natural samples using traditional biochemical methods. Our approach was to search for the genes responsible for the biosynthesis of storage polymers. Screening of the indigenous bacteria for the presence of these genes was performed in water samples from the Rio de la Plata using polymerase chain reaction (PCR) amplification combined with Southern blotting. PHA synthase genes and polyphosphate kinase (*ppk*) genes were detected using specially designed primers from template DNA from different laboratory and river isolated bacterial strains, and from total river water DNA. In this work we have shown that the genes responsible for the synthesis of reserve polymers are present in the natural bacterial community studied. This approach was also useful to asses the characteristics of a given community by their genetic information.

This work was supported by grants from UBA and CONICET.

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HYDROLYTIC ENZYMES SYNTHESIS BY ASPERGILLUS NIGER 3T5B8 UNDER DIFFERENT CONDITIONS. Pinto, G.A.S.[2]; Teixeira, M.V.V.G.[2] ; Bon, E.P.S.[2] and Couri, S.[1]

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The hydrolytic enzymes are largely employed on fruits and vegetables treatment for aid on extraction, maceration, liquefaction, filtration and clarification processes. An enzymatic complex production with high hydrolytic activity will benefit the brazilian biotechnology industry, which is in development state, and it shows the need of improvement on the country's technologies. This work's objective was to check over the effect of different carbon sources under two different operation conditions to obtain a pool of hydrolytic enzymes under submerged fermentation. The microorganism used was a mutant strain of *Aspergillus niger*. The fermentation was carried out in Multigen 1 of New Brunswick, containing 500 ml of culture media. At the first condition of operation (400 ppm and 0,5 vvm) 3 sources of carbon were tested: glucose, sucrose and starch plus inorganic salts at 32°C. In the second condition (500 rpm and 2.0 vvm), glucose and sucrose media were tested, at same temperature. All experiments have taken 72 hours, and samples were gotten each 12 hours. The cellular growth, the substract consumption and the activity of the following enzymes: polygalacturonase, cellulase, xylanase were determined. The results obtained have indicated that *Aspergillus niger* 3T5B8 produces polygalacturonase during the growth under glucose presence, therefore it is insensible to catabolic repression. It was a catabolic reoressor of cellulase and xylanase synthesis. The synthesis of this enzymes ocured only after total consumption of glucose.In the presence of sucrose, all the enzymes were produced There was no production of cellusase in starch.

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PURIFICATION AND CHARACTERIZATION OF ADP-GLUCOSE PYROPHOSPHORYLASE FROM *AZOSPIRILLUM BRASILENSE*. Polenta, G.A. and Iglesias, A.A. Instituto Tecnológico de Chascomús. INTECH. Casilla de Correo 164. Chascomús. 7130. Argentina.

Bacteria belonging to the genus *Azospirillum* are microaerophilic, nitrogen-fixing, Gram-negative rods, often associated with roots of cereals and plants, and playing a relevant role in agriculture as biological fertilizers. Studies on metabolic routes occurring in *Azospirillum* are scarce and the biosynthesis of polysaccharides in these bacteria is a subject poorly understood. In this work, it is determined the presence of ADPglucose pyrophosphorylase (EC 2.7.7.27; namely ADPG PPase) in extracts of *A. lipoferum* and *A. brasilense*. The enzyme from the later source was purified and characterized in its structural, kinetic and regulatory properties. Different compounds showed an allosteric regulatory effect on ADPG PPase from *A. brasilense*. P-enolpyruvate, Pi and ADP behaved as inhibitors; whereas fructose-6P, pyruvate, fructose-1,6-bisP, 3P-glycerate and, to a lesser extent, glucose-6P activated the purified enzyme. In the ADPG synthesis direction, fructose-6P, fructose-1,6-bisP and 3P-glycerate activated the enzyme about 10-fold with $A_{0.5}$ values of 0.13, 0.82 and 1.5 mM, respectively. Levels of ADPG PPase activity, together with its regulatory properties, suggest that the enzyme could play a relevant role in carbohydrates metabolism in the genus *Azospirillum*, and that these bacteria accumulate glycogen, in addition to poly-ß-hydroxybutyrate, as an intracellular storage polymer.

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ADAPTATION TO EXTREME pH IN THE CASE OF *Pseudomonas aeruginosa* AND OTHER MEMBERS OF THE GENUS *Pseudomonas*. G. Previtali and C. E. Domenech. Dpto. Biologia Molecuar, FCEFQN, Universidad Nacional de Rio Cuarto, Cba., Argentina.

The Gram negative bacteria belonging to the genus *Pseudomonas* represent a diverse collection of strains that may be found in a great variety of habitats and which are involved in many different processes. Therefore, these species contain members that can exist in the presence of different temperatures, pH, osmolarities, and other physical or chemical factors. The present work was focused upon the response of several members of the genus *Pseudomonas* when they were subjected to extreme conditions of pH. Experiments were carried out with bacteria grown in rich medium at different pH or with bacteria from the exponential phase suspended in isotonic sucrose solution. The results obtained may be summarized as follows: the extreme pH that support the growth of *P. aeruginosa* lie between 4.5 and 9.5; it was not possible to induce an adaptive system for conditions where the pH was below 4.5; when the initial pH of the culture medium was between 5 and 9 there were no appreciable differences in the lag period, in the doubling time of growth and in the cellular mass. With bacteria suspended in sucrose it was found that, at an alcaline pH, they released H^+ at a rate of 0.18 nmoles/min.OD; this system was inhibited by cyanide or arseniate in a reversible manner, N-ethylmaleimide was a powerful activator, but iodoacetamide, another blocking agent of -SH groups, did not affect this system; the ionofores CCCP or EDAC were also without effect. Bacteria suspended in acid media were able to increase the pH, but any of the above compounds were capable of modifying this system. At present, all the bacteria examined from the genus *Pseudomonas* contain a system similar to that described for *Pseudomonas aeruginosa*.

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ENZYMIC CHANGES IN *TRYPANOSOMA CRUZI* AFTER THEY HAVE INDUCED ERYTHROCYTE FUSION. M. S. Remedi, P. Scaraffia, M. Rodríguez, D. H. Bronia and N. M. Gerez de Burgos. Cátedra de Química Biológica. Facultad de Ciencias Médicas. Univ. Nacional de Córdoba, C.C. 35, Suc. 16, 5016 Córdoba, Argentina. E-mail: sremedi@biomed.uncor.edu.

Membrane fusion is the prerequisite for *Trypanosoma cruzi* invasion of host cells. It has been demonstrated that epimastigotes at declination phase in culture (day 14) are able to produce fusion of red blood cells in vitro (Calderón et al., Exp. Parasitol. 56: 159, 1983). We have used this model to study possible biochemical changes occurring in parasites during the phenomenon. The following enzymes were determined in *Trypanosoma cruzi* epimastigotes from 14 day old cultures exposed to eritrocytes: hexoquinase (HK), fructose-6-phosphate kinase (FPK), lactate dehydrogenase (LDH or isozyme II α-hydroxyacid dehydrogenase), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH), aspartate aminotransferase (AsAT), alanine aminotransferase (AlAT) and glutamate dehydrogenase - NADP (GDH).

Specific activity of the 3 glycolytic enzymes showed a moderate increase in parasites exposed to erythrocytes compared with those in the absence of red blood cells. Mean values were 17 % higher for HK, 20 % for FPK and 24 % for LDH. The Krebs cycle enzymes were decreased, 10 % for ICDH and 30 % for MDH. Enzymes related to amino acid metabolism did not show a uniform variation. There was a 50 % reduction in the GDH activity, and increases of 25 % for AsAT and of 40 % for AlAT.

Results suggest that fusogenic activity would be accompanied by an increment of glycolysis and a depression of the citric acid cycle. Enhanced LDH and AlAT activities could funnel the eventual pyruvate excess toward lactate or alanine production.

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CLONING AND CHARACTERIZATION OF THE GENES ENCODING THE BIOTINATED-SUBUNIT OF *Streptomyces coelicolor* A3(2) ACYL-CoA CARBOXYLASE. Rodríguez, E. and Gramajo, H. PROMUBIE (CONICET), Dpto. de Microbiología, Fac. de Cs. Bioquímicas y Farmacéuticas, Univ. Nac. de Rosario, Rosario, Argentina.

Streptomyces coelicolor A3(2) produces four known secondary metabolites, at least two of which (actinorhodin and undecylprodigiosin) are polyketide-derived antibiotics. Polyketide biosynthesis occurs by a series of reactions analogous to those by which fatty acids are made, so malonyl-CoA is the predicted substrate for all of the condensation reactions which yield these two antibiotics.

In all species, the malonyl-CoA used in the biosynthesis of fatty acids is produced by the carboxylation of acetyl-CoA by the action of the acetyl-CoA carboxylase (ACC). Studies made in several actinomycetes have shown that the ACC complex is formed by two subunits: a biotinated subunit of 64 kDa MW that has biotin carboxylase activity, and the carboxyltransferase component.

Using degenerated-oligonucleotides corresponding to the consensus sequence of the biotin-binding motif and the biotin carboxylase proteins, we amplified by PCR a DNA fragment of 1.4 kb. Single digests of *S. coelicolor* DNA were probed with this PCR-fragment, and two hybridizing bands appeared in all digests. Through a size-enriched library, we isolated, cloned and sequenced two copies of the gene corresponding to the putative biotinated subunit of the ACC complex. We are currently in the process of isolating mutants of these genes in other to understand their physiological role in the primary and secondary metabolism of this microorganism.

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Trypanosoma cruzi STRAINS **CA1** AND **RA** DIFFER IN POLYAMINE METABOLISM.

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Previously, we had found that *Trypanosoma cruzi* **RA** strain differs from other tripanosomatids in that putrescine is synthetised from arginine via agmatine. We wanted to extend this observation to **CA1** strain , which differs from **RA** in pathogenicity. This strain, grown in a polyamine free medium, showed a duplication time of about 32 hrs, with a polyamine content, at late logarithmic phase, of: putrescine 15, spermidine 22 and spermine 16 nmoles/10^9 parasites. No cadaverine could be detected.

Although we found growth inhibition when epimastigotes were grown in the presence of DFMO and DFMA -irreversible inhibitors of ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) respectively-, we could only detect $^{14}CO_2$ liberation from ^{14}C ornithine. ADC activity could not be found. DFMO showed a dose dependent action on the epimastigotes growth, with an ED_{50} =0.1 mM. Addition of putrescine restored both growth rate and putrescine content of parasites grown in the presence of DFMO. ODC activity could be detected 13 to 17 hrs after seeding the parasites, showing a maximum at 16 hrs. ODC K_M for ornithine was 0.5 mM.

These data show considerable differences, both in polyamine content and putrescine synthesis, between **RA** and **CA1** strains. **CA1** has a shorter duplication time, lower polyamine content, no cadaverine and synthetises putrescine from ornithine via ODC.

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INFLUENCE OF CHLORINE SUBSTITUTION ON THE DIHYDROXYLATION OF POLYCHLOROBIPHENYLS BY BIPHENYL-2,3-DIOXYGENASE FROM Pseudomonas sp. LB400.

Seeger, M.*#, Timmis, K.N.#, Jerez, C.A.* and Hofer, B.# *Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Santiago, Chile, #Department of Microbiology, GBF-National Centre for Biotechnology, Braunschweig, Germany.

Pseudomonas sp. LB400 is a gram-negative bacterium capable of oxidizing an unusually broad spectrum of polychlorobiphenyls (PCBs), one of the most widely distributed chlorinated pollutants in the environment. In order to determine the substrate spectra and the mechanism of biphenyl-2,3-dioxygenase (BDO) encoded by the *bph* locus of the strain LB400, PCB degradation products by *Escherichia coli* derivative overexpressing appropiate *bph* genes from LB400 was investigated. PCBs substituted in only one ring were hydroxylated by BDO at the *ortho* and *meta* positions of the non-chlorinated ring. In contrast, chlorinated congeners containing an *ortho*-monochlorinated ring after attack of BDO were found to undergo complete or partial dehalogenation at the *ortho*-chlorinated ring (Seeger, M., Timmis, K.N. and Hofer, B. 1995. FEMS Microbiol. Lett. 133: 259-264). All mono- and at least 8 of 12 diCBs were attacked by the BDO exclusively at *ortho* and *meta* carbons. Almost all the congeners chlorinated on both rings and with one ring mono-*meta*-chlorinated or chlorinated at positions 2 and 5 were attacked by the BDO also at *meta* and *para* carbons. These results indicate the relaxed regiospecificity of the BDO of LB400. The 3,4-dihydroxy derivatives of PCBs formed seem to be unable to be further degraded by subsequent enzymes of the pathway. The influence of the chlorine substitution pattern of the metabolized ring on the reactivity of initial dioxygenation resembled its influence on benzoate formation (Seeger, M., Timmis, K.N. and Hofer, B. 1995. Appl. Environ. Microbiol. 61: 2654-2658), suggesting that the rate of benzoate production may frequently be determined by the rate of initial attack by the BDO.

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THE MOLECULAR BASIS OF THE MAGNESIUM DEPRIVATION RESPONSE IN *Salmonella typhimurium*.

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The PhoP/PhoQ two-component system is essential for virulence in Salmonella typhimurium. This system controls expression of some 40 different proteins, yet most PhoP-regulated genes remain unknown. To identify PhoP-regulated genes, we isolated a library of 50,000 independent lac-gene transcriptional fusion strains and investigated whether production of ß-galactosidase was regulated by PhoP. We recovered 47 *lac*-gene fusions that were activated and 7 that were repressed when PhoP was expressed. Analysis of 40 such fusions defined some 30 loci including *mgtA* and *mgtCB*, which encode two of the three Mg^{2+} uptake systems of *S. typhimurium*; *ugd*, encoding UDP-glucose dehydrogenase; *phoP*, indicative that the *phoPQ* operon is autoregulated; an open reading frame with sequence similarity to VanX, a dipeptidase required for resistance to vancomycin; and *pmrCAB*, encoding another *Salmonella* two-component regulatory system that controls polymyxin resistance by modification of the net charge of the lipopolysaccharide. We established that transcription of PhoP-activated genes was regulated by the levels of Mg^{2+} in a PhoP-dependent manner, and that several PhoP-activated genes were essential for growth in low Mg^{2+} media. We identified that a subset of PhoP-regulated genes was under direct control of PmrA/PmrB system, and that these genes were essential to form colonies on low Mg^{2+} solid media. Cumulatively, our experiments establish that the PhoP/PhoQ system governs the adaptation of *Salmonella* to magnesium-limiting conditions by modifying both the rate of Mg^{2+} uptake and the requirements for the cation.

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GENOMIC ARRANGEMENT AND TRANSCRIPTIONAL ORGANIZATION OF GLYCOGEN (*GLG*) OPERON IN *AGROBACTERIUM TUMEFACIENS*.

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The structural genes for glycogen synthesis in *E. coli* are organized in two transcriptional units: *glgB-glgX* and *glgC-glgA-glgP(Y)*, (*glgB*: glycogen branching, *glgX*: glucan hydrolase, *glgC*: glycogen synthase, *glgA*: ADPGlc PPase and *glgP(Y)*: glycogen phosphorylase). Based on DNA sequence data, RT-PCR experiments and enzymatic assay of *glg* enzymes in three *glgB* polar mutants we concluded that *A. tumefaciens glg* cluster is organized as a single operon: *glgP-glgB-glgA-glgC*. Downstream of this operon and 8 bases of *glgA* mapped phosphoglucomutase (*pgm*). Insertion of a kanamycine resistant cassette in the *glgB* gene shows polar effect with no enzymatic activity of the downstream enzymes *glgA* and *glcC*. On the other hand, *pgm* activity was reduced by 80-20% depending on the mutant suggesting that an alternative promoter upstream of the *pgm* transcript might be present.

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EXTRACELLULAR METABOLISM OF OXALATE BY THE FUNGUS *Ceriporiopsis subvermispora*. Urzúa, U. y Vicuña, R. Laboratorio de Bioquímica, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile.

Several ligninolytic fungi secrete oxalic acid to the extracellular medium. Recent evidence suggests that this metabolite deriving from the metabolism of carbohydrates may play a direct role in lignin biodegradation.

Ceriporiopsis subvermispora is a white-rot basidiomycete with a ligninolytic system composed of Mn-dependent peroxidase (MnP) y laccase. As expected, oxalate was detected during the primary metabolism phase in liquid cultures of this fungus in salt medium containing glucose as carbon source.

In medium containing 11 ppm of Mn(II), the concentration of oxalate reached a maximum of 2 mM at day eight, whereas consumption of glucose ended at day ten. These cultures mineralized [^{14}C]-oxalate and produced about 0.6 units/ml of MnP. In contrast, when Mn(II) was ommited from the growth medium, glucose was only partially consumed, MnP was not detected and mineralization of [^{14}C]-oxalate was negligible.

In vitro experiments with an isoform of MnP showed that this enzyme oxidizes oxalate to CO_2 and hydrogen peroxide in a Mn(II)-dependent reaction. Based on previous information, it is proposed that trace amounts of Mn(III) trigger the oxidation of oxalate to CO_2 and formyl radical. The latter adds molecular oxygen to produce superoxide plus another molecule of CO_2. Then, in the presence of Mn(II), superoxide gives rise to hydrogen peroxide while Mn(II) is oxidized to Mn(III). This hydrogen peroxide oxidizes MnP peroxidase to compound I, which in turn oxidizes Mn(II) to Mn(III), thus accelerating the mineralization of oxalate.

Since *C. subvermispora* does not produce an extracellular oxidase such as glucose oxidase or glyoxal oxidase, the above information suggests that MnP itself generates the peroxide it requires to oxidize lignin in natural environments.

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PHOTOREACTIONS OF BIOLOGICAL POLYAMINES AND LYSINE SENSITIZED BY RIBOFLAVIN. J.L. Arroyo, L.Larrondo y E.Silva, Laboratorio de Química Biológica, Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile. jarroyo@hpc.puc.cl

Polyamines are present in cells up to millimolar concentrations and are known to play a series of fundamental roles in DNA replication, cell differentiation and growth regulation. Recently, the protective role of polyamines has been emphasized under oxidative stress conditions induced by a variety of stimuli. In this work the behavior of polyamine putrescine, spermidine, spermine and the basic aminoacid lysine is studied when exposed to the pro-oxidant action of riboflavin (RF) in excited state. The experiment was setup according to Silva et. al. (J.Photochem. Photobiol. B: Biol. 21, 197-201 (1993)). For all the amines studied an antioxidant effect was found when we irradiated with polychromatic light using RF as sensitizer. We found that oxygen consumption in the presence of the spermine and spermidine is 7,5 and 3,5 times higher respectively with respect to the observed oxygen consumed in the presence of putrescine. In reference to lysine that shows two amine groups as well as in putrescine, it was found that the oxygen consumption was similar to the amines. In all cases the amine groups interact with RF in the triplet state through charge transfer processes given rise to oxidized cation radicals together with RF anion radical. When these experiments were made in the absence of molecular oxygen, we were able to follow the kinetics for the photodescomposition of the sensitizer, which increases with increasing concentration of the amine. The reaction between RF in triplet state and the amines, was also favored with increased pH and therefore by uncharged amine groups. The present data indicate that polyamines, at physiological concentrations, could lead to a mild protection of substrate photo-oxidation induced by riboflavin.

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DETERMINATION OF ACID PHOSPHATASE AND DEHYDROGENASE ACTIVITIES IN SOILS FROM SOUTHERN CHILE AS MARKERS OF FERTILITY. Alvear, M., Ojeda, G. M. and Borie, F. Departamento de Ciencias Químicas, Universidad de La Frontera, Temuco, Chile.

The phosphatases present in the soil environment may play a significant role in P availability to plants from native soil organic P compounds and polyphosphate fertilizers. However, active dehydrogenases are considered to exist in soils as integral parts of intact cells and dehydrogenase activities are thought to reflect the total range of oxidative activities of the soil microflora.

The objective of the present work was to measure specific enzyme activities [acid phosphatase (P-ase) and dehydrogenase (DHG)] to evaluate the effects of agricultural practices on the residual microbiological fertility of soil ecosystems following their diverse utilization, that is, normal cultivation with crop rotation and as native undisturbed soil. P-ase was measured by means of p-nitrophenylphosphate method and DHG by quantification of red formazan produced.

Our results showed that intensive cultivation of soil affects negatively both enzyme activities, but not in native undisturbed soil. At present, the determination of specific enzyme activities, together with the use of chemical soil parameters, seems to be the best approach for evaluating the state of microbial activity and understanding its response to cultivation practices and environmental factors.

A major agricultural research priority is to sustain soil productivity and to develop better methods for monitoring the changes produced in physical, chemical and biological properties of soils, since they are affected by the habitually management systems used.

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BIOMARKERS OF ENVIRONMENTAL CONTAMINATION:
***Corbicula fluminea* IN THE RIO DE LA PLATA.**

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We have recently put forward a basis for using biochemical indicators to detect early effects of pollutants,in the Rio de la Plata,with particular reference to organic matter. *Corbicula fluminea* is a bivalve currently present in sediments of the river and potentially appropriate to be used in contamination surveys and monitoring programmes.

It was found after 72 hs exposure in sublethal 100 and 200 ppb solutions of rotenone or (PCP) pentachlorophenol that the biomarkers of the nervous system such as carboxylesterases and acetylcholinesterase were not significantly inhibited . Instead ,a general unspecific indicator like total protein level,was decreased in the range of 20% to 40%. The NADH-oxidase activity was significantly inhibited in individual basis ranging from 45% to 60% in rotenone 100 and 200 ppb solutions and 59% to 73% in 100 and 200 ppb PCP solutions. On the other hand, an importan xenobiotic metabolyzing system,such as Glutathione S-transferas did not show any significant inhibition in the conditions assayed.

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BIOSYNTHESIS OF SUCCINOGLYCAN IN *Agrobacterium tumefaciens*. Bassi, D., Mastronardi, L.O., Vojnov, A. and Dankert, M. Instituto de Investigaciones Bioquímicas, Fundación Campomar. Facultad de Ciencias Exactas y Naturales-U.B.A. y CONICET, Buenos Aires, Argentina.

Agrobacterium tumefaciens produces an extracellular polysaccharide (EPS) composed of repeating units (R.U.), each one containing glucose, galactose, pyruvate and succinate in a 7:1:1:1 molar ratio (Amemura et al. 1981, *Carbohydr. Res., 91, 59*). The proposed structure of the EPS. is shown below: -(-Glcβ1--4-Glcβ1--4Glcβ1--4Glcβ1--3Gal-)$_n$-

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Glcβ1--3Glcβ1--3Glcβ1--6Glcβ1

4 6

Pyr

As it has been established in other sistems, the biosynthesis takes place mainly in two stages (Ielpi et al. 1993, *J of Bacteriol*, 175, 2490). In the first one, the R.U. is made by tranferring sequentially the monosaccharides from the proper sugar nucleotides to a membrane bound prenyl phosphate lipid in an enzyme catalyzed reaction. Once the R.U. is completed, it acts as a monomer in a reaction of polimerization, being the EPS the final product.

The biosynthesis of the lipid-linked R.U. in *A. tumefaciens* has been studied in this Institute (Staneloni et al, 1984, *J. Gen. Microbiol*, 130, 869). Some lipid-linked sugars in which the saccharide moieties were structurally related with the R.U. were obtained and, in addition, the role of this lipids as intermediates of the biosynthesis of the R.U. was clearly demonstrated. However the "in vitro" polymer formation was not observed in these studies.

Our work was aimed at obtaining the "in vitro" synthesis of the EPS and demonstrating the participation in this process of the lipid linked sugars mentioned above. By performing incubations in the presence of UDP-[^{14}C]-Glc as sugar donor and EDTA-treated cells as enzyme source, the EPS was isolated from the aqueous supernatant. It was analyzed by gel filtration columns. The organic extract which contains the intermediate lipid-linked sugars was analyzed by TLC. In two steps incubations it was demonstrated that from preassembled lipophilic compounds and in the absence of sugar nucleotide donors, polymer was formed, confirming their role as intermediates. This work was supported in part by grants from SAREC and Shell CAPSA.

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PROPIONIC ACID INHIBITS "IN VITRO" CO PRODUCTION IN BRAIN OF SUCKLING RATS. Brusque, A. M., Malfussi, H. F. C., Rocha, M. P., Terracciano, S., Silva, A. R., Dutra-Filho, C. S., Perry, M. L. S. and Wajner, M. Departamento de Bioquímica, Instituto de Biociências, UFRGS, Porto Alegre, RS, Brasil.

Propionic acidemia is an inherited disorder of organic acid metabolism caused by a severe deficiency of propionyl-CoA carboxylase activity. It is commonly manifested in the neonatal period by severe neurological symptoms. In this study we investigated the influence of propionic acid on the "in vitro " oxidation of acetate and citrate by prisms of cerebellum, hyppocampus and cortex of 10-day-old Wistar rats. The prisms were incubated in Krebs-Ringer buffer in sealed flasks in the presence of one labeled substrate and propionate (1, 2.5 or 5 mM) for one hour in a metabolic bath. Control experiments did not contain propionate. ^{14}C-CO_2 was trapped by hyamine in filter papers after incubation, and the filters immersed in scintillation fuid in order to mensure radioativity, corresponding to CO_2 production. Our results show that propionate inhibit significantly CO_2 formation from different substrates in brain of young rats, indicating that this metabolite impairs cerebral energy metabolism. Therefore, it is possible that our findings may partly accout for the neurological clinical features of patients affected by propionic acidemia.

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EFFECT OF BRANCHED CHAIN AMINO ACIDS AND ITS α-KETOACIDS ON Na^+K^+ATPase ACTIVITY FROM CEREBRAL CORTEX OF YOUNG RATS. C. Bürger[1,2], C. G.Silva[2], A.T.S.Wyse[3], M.Wajner[2] and C.M.D.Wannmacher[2].1-FAQFAR - UNIVALI, Itajaí - SC ; 2-Depto de Bioquímica, Instituto de Biociências, UFRGS, Porto Alegre -RS; 3-Depto de Ciências Fisiológicas, FURG, Rio Grande - RS - Brasil.

Maple syrup urine disease (MSUD) is characterized by a severe deficiency of branched chain α-keto acid dehydrogenase complex (BCKD) activity. Branched chain aminoacids (BCAAs) and its α-ketoacids (BCKAs) accumulate in blood and tissues. Ketoacidosis and brain dysfunction are common symptoms in MSUD patients. The energy dependent exchange of cytoplasmatic Na^+ for extracellular K^+ in mammalian cells is due to a membrane bound enzyme system, Na^+K^+ATPase. The exchange sustains a gradient for Na^+ into and for K^+ out of the cell, and this is used as an energy source for creation of the membrane potential. We have previously demonstrated that compounds accumulated in PKU patients inhibit Na^+K^+ATPase activity "in vitro"and "in vivo". In this work, we studied the "in vitro" effect of valine, isoleucine and leucine and its 2-ketoacids on the enzyme activity. Synaptic plasma membrane was prepared according to Jones & Matus (1974) and Na^+K^+ATPase activity was measured by the method of Tsakiris & Deliconstantinos (1984). The results showed that the aminoacids and its ketoacids inhibit the Na^+K^+ATPase activity in the range observed in the blood of the patients (0,1 to 1,0 mM). These findings suggest that the brain dysfunction observed in MSUD patients may be related to the inhibition of the Na^+K^+ATPase.**FINANCIAL SUPPORT: CAPES, CNPq, FAPERGS and FINEP.**

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PG-240, THE PROTEOGLYCOGEN SPECIES HAVING GLUCOSYLTRANSFERASE ACTIVITY. Carrizo, M.E. and Curtino, J.A. CIQUIBIC, UNC-CONICET, Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina (e-mail: jcurtino@dqbfcq.uncor.edu).

We have described the purification of rabbit skeletal muscle proteoglycogen and isolation of glycogenin, and showed that the glucosylation of dodecyl-β-maltoside (DBM) carried out by glycogenin was independent of the degree of autoglucosylation of the enzyme ; the glucosylation of DBM continued even autoglucosylation reached plateau (SAIB 1996). This led us to consider that the reaction with DBM might occur with the whole proteoglycogen molecule acting as glucosyltransferase. The present results show that the assumption was incorrect ; the purified proteoglycogen preparation was unable to glucosylate DBM. However, a mild digestion of the polysaccharide moiety with α-amylase under controlled conditions produced proteoglycogen fractions of reduced size from which a fraction having glucosyltransferase activity was isolated by ion-exchange chromatography. Gel filtration chromatography on a Superose 12 column, SDS-PAGE, and glucosyltransferase activity measurement after incubation with UDP-[14C]glucose and DBM, revealed that a proteoglycogen fraction of 240 kDa (PG-240) and no free glycogenin was the active form glucosylating DBM. PG-240 represents the proteoglycogen species of higher size having glucosyltransferase activity, obtainable under controlled conditions of amylolysis.

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BLOOD GLUCOSE RESPONSE TO ACUTE AND CHRONIC STRESS IN MALE AND FEMALE RATS.
Gamaro G.D.[1]; Pilger,J.A.[2]; Ely,D.R.[2]; Corrêa,J.B.[1]; Xavier,M.H.[1]; Rosat,R.M.[3]; Dalmaz,C.[1] and Ferreira,M.B.C.[2] Dept. Bioquímica[1], Farmacol.[2], Fisiol.[3], Inst. Biociências, UFRGS, Porto Alegre, Brazil.

Acute exposure to stress induces the release of hormones which cause inhibition of insuline secretion and increased hepatic glucogenolysis. Chronic stress has not yet been thoroughlly studied and its consequences on hormone metabolism are not clear. Since serum glucose levels have been suggested as a measure of the degree of stress experienced by rats in aversive situations, and sexual steroids are reported to affect glucose metabolic control, the present study aimed to evaluate blood glucose response to chronic and acute stress in male and female rats. Adult Wistar rats were used. The animals were stressed by restraint during 40 days in the chronic model. In the acute model there was a single exposure. Animals were sacrificed at different times after stress, trunck blood was collected and plasma glucose was assessed by the glucose oxidase method. Acute restrained females showed decreased plasmatic glucose levels immediately after stress whereas restrained males showed increased glycemia. One hour after the end of restraint, glycemia had returned to control values in both, male and female rats. In chronically stressed females, there were increased plasmatic glucose levels immediately after the stress session. Two hours after the end of restraint, the levels were significantly below control values. Chronically stressed males showed increased plasmatic glucose levels immediately and two hours after the stress session. The present results suggest that male and female rats respond differentially to stress, maybe due to sexual steroid action on glucose levels. (CNPq, FAPERGS, PROPESP/UFRGS)

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A CHEMICALLY-INDUCED ANIMAL , MODEL OF HYPERLEUCINEMIA FOR NEUROCHEMICAL STUDIES

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Hyperleucinemia is the most prominent biochemical finding of patients with Maple Syrup Urine Disease (MSUD),an inherited metabolic disorder caused by the severe deficiency in activity of the branched chain α-keto acid dehydrogenase complex.Besides leucine, the other branched chain amino acids valine and isoleucine also accumulate secundarity in blood and other tissues of the affected individuals. Encephalopathy is the main clinical manifestation of this disease and usually appears in the neonatal period. We estabilisched a chronic chemically-induced model of, hyperleucinemia in rats by injecting increasing doses of leucine subcutaneously twice a day from 6th till the 28th day of life. The target was to achieve leucine plasma levels of 2-5 mM. Rats from 6-14 days of age received 3μmol/g body weight, whereas rats from 15-21 days and from 22-28 days were injected respectively with 5 and 7 μmol/g of the amino acid. Pharmacokinetic parameters were calculated anr revealed that the older the rats were the higher was the renal clearance and distribution volume and the lower the half life of the drug in plasma.

Support: CNPq, FAPERGS, FINEP, PROPESP/UFRGS.

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PURIFICATION OF ONE PROTEIN WITH NUTRITIONALLY BALANCED AMINO ACID COMPOSITION FROM *Amaranthus.*
Huerta, D., Arnao, I., Villanueva, O. & Sandoval, M. Centro de Investigación de Bioquímica y Nutrición. Facultad de Medicina. Lima, Perú.

The seed used as source of dietary protein for humans are generally deficient in at least one of the essential amino acids. However Amaranth (***Amaranthus caudatus***) has in its small grain a high protein content (14-16 g%) and it is rich in essential amino acids such as lysine, trytophan and sulfur containig amino acids.

We have purified one protein of the albumin fraction from the mature seeds of ***Amaranthus caudatus*** var. Oscar Blanco cultivated in Cuzco. We have used Sephadex G-75 and then the 35 kDa protein was electroeluted from a preparative gel on SDS-PAGE with a 5-20% acrylamide gradient.

The aminoacid content of the electroeluted protein was determined by using an LKB amino acid analyzer. The 35 kDa protein of the albumin fraction contains a balanced amino acid composition as compared to the World Health Organization recommended values for a highly nutritional protein.

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PROTECTIVE EFFECTS OF BOLDINE AND GLAUCINE UPON THE INACTIVATION AND OXIDATIVE MODIFICATION OF LYSOZYME.
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The 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH)-induced inactivation and oxidative modification of lysozyme (L), as determined by the increase in carbonyl groups (CO) and the loss of tryptophan-associated fluorescence, was studied in absence/presence of the free-radical-scavengers boldine and glaucine. In their absence, after 4 h incubation, 37 °C, AAPH (20 mM) caused a decrease in L activity (84%) and fluorescence (56%), and a concomitant rise in CO levels to 4.2 nmol/mg prot (from 0.2 nmol/mg prot). Boldine (2.5-30 μM) concentration-dependently protected L from inactivation and oxidative modification. After 2 h, only 26% of the activity remained in the AAPH-treated control, while the samples treated with 30 μM boldine retained 76% of their activity (68% protection). The increases in CO for L incubated with AAPH and boldine were time- and concentration-dependently diminished, with 30 μM boldine leading after 2 h to formation of only 1.9 nmol/mg prot (61% protection). At 2 h, L with AAPH alone had lost 49% of its fluorescence, while with 30 μM boldine this value was only 16% (65% protection). The effects of 100 μM boldine and glaucine on L inactivation by 20 mM AAPH were compared. While samples treated with boldine retained 77% of activity after 2 h, those treated with glaucine only showed 24% of their activity (thus, 73% and 10% protection). At 100 μM boldine, a 20 min lag was observed before any inactivation occurred. This concentration of boldine or glaucine led to identical degrees of protection (about 33%) against loss. For the sake of comparison, Trolox®, ascorbate (AA), and GSH (all CO formation, after 2 h. 100 μM Boldine afforded 84% protection against fluorescence 100 μM) were tested. Trolox® proved to be the most effective, not differing from boldine in the L activity assay, but affording a greater degree of protection against CO formation and protecting less than boldine against fluorescence loss. Nevertheless, AAPH-induced L inactivation, even in the presence of the scavengers, may proceed beyond the point at which no more CO is formed. On the other hand, upon incubation with Fe^{3+}/AA (0.1/25 mM), L underwent no loss of activity or fluorescence, though CO appeared rapidly and their formation could be inhibited by boldine, glaucine or Trolox®. In conclusion, formation of CO is clearly not always a good indicator of loss of protein function.

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EFFECT OF VARIABLE CHRONIC STRESS ON SWEET FOOD CONSUMPTION IN RATS. Manoli, L.P.; Gamaro, G.D.; Michalowski, M.B. and Dalmaz, C. Dept. Bioquímica, Inst. Biociências, UFRGS, Porto Alegre, Brazil.

Chronic restraint stress increases sweet food consumption in rats, probably as a result of a higher anxiety. Otherwise, chronic variable stress (CVS) is known to induce mood depression. This study shows the effect of CVS on sweet food consumption in rats submitted or not to fasting. Glycemia in this animals was also measured. Male Wistar rats were divided in three groups: control, handled and stressed. Stressed group was submitted to different stressors for at least 40 days. To evaluate sweet food consumption, the animals were placed in a lightened area in the presence of 10 pellets of sweet food (Froot loops), and this procedure was repeated during 4 days in order to habituate the animals to the new food. The number of ingested Froot loops was measured during a period of 3 min, in the presence or absence of fasting. On the contrary to our observations with chronic restraint, animals submitted to variable chronic stress showed a decreased ingestion of sweet food, suggesting a depressive state in these rats. The handled group (light stress) presented an increased consumption, maybe as a consequence of anxiety. These results were presented by fed rats. When using fasted animals, no effect was observed. One week after the interruption of the stress treatment, these effects were no longer present. There was no difference in glycemia between the groups. Thus, chronic variable stress decreases appetite for sweet food in fed animals, while the opposite effect is observed in handled rats, and this is not dependent on plasma glucose levels.

(Supported by CNPq, PROPESP/UFRGS, FAPERGS)

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STUDY OF WATER CHANNELS IN MAMMALIAN OVIDUCTAL EPITHELIUM. Morales, B. and Villalón, M. Departamento de Ciencias Fisiológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

The oviductal fluid plays an important role on providing the appropriate conditions for fertilization take place. The properties of this fluid are largely due the availability of water, supplied by the oviductal epithelium cells through an unknown mechanism. In this work we demonstrate the presence of water channels in mammalian oviductal epithelium; these channels may be involved in the formation of the oviductal fluid. Water channel activity was measured by the swelling and the change in the osmotic permeability coefficient (P_f) of *X. laevis* oocytes injected with mRNA isolated of: rat and human oviductal epithelia and, rat kidney cortex and skeletal muscle. The oocytes swelling time course was recorded using a Sony video camera and analyzed by image processing using the Imagepro software. In response to a gradient of 190 mOsm, the oocytes injected with mRNA from rat and human oviductal epithelium and rat renal cortex remarkably increased both the swelling rate and P_f, compared with oocytes injected with water or skeletal muscle mRNA. mRNA-injected oocytes swelled consistently to 1.2 to 1.5 times their initial volume and ruptured within 10 min, whereas oocytes injected with mRNA-free water swelled minimally and failed to rupture even after incubations of over 1 h. The swelling of oocytes injected with oviductal epithelium mRNA was inhibited by $HgCl_2$ (inhibitor of water channels). This effect was reverted with ß-mercaptoethanol. These results suggest the presence of water channels in the oviductal duct, possibly playing a fundamental role in the oviduct physiology. Moreover, it suggest that these water channels are of the constitutive type, since second messengers are not necessary for its activation.

Supported by Rockefeller Foundation

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RELATIONSHIP BETWEEN OXIDATIVE STRESS AND HEME OXYGENASE INDUCTION BY COPPER SULFATE. J.O. Ossola, MD. Groppa and ML. Tomaro. Departamento de Química Biológica. Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

The effect of copper sulfate on both, hepatic oxidative stress and heme oxygenase (HO) induction was studied. A strong increase in "in vivo" rat liver chemiluminescence (QLV) was observed 1 h after Cu(II) administration. To evaluate liver antioxidant enzymatic defenses, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities were determined. CAT and GSH-Px were found to be significantly declined 5 h after Cu(II) injection. On the contrary, SOD activity was increased. HO activity appeared 5 h after treatment, reaching a maximum value 18 h after Cu(II) administration. This induction was preceded by a decrease in the intrahepatic GSH pool and an increase in the generation of thiobarbituric acid reactive substances (TBARS), both effects taking place some hours before induction of heme oxygenase.

Administration of bilirubin, the end product of heme catabolism in mammals, and α-tocopherol, a widely employed antioxidant, completely prevented heme oxygenase induction as well as the decrease in hepatic GSH and the increase in QLV when administered 2 h before copper sulfate treatment.

Under the same experimental conditions, β-carotene showed a moderate preventive effect on both, heme oxygenase induction and oxidative stress parameters.

These data obtained with Cu(II) treatment are in agreement with our previous reports suggesting a correlation between heme oxygenase induction and oxidative stress.

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PIROXICAM ADMINISTRATION INCREASE ETHANOL OXIDATION RATE IN RATS. Riveros-Rosas, H., Julián-Sánchez, A., Guinzberg, G., Hernández-Tobías, A., Saavedra-Molina, A., Zentella de Piña, M. and Piña, E. Depto. de Bioquímica, División de Estudios de Posgrado e Investigación, Fac. Medicina, Universidad Nal. Autónoma de México. Ciudad Universitaria, México. 04510. MEXICO.

Previous studies showed that piroxicam (a non-steroidal anti-inflammatory drug), decrease blood alcohol concentration in rats treated acutely with ethanol (administered by orogastric *via*). In order to obtain more information about this effect, we analyzed with a mathematical model the farmacokinetic of blood ethanol in rats intoxicated with ethanol, administered either orogastrically or intravenously. The model was numerically solved, and predicted curves accurately fitted to experimental data. The results show that piroxicam produce: 1) a diminution on ethanol absorption rate from digestive tract, and 2) an increase on hepatic ethanol oxidation rate. To insight with more detail this last finding, we observe the effect of piroxicam on isolated rat hepatocytes incubated with ethanol. The obtained results show that piroxicam 10^{-6} M, increase 25% ethanol oxidation rate.

This last piroxicam effect, is related with an increase on rat liver mitochondrial oxidation of reducing equivalents generated by ethanol metabolism (the most important rate limiting step), as can be concluded from assays with isolated mitochondria and hepatocytes, measuring activities dependent of the transfer and oxidation of reducing equivalents, as malate-aspartate shuttle, citrulline synthesis, and oxygen consumption. Piroxicam does not change hepatic alcohol dehydrogenase activity. (Supported by Grant DGAPA UNAM IN212695).

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SOME BIOCHEMICAL PARAMETERS IN MACROPHAGES OF MICE AFTER EXPOSITION TO CADMIUM "IN VITRO".
Ramirez, D., Penna, F.*, Delgado, S.M*. and Gimenez, M.S. Laboratorio de Química Biológica Patológica y Cátedra de Bioestadística*, Facultad de Qca, Bioqca. y Fcia. Universidad Nacional de San Luis. Argentina.

It is know that cadmium is a toxic that produce biochemical alterations in different species. We studied the effect of Cl_2Cd on the lipoperoxidation of peritoneal macrophages from balb/c mice after 2, 6, 12 and 18 hs. of incubation in culture medium. The amount of TBAR'S in Cd(+) increase in relation to the Cd(-) at all time points. On the other hand the amount of Nitric Oxide (NO) in the culture medium is the same in Cd(+) and Cd(-) at 2 and 6 hs, but increases in Cd(+) in relation to Cd(-) at 12 and 18 hs. In Cd(+) macrophages the amount of NO increases with time, but in Cd(-) increases only between 2, 6 and 12 hs, but not between 12 and 18 hs. The glucose uptake not change between Cd(+) and Cd(-) at 2 and 6 hs but at 12 and 18 hs. Cd(+) increases in relation to Cd(-). LDH activity is the same in Cd(+) and Cd(-) at all studied times. In both groups the LDH activity increases with time. The activity ATPasa in Cd(+) increases in relation to Cd(-) at all time points. In both groups the ATPasa activity increases with the time. We conclude that Cadmium would modified the membrane permeability, faciliting the uptake of substrates and the production of NO in macrophages, by different ways as peroxide formation, alteration of enzyme activities and ion interchange.

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ANTITUMORAL ACTIVITY OF NAPHTHOQUINONES COULD BE MEDIATED BY PRODUCTION OF REACTIVE OXYGEN SPECIES.
Reis, H. J[1]., Dolabela, M. F[1]. , Oliveira, A. B[3]., Raslan, D. S[3]. , Salas, C. E[2]., Ortega, J. M[2]. and Lopes, M. T. P[1]. [1]Departamento de Farmacologia, [2]Departamento de Bioquímica e Imunologia, Instituto Ciências Biológicas and [3]Departamento de Química, ICEX, UFMG, Belo Horizonte, Brasil.

Lapachol from brazilian Bignoniaceae and its chemical derivatives α, β-lapachone, hydroxy-propil-naphtoquinone and two fluranonaphthoquinones show potent antitumoral activity in a tumor cell-line panel. The antitumoral effect was dose dependent.

In this tudy, the genotoxic effects of lapachol, α and β lapachone were evaluated to gain insight on the mechanisms underlying their antitumoral effects. We observed lethal effects in cultured Chinese hamster fibroblast (V79-M8), along with the induction of DNA strand breaks. In a parallel assay, a menadione (naphthoquinone) resistant cell line was unaffected by lapachol and α lapachone, suggesting that the mechanism of cellular death may involve damage by reactive oxygen species. Unexpectedly, β lapachone promoted similar levels of DNA damage in sensitive and resistant cells.

The oxidative stress promoted by naphthoquinones appears to involve release of reduced Fe(II) and Cu(I) from their cellular storage. This assumption was confirmed when a reduction of DNA strand breaks was observed in the presence of a specific chelator.

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CHANGES ON ETHANOL ABSORPTION RATE CAN MIMIC FIRST-PASS METABOLISM EFFECT ON BLOOD ETHANOL PHARMACOKINETIC.
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Differences on blood ethanol concentrations after orogastric *vs.* intravenous administration of equal ethanol doses, has been attributed to first-pass metabolism in the gastric mucosa. However, ethanol absorption rate, an important determinant of blood alcohol concentrations, is rarely taken in account on ethanol first-pass metabolism studies. Therefore, we simultaneously analyzed the effect of ethanol absorption rate and first-pass metabolism on blood ethanol pharmacokinetic, using a mathematical two-compartment model with either first-order or substrate saturable kinetics.

The proposed model was numerically resolved and fitted to experimental data, using the mathematical package *Mathematica Ver. 2.0* (by Wolfram Inc.).

The solved mathematical model shows that whenever first-pass metabolism is present, blood ethanol area under the curve ($_{b}e$-*AUC*) is modified by changes on ethanol absorption rate. Consequently, observed changes on $_{b}e$-*AUC* when ethanol is administrated orogastrically, can not be explained only by changes on first-pass metabolism, because alterations on ethanol absorption rate also produce similar effects. In this way, a significative overestimation on gastric first-pass metabolism of ethanol, can be obtained if changes on ethanol absorption rate are not considered.

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DEHYDROCROTONIN A NEW ANTIULCEROGENIC DRUG: *IN VITRO/IN VIVO* TOXICITY RELATIONSHIP.
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Dehydrocrotonin (DHC) is a nor clerodane diterpene obtained from *Croton cajucara* Benth. Recently has been demonstrated that DHC has a potent antiulcerogenic activity on several models of experimentally induced rat gastric ulcers. The aim of this work was to assess the toxicity of DHC and to establish the relationship between the in vitro and in vivo data. The citotoxicity assays were carried out on Chinese hamster lung fibroblast, V-79 cell line. Cells were plated into 24 wells plates and grown for 48 h. Then cells were incubated with DHC at doses from 80 - 400 μM in quadruplicate, during 24 h. Assays of neutral red uptake (NR), MTT reduction (MTT) and nucleic acid content (NAC) were performed. In another experiments, the effect of drug exposition time and the cell recovery capability after DHC treatment were assayed. Oral and intraperitoneally single-dose DHC toxicity was assessed in male Swiss albino mice. Our results showed a slight and similar citotoxicity effects for the three studied cellular endpoints (NR, MTT and NAC) with an IC_{50} ranging between 250 - 400 μM. The citotoxicity was dose and exposition time dependent effect. A reduced cell recovery capability after drug treatment was found. The LD_{50} values for oral and intraperitoneal DHC administration > 1000 mg/kg and > 250 mg/kg, respectively, are in good agreement with the theoretically expected values pre-calculated from the citotoxicity data.

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INVESTIGATION OF ANTIOXIDANT MECHANISM OF ALLIUM sativum (garlic) EXTRACTS. Suárez, S.; Ráez, J.E. and Marroquín, M. Centro de Invest. de Bioquímica y Nutrición. Facultad de Medicina. Universidad Nacional Mayor de San Marcos. Lima-Perú.

Several pharmacologics activities have been atributed to garlic for a long time. This bulbe is used as traditional herbal drugs, however exactly mechanisms have yet unknown. To gain further insight to the antioxidant mechanism(s), we have determined effect for aqueous and saline extracts on pulmonary homogenizates stressed with H_2O_2, measuring lipoperoxidation and superoxide dismutase (SOD), glutation peroxidase (GSPx) and glucose-6-phosphate dehydrogenase (G-6-PDH) activities. It is found a significant decrease in lipoperoxidation with garlic extracts. SOD activities are lightly increase with aqueous extract treatment in comparison with saline and stressed ones, samely results have observed in GSHPx activities. G-6-PDH activity in stressed pulmonary tissue is higher than extracts treatments.

These sugest that garlic extracts decrease significantly the lipoperoxidation through antioxidant enzymes. More discussion will be presented.

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INHIBITION OF NITRIC OXIDE SYNTHESIS IN RATS SUBJECTED TO TOURNIQUET SHOCK. Vega V.V.[a] Maldonado,M.[a], and Ward,P.H.[b]. [a] Depto. de Fisiopatología, Facultad de Cs.Biológicas, Universidad de Concepción. [b] Depto. de Fisiología, Facultad de Cs. Biológicas, Universidad de Concepción.

There is evidence which suggets that a relationship exists between nitric oxide (NO) production and irreversibility of the shock syndrome elicited by severe hemorrhage or endotoxin. NO has also been shown to plays a protective role in hepatic ischemia/reperfusion injury. We have previously shown that rats subjected to tourniquet shock, through the placement of bilateral rubber tourniquets to their hind legs for 5 hrs followed by a 2 hr reperfusion period, present severe liver oxidative damage after the 2 hr reperfusion period. In this work we have attempted to establish the role of NO on hepatic oxidative stress observed in this shock model. Nitric oxide synthase (NOS) inhibitors (L-arginine methyl ester, L-AME or N^w-nitro-L-arginine methyl ester, L-NAME) were administerd at different time periods. After the 2 hr reperfusion period, liver GSH levels decresed from 0.706 ± 0.04 μmoles/mg of protein found in non-pretreated animals to 0.139 ± 0.043 μmoles/mg of protein and to 0.137 ± 0.052 μmoles/mg of protein in rats livers pretreated with the NOS inhibitors. Lipid peroxidation levels, assessed in the form of TBARS, increased from 9.7 ± 2.4 nmoles/mg protein in rats subjected to tourniquet shock to 15.4 ± 3.8 nmoles/mg protein in rat pretreated with L-AME and to 17.4 ± 3.6 nmoles/mg protein those pretreated with L-NAME. Plasma transaminases (ALT and AST) rose from 95.4 ± 4.7 U/L and 228.3 ± 39 U/L, respectively, in non-treated animals to 135.6 ± 11.8 and 328.8 ± 30.8 U/L when L-AME was administerd and to 146.0 ± 7.4 U/L and 292.3 ± 39.5 U/L after L-NAME pretreatment. Nitrite plasma levels increased 5.5 times in animals subjected to tourniquet shock in comparison to control rats, but were lower than the control values in rats pretreated with NOS inhibitors. From the above results we can conclude that NO exerts a protective role on hepatic oxidative injury associated to hind limb tourniquet application followed by reperfusion..

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EFFECT OF CHRONIC RESTRAINT STRESS ON BINDING OF BENZODIAZEPINES MEMBRANES OF DIFFERENT BRAIN STRUCTURES. Xavier, M.H[1]; Wolfman, C.[2]; Medina, J.H.[2] and Dalmaz, C. [1]Dept. Bioquímica, Inst. Biociências, UFRGS, Porto Alegre, Brazil and [2]Instituto de Biologia Celular, Facultad de Medicina, UBA, Buenos Aires, Argentina.

Benzodiazepines (BZD) are among the most widely prescribed drugs. The psychotropioc effects of BZD are due to their binding to the benzodiazepine receptors (BZDR), present in neuronal membranes, which are part of a complex protein that includes the GABA A receptor and a chloride channel. Acute stress has been shown to release BZD-like substances in different regions of rat brain, and to induce changes in GABA/BZD receptor complex. Since the consequences of exposure to chronic stress on BZDR are not clear, the present study aimed to evaluate binding sites to benzodiazepines in hippocampus, amygdala and cerebral cortex of chronically restrained rats. Adult male Wistar rats were used. The stress group was restrained 1 h a day, 5 days/weed, during 40 days. There was also a manipulate and a control group. Membrane samples were suspended in Tris-HCl buffer, pH 7,3 and incubated with [^{3}H]flunitrazepam. Non-specific binding was determined in parallel incubations in the presence of 10 μM FNZ. Binding was terminated by rapid filtration through GF/B filters, which were washed, dried and transferred to vials and radioactivity was measured. An increased BZD binding was observed in hippocampal membranes of chronically stressed rats. No effect was observed in amygdala or cerebral cortex. The present results suggest an alteration concerning BZDR in this structure, maybe resulting from a change in the levels of endogenous benzodiazepines with the chronic treatment.(CNPq, FAPERGS, PROPESP/UFRGS)

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GLUTAMATERGIC MECHANISMS IN MEMORY CONSOLIDATION. *Zanatta M.S.[1], Schaeffer E.L.[1], Schmitz P.K.[1], Quevedo J.L.[2], Bianchin M.[1], Walz R.[1], Medina J.[3], Quilfeldt J.A.[2], and Izquierdo I.[1].* Centro de Memória - Departamento de Bioquímica[(1)] and Departamento de Biofísica[(2)]-I.B./UFRGS - Porto Alegre - Brasil, Fac. Medicina[(3)] Buenos Aires - Argentina.

Hippocampus and amygdala are interconected between themselves and with entorhinal cortex, and the latter has two-way connections with the posterior parietal area. Previous studies have suggested a participation of NMDA receptor-dependent mechanisms in these structures in diferent phases of memory consolidation which led to think of long-term potentiation (LTP) mechanisms operating within these circuits. AP-5 is a specific antagonist of glutamate NMDA receptors. The present study examines the efect of AP-5 infused bilaterally into the posterior parietal cortex at diferent times on retention of inhibitory avoidance in rats and compares it to the effect of the same drug infusions in hippocampus, amygdala, and entorhinal cortex. Male Wistar rats (3-4 months aged) have been bilaterally implanted with guide cannulae above CA1 regions of dorsal hippocampus, above the junction of central and lateral nuclei of amygdala, above both these sites (4 cannulae), above the surface of entorhinal cortex and below the sueface of posterior parietal II cortex. Once recovered from surgery, the animals were trained in a step-down inhibitory avoidance task and tested 24 hours later. They received bilateral infusions of saline or AP-5, 0 .or 30 min after training (rats implanted in hippocampus, amygdala or both) and 0, 30, 60 and 90 min. after training (entorhinal or parietal cortex).When infused into hippocampus, amygdala, or both 0 min. after training, AP-5 caused retrograde amnesia but had no effect when infused 30 min.after training in these structures. AP-5 had also no effect when infused into entorhinal cortex 0 min. after training and 0 or 30 min after training in parietal cortex. When given 30, 60 or 90 min post-training in entorhinal cortex, or 60 or 90 min post-training in parietal cortex, AP-5 caused pronounced retrograde amnesia for the avoidance task. These findings suggest that the mechanism of memory regulation in all these areas involves glutamate NMDA receptors, leading us to think in LTP. Furthermore, there is a sequential entry in operation of these structures in post-training memory processing which is presumed to be mediated by the pathways that interconect them.

Poster Session 2

Abstracts 289 - 457

Membranes

Lipids

Receptors-Hormones and Growth Factors

Signal Transduction

Cell Biology-Molecular Aspects

Membranes, Lipids, Receptors - Hormones and Growth Factors, Signal Transduction, Cell Biology - Molecular Aspects

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PHYSICOCHEMICAL LIPID PHASE PROPERTIES IN Na^+,K^+-ATPase RICH MEMBRANES. A FLUORESCENCE SPECTROSCOPY STUDY. Aguilar,L.F., Valdebenito, E, Sotomayor, C.P., Helms, M and Jameson, D. Instituto de Química, Facultad de Ciencias Básicas y Matematicas, Universidad Católica de Valparaíso, Chile and Department of Biochemistry and Biophysics, University of Hawaii at Manoa, USA.

The aim of this investigation is to study the influence of the physicochemical properties of the lipid bilayer on the membrane associated Na,K-ATPase. A series of fluorescence probes are used to undertake a systematic study to characterize the properties of the bilayer and the effects on them of physical and chemical interventions in order to correlate them with changes in the ATPase activity. Using Na.K-ATPase rich membrane preparation from pig kidney and large unilamellar vesicles from natural phospholipids as models, the effect of the incorporation of fluidizing agent and temperature were studied at different depths of the bilayers and at close and distant regions from the enzyme by steady state fluorescence measurements of different probes. Time resolved fluorescence measurements of DPH derivatives are used to study the properties of the lipid-protein interface. The effect of the fluidizing agent A_2C, sensed by different probes indicates that it exerts a greater perturbation on the acyl chain packing order at the central region of the bilayer. At the hydrophylic/hydrophobic interface level, sensed by the probe Laurdan, the molecular dynamics at low temperature is the same in the regions of lipid phase close and distant from the protein, while at higher temperature the former presents a slower dynamics than the latter and the effect of A_2C is greater in the region close to the proteins.

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Na/K/2Cl COTRANSPORT SYSTEM AND WATER TRANSPORT IN EEL INTESTINE BBMV

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The intestine is an important organ for the regulation of water and salt absorption in euryaline teleost fishes. Na/K/2Cl cotransport system is the main way that salt is absorped across the brush border membrane of the eel intestine. Na/K/2Cl cotransport and osmotic water transport was studied in a preparation of brush-border membrane vesicles (BBMV) from *Anguilla anguilla* intestine. In order to see if the Na/K/2Cl cotransport was present in this membrane vesicles, Na exchange experiments were preformed in the presence and in the absence of furosemide 1mM, an inhibitor of this cotransport. The uptake was measured at different times (from zero to 60 min) using the rapid filtration technique. When the experiments with furosemide were made, the uptake was only started after 1h of pre-incubation of the vesicles with the inhibitor. Preliminary results indicate that a decrease (~50%) in the uptake in the presence of furosemide related to control conditions.

Osmotic water transport was also studied across these membrane vesicles using the stopped-flow technique. Changes in total light scattering with time was observed when vesicles were exposed to hyper- and hypotonic cellobiose solutions. A non-linearity between the $\Delta I=I_\infty-I_0$ and tonicity of the osmotic shock was found when vesicles were loaded in 18mOsM cellobiose. Light sacterring data was converted to volume data and after this calibrationcould the estimations of osmotic water permeability coefficient (Pf) can be determined.

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BINDING OF PROTEINS TO *Cyprinus carpio* INTESTINAL BRUSH BORDER MEMBRANE. Miralles, M., Toledo, A., Villanueva, J., *Goicoechea, O. and Amthauer, R. Instituto de Bioquímica, *Instituto de Embriología, Facultad de Ciencias, Universidad Austral de Chile.

The internalization of intact protein in fish intestine is a well established process. It has been postulated that binding sites on the brush border membrane may play an important role in mediating the endocytosis of protein from the lumen, which eventually can reach the blood stream. In order to demonstrate the presence of binding sites in the intestinal epithelium, our group has developed an *in vitro* binding assay using isolated brush border membrane (BBM) and different proteins. Peroxidase (HRP) binds specifically to BBM, showing a saturation curve reaching a maximum at 50 nM with a Kd of 23 nM. Reducing the NaCl concentration in the assay results in an increased HRP specific binding, reaching a maximum in the absence of NaCl. Addition of polyions like polylysine or heparine inhibited the specific binding of HRP in ~80%. Other proteins tested like IgG, HDL and transferrin bind to BBM, but to a lesser extent than HRP. Whereas ferritin does not bind at all. However, cationized ferritin shows a high degree of binding to BBM which could be abolished by addition of polylysine or heparin to the assay. Taken together, these results strongly suggest that anionic binding sites on the membrane could be involved in the internalization of intact protein. Nevertheless, the participation of other binding sites in this process can not be ruled out. In fact, recent results demonstrated that purified carp intestinal alkaline phosphatase binds to immobilized IgG, but do not bind to the other tracer proteins tested in the binding assay.

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TRANSMEMBRANE FLUIDITY DISTRIBUTION IN THE NICOTINIC ACETYLCHOLINE RECEPTOR-RICH MEMBRANE AND ITS MODIFICATION BY FATTY ACIDS. S. S. Antollini and F. J. Barrantes. Instituto de Investigaciones Bioquímicas de Bahía Blanca, 8000 Bahía Blanca, Argentina.

The so-called generalized polarization (GP) of the fluorescent probe Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene) and the anisotropy of DPH and two of its derivatives were used to learn about the physical state of the lipids in the nicotinic acetylcholine receptor (AChR)-rich membrane and its modification by oleic acid (18:1). Laurdan GP decreased upon addition of 18:1. The wavelength dependence of Laurdan GP spectra did not change in the presence of 18:1, indicating the occurrence of a single, ordered-liquid lipid phase as previously reported for the native AChR membrane (Antollini et al., *Biophys. J. 70 (1996) 1275-1284*). Using energy transfer from the membrane protein fluorescence to Laurdan molecules, a minimal donor-acceptor distance r of 14±1 Å was calculated considering a distance $0<H<10$ Å for the separation of the planes containing donor and acceptor molecules, respectively. A monotonic decrease in DPH anisotropy was observed as a function of temperature, indicating that the hydrocarbon core of the AChR membrane is more fluid at higher temperatures. Since the cationic (TMA-DPH)) and anionic (PA-DPH) DPH derivatives selectively partition into the inner and outer hemilayers, respectively, the two derivatives enabled us to dissect the hemilayer-specific component of the fluidity parameter. The increase in the fluidity of the AChR membrane sensed by PA- and TMA-DPH at more superficial levels of the membrane upon addition of oleic acid was less marked than that occurring deeper in the hydrocarbon core of the membrane, as sensed by the parent compound DPH.

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INTERFACIAL FREE ENERGIES OF 1-ω-DIALKANOLS IN AQUEOUS SOLUTIONS, A.Aspee, and E.A.Lissi. Depto. Química, Facultad de Química y Biología, Universidad de Santiago de Chile.

In order to evaluate which factors determine the distribution of amphiphilic molecules betweeen different microenvironments we have measured the distribution of a series of alkanols of different size and/or topology between water and micelles, reverse micelles and LUVs. Also, we have evaluated the thermodynamics of their transfer to monolayers and the water/air interface. In the present work, we report data bearing in the transfer of 1-ω-alkanos from a bulk phase to the water/air interface. The surface area covered by each molecule is considerably larger than for 1-alkanols, suggesting folding of the alkyl chains in the dialkanols. This effect is strongly dependent upon the number of carbon atoms.

Acknowledgments: This work was supported by FONDECYT (194-1058). AA thanks CONICYT for a graduate fellowship.

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STIMULATION OF MEMBRANE-ASSOCIATED SERINE/THREONINE PHOSPHATASE IN ERYTHROCYTES BY HYDROGEN PEROXIDE AND STAUROSPORINE. Bize I[#*], Muñoz P[#], Canessa M[#], Dunham PB[*]*. [#]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile, and [*]Department of Biology, Syracuse University, Syracuse, NY, USA

It is known that the K-Cl cotransporter of human and sheep erythrocytes is activated by cell swelling and inhibited by okadaic acid a serine/threonine (S/T) phosphatase inhibitor; this finding has suggested that the transporter is activated physiologically by a S/T phosphatase. Under isotonic conditions, when the transporter is mostly silent, activation can be induced by H_2O_2 and by staurosporine, a kinase inhibitor. Activation by these agents was also inhibited by okadaic acid, thus suggesting that H_2O_2 and staurosporine act on the phosphatase.

In the present study we have assayed S/T phosphatase activity using phosphorylated glycogen phosphorylase as substrate. We found that human and sheep erythrocytes contain a membrane-associated S/T phosphatase with low affinity for inhibition by okadaic acid (PPase-1) as well as soluble S/T phosphatases with high affinity for okadaic inhibition (PP-2A). Membrane-associated phosphatase was stimulated by both H_2O_2 and staurosporine, while soluble phosphatase was unaffected. Stimulation by H_2O_2 in isolated membranes was observed both when intact cells were pretreated with H_2O_2 or when isolated membranes were treated directly. In contrast, staurosporine stimulation of membrane-associated phosphatase required pretreatment of intact cells. Therefore the actions of H_2O_2 and staurosporine are direct and indirect, respectively.

The results indicate that the S/T phosphatase regulating K-Cl cotransport is membrane-associated, inhibited by okadaic acid and that it may be regulated by both oxidation and phosphorylation by an non-identified protein kinase.

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ASYMMETRY IN THE NATIVE ACETYLCHOLINE RECEPTOR-RICH MEMBRANE FROM *DISCOPYGE TSCHUDII.* I. Bonini de Romanelli and F. J. Barrantes. Instituto de Investigaciones Bioquímicas de Bahía Blanca, 8000 Bahía Blanca, Argentina.

Selective enzymatic hydrolysis and lipid compositional analyses of acetylcholine receptor (AChR)-rich membranes from the electric ray *D. tschudii* indicate a marked asymmetry in the distribution of sphyngomyelin (SM) and its fatty acid molecular species in each bilayer leaflet. A fluorescent derivative of SM (N-(10-[1-pyrene]decanoyl) sphyngomyelin; Py-SM) was used to learn about AChR-SM interactions. The dynamics of the lipid acyl chains were explored by measuring excimer formation of Py-SM under direct and energy transfer conditions. We also studied the thermotropic behavior of excimerization, sensitive to the fluidity of the membrane, in the temperature range of 5 to 45°C. The presence of protein in the native AChR-rich membrane significantly reduced excimer formation and the broad thermal sensitivity of the SM analogue observed in pure lipid. Furthermore, excimer formation was 5- to 10-fold lower in the native AChR membrane than in the liposomes prepared from extracted endogenous lipid, suggesting restricted mobility of the probe in the presence of the AChR protein. We conclude that a subpopulation of Py-SM molecules lies within an average donor-acceptor distance *r* of ~20 Å from the AChR donor fluorophores, and a distance H of about 18 Å separates the donor-acceptor planes normal to the membrane surface. Asymmetry in transmembrane fluidity also occurs in the AChR membrane: the outer, exofacial leaflet of the AChR-rich membrane exhibits higher lipid fluidity than the inner, cytoplasmic leaflet.

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TAMOXIFEN (TMX) CYTOTOXICITY AGAINST HUMAN GLIOBLASTOMA CELL LINES IS MEDIATED BY INHIBITION OF PLASMA MEMBRANE-ASSOCIATED

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Aim. To assess the involvement of inhibition of protein kinase C (PKC) activity in Tmx cytotoxicity against cultured human glioma cells. Methodology. Cultures of the U-87, U-138, and U-373 human glioblastoma cell line variants were incubated for 4 days with serial dilutions of Tmx, and subsequently assessed for growth inhibition using the sulforhodamine B assay. In parallel, cytosolic and particulate PKC activity-containing fractions were prepared by repeated ultracentrifugation of homogenates of untreated or 4-days Tmx-treated cells, which were then assessed for their capacity to phosphorylate the PKC substrate myelin basic protein using ^{32}P-labeled ATP as phosphate donor. Results. Tmx inhibited the growth of the cells in a dose-dependent fashion with IC_{50} values of approximately 10 μM. The agent did not affect phosphor-ylation of myelin basic protein by the cytosolic cellular fractions, but phosporylation of myelin basic protein by the particulate cellular fractions was progressively inhibited by increasing concentrations of the drug. The latter phenomenon correlated linearly with Tmx-mediated cell growth inhibition. Conclusion. Our data suggest, that inhibition of the activity of plasma membrane-associated PKC, but not of that of cytosolic PKC, is the principle mechanism of cell growth inhibition by Tmx in our model. Immunohisto-chemistry studies to identify the PKC isoform(s) involved, are in progress.

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INHIBITION OF LIPOPEROXIDATION OF MICROSOMES AND MITOCHONDRIA BY CYTOSOLIC PROTEINS FROM RAT LIVER: EFFECT OF VITAMIN A A. Palacios, V. A: Piergiacomi and A. Catalá. Cátedra de Bioquímica, Facultad de Ciencias Veterinarias, UNLP, La Plata , Argentina.

Antioxidants are one of the body's primary defenses against free radicals and reactive oxygen molecules and they are considered to block carcinogen formation.Antioxidants as vitamin A may protect cells against neoplastic transformation, resulting from oxidative damage to DNA. Clinicals trials have shown the antioxidant role of vitamin A in cancer prevention. In order to provide an inside of the cellular mechanism, the role of vitamin A and one of its carrier proteins were studied during lipoperoxidation of hepatic microsomes and mitochondria . Long chain fatty acids and retinoid utilization is mediated by specific cellular proteins of low molecular weight (14-15KDa). The Fatty Acid Binding Protein (FABP) has been described as an intracellular carrier of fatty acids and retinol esters. The effect of hepatic FABP in the non enzymatic lipoperoxidation of hepatic microsomes and mitochondria was studied in control and vitamin A supplemented rats. One group of female rats Wistar AH/HOK were injected with vitamin A 100.000 UI / day / rat during 7 days, a second group was control. FABP was obtained from hepatic cytosol by gel filtration on Sephadex G-75 and it was concentrated by ultrafiltration. Later, retinol palmitate was measured by HPLC, that resulted 3,3 times higher on the supplemented group. Microsomal and mitochondrial RP concentration was higher in the supplemented group, 2,3 and 3,4 times respectively. A decrease of light emission was observed with the addition of cytosolic proteins that contain FABP, when microsomes and mitochondria were incubated at 37°C in a ascorbate-Fe++ system. FABP obtained from supplemented animals produced higher inhibition .

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LIPOPEROXIDATION OF HEPATIC MICROSOMES. EFFECT OF VITAMIN E. R. Zanetti, P. Leaden and A. Catalá. Cátedra de Bioquimica, Facultad de Ciencias Veterinarias, UNLP. La Plata (1900), Argentina.

In the cells, there are efficient defense systems that control lipid peroxidation and make sure the maintenance of cellular integrity and, therefore, an adequate metabolic and funtional efficiency Among these defense mechanisms, vitamin E (α-tocopherol) is one of the most important liposoluble antioxidant systems, because interfere the propagation reactions that leads to lipoperoxidation. In the present work, we study the effect of the intraperitoneal administration of vitamin E (100 mg/kg weight/24 h) on ascorbate (0.04 mM) induced peroxidation of rat liver microsomes . We also analyzed the effect of hepatic cytosolic proteins on microsome lipoperoxidation. The results indicate that the ascorbate induced light emission was 76% lower in microsomes (1 mg protein) obtained from vitamin E trated animals when compared with controls. In the presence of cytosolic protein (1 mg) the chemiluminescence of control microsomes diminished 40% and 60% when cytosol from controls and trated animals were used, respectively. By means of gas chromatography we analyzed the fatty acid content of native and peroxidated microsomes from both animal groups. The peroxidation affected principally araquidonic acid and its diminution was more evident in the control microsomes than in the microsomes from trated group. By HPLC we analyzed the vitamin E content in all subcellular fraction employed. In microsomes from vitamin E-group, the content of vitamin was 11 times higher than in the control ones (0.68 ± 0.1 vs 0.06 ± 0.004 μg α-tocopherol / mg protein, respectively), while in cytosol from vitamin E-group was only 2 times higher than in the control cytosol (0.057 ± 0.005 vs 0.025 ± 0.002 μg α-tocopherol/mg protein, respectively).

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Phosphorylation of the Yeast Plasma Membrane H^+-ATPase by casein kinase I. E. Estrada [1,3], P. Agostinis[2], J. Vandenheede[2], J. Goris[2], W. Merlevede[2], M. Ghislain[1] and A. Goffeau[1]. [1] Unité de Biochimie Physiologique, Université Catholique de Louvain, Louvain-la-Neuve, Belgium. [2] Laboratory of Biochemistry, Faculty of Medicine, Catholic University, Leuven, Belgium. [3] Instituto de Fisiologia, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile.

The plasma membrane H^+-ATPase of *Saccharomyces cerevisiae* is subjected to phosphorylation by a casein kinase I activity in vitro. We show this casein casein kinase I to result from the combined function of Yck1p and Yck2p, two highly similar and plasma membrane associated casein kinase I homologues. First, H^+-ATPase phosphorylation is severely impaired in the plasma membrane of Yckp-deficient yeast strains. Furthermore, the wild-type level of the phosphoprotein is restored by the addition of purified mammalian casein kinase I to the mutant membranes. We used the H^+-ATPase as well as a synthetic peptide substrate that contains a phosphorylation site for casein kinase I to compare kinase activity in membranes prepared from yeast cells grown in the presence or absence of glucose. The addition of glucose results in increased H^+-ATPase activity which is associated with a decline in the phosphorylation level of the enzyme. Mutations in both Yck1p and Yck2p affect this regulation, suggesting that H^+-ATPase activity is modulated by glucose *via* a combination of a "down-regulating" casein kinase I activity and another, yet uncharacterized, "up-regulating" kinase activity. Biochemical mapping of phosphorylated H^+-ATPase identifies a major phosphopeptide that contains a consensus phosphorylation site for casein kinase I.

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THE SINERGIC EFFECTS OF VANADATE AND OUABAIN IN ADRENAL GLAND OF MAMMALS. Fauth, M.G.*, Grillo, M.L., Wassermann, G.F. Inst. Biociências UFRGS, Porto Alegre, Brasil, *Inst. Química, PUCRS, Porto Alegre, Brasil.

The important metabolic event of aminoacid (aa) active transport in cells of adrenal glands of rats and cows takes place through the Na^+ and energy-dependent A system which is consequently potentiated by Na^+-K^+-ATPase. Low concentrations of the oligoelement vanadium (10^{-6}M) interact in the adrenal cells with citoplasmic sites of Na^+-K^+-ATPase, causing a decrease of aa transport. On the other hand, the "new" adrenal steroid ouabain inhibits the Na^+-K^+-ATPase acting on the surface of the cells. Therefore, the aim of the present work was to study the sinergic action of vanadium and ouabain on the active aa transport on adrenal of rats. Experiments were carried out with male adult Wistar rats. Whole adrenal glands were preincubated (90 min) and incubated (45min) with or without (control) different concentrations of vanadate or ouabain or vanadate plus ouabain, in Krebs-Ringer bicarbonate buffer in a Dubnoff metabolic incubator. The samples were maintained at 37°C, pH 7,4 and gassed with O_2:CO_2 mixture (95:5;v/v). At the beginning of the incubation period, 0,2 μCi of [^{14}C]MeAIB (methylaminoisobutyric acid) was added. The results were expressed as the tissue/medium (T/M) ratio. The aa transport was inhibited by vanadium (10^{-7}M; 3,5%) or ouabain (10^{-4}M; 16,8%). The action of the same concentrations of vanadium plus ouabain was more effective, showing a potentiation of the inhibitory action of the substances (28%).

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BENZYL ALCOHOL PROMOTES MEMBRANE FLUIDITY CHANGES WHICH MODULATE K^+ EFFLUX IN ERYTHROCYTES.

Celedón G.[1], Chicui J[1] , Montalar Y.[2], Valenzuela C.[1], Behn C.[3] and González G.[4] .[1] Departamento de Fisiología, Facultad de Ciencias, [2]Facultad de Medicina, Universidad de Valparaíso, [3] Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile, [4]Instituto de Química, Universidad Católica de Valparaíso, Chile.

Fluidity of biological membranes modulates the function of membrane receptors, enzymes and transporters.We have studied the effect of the membrane fluidifier benzyl alcohol on K^+ efflux and membrane fluidity of erythrocytes, using pyrene and 1-pyrenedodecanoic acid excimer formation to assess fluidity at different membrane depths. K^+ efflux was assesed in the effluents of a periperfusion system of a erythrocytes suspension. We found that benzyl alcohol enhances pyrene excimer formation but decreases that of 1-pyrenedodecanoic acid which is an indication of an increased fluidity of the lipid core and of the inner lipid monolayer. These findings correlates with an activation of K^+ efflux promoted by benzyl alcohol. We also detected an associated decreased fluidity of the outer monolayer. This can be interpreted as a differential expansion of the inner monolayer relative to the outer monolayer which would modulate K^+ efflux in erythrocytes.

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VESICLES ISOLATED FROM EPIDIDYMAL FLUID: AFFINITY SITES FOR N-ACETYL GLICOSAMINIDASE AND β-GALACTOSIDASE.

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The sperm maturation during the epididymal transit depends on the epididymal environment. This environment is maintained by the secretion of epididymal epithelium. Some components of the secretion is constituted by hydrolases. It was described that membrane bound vesicles that arise from principal ceils contained some of these enzymes (Fornés et al., 1994). In this work, we tested if two of these hydrolases, N acetyl glicosaminidase (Nag) and β galactosidase (βgal), posses high affinity sites in the vesicles isolated from rat epididymal fluid. Vesicles were isolated by differential centrifugation and washed with PBS containing 0.6M KCl to release endogenous enzymes. Then, vesicles were incubated with increasing concentration of NAG and βGal and the free and bound enzyme were assayed by a fluorometric methodology. In others experiments, the binding assay were performed with colloidal gold particles (20 nm) coated with βGal and observed by EM. The binding assay shows a saturation pattern and the electron micrograph of gold-βGal shows the dark particles located in patches on the membrane. The results indicate that NAG and βGal have high affinity sites on the membrane of the vesicles.

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RAPID CHARGE COMPENSATION UPON RELEASE OF CALCIUM FROM SARCOPLASMIC RETICULUM VESICLES.

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Changes in internal pH during Ca^{++} release from triad vesicles isolated from rabbit muscle sarcoplasmic reticulum were monitored by pyranin fluorescence. Triads were loaded with 1 mM $CaCl_2$ and 1 mM pyranin (a water soluble fluorescent pH-indicator) by incubation overnight. External pyranin was removed by passing the loaded triads over a Bio-Gel A column. Release of Ca^{++} was induced with ATP by mixing equal volumes of triads in buffer (pH 7.00) containing 1 mM $CaCl_2$, with buffer containing 2.5 mM HEDTA, 1 mM $CaCl_2$ and 2 mM ATP (pH 7.03) in a stopped-flow fluorimeter. Following mixing the external pH was exactly 7.00 and the external pCa was 5. Upon mixing the internal pH dropped about 0.5 units within 50 msec. Subsequently the pH gradient decayed slowly within about 3 min. When the experiment was repeated in the absence of ATP (i.e. slower Ca^{++} release), the pH_{in} dropped much slower (about 2 sec). No decrease in internal pH was observed when the Ca^{++} channels were blocked with 1 mM Mg^{++}. The kinetics of the decrease in pH_{in} were similar to the kinetics of Ca^{++} release observed with similar conditions using rapid filtration techniques (Donoso and Hidalgo, J. Biol. Chem. (1993), 268, 25432). We conclude that the influx of protons accounts for a fraction of the charge compensation needed for rapid efflux of Ca^{++} through the Ca^{++}-channels in the membrane of triads. *(Supported by Fondecyt grants 1940369, 1961226 and CEE grant CIICT940129).*

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IDENTIFICATION AND LOCALIZATION OF FUNCTIONAL WATER CHANNELS IN THE HUMAN DISTAL COLON

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Although the intestinal epithelia plays an essential role in the reabsorption and secretion of water, the existence of water channels or aquaporins (AQPs) in these cells rest to be clarified.

We demonstrate here the presence of functional water channels in the human distal colon by mRNA expression in *Xenopus* oocytes and immunolocalization studies. Osmotic water permeability (Posm) of *Xenopus* oocytes injected with adult and children distal colon mRNA, was significantly greater than control (water injected). The increase in Posm was inhibited by 0.3 mM $HgCl_2$ and the inhibition was reversed by β-mercaptoethanol. The AQP expression was detected in distal colon, but not in other tissues including stomach, jejunum and ileum. However, in all gastrointestinal sections, a 420 bp PCR product was amplified by RT-PCR and sequenced. The distal colon aminoacid sequence band showed an identity >90% when compared to human kidney AQP3 and rat kidney glycerol intrinsic protein (GLIP). We detected heavy labeling in the surface of colonic mucosa using a polyclonal antibody against a 26 amino acid synthetic peptide corresponding to the carboxyl terminus of kidney AQP3 (with an added NH_2-terminal cysteine).

We conclude that human distal colon possess functional water channels in their colonic epithelial cells probably localized in the apical membrane. Sequence analyses showed that these water channel proteins are very similar to AQP3.

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EFFECT OF MONOVALENT IONS ON CALCIUM RELEASE FROM SARCOPLASMATIC RETICULUM VESICLES. Marambio, P. Donoso, P. and Hidalgo, C. Depto. Fisiol. Biofís., Facultad de Medicina, U. de Chile, y C.E.C.S., Santiago, Chile.

In skeletal muscle, massive release of calcium from sarcoplasmic reticulum (SR) in response to transverse tubule depolarization elicits contraction. To maintain charge balance, and prevent the development of a membrane potential across the SR membrane that would limit further release of calcium, this large calcium efflux should be compensated by influx of cations or efflux of anions, or both. Electron probe microanalysis studies of whole muscle fibers have shown that K^+ and Mg^{++} are responsible for a significant fraction of the total charge compensation, leaving a charge deficit presumably due to protons that cannot be detected with this technique. Using isolated SR vesicles, we studied the role of several monovalent ions (K^+, Cs^+, $Tris^+$ and Cl^-) on calcium release kinetics measured by fast filtration. This method has a high time resolution (ms), not attainable by electron probe microanalysis. Calcium release from $^{45}Ca^{2+}$ loaded SR vesicles was induced by addition of a solution of 2 mM ATP, pCa 5.0, pH 7.0, with or without permeant ions. Very low rate constants of calcium release, $\approx$ 0.1 s^{-1}, were obtained in the absence of permeant ions. The highest rate constant values, 10 s^{-1}, were obtained with either 0.1 M K^+, $Tris^+$ or Cl^- as sole permeant ions. Cs^+ was significantly less effective than K^+ as counter-ion. These results demonstrate that Cl^-, not previously considered by electron probe microanalysis, is as effective as K^+ or $Tris^+$ in compensating the charge deficit generated during calcium release, and that H^+ by themselves at pH 7.0 compensate only a fraction of the deficit.

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STUDIES ON LIPID PEROXIDATION IN RAT LIVER NUCLEI AND CHROMATIN FRACTIONS M. Marmunti y A. Catalá. Cátedra de Bioquimica, Facultad de Ciencias Veterinarias, UNLP, La Plata, Argentina.

Lipid peroxidation process in cell nuclei has been the subject of relatively few studies, some of them presenting conflicting results. The juxtaposition of DNA to the nuclear membrane and the existence of chromatin-nuclear membrane attachment sites suggest the possibility that chromatin may be affected during the lipoperoxidation process. In the study reported here, the non enzymatic (ascorbic-fe++) peroxidation of hepatic nuclei and chromatin fractions were assayed. Chromatin obtained by sonication of nuclei suspended in 0.25M sucrose was fractionated by differential sedimentation according to the following schedule: 3000 , 9.000 , 11.000 g for 10 min each . The lowest density chromatin fraction was obtained by precipitation with cold ethanol of the supernatant obtained from the last centrifugation. Light emission (chemiluminescence- cpm/mg protein or cpm/mg DNA) decreased in the order heavy>low density chromatin during the peroxidation process. Analysis of fatty acids by GLC showed that heavy density chromatin fractions are enriched with 20:4n6 when compared with low density chromatin fraction. Our results seem to indicate that the selective damage of certain regions of the chromatin during non-enzymatic peroxidation is particularly active in those regions rich in arachidonic acid.

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BIOCHEMICAL CHARACTERIZATION OF A V-ATPASE OF AIRWAY SMOOTH MUSCLE PLASMA MEMBRANE FRACTION. Pacheco, G., Bécemberg, I. and Alfonzo, M. Sección de Biomembranas, Instituto de Medicina Experimental , Universidad Central de Venezuela, Caracas, Venezuela.

A biochemical characterization of a Mg^{2+}-ATPase activity associated with a plasma membrane fraction (P_1) isolated from bovine tracheal smooth muscle was performed. This V-ATPase was quantified by measuring the amount of Pi released using the procedure of Fiske-Subbarow. This enzyme is an integral part of the membrane and showed a cold inactivation in the presence of ATP and Mg^{2+}. Also, this ATPase was stimulated by monovalent anions being Cl^-, the best anion for such stimulation, even though Br^- and I^- were good substitutes and F^- was ineffective. This ATPase activity was not inhibited by ouabain, oligomycin C and vanadate indicating that neither P or F-ATPases were related to this enzyme activity. However, the existence of V-ATPase was shown by the significant inhibition caused by bafilomycin $A_1$and the inmunodetection of a 72 kDa polypeptide using a specific antibody against the A subunit (72 kDa) of V-ATPase from chromaffin granule demonstrated the presence of a V-ATPase in this plasma membrane fraction. Additionaly, this V-ATPase seems to be coupled to chloride conductor because duramycin inhibited this ATPase activity. The stimulatory effect produced by uncouplers such as FCCP and 1799, indicated the existence of a H^+-ATPase.

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INTERACTION BETWEEN LHI α(B870) POLYPEPTIDE WITH PHOSPHOLIPIDS DURING ITS INSERTION IN *Rhodobacter capsulatus*. Pucheu, N., Kerber, N., Rivas, E., Cortez, N and García, A. Cátedra de Microbiología, CIBYF-CONICET, Facultad de Agronomía, UBA, Buenos Aires, Argentina.

Membranes from in vivo labelled cells of *Rhodobacter capsulatus* U43[pTX35] grown photosynthetically carried 60% of the $[^{32}P]$-Pi in the heavy fraction (HM) after sucrose gradient sedimentation. Metal-chelating chromatography of either heavy or light (LM) membrane fractions after octyl-glucoside treatment rendered similar Bchl-protein complexes profile, including most of the radioactivity in the same corresponding elution fraction (Fr II). Similar labelling distribution of pigment-protein complexes was obtained for membranes of dark-grown cells induced by lowering oxygen tension. HM fractions carried most of the labelling associated to LHIα polypeptide and a low free-running phospholipids content. On the contrary, fractions from LM showed the higher content of $[^{32}P]$-Pi labelling on the free-running phospholipids. Phospholipid analysis showed a similar pattern for membranes isolated from cells photosynthetically or semiaerobically grown, being the most abundant: phosphatidylglycerol (PG), phosphatidylethanolamine (PE), cardiolipin (CL) and phosphatidylcholine (PC). Part of the phospholipids from HM comigrate with LHIα during SDS-PAGE and only dissociated from the complexes after solvent extraction and hydrophobic chromatography. However, a small amount remained always atached to LHIα indicating an unusual strong interaction. These results suggest the existence of two operationally defined membrane regions carrying LHIα complexes differing in phosphorylation status and protein-phospholipid interaction.

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SERUM PROTEIN AFFINITY TO SYNTHETIC MEMBRANES MODIFIED BY SUGAR INCORPORATION. Romero, E.L., Mena, M., Richard, S., Glikmann, G., Porro, S. & Alonso-Romanowski. *Departamento de Ciencia y Tecnología, CEI, Universidad Nacional de Quilmes (UNQ), Bernal, Buenos Aires, Argentina.*

Neural cell adhesion molecules (NCAMs) can undergo post-translational modifications, such as the addition of polysialic acid chains, thus generating PSA-NCAMs, which are mainly expressed during development. Since polysialylation considerably modifies NCAM adhesivity, we decided to investigate if multilamellar liposomes prepared in the presence of sialic acid have adhesive properties when reacting with rabbit serum proteins.
Lκ phase liposomes were incubated 30 minutes at 37°C with whole rabbit serum proteins or, alternatively, its glycoprotein fraction. The latter was obtained by column chromatography on Phenyl Sepharose G fast flow high sub, followed by affinity on Concavalin A-Sepharose 4B. Incubations were performed at physiological (PBS buffer, 0.14 M NaCl) and low ionic strength. Subsequently, the liposome-protein mixtures were washed extensively by centrifugation until there was no protein detectable by Bradford assay. The washed liposomes were dissociated by SDS and electrophoresed on 10% polyacrylamide-tricine-SDS gels. The proteins were analyzed by Western blot using horseradish peroxidase conjugated goat anti-rabbit IgG. These analyses showed that only a low molecular weight protein fraction binds to the membranes regardless of the ionic strength or the membrane structure (gel or Lκ phase). In conclusion, these results indicate that the changes induced in the interfacial membrane structure do not affect the in vitro interaction with low molecular weight rabbit serum proteins. On the contrary, other proteins such as albumin do not bind to these liposomes. The *in vivo* studies in rabbits will be carried out in order to assess the stability of the liposomes in the blood stream.
This work has been supported by a grant from UNQ.

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EFFECT OF OXIDATIVE STRESS ON FLUIDITY AND ENZYME ACTIVITY OF RAT LIVER MICROSOMES. Rosenbluth H., Fuentes O.R., Valdés E., Lissi E.[1] Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile. [1]Departamento de Química, Facultad de Ciencia, Universidad de Santiago, Chile.

The prevalence of polyunsaturated fatty acids makes the microsomal membranes potentially suceptible to peroxidation. Lipid peroxidation induces chemical transformation of lipids and may also alter membrane fluidity and the activities of membrane bound enzymes. The present study was undertaken to follow the time course of microsomal membrane lipid peroxidation by simultaneous monitoring physical(fluidity), biochemical (enzyme activity) and chemical (TBARS formation) indices of lipid peroxidation.

We have studied two microsomal enzymes of different topology: UDPG-T and aminopirina N-demetilase, which are located in the luminal and cytosolic side respectively. The fluidity was monitored by using two fluorescent probes: DPH and TMA-DPH. We also used two alkanols (2,6-dimethyl-4-heptanol and n-heptanol), which increase lipid phase mobility to evaluate the effect of fluidity changes on the UDPG-T and N-demetilase activities.

Both alkanols greatly enhanced the UDPG-T activity. On the other hand, the N-demetilase activity was decreased just by 2,6-dimethyl-4-heptanol. The induction of lipid peroxidation produced a slight membrane rigidization and a high increase on the UDPG-T activity. Whereas the N- demetilase activity was decreased.

These results suggest that the chemical and /or physical changes induced by lipid peroxidation affect mainly the enzyme located in the luminal side of the microsomal membrane (UDPG-T). The activity change of UDPG-T is probably mediated by annular lipid mobility alteration..
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LIPOPEROXIDATION OF ROD OUTER SEGMENT MEMBRANES IS MODIFIED BY CYTOSOLIC PROTEINS

A. Terrasa. M. Guajardo y A.Catalá. Cátedra de Bioquímica, Facultad de Ciencias Veterinarias, UNLP, La Plata, Argentina.

Rod outer segments (ROS) of bovine retina are highly enriched in docosahexaenoic acid (22:6n3) a fatty acid that is essential for optimal retinal function and comprises about 50 mol % of the fatty acids.This is the major polyunsaturated fatty acid of the photoreceptor membranes and is an excellent target for peroxidation with peroxide formation . When retinal tissues are exposed to reactive oxygen species docosahexaenoic acid is transformed with modifications in the physical and biochemical properties of the membrane. The retina contain several low molecular weight cytoplasmic proteins involved in the solubilization of lipophilic molecules such as long chain fatty acid and retinoids. It is known that these lipophilic molecules can act as antioxidants in many lipoperoxidation processes either "in vivo" or "in vitro". We have designed an experimental system in order to examine the inhibitory effect of these cytoplasmic proteins against lipoperoxidation ascorbate-Fe++ dependent of ROS membranes. The degradative process was followed simultaneously by determination of chemiluminescence and fatty acid composition of ROS. The unsaturation index was used to evaluated the fatty acid alterations observed during the process. After incubation of ROS in an ascorbate-Fe++ system, at 37 °C during 120 min, it was observed that the total cpm originated from light emission (chemiluminescence) was lower in those membranes incubated in the presence of soluble binding proteins for fatty acids. The fatty acid composition of ROS membranes was substantially modified when subjected to non-enzymatic lipoperoxidation with a considerable decrease of 22:6n3. These results indicate that soluble proteins with fatty acid binding properties may act as antioxidant protecting ROS membranes for deleterious effect.

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EFFECT OF VITAMIN A DEFICIENCY ON HEMATOLOGIC PARAMETERS IN RAT. Anzulovich A., Bianco M., Oliveros L., Giménez M.S. -Lab.Qca.Biol., Fac. Qca..Bqca.Fcia., UNSL - San Luis, Argentina -

Knowing that retinoids participate on the differentiation of hematopoietic cells and, by our previous results, that hepatic synthesis of fatty acids decrease in Vit.A deficients rats, we study the effect of Vit.A deficiency on the levels of seric lipids and populations of blood cells. Two groups of female Wistar rats al 21 d of age, were ramdomly weaned onto either a Vit.A deficient diet (-A) or the same diet with Vit.A. 4000IU/Kg diet, (+A). They were fed during 3 months before sacrifice. The body weight and consumed diet were registered daily. The Vit.A and circulating lipids [Triglicerides (TG), Cholesterol (Cho), Phospholipids (P), Total Lipids and Lipoproteins] concentrations, were measured by comercial kits. The number of blood cells was determined by autoanalizer. The concentration of Vit.A in serum and liver were significantly lower in the -A group. The TG, Cho and HDLc levels decreased compared with the +A group. The number and morphology of red blood cells and the hemoglobin concentration were not modified in the -A group, however, the number of white blood cells was significantly lower. The percent of distribution of lymphocytes, granulocytes and monocytes was not modified. These data indicate that Vit.A deficiency affects the number of white blood cells and the levels of circulating lipids.

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EFFECTS OF CLOFIBRATE ON LIVER AND PLASMA LIPIDS OF MICE FED FATTY ACIDS OF THE n-3 SERIES. **Pennacchiotti, G. L., Maldonado, E. N., Rotstein, N. P. and Aveldaño, M. I. Instituto de Investigaciones Bioquímicas de Bahía Blanca, UNS-CONICET, 8000 Bahía Blanca, Argentina**

Previous work showed that the administration of clofibrate to mice increased the amount of phospholipids (PL) and decreased that of triacylglycerols (TG) in liver. The stimulated membrane proliferation imposed by the drug increased the need for fatty acids, which was fulfilled mostly by TG acyl groups. The polyunsaturated fatty acids (PUFA) with the shortest (C_{18}) and the longest (C_{24}) chains decreased in TG, suggesting that they were metabolized to provide part of the required C_{20} and C_{22} polyenes of PL. The fatty acid composition of the expanded PL changed less than that of the consumed TG. In the latter, n-3 PUFA decreased much more than n-6 PUFA. Since the rodent diet used had a high n-6/n-3 ratio, the effect of the administration of clofibrate after adaptation to a diet supplemented with fish oil (n-3) was investigated. After one month of a daily dose of this oil liver amounts of PL and TG were not affected, although their composition changed, increasing 18:3, 20:5, 22:5 and 22:6 n-3 while decreasing 18:2, 20:4, 22:4 and 22:5 n-6. Plasma lipid changes resembled those of liver, reflecting variations in fatty acid composition with virtually unaltered levels of TG in the presence of n-3 PUFA. The administration of clofibrate to n-3 fed animals resulted in a similar increase in hepatic phospholipids, but a smaller decrease in TG, than had been previously observed with the standard diet. This supports the view that the n-3 PUFA required for PL synthesis originate in TG acyl groups. In the presence of an abundant supply of n-3 PUFA, the changes affecting n-6 PUFA were the ones to become more significant.

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FATTY ACID METABOLISM IN RAT RETINAL CELLS IN CULTURE. **Rotstein,N.P., Politi, L.E.. and Aveldaño, M.I. Instituto de Investigaciones Bioquímicas, UNS-CONICET, Bahía Blanca, Argentina**

We have recently shown that docosahexaenoic acid (22:6n-3) is essential for the survival of rat photoreceptor cells in culture, and may act as a trophic factor delaying the onset of apoptosis that otherwise starts in these cells after 3 days in vitro. Since in vivo the proportion of this fatty acid in retinal lipids increases steadily after birth, we have investigated the role the different retinal cells play in its supply and metabolism. The proportion of this fatty acid, as well as that of 22:5n-3, its metabolic precursor, in glial cell lipids was markedly increased when these cells were incubated in their presence. When [^{14}C]22:6 was used, phosphatidylcholine and -ethanolamine concentrated similar amounts of label. The results show that glial cells are able to take up and accumulate 22:5n-3 and 22:6n-3 in their lipids and could play a role in the provision of 22:6n-3 to neurons. In neurons incubated with 22:6n-3 and 22:5n-3, the proportion of these fatty acids in lipids increased several times (from 6% to 24%, and from 0.4 % to 2.5%, respectively). Neuronal fatty acid composition remained constant when 16:0 and 20:4 were added to the culture medium, which is consistent with the lack of effect these two fatty acids have on photoreceptor cell survival. [^{14}C]22:6n-3, [^{14}C]22:5n-3, [^{3}H]16:0 and [^{3}H]20:4n-6 were all incorporated and esterified in neuronal lipids, with a characteristic pattern of labeling for each fatty acid. This suggests that while 16:0 and 20:4 are turned over on preexisting molecules, 22:6n-3 is used for the synthesis of new molecular species of phospholipids, required for the formation of the outer segments of photoreceptor cells.

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CHARACTERIZATION OF HMG-CoA REDUCTASE ACTIVITY IN NUDE MICE LIVER, IN A HUMAN LUNG MUCO-EPIDERMOID CARCINOMA GROWN IN NUDE MICE AND IN HOST LIVER. Margarita G. de Bravo. Mónica Polo, and Cecilia Carbone*. Inst.de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET-UNLP, Fac. de Cs. Médicas, calle 60 y 120. *Fac. de Cs.Veterinarias, Bioterio. La Plata. Argentina. (E-Mail:Bravo@biolp.edu.ar)

A large body of evidence has been accumulated indicating that "the novo" cholesterogenesis plays a critical and essential role in cell growth. The conversion of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) to mevalonic acid, usually the rate-limiting reaction in cholesterol biosynthesis, is controlled by the enzyme HMG-CoA reductase. The production of mevalonic acid, in addition to serving as precursor for the structural cholesterol requirements of cell growth might regulate cell proliferation by playing a direct role in DNA replication. The aim of this study was to characterize HMG-CoA reductase activity in: nude mice liver, a human lung carcinoma (HLMC) grown in nude mice and host liver. Since the activity of hepatic HMG-CoA reductase is subjected to a marked diurnal cycle, it was measured at mid-dark and at mid-light by incubating hepatic or tumoral microsomes with ^{14}C-HMG-CoA. After 30 min at 37°C, the ^{14}C-mevalonate was converted into lactona that was isolated by thin layer chromatography. The enzyme activity expressed as mevalonolactone picomoles/min.mg microsomic protein was: HLMC = 64.67 ± 8.05, host liver = 121.95 ± 9.15 at mid-light and 63.41 ± 11.10 and 620 ± 99.54 at mid-dark respectively; liver = 158.82 ± 23.36 and 537.51 ± 60.61 at mid-light and mid-dark respectively. Mid-dark/mid-light ratio of host liver was significant greater than mid-dark/mid-light ratio of non-host liver. The difference in enzyme activity was due to differences in Vmax. and not in Km. Results showed an appreciable HMG-CoA reductase activity in HLMC and its influences upon host liver activity.This work was supported by grants from CONICET, CIC, UNLP (Arg.).

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LIPID BINDING CAPACITY OF SPIDER HEMOCYANIN. Cunningham, M., Gómez, C. and Pollero, R. Instituto de Investigaciones Bioquímicas de La Plata (UNLP-CONICET), La Plata, Argentina. E-Mail: Pollero@biolp.edu.ar

We have previously reported the presence of two lipoprotein fractions with a very high content of hemocyanin (Hc) in hemolymph of the spider *Polybetes pythagoricus*, fact that makes the respiratory pigment the most important plasma apolipoprotein. In this work, we studied *in vitro* the lipid binding capacity of Hc. The lipoprotein fractions were isolated by density gradient ultracentrifugation and delipidated using detergents. Then, lipoprotein particles were reconstituted by incubating Hc with different labeled lipids (PC, Chol., TG) and free fatty acid (FFA). The binding capacity of each lipid to the monomeric, hexameric and heptameric protein forms, which were separated by HPLC, was measured with a radiometer. Lipids were only bound to hexameric form of Hc. Hyperbolic curves (incubated lipid/protein versus bound lipid/protein) were obtained. Then double-reciprocal plots allowed to obtain linear plots showing the following parameters: **Mr** (maximal molar ratio of bound lipid/protein)was 8.0 for PC, 5.8 for Chol. and 34.1 for FFA. **Ka** (relative association constant) was 42.2 for PC, 59.5 for Chol. and 1992 for FFA. **Yo**, maximal yielding (initial) of the lipid/protein binding was 0.1, 0.2 and 0.02, respectively. The most suitable interpretation of the hyperbolic ratio is that the hydrophobic internal regions of Hc allow lipid binding whereas the available binding sites decrease progresively. The Mr calculated *in vitro* were higher than those ones found *in vivo*, suggesting that the respiratory pigment would be able to carry larger amounts of lipids under physiological conditions. This fact would enable the lipid transport system be adapted to changing situations in plasma lipid concentrations.This work was supported by grants from CONICET, CIC, UNLP (Arg.).

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Prostaglandin H Synthase (PGHS) activity in different subcellular fractions of rat renal papilla. María del Carmen Fenández-Tomé. Emir H. Speziale and Norma B. Sterin-Speziale. Dto de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, IQUIFIB-CONICET, Buenos Aires, Argentina.

PGHS, usually known as cyclooxygenase, is a rate-limiting enzyme in the biosynthesis of prostaglandins (PGs). PGHS is an integral-membrane protein and its activity has been detected mainly associated to the endoplasmic reticulum (ER) fraction. Although PGHS protein has been found in nuclear (N) and plasmatic membranes (PM) by hystochemistry, no activity has been demonstrated in these two fractions. Rat renal papilla possesses a very active PGs biosynthesis which is involved in its physiology. The aim of this work was to investigate PGHS activity in ER, N and PM membranes in rat renal papilla. ER, N and MP fractions were obtained from renal papillary homogenates (0.25 mM sucrose) by differential centrifugation and the activity of PGHS was measured by incubating aliquots of the different subcellular fraction with ^{14}C-arachidonic acid, 1 mM epinephrine and 1mM GSH at 37 ºC. Reaction was stopped by adding 1 mM citric acid up to pH 3 and radioactive PGs were extracted with chloroform and separated by TLC. Radioactivity was quantified and results expressed as ^{14}C-PG/ mg protein. In rat renal papilla PGHS activity was found not only in ER membranes but also in N and PM fractions. The highest PG biosynthesis activity was found in PM fraction followed by ER and N (89, 52 and 35 ^{14}C-PG/ mg protein.min respectively). The main PG synthesized in each zone were PGE_2 and $PGF_{2\alpha}$ while PGD_2 biosynthesis was almost undetectable. The results show that renal papilla PGHS activity is clearly compartmentalized and the highest activity is associated to PM fraction.

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[^{3}H]-H_2O INCORPORATION ON HEPATIC LIPIDS IN RATS INTOXICATED WITH CADMIUM.

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Cadmium (Cd) is a toxic that has acquired importance like environmental pollutant. In order to determine if the intoxication with Cd produces alterations on the turnover of hepatic lipids, the incorporation of [^{3}H]-H_2O, was measured. Wistar adult male rats were intoxicated with $CdCl_2$. 2.1/2H_2O, in the water of drinking (15 ppm of Cd), during 7 weeks. Controls and treated rats were injected with [^{3}H]-H_2O, 5mCi/ rat, intraperitoneally. After 1 hour the animals were sacrificed by heart punction and the livers were extracted. The lipids were extracted according Folch method, and in the dry extract, total lipids were determined. The differents lipids were separated by TLC and the incorporation of [^{3}H]-H_2O was determined in the corresponding strips to free cholesterol, sterified cholesterol, phospholipids and triglycerides. A minor incorporation of [^{3}H]-H_2O was observed in total lipids, sterified cholesterol, phospholipids and triglycerids. Simultaneously, cholesterol, triglycerides and phospholipids concentrations were determined, observing a significative decrease only in sterified cholesterol values These results suggest that Cd produces a decrease in the turnover of hepatic lipids.

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BIOSYNTHESIS OF TERPENOIDS, FATTY ACIDS AND POLYKETIDES IN *Gibberella fujikuroi*. Giordano, W.[1], Avalos, J.[2], Cerdá-Olmedo, E.[2] and Domenech, C.[1].

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The fungus *G. fujikuroi* is well known for its biotechnological importance in the production of gibberellins. In addition to these compounds, it produces other terpenoids such as carotenoids and kaurenolides and the derivatives from acetyl-CoA: bikaverins, fusarins, aflatoxins, etc. In experiments of isotope dilution utilizing leucine, mevalonate or acetate, we have shown that the biosynthesis of sterols, gibberellins and carotenoids occurred in different subcellular compartments. With the same experimental approach, utilizing the wild type and gibberellin mutants of *G. fujikuroi*, we have compared the flows of these precursors in the terpenoids, fatty acids and polyketides pathways. In all cases, the fungus was grown in a low-nitrogen minimal medium, with excess of glucose (444 mM) supplemented with 10 mM of radioactive leucine, mevalonate or acetate. After the extraction of the different compounds from the culture supernatants and mycelia, they were analyzed by TLC and HPLC. The results obtained are summatized as follows: In *G. fujikuroi*, mevalonate was utilized for the biosynthesis of fatty acids and polyketides in addition to the terpenoid synthesis; lovastatine, a known inhibitor of the HMG-CoA reductase, inhibited the synthesis of gibberrellins but did not affect the ergosterol and carotenoid pathways; leucine and mevalonate contributed in a similar manner to the synthesis of the polyketide bicaverine; of the three radioactive precursors, acetate was the least suited as a source of label for terpenoids, fatty acids and polyketides.

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MODULATION OF PHOTORECEPTOR MEMBRANE ACYL-CoA:LYSOPHOSPHOLIPID ACYLTRANSFERASE BY G-PROTEINS. GIUSTO, N.M. and CASTAGNET, P.I. INIBIBB, UNS-CONICET, Bahía Blanca, Argentina.

We have recently described an active oleoyl-CoA:lysophospholipid acyltransferase in photoreceptor rod outer segments (ROS). In this study we investigated the involvement of G-proteins in the regulation of ROS lysophospholipid acyltransferase (LPL-AT). Photoreceptor membranes were incubated with [1-^{14}C]oleoyl-CoA in the presence of increasing concentrations of $MgCl_2$, GTP or GTP analogs such as GTPγS and GMPP(NH)P (non hydrolyzable G-protein activators) and the GDP analog, GDPβS (a G-protein inhibitor). The reaction was stopped 5 min after incubation at 37 °C and the lipids were extracted and then separated by monodimensional thin layer chromatography. The bands corresponding to the lipids of interest were scraped off from the plates and the radioactivity was determined by liquid scintillation counting. Phosphatidylcholine (PC) incorporated the greatest amount of labeled oleate. When studying the effect of Mg^{2+} ions on AT activity we found that the chelator EDTA inhibited lysoPC (LPC) esterification by 20%. $MgCl_2$ stimulated LPC-AT up to a concentration of 1 mM and inhibited its activity at higher concentrations. GTP stimulated LPC acylation as a function of its concentration. In addition, non hydrolyzable GTP analogs stimulated whereas the G-protein inhibitor GDPβS inhibited AT activity as a function of the analog concentration. Light produced a 20% increase in LPC acylation as compared to the acylation measured in membranes incubated in the dark. These results suggest that G-proteins are involved in the regulation of LPC acylation.

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EARLY OCCURRENCE OF LIPID ALTERATIONS IN SPONTANEOUSLY DIABETIC RATS. Gómez Dumm, N.T. de, Igal, A., Montenegro, S., Tarrés, M.C. and Martínez S.M. INIBIOLP, Facultad de Ciencias Médicas, Universidad Nacional de La Plata and Cátedra de Biología, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Argentina.

Human and experimental diabetes mellitus extensively alters lipid metabolism in several tissues. The cSS (IIMe/Fm eSS) is an animal model that develops an spontaneous diabetes of slow evolution, resembling the non-insulin-dependent diabetes mellitus of young people. In the present work we have studied the biochemical disturbances of lipid metabolism in 5-month old eSS rats compared to aged-matched α-controls. Normal plasmatic glucose levels, in the fasted state, were found in all the animals studied, whereas a diabetic curve was evident for eSS rats after glucose load. Triglyceride concentration was elevated in plasma and in liver microsomal preparations of eSS animals. The diabetic strain revealed a significant fall in the amount of linoleic acid in the microsomal fraction of liver and kidney and in erythrocyte membranes. In the liver an increase in 22:6 (n-3) was also noted. The depression in linoleic acid as well as the enhancement of docosahexaenoic acid were detected in PC and PE phospholipid fractions of liver microsomes of eSS rats, compared to the α-controls. The fatty acid pattern of eSS rat testis showed a raise in the relative percentage of arachidonic and a decrease in 22:5 (n-6), 22:5 (n-3) and 22:6 (n-3) acids compared to the controls. In this tissue an increase in the fluorescence anisotropy of DPH was also shown. The current observations indicate the early alteration of lipid metabolism in eSS rats when the animals are still normoglucemic, fact that may contribute to the set establishment of the diabetic syndrome. This work was supported by grants from CONICET, CIC, UNLP (Arg.).

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EFFECT OF FENITROTHION ON MEMBRANE PHYSICO-CHEMICAL PROPERTIES IN *MACROBRACHIUM BORELLII*. M. González Baró and R. Pollero. Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), Fac. de Cs. Médicas, Calles 60 y 120, 1900, La Plata, Argentina.

The effect of the organophosphorous insecticide fenitrothion (Fen) on the physical state of hepatopancreas microsomal membranes of *M. borellii* was investigated by fluorescence polarization of 1,6-diphenyl 1,3,5-hexatriene (DPH) probing the bilayer core and its anionic propionic acid derivative (DPH-PA) probing the outer regions of the bilayer. Microsomes were obtained from animals exposed *in vivo* to sublethal doses of Fen (10^{-4} ppm) and from control animals. Microsomes added with different concentrations of Fen *in vitro* were also used.

The presence of sublethal water concentrations of Fen up to 15 days, does not exert significant membrane fluidity modifications either in the hydrophobic core or in the outer regions of the bilayer. When Fen was added *in vitro*, the insecticide strongly orders the fluid phase of both zones of the membrane studied. This ordering effect of Fen is directly dependent on the insecticide concentration. The rigidizing effect of Fen *in vitro* is greater in the control animales than in the *in vivo* Fen-treated ones. This fact may suggest the triggering of detoxifying mechanisms during the adaptation of the animals to the environment.

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INSULINEMIA AND LIPOPROTEINS IN OBESE PERSONS AT SEA LEVEL. *Jo, N., Pando, R., Garmendia, F., Losno, R., Jara, R. Instituto de Investigaciones Clínicas, Facultad de Medicina, Universidad Nacional Mayor de San Marcos, Lima, Perú.*

Recent studies have demonstrated that is the central distribution of lipid rather than overall obesity that is associated to diabetes, hypertension, and hypercholesterolemia. In orden to know the relationship between insulin and plasma lipoproteins, 36 obese persons from both sexes, between 19 and 54 years of age, were studied at sea level. An IMC higher than 25 for both sexes and an I.c/c higher than 0.8 for women, and higher than 0.9 for men, were considered as obesity. Glucose and insulin basal levels and levels after 120' of TTGO were dosed; as well as lipid and lipoprotein levels. Variations in function of sex, degree of obesity, and risk factors were observed. Also, a relationship between insulin and the alteration of lipoproteins was found. Obese with hyperinsulinemia show abnormalities in lipid and lipoprotein concentration, finding that might predispose to cardiovascular risk.

Proyecto FEDU UNMSM.

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EVALUATION OF LIPID SOLUBLE ANTIOXIDANTS BY A CHEMILUMINESCENT METHOD. Lissi, E., Cárdenas, G., Escobar, J. Departamento de Química, Facultad de Química y Biología, Universidad de Santiago de Chile.

A methodology based on the quenching of luminol chemiluminescence is proposed for the evaluation of lipid soluble antioxidants. Luminol chemiluminesce is promoted by the thermolysis of 2,2'-azobis(2-amidinopropane) in glycine buffer, pH 9.4. Lipid soluble antioxidants are solubilized by addition of 0.02 M dodecyltrimethylammonium bromide. Additions of a ethanolic solution of Vit. E produces a diminution in luminol chemiluminescence that is proportional to the additive concentration. The detection limit in the ethanolic solution is below 1 μM. The procedure takes less than 2 minutes and can be carried out in simple luminometers. The proposed methodology allows the evaluation of the levels of Vit. E in blood plasma and the amounts of total lipid soluble antioxidants in membranes or tissues.

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EFFECT OF THE *Amorphophallus konjac* ON THE PROFILE LIPIDS IN RATS. Llañez, S., Ore, R. Valdivieso, R. and Sandoval, M.

Centro de Investigación de Bioquímica y Nutrición, Facultad de Medicina, Universidad Nacional Mayor de San Marcos, Lima. Universidad Nacional José F. Sánchez Carrión, Huacho, Perú.

We have investigated the effect of the ingest of *Amorphallus konjac* Fiber (FSAK) on the plasma lipids parameters of experimentals obese rats. Animals were divided in to three groups, each of wich comprised six rats.

Group I: Control, received a normal diet of 440 Kcal; Group II: experimentals obeses received a hipercaloric diet of 497 Kcal; Group III: experimentals obesese received a control diet 100 mg/day of FSAK for three monts.

Following the dietary period rat were decapited and blood was collected.

Plasma triglycerides, total cholesterol, low density lipoproteins (LDLc) in group III disminished and were significancy higher in group II but the hight density lipoproteins (HDLc) disminished in group II and increased in group III. In group I the parameter plasmatic were unchanged.

This results suggest an important rol of FSAK on the profile of lipid in rats.

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EFFECT OF NEONATAL HYPOXIA-ISCHEMIA ON D-[6-^{3}H]-GLUCOSAMINE INCORPORATION IN GANGLIOSIDES OF RATS HIPPOCAMPUS. Madke,R.R.: Rotta,L.N.;da Silva,C.G.; Ruviaro,C.; Arteni,N.;Netto,C.A. and Trindade,V.M. Dep. Bioquimica-IB-UFRGS-Porto Alegre, RS, Brazil.

Neonatal hypoxia-ischemia (H/I) induces a wide spectrum of metabolic derangements and membrane structural damage. Gangliosides are glicosphingolipids present at relatively high concentration in neural cell membranes. They are synthesized in a stepwise manner by transfer of carbohydrates residues from sugar nucleotide donors to glycolipid acceptors, and the key glycosiltransferases have different pH profiles. The aim of present work was to investigate the effect of neonatal H/I on ganglioside biosynthesis in the hipppocampus rats. Seven-days old rat pups were exposed to H/I for 2.5 h according to the Levin model. After 30 min of hypoxia animals were killed by decaptation, hippocampus dissecaed out and incubated in KRB, 5mM glucose and 15μ Ci-[^{3}H]-glucosamine, at 34°C, for 150 min, in a metabolic incubator. The incubation medium was then separated from tissue by centrifugation and total lipids were extracted from the pellet with chloroform: methanol mixtures. Gangliosides were partitioned in aqueous phase, purified by silicic acid column, dialized and liyofilized. Residues were resupended in C:M (1:1). The radioactivity was measured in all experimental fractions. Partial results showed a decrease in radioactivity incorporation in total ganglioside fraction obtained from hypoxic animals. We suggest that this effect may be another consequence of oxigen deficit on cerebral metabolism. (CNPQ, FINEP,FAPERGS, PIBIC-UFRGS,PROPESP-UFRGS),

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NITROPRUSSIDE STIMULATES THE SYNTHESIS OF ^{14}C-ACETYLATED PHOSPHOLIPIDS IN ESTROGEN PRIMED MOUSE UTERINE HORNS.

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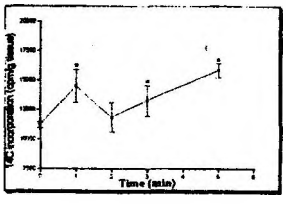
The effect of Nitroprusside (NP), a classic NO donor, on contractility and ^{14}C-acetylated phospholipids synthesis was analysed in estrogen primed mouse uterine horns. Tissue samples (65 mg) were preincubated with Sodium ^{14}C-acetate for 60 min and then stimulated with 300 µM NP for 1,2,3 and 5 min. Lipids were extracted by following a Folch modified method and developed in chloroform:methanol:water (65:35:6) in TLC and HPTLC. ^{14}C-acetylated phospholipids co-migrate with PAF standard in TLC, but after being extracted and rechromatographed in HPTLC with the same solvent system they migrate at a different Rf than PAF. No PAF was detected under these conditions. The synthesis showed a characteristic time course (Fig.1) that coincided with an increase in uterine contractions.

We propose that NP treatment of uterine horns from estrogen-primed mice generates PAF-like ^{14}C-acetylated phospholipids, which are mostly retained by the tissue.



Financed by Fondecyt 194-0955 and Univ. Concepción DI 195-3202, Chile.

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DIETARY GEOMETRIC AND POSITIONAL *TRANS* FATTY ACIDS ISOMERS FROM MARINE SOURCES: EFFECTS ON THE ACTIVITY OF SOME MEMBRANE-BOUND ENZYMES OF THE RAT LIVER. Morgado, N., Sanhueza, J., Garrido, A., Galleguillos, A., Nieto, S., and Valenzuela, A. Unidad de Bioquímica Farmacológica y Lípidos, INTA, Universidad de Chile, Santiago, Chile.

Dietary *trans* fatty acids (geometric and positional isomers) from fats obtained from the catalytic hydrogenation of vegetable or marine oils may produce some metabolic effects mainly in the structure of biological membranes and in the activity of membrane-bound enzymes. The biochemical effects of vegetable *trans* fatty acids are relatively well documented, but the information about those from marine origin is scarce because few countries allow the human consumption of hydrogenated fish oil. Chile is an important fish meal and fish oil producer and an important amount of the oil is hydrogenated for the manufacture of margarines and shortenings for human consumption and animal feeding. In this communication we describe the effect of a diet containig either, partially hydrogenated fish oil (high *trans* fatty acids content), highly hydrogenated fish oil (low *trans* fatty acids content), and vegetable oil (no *trans* fatty acids) as the lipid source, in the fluidity of erythrocyte and hepatic membranes and in the activity of some hepatic microsomal enzymes involved in the xenobiotic metabolism of the rat. Preliminary results are indicating that *trans* fatty acids from partially hydrogenated fish oil decrease the fluidity of erytrocyte and hepatic membranes when compared to controls (vegetable oil), and also change the biochemical activity of some microsomal enzymes involved in the xenobiotic conjugation process. The effect of highly hydrogenated fish oil (low *trans* content) in the parameters described is intermediate, but resembling closely to the effect of vegetable oil than of partially hydrogenated fish oil.

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OBTENTION OF STRUCTURED LIPIDS FOR NUTRITIONAL AND PHARMACOLOGICAL APPLICATIONS BY TRANSESTERIFICATION WITH STEREOSPECIFIC LIPASES. Nieto, S., Sanhueza, J., and Valenzuela, A. Unidad de Bioquímica Farmacológica y Lípidos, INTA, Universidad de Chile.

The type and position of the fatty acids (FA) forming the triacylglycerols (TAG) of fats and oils used for human and animal nutrition are important from the metabolic point of view. FA in the positions sn-1 and sn-3 of the TAG are transported to the liver for oxidation, mainly the short- and medium-chain FA (C6-C12). TAG containing FA in the sn-2 position are reesterified and transported to the extrahepatic tissues as chylomicrons. Therefore, the position and the type of the FA in the TAG is of metabolic importance. Structured lipids obtained by the modification of TAG allow the obtention of FA por specific metabolic destination and nutritional applications. In this work we describe the obtention of structured lipids (TAG) containing medium chain FA at the sn-1 y sn-3 positions and long-chain polyunsaturated FA at the sn-2 position by the controlled transesterification with a stereospecific immobilized microbial lipase (Lipozyme IM-20) obtained from Mucor miehei. Lipases when acting in a medium with low water activity catalyze transesterification rather than hydrolysis. Lauric acid (C12:0) obtained by the enzymatic hydrolysis and selective fractionation of coconut oil was used as medium-chain FA for transesterification. Monoacylglycerols having long-chain polyunsaturated FA at the sn-2 position were obtained by the stereospecific hydrolysis of a fish oil concentrate containing 80-90% of C20:5 and C22:6. The structured TAG obtained after the transesterification with Lipozyme IM-20 were stabilized to oxidation by synthetic and natural antioxidants.

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THE POTENCIAL ROLE OF VITAMIN E IN HYPERCHOLESTEROLEMIC RATS. Ore, R; Haak, H and Valdivieso, R. Centro de Invest. de Bioquimica y Nutrición. Fac. de Medicina. Universidad Nacional Mayor de San Marcos. Lima-Peru.

We have investigated the effects of high-cholesterol diet in the presence and absence of Vitamin E on the lipid peroxidation measured as malondialdehyde in blood and aortic tissue and lipid profile in rats.

The animals were divided into four groups. In group I received a control diet; those in group II received Vitamin E; those in group III received high cholesterol + Vitamin E and those in group IV received a high cholesterol diet. Blood concentrations of triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-C); high density lipoprotein cholesterol (HDL-C); and malondialdehyde (MDA) were measured. In the aortic tissue MDA were estimated.

Serum triglycerides, total cholesterol LDL-C and HDL-C increased in groups III and IV but remained unchanged in groups I and II. Blood and aortic tissue MDA increased in group IV but decreased in groups II and III. This results suggests a role hypercholesterolemia induced atherosclerosis. The protection afforded by vitamin E wich was associated with decreased in blood and aortic tissue malondialdehyde concentrations. Proyecto FEDU Nº 6010508-96

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ETHER PHOSPHOLIPIDS SYNTHESIS DURING *IN VITRO* INDUCTION OF LIPOCYTE PHENOTYPE IN HEPATIC CONNECTIVE TISSUE CELLS. Rosa, T. Galetto; Borojevic, R.; Guaragna, R.M. Departamento de Bioquímica, Instituto de Biociências, UFRGS, Porto Alegre, Brasil. Connective tissue cells associated with hepatic fibrosis and cirrosis were identified as miofibroblasts and fat-storing (Ito-cells). The GRX as a myofibroblastic cell line, representative of liver connective tissue cells (LCTC). This cell line can be induced *in vitro* a lipocyte phenotype by insulin and indometacin. This drugs act synergistially inducing, after 4-6 days, synthesis and accumulation of neutral lipids: triacylglycerols, monoalkyl-diacylglicerol and cholesterol. This results indicates that the synthetic pathway of long-chain alcohol formation and their incorporation into dihydroxyacetone phosphate exists in LCTC. Ether phospholipids have biological significance as membrane components and as cell mediators. This research identified ether-phospholipids in GRX cells. The cells were radiolabeled either [U-^{3}H] - inositol (10μCi/5ml) or [^{32}P]-phosphate (20μCi/5ml) 24h. The lipids extrated with chloroform/ methanol (2:1,v/v) were resolved by two dimensional TLC: Chloroform/methanol/ acetic ac. (65:35:10; v/v), first dimension, and chloroform/methanol/ formic ac.(65:35;10; v/v), in the second. Betweens dimensions the plates were sprayed with 10nM $HgCl_2$. Base hydrolysis of phospholipids radiolabeled with [U-^{3}H]-inositol was achieved with 0,5ml of 0,1N KOH (in methanol) for 1h at 37°C.The lipids were resolved by TLC:chloroform / methanol/ aq.NH_4OH /1M ammonium acetate /water (90:70:4,5:4:11,5:,v/v). Choline and ethanolamine plasmalogens were identified in both phenotypes, but the syntesis was increased 2 and 3 fold in the lipocytes respectively. The PI synthesized was base-stable in both phenotypes, suggesting that this cells were deficient in alkyl-PI. Our results indicated the interest of frurther studies the participation of ether-phospholipids during conversion of LCTC. FINEP/CNPq/CAPES/FAPERGS.

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EFFECTS OF A SUCROSE-RICH DIET ON MORPHOLOGICAL AND FUNCTIONAL ASPECTS OF RAT ADIPOSE TISSUE. Soria A., Chicco A., Lomberdo Y.B. Departamento de Ciencias Biológicas, Facultad de Bioquímica. y Cs. Biológicas, U.N.L. - Santa Fe, ARGENTINA.

Wistar male rats fed for 3-4 months on a 63% w/w sucrose-rich diet (SRD) developed a metabolic-hormonal syndrome which evolved from normal glucaemia and high insulin levels (Induction Period -IP-: 3-4 weeks of feeding) to high glucaemia and normal insulin levels (Recurrence Period -RP-: 10-15 weeks of feeding). High triglyceride (TG) and NEFA levels were found in both periods. Taking account of the relationship between NEFA plasmatic concentration, insulin resistance and hypertriglyceridemia, and being aware of the key function of the adipose tissue (AT) in NEFA delivery to the plasma, the aim of this work was to evaluate the following parameters in epididymal AT of animals fed a SRD: 1) basal and stimulated (10^{-6} M Isoproterenol) lipolysis; 2) antilipolytic insulin (1,7 nM) action expressed as the percentage (%) of induced lipolysis (10^{-7} M Isoproterenol) inhibition; 3) Lipoprotein Lipase activity (LPL); and 4) cellularity and cellular TG content during IP and RP. The results ($\bar{X} \pm$ SEM) were compared with animals which received a standard diet (STD) during a identical experimental period. (*$p < 0,05$ SRD vs STD; $n \geq 4$).

DIET	GLYCEROL RELEASE BASAL (nmol / 10^5 cells . hour)	GLYCEROL RELEASE ISOP. 10^{-6} M (nmol / 10^5 cells . hour)	% OF INDUCED LYPOLISIS INHIBITION	LPL (pKatal / total organ)
STD-IP	69.3 ± 9.7	663.7 ± 56.4	47.5 ± 2.,3	12920 ± 762
SRD-IP	127.5 ± 6.2*	417.7 ± 49.8*	32.8 ± 3.5*	11633 ± 728
STD-RP	81.3 ± 12.9	626.8 ± 61.2	47.2 ± 4.9	18710 ± 1037
SRD-RP	373.1 ± 66.0*	744.3 ± 52.6	8.8 ± 4.2*	35677 ± 1613*

A higher weight (SRD: 8,1 ± 1,0 vs STD: 4,7 ± 0,9 gr) and cellular TG content (SRD: 0.55 ± 0.19 vs STD: 0.27 ± 0.08 nmol of trioleyn / cell) was found in the epididymal AT of the SRD group in the RP, but not in the IP. The number of adipocytes/ total organ was not statistically different in the analysed groups. From these data, it can be inferred that the administration of a SRD: 1) modifies the lipolytic activity of the epididymal AT in IP and RP, so that the higher basal lipolysis and the lower insulin antilipolytic action (more noticeable during RP) would contribute to glucose intolerance and hypertriglyceridemia present in both periods; and 2) it leads to cellular hypertrophy, higher epididymal weight y higher LPL activity when a long administration period is considered. The increased LPL activity would promote, at least partially, the rise in adipocyte TG content in spite of the higher lipolysis that characterises the RP.

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SPHINGOMYELIN METABOLISM IN SERTOLI CELLS: EFFECT OF RETINOL TREATMENT. Souza, I.C.C.; Raiman, P.E.; Casali, E.,A.; Bernard, E.A. and Guma, F.C.R.
Dpto. de Bioquímica, IB, UFRGS, Porto Alegre, Brasil.

In previous studies we described that the treatment of Sertoli cells with 10 μM retinol modified the turnover and decreased the mass of sphingomyelin (SM). Sertoli cells are the proposed target of important modulatory effects of vitamin A upon normal spermatogenesis. When the SM metabolism was followed by labeling with ^{32} Pi, SM labeling showed a significant decrease to 78 ± 5% of control during the frist 1h after addition of retinol. When the phospholipid concentration was determined by measuring of phosphate content, or the cells were labeled with [^{14}C] choline, retinol treatment for 30 or 60 min induced a decrease in the content and in the labeling of one of the SM bands detected by TLC.To better understand the mechanism of retinol action on SM metabolism, we have developed a cell-free sphingomyelinase (SMase) assay for Sertoli cells. Sertoli cell cultures obtained from 19-day-old Wistar rats were maintained for 5 days and than used as enzyme source. Sertoli cells crude homogenate, citosolic and microsomal fractions displayed SM hydrolysis within the range pH 4-7.5. The highest activity was detected at pH 4-5. The acidic enzyme activity, was much higher in citosolic fraction. The release of phosphorylcholine was time- and enzyme concentration-dependent until 45 min and 150μg protein. Studies are underway to explain the biochemical mechanisms involved in the retinol induced SM hydrolysis in Sertoli cells.

Supported by: FINEP, CNPq, FAPERGS and PROPESP-UFRGS.

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Effect of protein-calorie malnutrition during gestation and lactation upon Δ5 and Δ6 desaturase activities in rats fed different oils. María C. Marín, María E. De Tomás and María J.Tacconi de Alaniz. Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP) CONICET-UNLP. Fac. de Cs. Médicas. UNLP. E-Mail: mtacconi@isis.unlp.edu.ar

In earlier experiments it was demonstrated the adverse influence of protein-calorie malnutrition during gestation and lactation upon the fatty acid desaturase activities, using corn oil in the dam's diet.

We undertook this study to determine the effect of αlinolenic acid in the maternal diet upon the liver Δ5 and Δ6 desaturase activities of malnourished weaning rats and their pups, using soy oil or corn oil.

18 days after parturition the dam and their pups were killed, the livers were removed and used for microsomal isolation by differential centrifugation. The microsomal protein was estimated by the method ef Lowry. The rate of Δ6 and Δ5 desaturation was measured using 1^{14}C 18:2n6 and 1^{14}C 20:3n6 as substrate in presence of NADH, ATP and Coenzime A. The conversion of precursor to product was measured by reverse phase HPLC.

Protein-calorie malnutrition produced a reduction in Δ5 and Δ6 desaturase activities. A lesser severe effect in the Δ6 desaturase was observed in both diet treatments (corn oil and soy oil). The desaturation activities were less affected in those animals which received soy oil. According to the results of the present experiment we can conclude that either α18:3n3 or their elongation-desaturation products may be responsible for the results obtained

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MALNUTRITION DECREASES BIOCHEMICAL PARAMETERS OF RAT HIPPOCAMPUS. Saldanha,T.F.R; Rotta,L.N.; Silva,C.G. da; Madke.R.R.:Trindade,V.M.T. Dep. Bioquímica - IB - UFRGS-Porto Alegre, RS, Brasil.

It is well recognized that undernutrition during the vulnerable period of growth impairs brain maturation. Morphological studies have shown that migration of cells is retarded and proliferation of neuronal fibres decreased in undernourished rats. Biochemical investigations showed significantly lower levels of myelin lipid components in undernourished whole rat brains. The present work describes the changes of total gangliosides and phospholipids contents in hippocampus of normal (diet: 25% casein) and pre- and postnatal undernourished rats (diet: 8% casien). Hippocampus plays a major role in spatial memories and thys ability is affected by early undernutrition. Animals were killed by decaptation and hippocampi dissecated out for biochemical evaluations. Total lipids were extracted with chloroform-methanol mixtures. Gangliosides were partitioned in the aqueous phase and determined by tiobarbituric acid procedure. The organic phase was evaporated and lipid phosphorus measured after samples mineralization. Significant increases were found on the gangliosides and phospholipids contents during the development. Both parameters were diminished in undernourished rats. These results are in accordance with similar studies on hyphotalamus, and have been interpreted taking into account the reduction of synaptogenesis and myelinogenesis by early undernutrition. (CNPQ, FAPERGS, FINEP, PIBIC-UFRGS, PROPESP-UFRGS, ROCHE, BLANVER).

Membranes, Lipids, Receptors - Hormones and Growth Factors, Signal Transduction, Cell Biology - Molecular Aspects

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EFFECT OF ETHANOL ON THE KIDNEY MICROSOMAL AND PEROXISOMAL FATTY ACID OXIDATION. Valdés, E., Rodrigo, R. and Orellana, B.M. Dpto. de Bioquímica y Med. experimental, Fac. de Medicina, U. de Chile.

Long-term alcohol consumption induces the cytochrome P-450 microsomal activity of several xenobiotics and inhibits mitochondrial fatty acid oxidation. The microsomal cytochrome P-450 catalizes the ω-hydroxylation of fatty acid. The hydroxylation in a terminal carbon and its last oxidation catalized by an alcohol dehydrogenase, generates the corresponding dicarboxilic acid, preferencially chain-shortened by peroxisomes, a minor pathway for fatty acid oxidation.

In this work we study the effect of long-term ethanol consumption on the kidney microsomal cytochrome P-450 and the peroxisomal fatty acid oxidation. Mature male wistar rats received ad libitum an aqueous 20% (v/v) ethanol solution as sole drinked fuid for 10 weeks. It was determined the microsomal lauric acid ω- and ω1-hydroxylation and the aminopyrine N-demethylation. Also the peroxisomal fatty acid β-oxidation and the catalase activity was measured. In the long-term ethanol treated rats only the microsomal aminopyrine N-demethylation was increased. The palmitoyl CoA β-oxidation was not modulated by ethanol, in contrast, the kidney catalase activity was enhanced. It is conclude that long-term ethanol treatment don't modulate the kidney microsomal and peroxisomal fatty acid oxidation. Moreover, the kidney response to a long-term ethanol treatment is different to the hepatic one.

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INCORPORATION OF 20:4n-6 ACID INTO NUCLEAR LIPIDS OF RAT LIVER CELLS. Ves Losada, A. and Brenner, R.R. Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET-UNLP, Facultad de Ciencias Médicas, calle 60 y 120 y Dto. de Cs. Biológicas, Fac. de Cs. Exactas, UNLP. 1900-La Plata, Argentina. E-mail: avlosada@biolp.edu.ar

The most abundant polyunsaturated fatty acids in liver cell nuclei are those belonging to the n-6 series (46%), and among them arachidonic acid (20:4n-6) is found in a greater proportion (23%). This acid is not only an important structural component in nuclei, but also the substrate for the eicosanoid synthesis. Nuclear arachidonic acid may be of two origins: it would be either synthesized from 20:3n-6 by nuclear Δ5 desaturase or come from citoplasm. The aim of the present work was to study the incorporation of 20:4n-6 acid into nuclear lipids. For this purpose, nuclei either with or without cytosol were incubated with [1-^{14}C]20:4n-6. After 5, 10 and 20 min, nuclei (N) were separated from the incubation medium (S) by centrifugation. Lipids were extracted from both fractions using Folch technique, then separated by TLC and the radioactivity was measured by scanning. After 5-min incubation, 70% of 20:4n-6 acid was incorporated into the supernatant: 65% as free acid and 5% as acyl-CoA. In the nucleus, 8% was incorporated as free acid, 10% was esterified in PL, 9% in TG and 2% in DG. The 20:4n-6 acid decreased in the incubation supernatant (45%) after 20 min incubation since the free acid and the acyl-CoA also evidenced a fall. The remaining 20:4n-6 continued its incorporation into PL (27%) and TG (19%). 20:4n-6 acid was linearly incorporated into nuclear ethanolamine glycerophospholipids (85%) and into phosphatidyl inositol (15%) for 10 min of incubation.

This work was supported by grants from CONICET, CIC and UNLP.

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IN VITRO HYPOTHALAMIC RELEASE OF SOMATOSTATINE IS MODIFIED BY ESTROUS CYCLE AND BY STRESS DELIVERED TO DONOR RATS. *Belmar, J., Estupina, C., Pesco, J., Tapia-Arancibia, L. and Arancibia, S. *Departamento de Biologia Celular y Molecular, Pontificia Universidad Católica de Chile, Santiago-Chile and Laboratoire de Plasticité et Adaptations Cellulaires, Université de Montpellier 2., Montpellier, France.

Stress and sexual cycle can modify chemical neurotransmission in the central nervous system. neurons of hypothalamic nuclea are specially involved in this process. In this work we studied the effect of depolarizing stimulus (K^+, 40 mM) on spontaneous release of somatostatine (SS) from blocks of hypothalamic regions dissected from cycling donor rats having undergone prior stress, 30 minutes of cold exposure (4°C).

The blocks were incubated in Krebs-Ringer Buffer (pH 7,4 at 37° C, saturated with 0_2 95%, and CO_2, 5%). In no flowing conditions. When submitted to K^+, (10 min) no significant differences were found whatever was the stage of the ovarian cycle of the donor rats. However, significant differences appeared after ovariectomy an pregnancy or in some stages of the sexual cycle (proestrus and estrous) when donor rats underwent a previous cold stress.

Our data show that SS release from hypothalamus obtained from rats under stress conditions is modified by the sexual cycle and suggest interactions between steroids of gonadal origin and those derived from the adrenal cortex operating under stress conditions.

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LYSOSOMAL ENZYMES SECRETED BY CULTURED CELLS OF CAPUT, CORPUS AND CAUDA OF RAT EPIDIDYMIS. **Belmonte, S., Sosa, M., Bertini, F. and Castellón, E.* IHEM-CONICET. Fac. de Cs. Médicas. Univ. Nacional de Cuyo. Mendoza, Argentina and *Departamento de Biología Celular y Genética, Facultad de Medicina. Univ. de Chile, Santiago, Chile.**

Spermatozoa leaving the mammalian testis are inmotile and infertile; post-testicular sperm maturation takes place in response to the environment provided by the caput and corpus epididymidis. Sperm maturation involves an interaction of the epididymal epithelium and sperm. Mammalian epididymis has a rich distribution of lysosomes in the epithelial cells and high activity of glycosidases in the lumen. We use cell cuture to study the secretion of these cells. Epididymides from 20, 40 and adult Sprague-Dawley rats were removed aseptically and dissected free of fat. After division into regions corresponding to caput, corpus and cauda, the tissue was minced into fragments and enzymatically dissociated. The cells were washed with buffer and suspended in Dulbecco's modified Eagle's Medium (DMEM) and Ham's nutrient mixture from Sigma, suplemented with hormones, vitamins and growth factors. The cells were plated in plastic tissue culture dishes and incubated at 34 °C, with 3% of CO_2. Medium was changed every 48 h as culture continued. Cell-free supernatant was collected and stored at -20 °C. The cells were treated with trypsin and then collected. Activity of β-galactosidase, β-N-acetyl-glucosaminidase, α-mannosidase, β-glucuronidase, β-glucosidase and arilsulfatase was determined by fluorometric assays.

In the present study we standarized a new method for the culture of epithelial cells from rat epididymis. In addition, we demonstrated that epithelial cells are a major source of glycosidase activities in the epididymal lumen and that there are regional differences in the level of glycosidase activities in epididymal cells and in the dynamics of secretion.

Membranes, Lipids, Receptors - Hormones and Growth Factors, Signal Transduction, Cell Biology - Molecular Aspects

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A STUDY OF THE EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTOR (mGLUR1) mRNA IN RAT BRAIN BY NON-ISOTOPIC IN SITU HYBRIDIZATION.

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The neurotransmitter glutamate (GLU), play a function in plastic changes associated to memory, learning and neuronal damage. Glu receptors have been classified in two groups: ionotropic and metabotropic (mGLUR). The recent cloning of the mGLUR has permitted to identify eight different types of these receptors (mGLUR1-mGLUR8). This work uses non-isotopic hybridization techniques to look at the expression of mGLUR1 mRNA in different brain regions of rats kept in normal conditions, as well as following partial or total lesions of the nigro-striatal dopamine pathway.

Tissue brain slices (15 µm) from male Sprague-Dawley rats, were incubated in the presence of 30-mer oligodeoxynucleotides (oligo) complementary to the mRNA of mGLUR1. The oligo probes were labeled with digoxigenin (DIG). Hybridization signals were detected with anti-DIG conjugated with alkaline phosphatase.

Hybridization signals corresponding to the mRNA were observed in the cerebellum, substantia nigra and brain stem region. A brain-region specific decrease of the mRNA label was observed in substantia nigra following destruction of dopamine neurons with 6-OH-dopamine. No hybridization signals were observed in the striatum in either experimental condition. This study indicates that non-isotopic hybridization may be suitable to study regulation of mGLUR1 expression in the brain.

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THE EXPRESSION OF ECDYSONE RECEPTOR GENE IN THE SALIVARY GLAND OF *Bradysia hygida*.

Carvalho, D.P. and Almeida, J.C. de, Departamento de Morfologia, Faculdade de Medicina de Ribeirao Preto, Universidade de Sao Paulo, Brasil.

Gene amplification during development is described in only two cases: DNA puffs of Sciaridae salivary glands and the domains that contain the chorion genes in ovarian follicle cells of *Drosophila*. Gene amplification in Sciaridae is triggered by the molt hormone, ecdysone. In *Bradysia hygida* the salivary gland presents three morphologically distinct regions: anterior (S1), granulosa (S2) and posterior (S3). Eight chromosomal sites form the more prominent DNA puffs, which expand in two different groups, in a period of time of about 24 hours, solely in the gland regions S1 and S3. We are currently investigating the role of ecdysone receptor gene (*EcR*) expression on the control of DNA puff formation. Initially we generated by RT-PCR a fragment of about 600 bp, that should contain at the 3 end the DNA-binding domain (P-box), which is conserved in the thyroid hormone/retinoic acid receptor subfamily, and at the 5 end the hormone-binding domain of *Drosophila EcR* (Cho *et al.*, Insect Biochem Molec Biol 25,19-27, 1995). The 600 bp PCR amplification product was cloned in the vector Bluescript KS+. We have sequenced 123 nt at the 3 end and 190 nt at the 5. Both segments present 70% of nucleotide and 80% of aminoacid identity with the sequence of Aedes aegypt EcR cDNA. With this clone now it is possible to characterize the *Bradysia hygida EcR* (BhEcR) mRNA. In a preliminary experiment a Northern blot containing total RNA, from S1region, from larvae at ages E3 (when gene amplification begins) to E7+20hours (about 40 hours after E3, when the second group of DNA puffs arestill active), and RNA from pupae at the pupal molt (PM), PM+10 hours andPM+20 hours, was probed with the PCR product labeled with a32P-dCTP. At all stages analyzed a 4.2 kb RNA was detected which size is similar to one of the *Aedes* transcripts. S1, S2 and S3 total RNA was isolated from larvae at the ages E1 (before gene amplification starts), E3 (beginning of amplification) and E7 (first group of DNA puffs in activity). The RT-PCR showed the presence of EcR RNA in all the samples. Therefore, the expression of EcR in the S2 region is not sufficient to activate gene amplification at the same levels as in the other gland regions. We are looking for other factors involved in such control. Financial support: FAPESP, CNPq, FAEPA.

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CELLULAR INTERACTIONS IN THE HUMAN CORPUS LUTEUM

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The corpus luteum (CL) of several species is constituted by macrophages, neutrophils, lymphocytes, vascular cells and fibroblasts, in addition to steroidogenic cells. Quantitative and qualitative variations of the luteal cellular types suggest that cellular interactions in the CL would be determinant of their function. The aim of the present study is to understand the mechanisms involved on luteal cell interactions using a human luteal cell culture. The CL was obtained from normal women undergoing mini laparotomy for tubal sterilization in the San Borja-Arriaran Hospital, University of Chile, during mid luteal phase. The study was approved by the Institutional Review Board. Informed written consent to remove the CL was obtained from each patient. To assess the cellular subpopulations present in CL, a batery of monoclonal antibodies (mAbs) and flow cytometry were used. To determine if the presence of Leucocytes and endothelial cells modulate the steroidogenic function, luteal cultures was depleted of these cellular types with the specifics mAbs and immunobeads, and the production of progesterone and estradiol was evaluated by RIA. Endothelial cells (12%) and leukocytes (20%) were present in dispersed luteal cells; macrophages were the principal leukocyte subpopulation determined. Functional studies revealed a 3 fold increase in basal progesterone and estradiol production when luteal cultures were depleted of leukocytes. hCG-stimulated steroid synthesis was not affected.

In summary, this data suggest that non-steroidogenic luteal cells subpopulations have a regulatory role on human luteal function.

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MOLECULAR INTERACTIONS BETWEEN CYTOKINES AND GLUCOCORTICOID RECEPTOR.

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The interaction between transcription factors can induce the transactivation or transrepression of their respective target genes. The glucocorticoid receptor (GR) presents a complex functional interaction with NFκB which dampens cytokine-induced gene expression. We have analyzed the interaction between NFκB and GR by EMSA, on the TNF-α target cell line L-929 at 15, 45, 60 and 120 min. post-stimulation with 0.02 ng/ml TNF-α and 10 nM DEX and found for all time points, that GC did not change TNF-α-induced NFκB activity. However, at the same time points TNF-α increased (1.8 fold, at 45 min) the GC-induced GR binding to GC response elements (GRE), while not shift or extra bands were observed. This correlates with a TNF-α-induced increase of the transcriptional activity of the GR in different types of target cells transfected with a GC-inducible reporter plasmid. As a functional correlate of this mechanism, priming of L-929 cells with this low non-cytotoxic dose (0.02 ng/ml) of TNF-α significantly increased ($p<0,001$) the sensitivity to GC inhibition of TNF-α (60 ng/ml)-induced apoptosis. Both the long-term inhibition of NFκB and the potentiation of GR through GRE act to inhibit TNF-α overshooting.

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Prevalence of autoantibodies against cardiovascular receptors in Chagas and other cardiac diseases.

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The prevalence of autoantibodies recognizing the second extracellular loop of the human Beta1, Beta2 adrenergic and the M2-muscarinic receptors was determined in patients with Chagas'heart disease, idiopathic dilated cardiomiopathy, primary electrical abnormalities and healthy individuals.

TABLE: Percentage of sera recognizing the cardiovascular receptors.

	N	Beta1	Beta2	M2
Healthy controls	25	02.0 %	02.0 %	02.0 %
Chagas	200	37.5 %	31.25 %	47.9 %
IDC	25	31.5 %	25.3 %	15.0 %
E1	35	41.2 %	20.5 %	17.5 %

In order to test the functional activity, the IgG fraction of these sera were incubated with cardiomyocites from neonatal rats and their chronotropic effect was measured. IgG antibodies from patients seropositive for beta-receptor produce an increase in the beating rate of cardiomyocites. Nevertheless, the IgG fraction from some chagasic patients decreased the frequency of contraction due to anti-M2 receptor antibodies. The specificity of these chronotropic effects was confirmed pharmacologically and by measurement of the effects of affinity purified anti-receptor autoantibodies.

The autoantibodies with colinergic agonist effect inhibit partial or totally the stimulating effect of the anti-adrenergic autoantibodies. Therefore anti-M2 antibodies might protect against arrythmias and myocardial damage generated by the sympathetic stimulation.

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HORMONAL REGULATION OF ISOCITRATE DEHYDROGENASE NADP- DEPENDENT IN CULTURE MALE RAT HEPATOCYTES.

Zirulnik, F., Larregle, E., Anzulovich, A.D. and Gimenez, M.S. Area de Química Biológica. Fac. de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. San Luis, Argentina.

Previous results obtained in our laboratory, showed that castration and testosterone (T) administration affect cytosolic Isocitrate dehydrogenase NADP- dependent (NADP- ICDH) (E.C.1.1.1.4.2), in male rat liver. Working in vivo, the results showed that the enzymatic activity (EA) in control (Co) rats increased significantly after the administration of different antiandrogens and when androgens or prolactin (PRL) are administered to castrated (Cx) rats the EA decreased to Co values. The objective of this work is study the same phenomenon in vitro, with hepatocytes cultures, obtained by the Perfusion technique with collagenase (Seglen, 1976, with modifications). The EA was determined in isolated hepatocytes and in cells incubated during 3 hours, in Co and Cx rats. The differences observed in vivo, are repeated in the isolated cells like in the cultivated ones, presenting this activity an increment of more than the 50%, in hepatocytes of Cx rats. The effect of the compounds proved in vivo, was also studied adding to the incubation medium of Co hepatocytes, concentrations of 10^{-7} M of antiandrogens like flutamide and ciproterone acetate, and 2-Bromo-ergocryptine (an inhibitor of the serio levels of circulating PRL), and any significant differences with the Co values, with 3 hours of incubation, were observed. On Cx hepatocytes, androgens like T and dihydrotestosterone (DHT), were added, observing that the EA doesn't diminish to Co values. When the effect of PRL was studied (in vivo, it would be involucrated in the enzymatic regulation), differences were not observed in relation to Cx hepatocytes, after 3 hours incubations. The same occur when ciclohexymide was added to the medium (in order to determine if the increase of EA is due to a stimulation of the protein synthesis). Simultaneously the EA of Glucose 6- phosphate dehydrogenase was determined, and a similar behavior to NADP- ICDH, was observed. From this preliminary study, we could conclude that the phenomenon observed in vivo, it don't reproduce in vitro, when short time incubations are used.

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CULTURED PITUITARY CELLS OF DEVELOPING RATS AS MODEL SYSTEMS FOR STUDYING MOLECULAR MECHANISMS OF GROWTH REGULATION BY HORMONES AND NEUROHUMORAL FACTORS.

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According to our previous investigations, glucocorticoid-induced growth retardation in rats (Goudochnikov, V.I. et al., 1994) may be partially explained by direct, age-dependent inhibition of proliferative activity in pituitary gland (Gudoshnikov, V.I. & Fedotov, V.P., 1993). In the present work we used primary cultures of pituitary cells obtained from developing rats of different ages, in order to study the interactions of glucocorticoid hormone with other bioregulators. Dexamethasone (0.1-1.0 μM) alone inhibited DNA, total RNA and protein syntheses in pituitary cells of rats of various ages (N = 5-6, $P < 0.01$ in most of cases). L-triiodothyronine (0.1 μM), L-norepinephrine (10 μM), γ-aminobutyric acid (10 μM) and hypothalamic peptides (thyroliberin, gonadorelin, somatostatin) diminished also macromolecular biosyntheses in rat pituitary cells of some (but not all) age groups when used alone, and interacted with glucocorticoid in regulation of these processes. Calcium ionophore A23187 (5 μM) and dibutyryl-cyclic AMP (1.25 mM) inhibited DNA and total protein syntheses, when given alone or in combination with dexamethasone. Our data allow to suggest that maturation of hypothalamo-pituitary connection and peripheral endocrine glands in early postnatal development is accompanied by calcium- and cyclic AMP-mediated inhibition of macromolecular biosyntheses in pituitary gland.

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MUSCARINIC ACETYLCHOLINE RECEPTOR AFFINITY IS MODIFIED IN RAT STRIATUM AFTER MOTOR ACTIVITY DURING A CRITICAL PERIOD OF POSTNATAL DEVELOPMENT.

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Recently, we have described a drop in receptor number of the cholinergic system in rat striatum after a specific motor behavior, the circling training test (CT) (1). This alteration was only observed after training during a limited period of postnatal development (30 to 37 days of age). To further investigate these findings, we evaluated the effect of CT in the pattern of binding of 3H-QNB to muscarinic acetylcholine receptor (mAchR) immediately after motor activity. Male rats were trained in CT for three days starting at 20, 30 and 60 days of age and sacrified 30 minutes after the last session. Synaptic plasma membrane was isolated and Scatchard analysis performed for mAchR. These experiments showed that training starting at 30 days of age induced an increase of receptor affinity of 300% in trained animals (Kd controls: 0,75 nM, Kd trained: 0,26 nM). Changes in Bmax was not detected. Training starting at 20 or 60 days of age did not show modification neither in Kd nor in Bmax. Since this early alteration of mAchR after CT was only detected during the critical period described for permanent alterations, these two processes could be related. Also, affinity variation could be the first step before receptor desensitization, internalization and degradation as it was reported by other authors (2). Both hypothesis are under further investigation yet.

(1) Ibarra et al. Brain Res 705:39-44, 1995.
(2) Liles et al. JBC 261:5307-5313, 1986.

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EFFECT OF ANGIOTENSIN II ON PROGESTERONE SYNTHESIS BY HUMAN GRANULOSA CELLS (GC). EXPRESSION OF AT_2 RECEPTOR.

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In previous reports, we observed that angiotensin II (AngII) has a regulatory role on the ovarian steroidogenesis. To stablish an AngII mechanism on progesterone (P) synthesis, GC obtained from women from IVF Program were cultured for 2 or 4 days (d) in M199-5%FCS. The medium was changed for a serum-free medium, and the cells were incubated without (basal) or with 10^{-7} M AngII and/ or 10 IU hCG during 3 or 24 h. The 3-ß hydroxysteroid dehydrogenase (3ßHSD) activity was determined by convertion of 17-OH pregnenolone to 17-OHP, and its expression for reverse transcription and PCR (RT-PCR) of RNA prepared from GC in 2 and/or 4 d cells culture. The effect of AngII receptor subtype on P production was studied with 10^{-8} M [Sar^1,Ala^8]AngII (SA); and 10^{-5} M PD123177 (PD). AT_2 expression was analyzed by RT-PCR with specific AT_2 primers; a 586-bp of PCR fragment was obtained from cells cultured cDNA, and NlaIII digested the PCR fragment in the expected 131-, 192-, and 263-bp products, indicating the presence of AT_2 receptor in human GC. The addition of hCG (3 h), with or without AngII, increased P secretion by 126% respect to basal in 4 d culture, and no effect was observed in 2 d culture. In contrast, in basal conditions, AngII affected the P secretion in a culture-time dependent manner: in 2 d culture, P production and 3ßHSD activity were inhibited by 32%, and 87% ($p<0.05$), respectively; and in 4 d culture, P secretion was increased by 56% ($p<0.05$). 3ßHSD mRNA expresssion was not affected by AngII (24 h) in 4 d culture. Both, the inhibitory and stimulatory effects of AngII on P secretion were dose-dependent, and they wrere blocked by SA and PD. In conclusion, these data suggest that AngII regulates the P synthesis in human GC modulating the 3ßHSD activity, and this action may be mediated by AT_2 receptors. On the other hand, the P response of GC to AngII and hCG changed during culture, suggesting a cellular differentiation process involved.

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HEMIN ACTION ON ALDOSTERONE PRODUCTION IN RAT ADRENAL. Martini, C., San Martín de Viale, L. C. and Vila, M. C. Depto. Química Biológica . FCEyN, UBA, Buenos Aires. Argentina.

Steroid biosynthesis involves cytochome P-450 enzymes which are heme proteins. Therefore, alterations in heme biosynthesis might affect steroid production. We previously reported that aldosterone production is stimulated by hemin in primary cultures and homogenates of calf adrenal glomerulosa. 3,5- Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethyl-pyridine (DDC) is a potent inhibitor of liver ferrochelatase, the enzyme that catalyzes the formation of heme by insertion of Fe onto protoporphyrin IX. DDC blocked the stimulation of aldosterone mediated by ACTH. Addition of hemin after treatment with DDC partially restored ACTH action. These results suggest that DDC inhibits heme biosynthesis in calf adrenal. In this work we tested the ability of DDC to alter heme pathway in rat adrenal. We determined the activity of ferrochelatase and ALA-Synthase, the rate-limiting enzyme of heme pathway, in control and DDC-treated animals. We found that DDC treatment inhibited ferrochelatase and increased ALA-Synthase activity in rat adrenal. This is consistent with the effect of DDC on these enzymes in liver and proves that this agent is also able to inhibit heme production in adrenal. On the other hand, we found that hemin stimulated aldosterone production in rat adrenal homogenates in a dose-dependent fashion. This is consistent with our previous findings in calf.

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CARDIAC BASIC FIBROBLAST GROWTH FACTOR (BFGF) EXPRESSION IN DIFFERENT IN VIVO MODELS OF HEART HYPERTROPHY. Meléndez J, Wilson I, Jalil J, Lavandero S. Fac. Cs. Qcas y Farmacéuticas, U. Chile y Fac. Medicina, PU Católica de Chile. Santiago-Chile.

Basic fibroblast growth factor (bFGF) is a potent mitogen and angiogenic agent that has been localized both in extracellular matrix as in cardiomyocyte nuclei being able to induce reparative and regenerative process. However, little is known if bFGF is involved in the genesis and development of left ventricular hypertrophy (LVH). Our aim was to characterize *in vivo* the cardiac bFGF expression in adult rats in three models of cardiac hypertrophy: (1) renovascular hypertension (GB), (2) treatment with deoxicorticosterone (DOCA) and (3) administration of isoproterenol (ISO). For (1) and (2), the bFGF expression was evaluated at 1, 4 and 9 weeks. For (3), the study was conduced for 2, 15 and 30 days post-treatment. Rats underwent sham surgery or treated with vehicule served as controls. bFGF was isolated from homogenized left ventricle and separated by heparin-sepharose chromatography. bFGF eluted fractions and serum samples were measured by RIA. Cardiac bFGF was also characterized by Western Blot (WB) using an anti bFGF polyclonal antibody.

The results showed that in all experimental model of LVH, cardiac bFGF levels did not change throughout the study as compared to controls. Serum bFGF was indetectable in all LVH models. Cardiac bFGF was visualized as two bands (18 and 24 KDa) by WB.We concluded that bFGF may participate in other cellular events rather than in the genesis and development of LVH in adult rats.

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EXPRESSION OF ESTROGEN RECEPTORS IN HUMAN UTERINE MAST CELLS. AN IMMUNOHISTOCHEMICAL STUDY. Nicovani S, Campos A, Martínez L,Rudolph M.I. Departamento de Farmacología, Facultad de Ciencias Biologicas, Universidad de Concepción, Concepción, Chile.

Recent studies have focus the attention towards the possible role of uterine mast cells in the regulation of gestation and parturition. It has been found that upon stimulation uterine mast cells, which are localizaed in close apposition to smooth muscle cells, release a number of biologically active products that may affect contractility. Besides, it is known that parturition has certain similarity to an inflammatory reaction. Histological studies describe the presence of inflammatory cells, and inflammatory mediators as eicosanoids and cytokines are involved. The mast cell is central for a number of inflammatory reactions. It contains many potent mediators that induces leukocyte infiltration of the myometrium.

It has been found that estrogens may facilitate uterine mast cell activation. Nevertheless, direct evidences related to the presence of estrogen receptors in those cells are lacking.

The aim of this study was to analyse the experimental variables that could affect the expression of estrogen receptors in uterine mast cells by using a monoclonal antibody on frozen and formalin-fixed, paraffin-embedded tissues of human myometrium.

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EARLY ACTIVATION OF THE TRH-PRL AXIS DURING A T-CELL-DEPENDENT IMMUNE RESPONSE. Perez Castro, C.[1]; Páez Pereda, M.[1,2]; Peñalva, R.[1]; Renner, U.[2]; Stalla, G.[2]; Arzt, E[1]. [1]Instituto de Investigaciones Médicas and Dpto de Biología, FCEyN, Universidad de Buenos Aires, Argentina. e-mail: postmaster@arzt.ba.ar
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The immune response is accompanied by homeostatic changes in the neuroendocrine system. In this work, we studied the expression of hypothalamic-pituitary genes during the T cell-dependent immune response. Male rats Wistar (180-200 g) were injected intraperitoneally with Sheep Red Blood Cells (SRBC) ($5x10^9$ cells/ml) and samples obtained at 2 h, 6 h, 24 h, 4 d and 7 d post-injection. The increase of IL-2 at 6 h and 24 h (162% and 490%) in the splenocyte supernatant stimulated by Con A and the peak of anti-SRBC antibody production at 7d are accompanied by: a) a high level induction of hypothalamic TRH gene at 2-4-6-18 h (which is different compared with the decrease of TRH mRNA induced by LPS) without changes at 4-7 d; b) an increase of pituitary TRH receptor (TRHR) mRNA at 6 h (91%), and a further decrease at 24 h (40%) and 4 d (48%); c) an increase of plasmatic PRL levels (by RIA) at 2 h: 242%, 6 h: 232% and 24 h: 223% without changes at 4-7 d. These results show that: a) there are different neuroendocrine responses depending on T cell-dependent or independent antigens (LPS); b) there is an early onset of TRH-TRHR-Prolactin axis which may be, considering the TRH and PRL activation of T lymphocytes, important in the initial stages of the T-cell dependent immune response.

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RAPID MODULATION OF INTESTINAL CELL CALCIUM HOMEOSTASIS BY 17ß-ESTRADIOL, PTH AND 1,25-DIHYDROXYVITAMIN D_3 BY SECOND MESSENGER PATHWAYS. Picotto G., Massheimer V., Vazquez G., Boland Ana R. de , Boland R. Dto. de Biología y Bioquímica, Universidad Nacional del Sur, Bahía Blanca, Argentina

Recent studies have provided evidence for rapid and direct modulation of calcium influx in rat intestinal cells mediated by the steroid hormones 1,25$(OH)_2$-vitamin D_3 [1,25$(OH)_2D_3$] and 17ß-estradiol [17ß-E_2] and the peptide hormone PTH which involves the regulation of Ca^{2+} channel-opening through the cAMP pathway. We have further investigated the participation of this second messenger system by measuring direct effects of the hormones on adenylate cyclase activity in isolated enterocyte microsomal membranes. Three minutes treatment with 17ß-E_2 (10^{-10} M), PTH (10^{-8} M) and 1,25$(OH)_2D_3$ (10^{-10} M) induced significant elevations in the enzyme activity (183%, 147%, 145%, respectively). The specificity of these hormone action was tested using 17α-estradiol, dihydrotestosterone, progesterone, PTH fragment (7-34), 1α$(OH)D_3$ and 24,25$(OH)_2D_3$. All of them were devoid of activity. Only the monohidroxylate vitamin D_3 metabolite, 25$(OH)D_3$, elicited significant increment of the enzyme activity (7.41 vs. 2.46 pmol cAMP/ mg prot.). We examined direct effects of 17ß-E_2 and 1,25$(OH)_2D_3$ on the intracellular Ca^{2+} concentration of isolated rat enterocytes loaded with the fluorescent Ca^{2+} dye fura-2. Treatment with both hormones (10^{-10} M) increased intracellular calcium concentration since 30 sec. The rise in intracellular calcium evoked by 1,25$(OH)_2D_3$ directly correlates with increments in IP_3 content previously observed. To evaluate the possibility that estrogens and PTH stimulate intestinal Ca^{2+} fluxes through other second-messenger pathways (the PLC-mediated system), we measured calcium uptake in the presence or absence of PLC specific inhibitors (0.5 mM neomycin and 2 μM U-73122). The increment elicited by 17ß-estradiol (10^{-10} M) and PTH (10^{-8} M) was completely supressed with these blockers. The results further confirm the participation of the adenylate cyclase pathway and also suggest the involvement of the PLC messenger system in the rapid modulation of calcium homoestasis in intestinal cells by 17ß-E2, PTH and 1,25$(OH)_2D_3$.

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THE USE OF RECOMBINANT HUMAN ERYTHROPOIETIN IN HIV INFECTION. EXPERIENCE ON AN ARGENTINIEAN POPULATION.

Troncoso, A.[1], Iacono, R.[2], Ostrowski, H.[3], and Poskus, E.[2]. [1]Hospital de Infecciosos F.J. Muñiz, [2]Cátedra de Inmunología-IDEHU, FFyB, UBA-CONICET, and [3]Lab. ELEA SACIFYA.

Human immunodeficiency virus (HIV) infection, AZT, and other drugs treatment are associated with defects in hematopoiesis. These defects include decreasing of hematopoietic progenitors cells and mature cell destruction. In those cases hematopoyetic cytokines are administered. The objective of our study was the follow up and control of patients with AIDS under recombinant human erythropoietin (r-h-EPO obtained by transfection of CHO cells) treatment and some with combined EPO and G-CSF therapy, in order to optimize their clinical management. Radioimmunoassay for parallelism control of r-h-EPO preparations and ELISA for monitoring circulating EPO were used. Hemoglobin, hematocrit, total and differential leukocyte counts, were determined weekly in order to evaluate the efficiency of therapy. Patients (n=15) with symptomatic HIV-infection, were eligible for the study when they had a hematocrit less than 29% and/or hemoglobin less than 9.5 g/dl. r-h-EPO (100 UI/Kg) was administered subcutaneously three times for week during a month. Results: There were significant increases in mean hemoglobin (baseline: 8,6±0,6 g/dl; 7th week: 12,5±0,1g/dl) and hematocrit (baseline: 27±2%; 7th week: 37±2%). Mean endogenous immunoreactive EPO level was 25±16mIU/ml, and increased to 47±10 mIU/ml after 1 hr r-h-EPO injection and to 90±21 mIU/ml after 24hr. Conclusions: To date all patients enrolled in our study exhibited amelioration of the hematopoietic parameters. Baseline of endogenous EPO and pharmacokinetic parameters of the injected hormone may be useful in therapy modulation.

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MECHANISM OF hCG-INDUCED SPERMIATION IN *Bufo arenarum*: EFFECT OF STEROIDS BIOSYNTHESIS INHIBITORS. Pozzi, A.G.*, Lantos, C.P.# and Ceballos, N.R.*. * Dpto.de Ciencias Biológicas, # Dpto de Química Biológica and PRHOM-CONICET, Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires, Buenos Aires, Argentina

Spermiation is the process in which vertebrate spermatozoa are detached from Sertoli cells into the lumen of seminiferous tubule. Amphibians spermiation has been used extensively for studying the physiology of gonadotropins of various vertebrate species. In this study we have used an *in vitro* system to identify the steroidal mediator of spermiation in *Bufo arenarum*. Spermiation was induced by different concentrations of hCG (0.1 to 10 IU) and the effect of different steroids biosynthesis inhibitors was analyzed Testis fragments were incubated for differet times at 28 C. Cyanoketone (10^{-4} to 10^{-6} M,) an inhibitor of 3-oxo-4-ene steroids biosynthesis was no effective to block hCG-inducting activity even when 3ß-hydroxysteroid dehydrogenase-isomerase activity was reduced in 95 %. No spermiation-inducing activity was found with differents 3-oxo-4-ene-steroids (progesterone, 17α-hydroxyprogesterone, 17α, 20α-dihydroprogesterone, 17α,20ß-dihydroprogesterone, estradiol, testosterone, etc). When spironolactone was employed in a concentration that inhibits 17α-hydroxylase activity (10^{-5} M, 50% inhibition), hCG action was diminished in 50 %. The effect of spironolactone was reverted by the addition of 17α-hydroxypregnenolone (20 ng/ml). This results suggest that the spermiation induced by hCG requires steroids biosynthesis in Leydig cells. It is possible to speculate that the steroid involved in hCG inducing-spermiation is a 3ß-hydroxy-5-ene steroid.

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EFFECT OF OVIDUCTAL RNA FROM ESTRADIOL-TREATED RATS ON PROTEIN SYNTHESIS IN THE OVIDUCT Rfos. M.. Croxatto, H.B. Unidad de Reproducción y Desarrollo , F. CC BB, P. U. C., Santiago, Chile.

Estradiol (E2) accelerates oviductal transport of ova in rats through a genomic mechanism in the oviduct. Previously we demostrated that intraoviductal (i.o) administration of oviductal RNA obtained from E2-treated rats was able to mimic the effect of E2 upon oviductal egg transport. We proposed that an estrogen-induced RNA can enter cells, be translated and lead to a cell response. Therefore, a change in protein synthesis should be detectable in these cells. Other work done in our laboratory showed that E2 administered in vivo is capable of stimulating protein synthesis in the oviduct in vitro, so we used this model to assess the ability of the active RNA extract to mimic E2-induced protein synthesis.

Rats on day 2 of pregnancy were injected subcutaneously (s.c.) with E2 (10ug in 0.2 ml of propilene glycol) or its vehicle and 3 hours later they received an i.o. injection of vehicle or oviductal RNA (70 ug in 3 ul of saline) obtained 4 hours after a s.c. injection of E2, respectively. Controls received only vehicles. 4 hours after the s.c. injection, the recipient rats were killed, the oviducts were removed and incubated in medium supplemented with ^{35}S-Methionine. 8 hours later, the oviducts were homogenized, proteins were precipitated with TCA and the radioactivity was determined. Part of the homogenate was analysed by PAGE followed by fluorography.

E2 and RNA increased the incorporation of ^{35}S-metionine into oviductal proteins in comparison with vehicle treated controls. Fluorography showed that both treatments increased significantly the radioactivity of the majority of the protein bands. The magnitude of this increase varied significantly among the bands and followed an identical pattern for both treatments. These results are consistent with the hypothesis that the RNA placed in the oviduct lumen can enter the cells and be translated.

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EFFECTS OF THE 13-36-FRAGMENT OF THE NEUROPEPTIDE TIROSINE ON ^{3}H-NOREPINEPHRINE RELEASE FROM CEREBRAL CORTEX SLICES OBTAINED OF RATS DURING THEIR ESTROUS CYCLE. Saragoni. M.L.. González. A.. Llona I. and Belmar. J. Departamento de Biologia Celualr y Molecular. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de chile. Santiago, CHILE.

The effects of neuropeptide tirosine (NPY) on chemical neurotransmission have been studied on central and peripheral sinapsis. In the central nervous system exist at different regions of the cerebral cortex (CC) a well defined network of NPY neurons. Their interactions with other neuroactive agents and functional roles are poorly understood. In this work we studied the influence of the estrous cycle of the rat on the NPY 13-36 fragment effect on ^{3}H-norepinephrine (^{3}H-NE) release from CC slices obtained from the occipital (OC) and frontal (FC) regions.

Slices of 225 μm thickness were superfused with Krebs-Ringer-Bicarbonate buffer, pH 7.4 at 37°C, gassed with a mixture of O_2 (95%) and CO_2 (5%) and stimulated with K^+, 20 mM. Peptides concentrations from 5 to 50 mM were used. NPY inhibited the release of ^{3}H-NE both at OC and FC being its effects dependent of the stage of the cycle and of the region considered. At estrus the inhibition was stronger at CO while at diestrus-II (D-II) the stronger inhibition was found in FC. The 13-36 fragment showed a higher inhibitory effect than NPY at D-II and in the frontal cortex. No differences were found qith the fragment in OC. The results suggest a modulatory fole for NPY on EN release at the CC. This role could be also under the influence of the estrous cycle possibly by modulating the Y_1 or Y_2 subtypes of NPY receptors.

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TWO DIFFERENT SIGNAL TRANSDUCING MECHANISMS REGULATED MEMBRANE BOUND GUANYLYL CYCLASE. Alfonzo, M; Napoleón, V., Misle, A., Villarroel, R.S., Alfonzo, R. and Bécemberg, I. Sección de Biomembranas. Instituto de Medicina Experimental. Universidad Central de Venezuela. Caracas. Venezuela.

A guanylyl cyclase (GC) coupled to muscarinic receptors has been described by us in airway smooth muscle (FEBS. Lett. 253: 16-22. 1989). Muscarinic receptors have been claimed to be a mixture of M_2 and M_3 mAChRs in this tissue. Muscarinic actions are "dual", an activation from 10^{-10} to 10^{-8} M CC follows by an inhibition from 10^{-7} to 10^{-4} M were found. To understand the molecular mechanisms behind these responses, GC was assayed with specific muscarinic antagonists using a plasma membrane fraction P2 from bovine tracheal smooth muscle, isolated from discontinuous sucrose gradient and cGMP was determined by RIA. The CC activation seems to be mediated by a M_3 mAChR showing a pharmacological profile (4-DAMP> HDD> PZ). Pertussis toxin treatment abolish the inhibition process of CC and produced a significant augmentation of the CC activation. Thus, this GC seems to be regulated by two different signal transducing pathways. A M_3 MAChR coupled to a PTX insensitive G protein associated with the muscarinic activation while the inhibition may be mediated by a M_2 AChR coupled to a PTX sensitive G-protein. Grants from CONICIT S1-2749 (IB) and CDCH-09.33.2942/95 (MA) 09.33.3436/95 (IB).

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CALCITRIOL-DEPENDENT PHOSPHORYLATION OF RAT DUODENAL PROTEINS: EFFECTS OF SENESCENCE. Gabriela Balogh. Ricardo Boland and Ana R. de Boland. Departamento de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur. 8000 Bahía Blanca, Argentina.

The effects of senescence on phosphorylation of intestinal proteins by calcitriol, the hormonal form of Vitamin D_3, was evaluated in duodenum from young (3 months) and aged (24 months) rats prelabelled *in vitro* with ^{32}P-orthophosphate. Exposure of duodenum to 1 nM calcitriol caused a stimulation of ^{32}P incorporation into the whole homogenate from young rats (+110%), the response was greatly disminished in aged animals (+20%). The effects of the hormone varied with time of exposure (15 sec-5 min.), being maximal at 30 sec, were mimicked by dioctanoylglycerol (DOG) and effectively supressed by the protein kinase C inhibitor H7 in both young and aged animals. Phosphoproteins in subcellular fractions obtained by differential centrifugation from duodenal mucosae were resolved by SDS-polyacrylamide gel electrophoresis and indentified by autoradiography. In brush border (BBM) and basolateral (BLM) membranes isolated from young duodenum, calcitriol clearly potentiated (1.5-2 fold increase) the phosphorylation of several proteins of relative molecular masses ranging from 14 to 80 kDa. In senescent rats, calcitriol increased BBM protein phosphorylation to a lesser extent than in young rats, but did not alter the phosphorylation of BLM proteins. This study indicates that calcitriol-induced protein phosphorylation of duodenal proteins is impaired with senescence.

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ROLE OF PROTEIN KINASE C IN THE REGULATION OF IODIDE UPTAKE BY CALF THYROID PRIMARY CULTURE.

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The present research was performed in order to investigate the effect of protein kinase C on a typical functional parameter and limiting step in thyroid hormone biosynthesis: the iodide uptake. Primary cultures of calf thyroid were used as a model. In the first studies, confluent cells were maintained with TSH (0.5 mU/ml) for 72 h and incubated with 100 nM PMA for 60 minutes. Then ^{125}I uptake was assayed. PMA caused an inhibition by around 70% in cells treated with TSH ($p<0.001$). This effect was mimicked by 10 µM forskolin (53% $p<0.001$) or 100 µM Bu_2 cAMP (51% $p<0.01$). PMA inhibitory action was blocked by a PKC inhibitor: bisindolylmaleimide (BS), suggesting that it is mediated by PKC pathway. In order to determine whether the inhibitory effect of PMA take place at level of iodide efflux or influx, we studied the halogen liberation and Na^+/K^+ ATPase activity. No change on iodide efflux was observed, however PMA caused a significant inhibition of TSH effect on the ATPase (65%, $p<0.01$). This effect was blocked by BS. In summary, PMA inhibits the TSH effects on iodide uptake, this action is mediated by PKC pathway and it takes place at level of Na^+/K^+ ATPase activity.

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1α,25-DIHYDROXY-VITAMIN D_3 STIMULATION OF THE ADENYLYL CYCLASE/cAMP PATHWAY IN MUSCLE CELLS : STUDIES ON THE UNDERLYING MECHANISM. G. Vazquez; AR de Boland. and RL Boland. Depto. Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. (8000) Bahía Blanca, Argentina.

We have recently established that the hormonally active derivative of vitamin D_3, 1α,25-dihydroxy-vitamin D_3 ($1,25(OH)_2D_3$), rapidly (1-5 min.) stimulates voltage-dependent Ca^{2+}-channel mediated Ca^{2+} influx into both chick and rat cultured embryonic muscle cells (myoblasts) by a non-genomic action which involves hormone-induced inhibition of a 40 kDa pertussis-sensitive G protein (Gi) with accumulation of cyclic AMP (cAMP) cellular levels, these events leading to Walsh inhibitor-sensitive phosphorylation of several membrane proteins. To further characterize the mechanism underlying $1,25(OH)_2D_3$ activation of the cAMP pathway, in the present study the effect of the hormone on both adenylyl cyclase (AC) and protein kinase A (PKA) activities as well as on the phosphorylation state of Gi was investigated. In membrane preparations from chick embryo myoblasts, $1,25(OH)_2D_3$ stimulated AC activity (% above basal) at 0.1 (31), 1 (54) and 10 nM (27) in a time (1-5 min.)-dependent fashion, provided GTP (0.1 mM) was included in the assay buffer. In the absence of GTP or in the presence of Mn^{2+} (20 mM) $1,25(OH)_2D_3$ stimulation of AC was completely abolished. Additionally, in hormone pretreated myoblasts (1 nM $1,25(OH)_2D_3$, 5 min.) in vitro PKA activity was increased (21,0 ± 2,0 vs. 36,0 ± 9,0 pmol ^{32}P/min/mg of protein, control vs. treated cells). Immunoprecipitation of Gαi from ^{32}P-labeled myoblast microsomal membranes shows that 2 min. exposure to $1,25(OH)_2D_3$ (1 nM) results in a marked increase (2-2.5 fold) in the phosphorylation of its α subunit. The present data suggest that in muscle cells, $1,25(OH)_2D_3$ activates AC by a non-direct, GTP-dependent action which in part implies amelioration of Gi function by hormone-induced αi phosphorylation. Augmented cAMP levels and in turn, PKA activation, are sequential events mediating $1,25(OH)_2D_3$-dependent membrane protein phosphorylation.

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ACTIVATION OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE SUPPLY BY CARBACHOL. Marchesini, N., Hernández, G., Bollo, M., Racagni, G., Garrido, M. and Machado-Domenech, E.E..Química Biológica, FCEFQN, Universidad Nacional de Río Cuarto, Córdoba, Argentina. e.mail: emachado@unrccc.edu.ar

PtdIns(4,5)P_2 serves as precursor of a diverse family of signalling molecules, including diacylglycerol, InsP_3 and PtdIns(3,4,5)P_3. The production of these messengers can be activated by agonists, and therefore the rate of utilization of PtdIns(4,5)P_2 can vary dramatically. In our previous study in *Trypanosoma cruzi* epimastigotes, carbachol-induced phosphoinositide turnover was demonstrated (Machado-Domenech et al. (1992) FEMS Microbiol. Lett. 95: 267-270). In further experiments, we found that carbachol influences rapid changes in generation of InsP_3 which would be through a receptor-mediated process linked to phospholipase C (PLC) by a G-protein (Garrido et al. (1996) Cell. Mol. Biol. 42: 221-225). In accordance with this, our aim was to study the PI cycle after parasite stimulation with carbachol in reference to its modulation during stimulation, for a period of at least 20 minutes. Endogenous phospholipids were phosphorylated with [γ-^{32}P] ATP at 30 °C for 5 minutes. The products were isolated by TLC and quantified by scintillography. The Ins*P*s were separated by anion-exchange chromatography on Dowex columns (formate form). $[Ca^{2+}]_i$ was measured in suspensions of Fura-2-loaded cells.
In the present study we demonstrate a rapid mobilization of polyphosphoinositide in *T. cruzi* epimastigotes stimulated with carbachol. It causes a rapid release of Ins*P*s, diacylglycerol and a transient rise in the cytoplasmic free Ca^{2+} concentration. Under this circumstance, the demand for PtdIns(4,5)P_2 can rise subtantially and an increase in its net synthesis might occur. The latter could be supplied by an increase in the rate of PtdIns 4*P* 5 kinase and/or a decrease in the rate of a PtdIns(4,5)P_2 degrading reaction.

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PHYTOHEMAGLUTININ STIMULATES THE T-LYMPHOCYTES ACTIVATING PROTEIN KINASE C: Bustamante, M., Gatica, A., *Olate, J. & §González, M.. Dpto de Biología Molecular, * Dpto de Fisiopatología, Facultad de Ciencias Biológicas, § Dpto. de Química Clínica e Inmunología, Facultad de Farmacia, Universidad de Concepción, Concepción, CHILE

The protein kinase C (PKC) family of isoenzymes is participating in a wide range of signal transduction pathways in many different cell types, particularly in haematopoyetic cells like T-lymphocytes. The effects of activators (Phytohemaglutinin (PHA) and the phorbol ester (PMA)) and inhibitor (RO 31-8220) of PKC on the T-cells proliferation were studied. Peripheral blood T-lymphocytes from normal donors were isolated, cultured and stimulated. The cell proliferation was measured using ^3H Thymidine uptake. 500 nM of RO 31-8220 was able to inhibit the PHA and PMA induced T-cell proliferation. This effect is reversed by fetal calf serum (FCS) suggesting that FCS do not stimulate T-cells using the PKC pathway. The PKC inhibitor and the solvent of PMA and RO 31-8220, Dimethylsulphoxide, at concentration higher than 3 µM and 0,5% respectively were toxic for the T-cells cultures. These results show that the PKC inhibitor can be very usefull for disection of PKC mediated signal transduction pathways.
Proyecto Nº 96031069-1.1 D y Proyecto 96072014-1 de la Dirección de Investigación de la Universidad de Concepción.

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BLOCKADE OF GERM TUBE FORMATION IN *CANDIDA ALBICANS* BY IN VIVO INHIBITION OF PKA.. R. Castilla, S. Passeron and Maria L. Cantore. Catedra de Microbiologia, Facultad Agronomia, Universidad de Buenos Aires and CIByF(CONICET).

Previous work from our laboratory has provided firm evidence on a role for cAMP as second messenger on N-Acetyl-D-Glucosamine(GlcNAc) or serum induced yeast to hypha transition in the dimorphic pathogenic fungus *Candida albicans*. Since cAMP-dependent protein kinase (PKA) regulatory subunit is the main receptor of cAMP inside the cell, we have explored the *in vivo* involvement of PKA activity on *C. albicans* germ tube formation. We investigated the effect of the *in vivo* PKA inhibition on germination: the presence of the cell permeable PKA inhibitor Myristoilated PKI (14-24) amide (MyrPKI), which efficiently inhibited *Candida* PKA *in vitro* (Ki 4μM), completely blocked germ tube formation in yeast cells induced to germinate by GlcNAc; the inhibition is dose-dependent and could not be reverted by GlcNAc, cAMP nor by any cAMP enhancing compound. Serum induced cells behaved differently: MyrPKI failed in inhibiting serum induced germination; on the other hand addition of serum to MyrPKI blocked GlcNAc induced cells did revert the effect of the PKI inhibitor, suggesting that different biochemical mechanisms underlie differentiation induced by serum and by GlcNAc. Control experiments showed that MyrPKI neither altered cellular cAMP levels compared with untreated cells nor inhibited protein kinase CK2 or cGMP-dependent protein kinase. Our results provide the first evidence that germ tube outgrowth, at least when induced by GlNAc, is mediated by PKA, reinforcing previous evidence on the existence of a cAMP mediated pathway involved in morphogenesis in *C. albicans*.
Supported by Grants from UBA, CONICET and ICGEB

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CHARACTERIZATION OF AP-1 FACTORS IN *Trypanosoma cruzi*. Espinosa, J., Martinetto, H., Paveto, C., Pereira, C., Díaz Añel, A., Portal, D., Torres, H. and Flawiá, M. INGEBI (CONICET and FCEN-UBA), Obligado 2490, Buenos Aires, Argentina.

Two signal transduction pathways have been described by this laboratory in *T. cruzi* epimastigotes. One of these involves cyclic AMP as second messenger and the other nitric oxide and cyclic GMP. The presence in *T. cruzi* of AP-1 transcription factors, as effectors of such pathways was analyzed.

Using murine cDNA probes of c-Fos and c-Jun, hybridization signals at low stringency were detected in restriction fragments of *T. cruzi* DNA. In addition, cytosolic or nuclear extracts were electrophoresed on SDS-PAGE gels and analyzed, by western blotting, employing polyclonal sera raised against human c-fos and c-jun. These procedures led to the identification of specific polypeptide bands of about 37 and 62 kDa respectively. Since a common feature of these factors is their ability to bind a specific DNA sequence named TRE, electrophoretic mobility shift assays using a TRE probe were performed. Three complexes were detected; the two faster migration complexes were specific.

T. cruzi AP-1 proteins seemed to be under the control of the nitric oxide pathway because after cell treatment with NMDA, the polypeptide recognized by the c-fos antiserum was increased. These results suggest that there is some degree of structural and functional conservation between *T.cruzi* and higher organisms at the level of AP-1 factors. (Supported by CONICET, UBA, WHO, F. Antorchas and ICGEB).

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AGE-RELATED LOSS OF CALCITRIOL STIMULATION OF PHOSPHOINOSITIDE HYDROLYSIS IN RAT SKELETAL MUSCLE. Maria Marta Facchinetti and Ana R. de Boland. Departamento de Biologia y Bioquimica. Universidad Nacional del Sur. 8000 Bahia Blanca, Argentina

We have examined the *in vitro* effects of calcitriol [1,25$(OH)_2D_3$], the hormonal form of vitamin D_3, on the breakdown of membrane phosphoinositides in rat skeletal muscle during ageing. Calcitriol 10^{-9} M induced a rapid and transient release of inositol phosphates and diacylglycerol (DAG) from muscle slices prelabeled with [^{3}H]myo-inositol and [^{3}H]arachidonate, respectively. Inositol phosphate release was maximal at 15 s and then declined. The effects of the hormone exhibited specificity, as the monohydroxylated derivative of vitamin D_3, 25OHD_3, did not alter muscle inositol phosphate levels. The stimulation of DAG was biphasic, the early phase (15 s) being abolished by neomycin (0.5 mM), an inhibitor of phosphoinositide hydrolysis, consistent with a role of phospholipase C (PLC) in intracellular signal generation.. Neomycin had no effect on the second DAG peak (2 min) induced by calcitriol, suggesting that the late phase of DAG formation is independent from the hydrolysis of phosphoinositides. Hormone generation of inositol phosphates and DAG was significantly reduced in muscle from senescent rats (80% and 60% for inositol phosphates and DAG, respectively). Calcitriol stimulation of PLC was mimicked, in both young and old rats, by GTPγS while GDPβS suppressed the effect of the hormone. Bordetella pertussis toxin abolished by 85% the effects of calcitriol on inositol phosphate release in young rats but was without effect in aged animals. These results suggest that the activation of phosphoinositide-PLC by calcitriol in rat skeletal muscle involves a pertussis-sensitive G protein and that the effects of the hormone are severely altered with senescence.

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CALCITRIOL-TRANSMEMBRANE SIGNALLING: REGULATION OF MUSCLE PHOSPHOLIPASE D ACTIVITY. Maria Marta Facchinetti and Ana R. de Boland. Departamento de Biologia y Bioquimica. Universidad Nacional del Sur. 8000 Bahia Blanca, Argentina

In rat skeletal muscle membranes, calcitriol, the hormonal form of vitamin D_3, rapidly stimulates the biphasic formation of diacylglycerol (DAG), being the second phase independent of phosphoinositide hydrolysis driven by phospholipase C. In this work we showed that the effect of calcitriol on the second phase of DAG formation was totally inhibited in the absence of extracellular Ca^{2+} (+EGTA) and by the Ca^{2+} channel blockers nifedipine (5 uM) and verapamil (50 uM) whereas the Ca^{2+} ionophore A23184 (1 uM), similarly to calcitriol, increased by 100% DAG formation. The involvement of G proteins in the second phase of calcitriol-induced DAG was evaluated. GTPγS (100 uM), which activates G protein-mediated signalls, mimicked the effects of the hormone while GDPβS, an inhibitor of G proteins, suppressed calcitriol-induced DAG formation. To elucidate the pathway of the late phase of DAG production, we examined the contribution of phospholipase D (PLD), which acts on phosphatidylcholine (PC) generating phosphatidic acid than is converted to DAG by a phosphohydrolase. In [^{3}H]arachidonate labeled muscle membranes, calcitriol increased [^{3}H]phosphatidylethanol (PEt) formation in the presence of 1.5% ethanol, a transphosphatidation reaction, specific of PLD activity. The effects of the hormone were dose-dependent (10^{-11} to 10^{-8} M) with higher PEt levels achieved at 10^{-9} M.
These results suggest that calcitriol triggers the hydrolysis of PC in rat skeletal muscle through a Ca^{2+} and G protein-dependent PLD-catalyzed mechanism.

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Histamine Receptors Signaling in Mouse Epidermal Keratinocytes Harboring H-*ras* Gene Alterations. Fitzsimons C., Durán H., Labombarda F.,Molinari B. and Rivera E. Lab. Radioisótopos, Fac. Farm. y Bioquímica, UBA and Dept. Radiobiología, CNEA; Argentina.

A role for histamine in carcinogenesis process has been widely postulated. We have described an atypical association of histamine receptors to signal transduction pathways, which could be an important feature for the action of histamine in multistage mouse skin carcinogenesis. In this model, a crucial role for *ras* protooncogene activation has been proposed. The aim of the present work was to study histamine receptors signaling in cultured cell lines derived from mouse keratinocytes harboring H-*ras* alterations. Cell lines transfected with plasmids containing human T24 mutant H-*ras* gene (AT/5) or transformed *in vitro* by a chemical carcinogen (PDV and PDVC57), all of which have altered H-*ras* genes and express different amounts of mutated H-*ras* p21, were used for all experiments. Cyclic AMP and inositol phosphates levels were measured after stimulation with H_1 or H_2 agonists. Whole cell binding experiments with histaminergic radioligands were also performed to compare the expression levels of H_1 and H_2 receptors in these cell lines. In PDVC57 cells, which have an increased expression of mutant *ras* p21, we found H_2 receptors coupled to inositol phosphates production and histamine, H_1 and H_2 agonists failed to produce modifications in cAMP concentration. In AT/5 cells, which express low levels of mutated p21, inositol phosphates production was mediated by H_1 receptors while H_2 receptors failed to induce inositol phosphates production. Moreover, H_2 receptors mediated cAMP production in AT/5 cells. A correlation between histamine receptors levels and p21 was also observed. These results suggest a relation between mutant *ras* expression and histamine receptors coupling and expression levels in these cell lines.

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TRANSIENT EFFECTS OF A PHORBOL ESTER ON GROWTH RESPONSES OF CULTURED SWISS 3T3 FIBROBLASTS.

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Phorbol esters, like tetradecanoyl phorbol acetate (TPA), activate protein kinase C (PKC) and induce cell proliferation. They also interact synergistically with insulin to stimulate proliferation in Swiss 3T3 cells. On the other hand, sustained exposure to TPA evokes down-regulation of PKCα. In this study, we examined how these two events relate in time. Confluent Swiss 3T3 monolayers were exposed to TPA (1 μM) or vehicle, 48 h before, 24 h before, simultaneously or 8 h after the addition of 5 μg/ml insulin. Mitogenesis was assayed 20 h after addition of insulin by an 8 h pulse of [^{3}H]thymidine. We observed that synergism between TPA and insulin only took place when both agents were added simultaneously, indicating that overlapping of the two signaling pathways is required for the synergistic effects. Outside this time frame, insulin proliferative effects are similar in both vehicle- or TPA-treated cells, in which PKCα is down-regulated. This suggests that this kinase is not involved in insulin action. Similar effects were observed when vanadate, instead of insulin, was used. However, while prolonged exposure to insulin abrogated the response to further addition of this hormone, suggesting down-regulation of insulin receptors, the response to vanadate was not affected. This indicates that proliferation induced by vanadate does not appear to require enhanced tyrosine phosphorylation of insulin receptors.

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INSULIN LIKE GROWTH FACTOR-I STIMULATES TYROSINE PHOSPHORYLATION OF CARDIAC PROTEINS IN NEONATAL VENTRICULAR MYOCYTES IN CULTURE.

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Cardiomyocytes are target for the action of peptide growth factors such as Insulin like growth factor-I (IGF-I) which can regulate the hypertropic growth on these cells. The mechanism by which IGF-I exerts this effect has not been elucidated. In other cell types, it has been shown that activation of the tyrosine kinase of IGF-I receptor is necessary for its biological effects and leads to the tyrosine phosphorylation of different intracellular substrates.

Our objective was to study the early tyrosine phosphorylation involved in the IGF-I pathway on neonatal ventricular cardiomyocytes (NVC), specifically at the level of IGF-I receptor (IGF-IR), insulin receptor substrate-1 (IRS-1), phosphatidylinositol 3 kinase (PI3K) and phospholipase Cγ1 (PLC-γ1) using immunoprecipitation and immunowesternblot. NVC were stimulated with IGF-I (1-100nM) for 0-10 min. Results showed that both the tyrosine phosphorylation of β subunit of IGF-IR as well as IRS-1 were time- and IGF-I concentration- dependent. Maximal response was obtained after 1 min with IGF-I (10nM). PI3K and PLC-γ1 were also activated with IGF-I but maximal effect was seen at 2 min.

We conclude that IGF-I produces IGF1R autophosphorylation and the early transphosphorylation of signaling molecules associated to the IGF-IR on cardiomyocytes which may represent new targets for understanding the developmentof heart hypertrophy.

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SIGNALING FROM CELL SURFACE RECEPTORS TO THE C-*JUN* PROMOTER INVOLVES THE MEF2 TRANSCRIPTION FACTOR: EVIDENCE FOR A NOVEL JNK INDEPENDENT PATHWAY.

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Mitogen-activated protein kinases (MAPKs) convert extracellular stimuli to intracellular signals which, in turn, control the expression of genes essential to many cellular processes, including cell growth and differentiation. The c-Jun NH_2-terminal kinases (JNKs) are a subfamily of MAPKs that phosphorylate c-Jun and ATF2. The function of JNK remains poorly understood, but it has been postulated that phosphorylated c-Jun enhances its own expression through two AP-1 sites on the c-*jun* promoter. Thus, we asked whether signals activating JNK regulate the c-*jun* promoter, using NIH 3T3 cells expressing G protein-coupled m1 acetylcholine receptors as an experimental model.

In these cells, we have recently shown that the cholinergic agonist carbachol, but not PDGF, potently elevates JNK activity. Consistent with these findings, carbachol, but not PDGF, increased the activity of a c-*jun* promoter-chloramphenicol acetyltransferase (CAT) reporter gene. However, coexpression of MEKK effectively increased JNK activity, but resulted in surprisingly limited induction of the c-*jun* promoter. This raised the possibility that pathway(s) distinct from JNK control the c-*jun* promoter, and prompted us to explore which of its elements regulate transcriptional control. Surprisingly, whereas deletion of the 5' AP-1 site had no effect, deletion of the 3' AP-1 site diminished CAT activity by only 30%. In contrast, deletion of a MEF2 site dramatically reduced expression by 70%, and deletion of both the MEF2 and 3' AP-1 sites abolished induction. Furthermore, coexpression with MEF2D and MEF2C cDNAs potently enhanced expression from the c-*jun* promoter, and carbachol, but not MEKK, induced expression of a MEF2-responsive plasmid. Taken together, these data strongly suggest that MEF2 mediates c-*jun* promoter expression through a yet to be identified pathway, distinct from that of JNK. We are currently further characterizing this pathway and exploring a potential role for JNK in other functions, such as in mediating the stability of the c-Jun protein.

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VASOPRESSIN (AVP) INCREASES THE PHOSPHORYLATION OF MAPKS AND INDUCES THEIR TRANSLOCATION FROM THE CYTOPLASM TO THE NUCLEUS IN SMOOTH MUSCLE CELLS. Gonzalez, C.B., Reyes, C.E., Figueroa, C.D. Barra, V., Troncoso, S. Department of Physiology and Histology & Pathology, Universidad Austral de Chile, Valdivia, Chile.

Since AVP is a potent mitogenic hormone we are interested in the tranduction mechanism of this activity. We have characterized the V1 receptor using a tritiated AVP and an iodinated V1 antagonist in A-10 cells derived from rat aorta smooth muscle. The activation of AVP receptors increased the tyrosine phosphorylation of proteins in a dose- and time- dependent manner. The maximum phosphorylation was reached between 15 to 30 min after incubation with AVP and decreased thereafter. The incubation of cells with AVP in the presence of a V1 antagonist inhibited phosphorylation, whereas the incubation with a V2 analogue did not increase phosphorylation. These results suggest the participation of V1 receptors in the tyrosine phosphorylation process. Immunoblotting using specific antibodies raised against the phosphorylated MAPKs showed the rapid phosphorylation and dephosphorylation of the 41K and 42K MAPKs within 5 to 15 min after incubation of cells with AVP. Densitometric analysis showed that MAPKs increased 4 to 5 times their phosphorylation state. Immunofluorescence microscopy using a MAPKs antiserum showed that MAPKs begin to concentrate in the nucleus shortly after giving AVP and remain there for more than 30 min. These results indicate that the kinetic of phosphorylation is different from that of translocation of MAPKs. The phosphorylation and translocation of MAPKs might be associated with the mitogenic activity of AVP. (FONDECYT 1961171 and DIUACH).

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EFFECTS OF OCTANOL ON SECRETORY AND CONTRACTILE FUNCTIONS REVEAL POSSIBLE INTERACTIONS BETWEEN SECOND MESSENGERS AND GAP-JUNCTIONAL INTERCELLULAR COMMUNICATION. Goudochnikov, V.I.* and Dalmora, S.L. Depto. de Farmácia Industrial, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil. * - *Pesquisador Visitante*

According to previous data, octanol - the well-known blocker of gap-junctional communication - inhibited prolactin and growth hormone secretion stimulated by dibutyryl-cyclic AMP and thyroliberin, but did not modify prolactin release induced by calcium ionophore A23187, or growth hormone release induced by dibutyryl-cyclic GMP in primary cultures of pituitary cells obtained from rats of different age groups (Fedotov, V.P. et al., 1992-1994). In recent study on isolated uterus of adult, hormonally pretreated rat octanol interacted with Ca^{2+} in the regulation of isotonic contractions stimulated by acetylcholine, oxytocin, K^+ (29 or 54 mM) or Ba^{2+}. The interactions between Ca^{2+} and verapamil or epinephrine were also demonstrated (Goudochnikov, V.I., 1996). In the present work, using isolated uterus of prepubertal, hormonally pretreated rat, we confirmed that octanol (0.3 or 0.6 mM) inhibited isotonic contractions induced by K^+ or Ba^{2+} (0.5 or 1.0 mM). Moreover, we have shown again that the increase in Ca^{2+} level in the incubation medium from 1.4 to 3.4 or 5.4 mM was able to attenuate the inhibitory octanol action. We suggest that octanol-treated isolated rat uterus may be efficiently used for studying the development of hormonally regulated interactions between second messengers and gap-junctional communication in the myometrium.

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PLC IN *Catharanthus roseus* TRANSFORMED ROOTS. S.M.T. Hernández-Sotomayor. De Los Santos-Briones C., Muñoz-Sánchez J. A., Chin-Vera J., Sánchez-Cach L., Piña-Chable M.L. and Loyola-Vargas V. M. CICY, Apdo. Postal 87, Cordemex 97310, Yucatán México.

Phospholipase C (PLC) has a key role in the signal-transduction pathway in different cells. PLC catalyses the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), generating two potential intracellular second messengers: diacylglycerol and inositol 1,4,5 trisphosphate (IP_3). Purification and biochemical characterization of PLCs have been carried out mostly with materials of animal origin. The possible ways of activation of plant PLC(s) is central, especially if exists any analogy to mammalian system. *C. roseus* transformed root culture constitute a very important model for biotechnological purposes due to their high growth rates and their degree of cell differentiation. In the present work, different aspects of the phosphoinositide metabolism pathway particularly PLC in *C. roseus* transformed roots will be presented. A PIP_2 PLC activity was identified in different tissues from *C. roseus*. The specific activity of the enzyme was 10 times higher in a membrane fraction than in cytosol. In three different root lines, PLC-activity in cytosol reached a maximum that precedes the higher activity in membranes. Increase in thymidine incorporation into DNA and IP_3 levels correlate with changes in PLC activity. Supported by grants from FIRCA (RO3TW00263),CONACYT (3016-N9306) and IFS (C/2236-1).

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ACCUMULATION OF JASMONIC ACID AND JASMONIC ACID-INDUCED PROTEINS AFTER INFECTION WITH TOBACCO MOSAIC VIRUS IN TOBACCO. Hidalgo, P.[1]. Ramírez, I.[1], Peña-Cortés, H.[2] and Holuigue L. [1] Departmento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile. P.O. Box 114-D, Santiago, Chile. [2] Institut fur Genbiologische Forschung, Ihnestr. 63, D-14195 Berlin, Germany.

Jasmonic acid (JA) is one of the hormones acting as intracellular mediators of gene activation triggered by environmental signals in plants. Accumulation of JA and JA-induced proteins in response to abiotic stress like wounding, desiccation and water deficit has been well documented. It has been also postulated a role for JA in defense reactions against pathogens, but a direct evidence of its accumulation after pathogen infection is lacking. In order to study this relation, we analyzed the response of tobacco plants infected with the tobacco mosaic virus (TMV). The endogenous levels of JA, salicylic acid (SA) and mRNA for JA- and SA-inducible genes were determined in the infected tissues, where the local hypersensitive defense response is developed. We detected an increase in the level of JA after 24 hours of infection with TMV. This increase was also observed in shift-temperature experiments, were the hypersensitive response is more dramatic. In both cases, a clear increase in the endogenous level of SA was produced. We also detected a transient increase in the mRNA for the allene oxide synthase (AOS), one of the enzymes of the biosynthetic pathway of JA, and phenylalanine ammonia-lyase (PAL), a JA-inducible protein. The peak for AOS was detected between 8-12 hours, while the peak for PAL was detected around 24 hours post infection with TMV. The levels of the mRNA for the pathogenesis-related protein (PR1a), a SA-inducible protein, showed an increase after 48 hours post infection, as it has been previously reported. This results indicate that the JA-mediated signal transduction pathway is activated by pathogen infection in tobacco plants. The importance of this pathway and its correlation with the SA-mediated pathway will be discussed.

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TWO-HYBRID SYSTEM SCREEN WITH THE *XENOPUS LAEVIS* Gαs. HINRICHS, M.V. and OLATE, J. Laboratorio de Genética Molecular., Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

G proteins have a heterotrimeric structure composed of α, β and γ subunits, that couple seven transmembrane receptors to intracellular effector systems. Upon binding of agonist, the receptor interacts with the G protein oligomer and catalyzes the exchange of bound GDP for GTP on the α subunit, wich in turn causes the dissociation of the heterotrimer. The activated GTP bound α subunit and the βγ dimer are then able to activate different effector systems. Several regulatory proteins and effectors have been described to interact with different Gα subunits. In our laboratory, we have been studying a *Xenopus laevis* Gαs (XlGαs) and until now, we have been unable to detect adenylyl cyclase stimulation in reconstitution assays with cyc⁻ membranes. In order to identified the regulatory or effector proteins that interact with the XlGαs, we performed an interaction screening using the two-hybrid system in yeast. The yeast reporter strain Y190 was transformed with the bait plasmid pAS1-GαsXl and a *Xenopus laevis* oocyte cDNA library inserted 3´ to the GAL4 activating domain in the pGAD plasmid. Approximately 3×10^6 yeast transformants were screened, from which 36 colonies were found to grow on histidine-free plates and among them 6 displayed β-galactosidase activity. These 6 histidine$^+$/β-galactosidase$^+$ clones were further purified and analyzed. (Proyectos FONDECYT 1940256 y ECOS)

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INSULIN LIKE GROWTH FACTOR-I (IGF-I) STIMULATES THE MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) SIGNALING CASCADE IN CARDIAC MYOCYTE* Lavandero S, Foncea R., Ketterman A, Andersson M, Sudgen P. Fac. Ciencias Quimicas y Farmacéuticas, U. de Chile, Santiago, Chile e Imperial College -National Heart & Lung Institute, London, UK.

In response to insulin like growth factor (IGF-I), neonatal rat ventricular myocytes (NVM) exhibit a hypertrophic response that is characterized by induction of cardiac specific genes and increased myocardial cell size.

In this work we have studied the transduction pathway of IGF-I in the hypertrophic process at the level of MAP kinase cascades (c-Raf, MEK and ERK) on NVM in culture. Serum starved NVM were exposed to IGF-I (0-30nM) in serum-free medium for 0-60min. A- and C-Raf were immunoprecipitated and the activities were measured both in a couple assay as MEK activating activity (GST-MEK1 y GST-ERK2) as by the phosphorylation of GST-MEK1 (triple mutant). The exposure of cultured NVM to IGF-I activated c-Raf but not A-Raf. ERK activity was characterized by the *in situ* myelin basic protein phosphorylation assay and by immunowesternblot using an antibody that recognized both phosphorylated and not phosphorylated p42. Maximally effective concentration of IGF-I (10nM) activated ERK1 and ERK2 by 8 and 13 fold in crude extracts of NVM. Maximal activation was achieved after 5 min. Both ERK and MEK were resolved in two peaks each by FPLC after stimulating NVM with IGF-I.

We conclude that MAPK signaling cascade is activated by IGF-I on neonatal cardiomyocytes and this may be revelant to the hypertrophic response of the heart.

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H_2 RECEPTORS DESENSITIZATION AND SIGNAL TRANSDUCTION MECHANISM IN U937 CELLS. Lemos Legnazzi, B., Monczor F., Martín, G., Cocca C., Rivera E. and Davio, C. Lab. Radioisótopos, Fac. Farm. y Bioquímica, UBA. Buenos Aires. Argentina.

In the present work we studied the histamine H_2 receptor-G protein-effector system in the monocytic cell line U937. Binding experiments using [^{3}H]tiotidine showed the presence of a double site: one of high affinity *(Q_1= 2790 ± 430 sites/cell; Kd_1= 2.2 ± 0.8 nM)* and the other of low affinity *(Q_2= 15500 ± 1800 sites/cell; Kd_2= 20 ± 3 nM)*. The high affinity site disappeared after pre-treatment with GTPγS, although no modification was observed in the total number sites *(Qtotal= 21800 ± 2114 sites/cell)*. This indicates the existence of a high affinity G protein-receptor complex in cell membrane. No modifications in the binding profile were observed when cells were pre-treated with *cholera toxin;* furthermore, cAMP production through H_2 receptors was not potentiated by this treatment, suggesting that the receptor is coupled to a G protein not sensitive to *cholera toxin.*

BU-E-75 was used to perform desensitization assays in order to avoid cross-talk with histamine H_1 receptors as already described for this cell line. cAMP production decreased rapidly (half time of approximately 20 min.) and this effect was specific for H_2 receptors. The pre-treatment did not induce modifications in the response to PGE_2 and forskolin, indicating an homologous desensitization. When cells were pre-exposed to H_2 agonist for 2 hours (total desensitization) the binding assays showed a loss of the high affinity component with no modification in the total number of H_2 receptors.

We conclude that desensitization is a consequence of the uncoupling of the G protein-receptor system and that during this process receptor sequestration does not take place.

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MOLECULAR CHARACTERIZATION OF *Trypanosoma cruzi* ADENYLYL CYCLASE. Montagna, A.E., Iribarren, A.M., Farber, M.D., Torres, H.N. and Flawiá, M.M. INGEBI (CONICET and FCEN-UBA), Obligado 2490, Buenos Aires, Argentina.

Metacyclogenesis is the process leading to *Trypanosoma cruzi* differentiation from epimastigotes (non-infective form) to metacyclic trypomastigotes (infective form). Studies by our group demonstrated that a α globin chain fragment activates adenylyl cyclase (AC) activity, triggering differentiation.

We have designed a molecular strategy to obtain the adenylyl cyclase coding sequence. Using oligonucleotides that recognize a conserved region within the catalytic domain of other *Trypanosomatidae* AC, and *T. cruzi* DNA as template, a single PCR band of the expected size was obtained. The 600 bp PCR product was subcloned in a Bluescript vector, sequenced, and compared with the BLAST data base (NCBI). Characterization of genomic and RNA sequences by Southern and Northern blots are now being performed.

Results indicated a high amino acidic identity (61-72%) between our sequence and the catalytic domain of adenylyl cyclase from several trypanosomatids, including *T. brucei, T. equiperdum, T. congolense* and *Leishmania donovani* This type of homology corresponds to the catalytic domain of Type III adenylyl cyclases. (Supported by CONICET, UBA, WHO, F. Antorchas and ICGEB).

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OVEREXPRESSION OF VARIANT REGULATORY SUBUNITS FROM *SACCHAROMYCES CEREVISIAE*. Zaremberg,V. Mormina, M.E., and Moreno, S. Departamento de Química Biológica, Facultad Ciencias Exactas y Naturales, UBA, Argentina.

The regulatory subunit (R) of the protein kinase A (PK A) from *Saccharomyces cerevisiae* is encoded by the BCY1 gene. We are studying five bcy1 mutants previously described . The mutations are located in cAMP-binding domains A or B or proximal to the PK A-phosphorylation site. In a previous study, we have shown that these mutated R subunits yielded, *in vivo*, variant holoenzymes partially active in the absence of cAMP. A number of gradually increasing PKA-dependent traits affected reflected, *in vivo*, the cAMP dependence of each mutant holoenzyme. We would like to understand the molecular mechanism of the activation of PK A. For studying biochemically these mutant holoenzymes, we outlined a strategy for the cloning of each variant R, overexpression in adequate yeast strains and final purificaton in order to reconstitute *in vitro* holoenzymes with purified yeast catalytic subunit (C). The variant *bcy1* alleles were cloned first in a Bluescript SK- vector containing the NcoI-BCY1 gene (NcoI sequence recognition, including the first BCYI AUG was created by site directed mutagenesis). Finally each mutant allele was subcloned in the YEp51 shuttle vector under the control of the GAL10 promoter .The different constructions were checked by PCR and restriction map analysis. The overexpression and functionality was studied by SDS-PAGE, Coomassie Blue staining, westerns blots, northern blots, and phenotypic analysis of different strains transformed with the *bcy1*-YEp51 constructions grown in presence or absence of galactose.

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YEAST Ca^{2+}/CALMODULIN BINDING PROTEINS INDUCED BY MALTOSE .

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Ca^{2+}/calmodulin is involved in the regulation of a variety of cellular processes in eucariotic cells by interacting with key regulatory proteins. In yeast, although a large number of proteins has shown affinity for calmodulin, still little is known about them. As an initial attempt, we studied the pattern of expression of the Ca^{2+}/calmodulin-binding proteins. We observed that some of them are subject to catabolite repression, whereas others have their expression induced by maltose or galactose. In order to investigate the Ca^{2+}/calmodulin-binding proteins that are induced by maltose, strains harbouring either an inducible *MAL6* gene or a constitutive *MAL4* gene were compared. Cell free extracts of these strains grown on glucose or maltose were chromatographed on a Ca^{2+}/calmodulin affinity collumn; the binding proteins were fractionated on SDS-Page and the protein profiles were compared. Polypeptides of molecular masses 82 and 46.5 kDa were expressed at higher level during growth on glucose while another set of polypeptides of 45, 44 and 27.5 kDa had their synthesis induced by maltose. The 27.5 kDa protein is under investigation.

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SUBSTRATES FOR PROTEIN PALMITOYLATION IN AXON TERMINALS ALTER WITH GROWTH STATE. Sean I. Patterson[1,2] and J.H. Pate Skene[2]. (1) Departamento de Fisiologia, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina and (2) Department of Neurobiology, Duke University, Durham, North Carolina, USA.

Ongoing cycles of posttranslational protein palmitoylation in growth cones are necessary for normal neuritic extension (J. Cell Biol. 124: 521). Incorporation of ^{3}H-palmitate by isolated axon terminals shows that the protein substrates available for palmitoylation change profoundly as developing growth cones mature into synaptic terminals. Among the developmentally regulated changes are decreased GAP-43 and increased SNAP-25. Both of these proteins have been implicated in some forms of neurite extension. Furthermore, SNAP-25 is part of the membrane fusion apparatus that mediates synaptic vesicle cycling in synaptic transmission. Other palmitoylated proteins whose expression is developmentally regulated in nerve terminals include synaptobrevin, nitric oxide synthase, and members of the src family kinases (fyn) and small G-protein superfamily (ras, rac, rho). Thus the palmitoylation substrates of nerve terminals include respresent several classes, including proteins functionally specialized for signal transduction, neurite growth and for synaptic transmission. The expression of the proteins specialized for growth and synaptic transmission show an intermediate developmental period when both sets of proteins can be found at high levels in the immature synaptic terminals, during the time that correlates with the activity-dependent remodelling of cortical synapses.

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CONTROL OF *Trypanosoma cruzi* EPIMASTIGOTE MOTILITY THROUGH THE NITRIC OXIDE (NO) PATHWAY. Pereira, C. Paveto, C., Espinosa, J., Alonso, G., Flawiá, M. and Torres, H.. INGEBI (CONICET and FCEN-UBA), Obligado 2490, 1428 Buenos Aires, Argentina

In neural cells, NO, which is synthesized from L-arginine by a Ca^{2+}-calmodulin-dependent synthase, activates a soluble guanylyl cyclase, leading to a rise in cyclic GMP levels. Moreover, stimulation of L-glutamate/N-methyl-D-aspartate (NMDA) receptors in neural cells leads to the influx of Ca^{2+}, which in turn activates nitric oxide synthase. Studies from this laboratory shows that the nitric oxide pathway is also present in the lower eukaryotic organism *T. cruzi*.

A NO synthase was purified from epimastigote soluble extracts. The enzyme required NADPH, was activated by Ca^{2+}, calmodulin, tetrahydrobiopterin and FAD, and inhibited by N-methyl-L-arginine. Glutamate and NMDA stimulated *in vivo* conversion of arginine to citrulline by epimastigote ceils and enhanced cell motility. These stimulations could be blocked by, MK-801 and ketamine and enhanced by glycine and serine. A Na nitroprusside-activated guanylyl cyclase was detected in cell-free, soluble epimastigote preparations. L-glutamate, NMDA and sodium nitroprusside increased, *in vivo*, cyclic GMP levels and also enhanced cell motility. This evidence indicates that in *T. cruzi* epimastigotes, L-glutamate controls cyclic GMP levels and cell motility through a pathway mediated by NO. (Supported by CONICET, UBA, WHO, F. Antorchas and ICGEB).

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DIFFERENTIAL DISTRIBUTION OF PROTEIN KINASE C (PKC) ISOFORMS MEDIATED BY INSULIN LIKE GROWTH FACTOR-I IN CULTURED NEONATAL CARDIOMYOCYTES* Pérez V. Foncea R, Ketterman A, Sapag-Hagar M, Lavandero S. Fac. Cs Qcas y Farmacéuticas, U.Chile, Chile Imperial College-National Heart & Lung Institute, London, UK.

Insulin like Growth Factor-I (IGF-I) has been associated to the genesis of cardiac hypertrophy, however its signalling pathway is still unknown. The activation of IGF-I receptor triggers multiple signalling pathways including the activation of several protein kinases. Protein kinase C (PKC) has been implicated in the cardiomyocyte hypertrophic response.

Our aim was to study the effect of IGF-I in the traslocation of the different PKC isoforms from the soluble to the particulate fraction in cultured ventricular cardiomyocytes (VC) by immunofluorescence and westernblot using specific antibodies against PKC isoforms (α, β, δ, ε, ζ). VC were isolated from neonatal rat hearts by digestion with proteases and cultured for 24h in serum free medium before the incubation with IGF-I (10nM) or TPA (1uM). Phosphoinositide (PI) turnover was assessed adding [^{3}H]-myoinositol (5μCi) one day before IGF-I stimulation.

Our results showed that PKC isoforms changed their subcellular distribution when cardiomyocytes were incubated with IGF-I (10nM) between 0-5 min. IGF-I modified only the subcellular distribution of β, δ, ε PKC isoenzymes although did not stimulate PI turnover on VC. We concluded that IGF-I activated differentially the PKC isoforms on neonatal cardiomyocytes.

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PARTICIPATION OF PROTEIN KINASES A AND C IN THE STIMULATION OF PROTEIN PHOSPHORYLATION BY 1,25(OH)$_2$D$_3$ IN CARDIAC MUSCLE. Graciela Santillán and Ricardo Boland. Departamento de Biología, Bioquímica y Farmacia. Universidad Nacional del Sur. (8000) Bahia Blanca, Argentina.

1,25(OH)$_2$D$_3$, the active hormonal derivative of vitamin D$_3$, rapidly (1-5 min) stimulates microsomal protein phosphorylation, calcium influx and cyclic AMP (cAMP) production in cardiac muscle. This work investigates the participation of cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) in 1,25(OH)$_2$D$_3$-induced protein phosphorylation in chick heart muscle.

Incubation with [γ-^{32}P]ATP of microsomes from left ventricle thin slices pretreated with very low 1,25(OH)$_2$D$_3$ concentrations (0.01-0.1 nM) showed an increase in the phosphorylation of mainly three proteins of 45, 55 and 70 kDa. The hormone effect was dose- and time-dependent, being maximal at 3 min and at a concentration of 0.01 M. The 1,25(OH)$_2$D$_3$ action on microsomal protein phosphorylation was reproduced by Sp-cAMPS (50 μM), a specific PKA activator, as well as TPA (10-100 nM) and DOG (10-100 μM), well known PKC stimulators.

In vitro treatment of microsomes with the catalytic subunit of PKA (3.3 U/μl) stimulated the phosphorylation of the same proteins as 1,25(OH)$_2$D$_3$ whereas Rp-cAMPS, a cAMP antagonist, inhibited the hormone effects on protein phosphorylation. In contrast, bis-indolylmaleimide (40 nM), a specific PKC inhibitor, potenciated the action of 1,25(OH)$_2$D$_3$.

The results obtained indicate that 1,25(OH)$_2$D$_3$ target cardiac muscle phosphoproteins contain both PKA and PKC sites and suggest that the latter attenuate hormone stimulation of PKA-dependent phosphorylation.

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STIMULATION OF PHOSPHOLIPID BIOSYNTHESIS BY HIGH GLUCOSE CONCENTRATION. C.P.Setton-Avruj and N.B. Sterin-Speziale. Dpto. Química Biológica, F.F.y B., UBA, IQUIFIB-CONICET. Buenos Aires, ARGENTINA.

Glucose, once considered biologically inert, is a harmful agent through at least two independent mechanisms: a permanent alteration of proteins by nonenzymatic glycosilation, and by the accumulation of sorbitol via an aldose reductase dependent mechanism.

We had previously demonstrated that extracellular high glucose concentration produces the same effect as the diabetic state in papillary slices in regard to phospholipids "de novo" biosynthesis, through the activation of phospholipase C (PL C). As respect the polyol pathway phospholipid "de novo" biosynthesis is independent of sorbitol accumulation.

To further elucidate the high glucose concentration effect on renal papillary PC de novo biosynthesis, we evaluated the effect of prostaglandins (PGs) synthesis measuring ^{32}P incorporation to PC in the presence of indomethacin and the effect of exogenous PGs.

Indomethacin inhibits papillary phospholipid biosynthesis (6.8±1.1 vs 3.1±0.6), and is reverted by the addition of PGE_2 and $PGF_{2\alpha}$ (5.1±0.4 and 4.3±0.5, respectively).

High glucose concentration produced a change in the PGs-pattern being ($PGF_{2\alpha}$: 30%, PGD2:18% at 25 mM glucose vs PGF2α:16.5, PGD2:25.2% at 5 mM glucose). The use of two PKC inhibitors showed that H7 promoted a generalized decrease in PGs biosynthesis, meanwhile no changes were observed in the presence of staurosporine.

These results demonstrate that the effect of 25 mM glucose on papillary phospholipid biosynthesis is mediated through PGs biosynthesis.

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DECAPACITATION BY SEMINAL PLASMA INHIBITS TYROSINE PHOSPHORYLATION IN HUMAN SPERM. Claudia N. Tomes[1,3], Rosa Carballada[1], DF Katz[2] and Patricia Saling[1]. (1) Departments of Obstetrics and Gynecology and Cell Biology, (2) Departments of Biomedical Engineering and Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA. (3)Present address: Instituto de Histología y Embriología (IHEM). Facultad de Ciencias Médicas. Universidad Nacional de Cuyo. Mendoza. Argentina

Freshly ejaculated sperm are not capable of fertilizing eggs. In most species, sperm acquire this ability several hours after mating or after incubation in vitro in an appropriate medium, in the absence of seminal plasma components. Many modifications take place during this period, collectively termed capacitation. Seminal plasma, the vehicle for spermatozoa at the time of ejaculation, contains factors that influence sperm's fertilizing capacity. Little is understood, however, of the biochemical cascade triggered when sperm and seminal plasma interact. In vitro capacitation of human sperm has been associated with increase in the phosphorylation of several proteins on tyrosine residues. Here we demonstrate that prevention of capacitation by seminal plasma, correlates with the lack of phosphorylation of sperm proteins on tyrosine residues. Furthermore, addition of seminal plasma to a suspension of previously capacitated sperm (decapacitation), decreases the level of tyrosine phosphorylation in all the proteins. The effect is dependent on the concentration of seminal plasma. Conversely, the removal of seminal plasma leads to recapacitation and partial recovery of the phosphotyrosine signal. Seminal plasma affects neither the percentage of motile nor viable cells, ruling out that toxicity could be responsible for the lack of tyrosine phosphorylation. In addition to its effect on tyrosine phosphorylation, seminal plasma also alters sperm motility, reverting the typical pattern of the washed/capacitated sperm to the one characteristic of the sperm in semen. To our knowledge, this is the first study reporting a link between seminal plasma and a signal transduction cascade in sperm.

Membranes, Lipids, Receptors - Hormones and Growth Factors, Signal Transduction, Cell Biology - Molecular Aspects

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CLONING AND EXPRESSION OF A *Xenopus laevis* OOCYTE ADENYLYL CYCLASE. Torrejón, M. and Olate, J. Lab. de Genética Molecular, Fac. de Cs. Biológicas, Universidad de Concepción, Chile. (FONDECYT : 1940256)

In eukaryotic cells, adenylyl cylclase (AC) is a widely distributed effector system regulated by G-protein coupled receptors (GPCR), which in turn regulate the synthesis and levels of the second messenger cAMP. Until now 9 different mammalian AC have been cloned and characterized from different sources. They can be distinguished by their modes of regulation, for example Mg^{++}, GTPγS, forskolin and Ca^{++}-calmodulin. Our group is interested in the characterization of the adenylyl cyclase system present in *Xenopus laevis* oocyte. As the first step to understand the function of this enzyme, we have cloned a full-length cDNA for a novel member of the AC family from an oocyte *X. laevis* library (XLAC). The cDNA is 4,372 bp long with a open reading frame of 4,062 nucleotides that encodes a protein of 1,355 amino acids. Comparison of XLAC deduced amino acid sequence with previously mammalian cloned AC, showed a high homology with a mouse AC recently cloned by Premont et al. (J.B.C. 23 : 13900-13907,1996). As a second step in the understanding of XLAC function and regulation we expressed it in HEK293 and COS-7 cells. The expressed enzyme presents similar features for all the mammalian AC, being the enzyme activated by forskolin, Gpp(NH)p and aluminium fluoride. Through the reverse PCR technique (RT-PCR) we found that the XLAC gene is expressed only in oogenesis and not in the early steps of embriogenesis morula and blastula, indicating the importance of the XLAC in the oocyte meiotic maturation process.

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IPG SIGNALLING IN ACTH ACTION IN BOVINE AND RAT ADRENAL. Vaena, S., Lima, C., Lederkremer, R. and Vila, M. C. Departamento de Química Biológica y Departamento de Química Orgánica, FCEyN, UBA, Buenos Aires. Argentina. *silvae@quibiol.qb.fcen.uba.ar*

We previously reported that ACTH increases the hydrolysis of glycosylphosphatidylinositol (GPI) in bovine adrenocortical cells. We found that ACTH increases: a) the release of alkaline phosphatase, a GPI-anchored enzyme, to the extracellular medium, b) the release of ceramide from a glycoinositol-phosphoceramide (LPPG) purified from *T. cruzi*. These data suggest that ACTH activates a PLC that is able to hydrolyze GPI, which would result in the production of inositol phosphoglycan (IPG). We also reported that an IPG, purified from *T. cruzi*, inhibited ACTH-mediated aldosterone accumulation in these cells. In the present work we tested if IPG is also able to inhibit the stimulation of glucocorticoid production by ACTH in bovine adrenocortical cells preparations enriched in fasciculata. We found that IPG purified from *T. cruzi*, inhibits ACTH mediated cortisol accumulation. We also proved that in this preparation, ACTH increases the release of alkaline phosphatase. On the other hand, we evaluated the activation of a GPI-PLC by ACTH in rat adrenal core cells. An increase by ACTH treatment in the release of alkaline phosphatase and of ceramide from LPPG was observed. Since the activation of a GPI-PLC by ACTH occurs both in bovine and rat adrenal, this may be a conserved mechanism in mammals. Besides, the IPG released proved to be able to inhibit the accumulation of mineralocorticoids as well as glucocorticoids.

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GUANINE NUCLEOTIDES REGULATED MEMBRANE BOUND GUANYLYL CYCLASE . Villarroel, S., Bécemberg, I.L. and Alfonzo, M. Sección de Biomembranas. Instituto de Medicina Experimental. Universidad Central de Venezuela. Caracas. Venezuela.

Guanylyl cyclases (GC) are responsible for the formation of cGMP and two different GC have been described. A hemoprotein located in the cytosol, wich is involved in the nitric oxide (NO) biological effects and glycoproteins being associated to particulate fractions. A plasma bound guanylyl cyclase activity has been described by us (FEBS.Lett. 253: 16-22, 1989), which is regulated by muscarinic compounds and more recently, we have been shown that this GC is regulated by G-proteins (Arch. Biochem. Biophys. 324: 209-215, 1995). In order to understand the role of G-proteins, several GTP analogs and related compounds were assayed on the GC activity presents in a plasma membranes fraction P2 isolated from bovine airway smooth muscle, using discontinuous sucrose gradient, and the cGMP was determined by RIA. When GTP is titrated in the presence of NaCl, this salt behaves a "allosteric inhibitor" for this GC activity. This NaCl effect is reverse by GTP or GDP analogs such non-hydrolysable GTPγS and GDPβS respectively. These data supported the fact about G-proteins are involved in the regulation of the membrane bound guanylyl cyclase activity. Grants from CONICIT S1-2749 (IB)-CDCH-09.33.2942/95 (MA) and 09.33.3436/95 (IB).

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PHOSPHORYLATION OF PHOSPHOLAMBAN IN THE INTACT HEART BY THE Ca^{2+}-CALMODULIN-DEPENDENT PROTEIN KINASE CASCADE. ROLE OF PHOSPHATASES. Vittone, L., Mundiña, C., Ortale, M., Chiappe de Cingolani, G. and Mattiazzi, A. Centro de Investigaciones Cardiovasculares, Universidad Nacional de La Plata, Argentina. Email: cicme@isis.unlp.edu.ar.

Phospholamban, the regulator of the cardiac sarcoplasmic reticulum (SR) Ca^{2+}-ATPase, is phosphorylated at serine 16 (Ser^{16}) by cAMP-dependent protein kinase (PKA) and at threonine 17 (Thr^{17}) by Ca^{2+}-calmodulin-dependent protein kinase type II (CaMKII). In SR membrane vesicles, both phosphorylation pathways are independent of each other. In contrast, in the intact heart, CaMKII phospholamban phosphorylation is only detected when intracellular cAMP increased. The reason for this discrepancy remains unknown. The availability of phosphorylation site-specific antibodies to phospholamban prompted us to reexamine the issue in Langendorff-perfused rat hearts. Immunological detection showed: 1) 30 nM isoproterenol (Iso) increased the phosphorylation of both Ser^{16} and Thr^{17}; 2) Lowering Ca^{2+} supply to the myocardium (low $[Ca^{2+}]_o$ and/or 0.4 μM nifedipine) in the presence of Iso did not affect Ser^{16} phosphorylation but decreased phosphorylation of Thr^{17}; 3) Increasing Ca^{2+} supply by cAMP-independent mechanisms (high $[Ca^{2+}]_o$) failed to increase Thr^{17} phosphorylation; 4) The same increase in $[Ca^{2+}]_o$ in the presence of 1 μM okadaic acid, an inhibitor of the protein phosphatase-1 (PP1), increased Thr^{17} phosphorylation. The results suggest that CaMKII pathway requires in the intact heart simultaneous activation of the protein kinase and inactivation of PP1. The fact that PP1 is inhibited by PKA-dependent mechanisms may explain that the CaMKII pathway is detected at high cAMP levels. (Supported by British Council-Fundación Antorchas and CONICET).

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A PHORBOLESTER- AND ZINC- BINDING PROTEIN IS NECESSARY FOR THE RAB 5 EFFECT ON ENDOSOME FUSION. Aballay, A., Arenas, N.G., and Mayorga, L.S. IHEM-CONICET, Facultad de Ciencias Médicas, U.N. Cuyo, Mendoza, Argentina.

It has been suggested that the transport of proteins from the endoplasmic reticulum to the Golgi apparatus is regulated by a protein containing the regulatory domain of protein kinase C (PKC). This domain has two zinc finger motifs necessary for diacylglycerol and phorbol ester (PMA) binding. Recently, we have observed an inhibitory effect of several zinc chelators on the endosome fusion reconstituted in an *in vitro* system. The factor that requires zinc for its activity is still unknown. A possibility is that PKC or a PKC-like protein might be that factor. To test this hypothesis we studied the effect of calphostin C (CPC) -a specific inhibitor of PKC that interacts with the highly conserved cysteine-rich motif present in the regulatory domain- and PMA on endosome fusion. We observed an inhibitory effect of CPC on the fusion process whereas PMA presented a stimulatory effect. In order to know if the effect of CPC and PMA was due to the respective inhibition or activation of the kinase activity, inhibitors of the catalytic domain of PKC were studied. The results indicate that the kinase activity is not required by fusion between endosomes. Besides, we noticed that rab 5 activation of endosome fusion requires zinc, suggesting that the Zn^{++}-binding protein is necessary downstream of rab 5. These results provide evidence that a phorbol ester-binding protein that can be PKC, is needed for the rab 5 effect on endosome fusion.

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GLUTATHIONE DEPLETION DECREASES INTESTINAL CALCIUM ABSORPTION AND ALKALINE PHOSPHATASE ACTIVITY IN NORMAL CHICKS. A. Alisio, A. Marchionatti, G. Díaz de Barboza, V. Baudino and N. Tolosa de Talamoni. Cátedra de Química Biológica. Facultad de Medicina.Univ. Nac. Cba., C.C. 35, Suc.16, 5016, Córdoba,Argentina. E-mail: aalisio@biomed.uncor.edu

Recent studies from this laboratory demonstrated, by using DL-buthionine-S,R-sulfoximine (BSO) as a tool to decrease the intestinal glutathione (GSH), that calcium transfer from lumen-to-plasma was inhibited in vitamin D-deficient chicks treated with cholecalciferol, but not in vitamin D-deficient chicks (Tolosa de Talamoni et. al., Comp. Biochem. Physiol., in press). BSO is used in cancer therapy of tumors that exhibit multi-drug resistance. In order to know potential complications in the intestinal function, we have investigated the intestinal Ca absorption and associated variables in normal chicks after one i.p. dose of BSO. Intestinal Ca absorption was decreased half an hour after injection of one mmol of BSO/kg returning to the control values 30 min later. The activity of intestinal alkaline phosphatase (AP) was also reduced by the same dose but other brush border membrane enzyme activities, such as sucrase and γ-glutamyltranspeptidase, were not affected. Doubling the BSO dose did not produce a further decrease in Ca absorption but the activity of AP was highly decreased. This inhibition lasted for 12 hours. Kinetic studies revealed that BSO decreased V_{max} while the K_m for the p-nitrophenyl phosphate as substrate remained unchanged. Addition of GSH monoester to the duodenum lumen sac reversed the inhibitory effect on both Ca absorption and on intestinal AP activity. These results indicate that GSH depletion caused by BSO modifies the cellular redox state altering probably -SH groups of proteins involved in the intestinal Ca absorption and AP, an enzyme closely related to the Ca homeostasis.

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INCORPORATION OF SOLUBLE AND PARTICULATE ANTIGENS INTO THE EPITHELIUM OF THE HUMAN OVIDUCT. Imarai C, Acuña C, Rocha A, Nelson P, Fuhrer J*, Vargas R*, Cardenas H. Departamento de Ciencias Biológicas, Facultad de Química y Biología, USACH. *: Hospital San José, Serv. Ginecología y Obstetricia.

The epithelium of the human oviduct expresses HLA class II that suggests it could play a role in antigen presentation. As a first step towards the elucidation of this putative role, we are currently studying the endocytic and phagocytic properties of the tubal epithelium. Oviducts were obtained from women (n=27) submitted to hysterectomy after informed consent. Protocols were approved by the Ethical Committee of the University. After stripping of the muscle layer, fluorescein isotiocyanate-labeled bovine serum albumin (BSA-FITC) or *Escherichia coli* (*E. coli*-FITC) were infused into the lumen. One centimeter pieces were incubated (37º C, 5% CO2) for 2 h and processed for fluorescence or confocal scanning microscopy. Incorporation of both BSA-FITC and *E. coli*-FITC was observed into secretory and ciliated epithelial cells, in oviducts from women operated during the follicular or luteal phase. Similar BSA-FITC endocytosis was demonstrated in the oviductal epithelium of the rat. Our results are the first demonstration of incorporation of soluble and particulate antigens by the epithelium of the human oviduct, and support its role in some stages of antigen processing.

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ACETYLCHOLINESTERASE PROMOTES THE AGGREGATION OF THE AMYLOID-β-PEPTIDE FRAGMENTS. Alvarez A. and Inestrosa N.C. Dpto. Biología Celular y Molecular, Fac. de Ciencias Biológicas, P. Universidad Católica de Chile.

Alzheimer's disease (AD) is characterized by the presence of amyloid plaques where amyloid-β-peptide (Aβ) is the major component. Previously we have reported that the enzyme acetycholinesterase (AChE) accelerates assembly of the Aβ(1-40) peptide into Alzheimer's fibrils *in vitro*.

Fragments of Aβ have proved being interesting because some of them aggregate and lead to amyloid fibril formation *in vitro*. In order to evaluate the domains and structural elements of Aβ involved in the aggregation induced by AChE, we have studied the effect of AChE on such fragments.

Thioflavine-T fluorescence, turbidometric and congo red measurements of the polymerization process shown an enhancement of the aggregation of the fragments Aβ12-28 and Aβ25-35 in the presence of the AChE. However AChE was not able to induce the aggregation of the fragments Aβ1-16 and Aβ9-21. Because the effect of AChE on Aβ12-28 was higher than the one observed with the Aβ25-35 fragment, we have studied the effect of AChE on the aggregation of the mutants fragments, Aβ12-28Val18→Ala and Aβ12-28Glu22→Gln. AChE promotes the aggregation these fragments in a differential way. These results suggest that the elements required for the interaction of AChE witht the Aβ peptide are present in the region between residues 22-28. Besides, we have observed an inhibitory effect on the Aβ aggregation induced by AChE with a specific AChE monoclonal antibody.

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INFLUENCE OF ELICITATION ON PEROXIDASE AND PHENYLALANINE AMMONIALYASA ACTIVITIES OF *ARMORACIA LAPATHIFOLIA* HAIRY ROOTS M.A.Alvarez MA, C.Flocco, A.M.Giulietti.Cátedra de Microbiología Industrial y Biotecnología, Facultad de Farmacia y Bioquímica, Universitdad de Buenos Aires, Junín 956 6° (1113),Buenos Aires, Argentina.

We have established several clones of *Armoracia lapathifolia* hairy roots that show a peroxidase specific activity higher than *A. lapathifolia* roots of the field-grown plant (62 U/g FW and 48 U/g FW to 19U/g FW respectivelly). *A. lapathifolia* hairy roots were obtained by infection with *Agrobacterium rhizogenes* LBA 9402. The clone established produced showed an active and strong growth and produced almost exclusively basic peroxidase isozyme. In this work we studied the influence of biotic elicitors on growth and on the relationship between peroxidase activity, phenylalanine ammonialyasa (PAL) production and total phenol concentration by *A. lapathifolia* hairy roots. It is well known that elicitors induce the synthesis of PAL which is the first enzyme involved in the phenilpropanoid pathway . The phenolic compounds produced, which have toxic effects on the growth of cells, induced peroxidase biosynthesis. In this study we used as elicitors chitosan ,*Rhizopus* sp., *Aspergillus niger, Monodyctis cataneae* and *Verticillum* sp(at different concentrations). The results obtained shown that PAL and peroxidase activity increased inmediatelly after elicitation. No peroxidase activity was detected in the culture medium. Total phenolic concentration remained at a relatively constant level during the treatment. Index growth was only affected by the highest concentration of chitosan (10 mg L^{-1}).

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HEXOSE TRANSPORTER EXPRESSION IN HUMAN SPERMATOZOA AND TESTIS. Angulo, C., Rauch, C., Golde, D.W¶, Vera, J.C.¶ and Concha, I.I. Instituto de Bioquímica. Universidad Austral de Chile, Valdivia, Chile and ¶Memorial Sloan-Kettering Cancer Center, New York, USA.

We analyzed the expression of facilitative hexose transporters in mature human spermatozoa and in pre and post puberal human testis. Using immunoblotting and immunolocalization, we identified the proteins GLUT1, GLUT2, GLUT3 and GLUT5 as the hexose transporters expressed in human spermatozoa. The four transporters were distributed similarly along the sperm tail, but they showed a differential distribution at the sperm head. GLUT2, GLUT3 and GLUT5 were mainly present in the posterior half of the head, whereas GLUT1 was present at the acrosomal level. Immunoblotting experiments confirmed the presence of proteins reactive with anti-GLUT1, GLUT2, GLUT3 and GLUT5 in membranes prepared from human spermatozoa. The proteins GLUT1, GLUT2 and GLUT5 were the main hexose transporters expressed during prepuberal testicular development, while adult testes also expressed the isoforms GLUT3 and GLUT4. Our data indicate that human spermatozoa express hexose transporters of the facilitative type that endow these cells with the capacity to transport fructose, glucose and vitamin C. The presence of the high affinity glucose transporter GLUT3 in human sperm and testis may be related to the low glucose content of the seminiferous tubules. Our findings indicated that two different fructose transporters are expressed during testicular development, consistent with the notion that testicular cells may have a specialized need for fructose, a substrate that has not been identified as a source of metabolic energy during human development.

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RECRUITMENT OF COP-PROTEINS TO PHAGOSOMAL MEMBRANES: PUTATIVE ROLE IN VESICLE FORMATION FROM THE PHAGOSOMAL COMPARTMENT. (W. Berón, M.I. Colombo, and P. D. Stahl). IHEM-CONICET, Fac. Cs. Médicas, U.N. de Cuyo, Mendoza, Argentina and Department of Cell Biology and Physiology, Washington University, School of Medicine, St. Louis, MO, USA.

Particle internalization in macrophages is followed by a complex maturation process involving membrane and protein traffic between the maturing phagosome and organelles of the endocytic pathway. We have previously observed that phagocytosed proteins are sorted from phagosomes into a heterogeneous population of vesicles that fuse with endosomes. However, the mechanism and the protein machinery involved in the formation of these phagosome-derived vesicles is unknown. Three types of coated transport vesicles have been involved in intracellular trasport pathways: clathrin -coated vesicles, COPII- and COPI-coated vesicles. The latter are involved in anterograde transport from the endoplasmic reticulum (ER) to Golgi and between Golgi cisternae. To address the possibility that COP-proteins may participate in the formation of phagosome-derived vesicles we studied the binding of β-COP and β'-COP to highly purified phagosomes. Binding was detected by western blot assays after incubating purified phagosomes with cytosolic proteins. It has been shown that coatomer binding depends on the interaction of the small GTP-binding protein ARF with the membranes, a process that is inhibited by the fungal metabolite brefeldin A (BFA). BFA appears to inhibit an ARF guanine-nucleotide exchange factor that regulates ARF recruitment. β-COP binding to phagosomal membranes was regulated by nucleotides and inhibited by BFA, similar to that observed with Golgi membranes. An ARF mutant defective in GTP hydrolysis, supported binding of β-COP to phagosomes independently of added nucleotide. Heterotrimeric G proteins may mediate recruitment of ARF and coatomer to intracellular membranes. AlF and Gβγ subunits, agents known to modulate G protein activity, were tested in the binding assay. AlF in the presence of GTP increased β-COP association; binding was inhibited by Gβγ subunits. Given that coat proteins play an essential role in vesicle budding events our findings of COP interaction with phagosomal membranes suggest that these proteins may be involved in the recycling of components from the phagosomal compartment.

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PARTITION OF ABSORBED GLUCOSE BETWEEN TRANSPORT AND METABOLISM IN PERFUSED EVERTED RAT INTESTINAL SEGMENTS. Carmona, A., Instituto de Biología Experimental, Universidad Central de Venezuela.

Most of the liver glycogen appears to be synthesized from gluconeogenic precursors. It is not clear to what extent absorbed glucose gets intact into blood or is metabolized to lactate. Partition of glucose between translocation and metabolism was studied in an *in vitro* perfusion system for intestinal segments developed in our laboratory. After glucose addition to the luminal medium, translocation of glucose followed a sigmoidal course and reached a plateau stable for up to one hour. Cumulative sugar uptake was linear after a lag phase of 3-6 min. That is the time required to build up the intracellular concentration of absorbed solute. Lactate output followed a similar trend. Glucose output was larger in jejunal segments from rats fed glucose than in those which received a commercial diet. For lactate, the opposite trend was observed. Ileal segments translocated less glucose and released less lactate than those from the jejunum. In chow fed rats, lactate production accounted for 42% of absorbed glucose, increasing to 75% in segments from rats receiving 5% glucose as drinking solution. This treatment increased the thickness of the intestinal walls thereby diminishing oxygen availability. Therefore, intestinal partition of glucose was influenced by the age and type of diet fed to donor animals, the route of glucose administration and by conditions which favor glycolysis (anoxia, NH_3 addition). These variables should be controlled when evaluating the initial metabolism of glucose within the intestinal tissue.

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SCAVENGING OF TOXIC OXYGEN DERIVATIVES BY FERREDOXIN-NADP$^+$ REDUCTASE. A. R. Krapp, N. R. Cortez, J. Palatnik, V. B. Tognetti, A. Acevedo*, E. M. Valle and N. Carrillo. PROMUBIE, Biochemistry Faculty. University of Rosario, Suipacha 531. 2000 Rosario; and *INTA-Castelar, PO Box 25, 1712 Castelar, ARGENTINA. e-mail: carrill@unrobi.edu.ar.

Propagation of superoxide radicals induced by viologens in *Escherichia coli* is largely mediated by the NADPH activity of the flavoprotein ferredoxin-NADP$^+$ reductase (FNR). Unexpectedly, different *E. coli* mutant strains devoid of FNR are extremely sensitive to oxidative stress, and do not grow aerobically in the presence of methyl viologen (MV) or hydrogen peroxide. A wild-type phenotype could be restored to the mutant cells by expression of a functional FNR from a cloned plant gene. Then, FNR appears to play a double role as an oxygen radical propagator and scavenger, with the latter activity overcoming the former. FNR is as good a scavenger as the two major *E. coli* superoxide dismutases (SOD) combined, as judged by the aerobic performance of FNR-deficient cells and of a *sodA sodB* double mutant. FNR failed to complement SOD-deficient *E. coli* cells, whereas expression of cloned plant SOD and catalase genes prevented killing of FNR-deficient bacteria by MV. The results suggest that the two scavenger systems operate at different levels of the oxidative pathway initiated by MV reduction. Neither ferredoxin nor flavodoxin appear to play any significant role in FNR-mediated scavenging. We discuss possible mechanisms to account for the contribution of FNR to the cell concerted defense against oxidative damage. We also describe the use of this bacterial system to clone eucaryotic genes involved in antioxidative functions by genetic complementation of FNR-deficient *E. coli* cells.

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HUMAN ERYTHROCYTE DAMAGE INDUCED BY AZOCOMPOUNDS

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The radicals produced in the thermal decomposition of water soluble azocompounds in a red cell suspension lead to hemoglobin oxidation, lipid peroxidation, hemolysis and changes in the structure and function of membrane proteins. The pattern of changes strongly depends upon the employed azocompound. The radicals produced in the thermolysis of 2,2'-azobis(2-amidino propane) (AAPH) produces significant amounts of TBARs and hemolysis prior to hemoglobin oxidation. On the other hand, 4,4'-azobis(4-cianovaleric acid) (ACV) produces extensive hemoglobin oxidation prior to hemolisis and/or TBARs accumulation. These differences can be due to a faster penetration of ACV to the red cell interior, as a consecuence of its larger hidrophobicity. However, ACV derived radicals also oxidizes hemoglobin faster than AAPH in red cell lysates. These results would indicate a particular sensitivity of the hemoprotein to the ACV derived radicals. Possible explanation of this effect will be discussed.

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POLYAMINE LEVELS IN RATS TREATED WITH TWO PORPHYRINOGENIC AGENTS. Cochón, A.C., Aldonatti, C., González, N. and San Martín de Viale, L. Depto. de Qca. Biológica e Instituto de Investigaciones Bioquímicas, FCEyN, UBA, Buenos Aires, Argentina. E-mail: adcris@quibiol.qb.fcen.uba.ar

It has been suggested that tissular polyamine levels may play a role in the toxic effects of two porphyrinogenic agents: malathion and 2,3,7,8-tetrachlorodibenzo-p-dioxin. In addition, it has been reported that porphyrin derivatives are able to inhibit polyamine catabolism. Thus, we evaluated the alterations in putrescine (Put), spermidine (Spd) and spermine (Spm) hepatic levels in rats treated with the porphyrinogenic agents hexachlorobenzene (HCB) and 3,5 diethoxycarbonyl-1,4-dihydrocollidine (DDC). Polyamine levels were determined by HPLC after pre-column derivatization with dansyl chloride. Female Wistar rats treated with HCB (1.4 g/kg 24 and 4 hs before sacrifice) showed normal hepatic polyamine levels: Put (control=20,2 ± 1,2; HCB=23,8 ± 2,9 nmol/g), Spd (control=443,8 ± 13,4; HCB=421,0 ± 26,3 nmol/g) and Spm (control=524,2 ± 43,8; HCB=457,0 ± 32,9 nmol/g). Rats treated with different doses of HCB (0,12; 0,25; 0,5 or 1g/kg/day) during 1, 2 or 3 weeks also showed normal hepatic polyamine levels. On the other hand, rats treated with DDC (0.3 g/kg, 5 hs before sacrifice) showed increases of 179% and 23% in Put and Spd levels respectively. Spm levels were not modified. The results obtained suggest that DDC may induce ornithine decarboxylase and elicit promotor-like responses.

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Characterization of the inhibitory effect of notochord on the neural crest induction in the *Xenopus leavis* embryos. Cofré, C. y Mayor, R. Laboratorio de Biología del Desarrollo, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Our laboratory has been involved in the study of molecular mechanisms present in the induction of neural folds at the borders of the neural plate in *Xenopus leavis* embryos. Several hypothesis have been proposed to explain how the neural crest are determined, but the experimental analysis has been difficult because of the absence of any specific early marker for the neural crest. In order to analyze this problem we have used the expression of the zinc finger gene Xslug as a molecular marker for the prospective neural crest.

Using whole mount in situ hybridization for the Xslug gene, we have analyzed the role of the notochord on the induction of the neural crest. By grafting notochords in the prospective neural fold region we have been able to identify and characterize an inhibitory activity on neural crest induction. When notochord was taken from an early neurula embryo and grafted into the prospective neural folds of an early gastrula embryo, we found an inhibition of the Xslug expression in the side that contained the graft.

Currently we are studing if this effect is due to a direct inhibition, or wheter this could be consequense of another tissue induced by the notochord. We are analyzing the expression of others neural markers in the grafted region.

Our final aim is characterize all the inductive activities of the notochord and propose a model that explain how the neural crest are induced at the border of the neural plate.

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BODIPY-VSV AS A PROBE TO STUDY VIRAL INFECTION. Da Poian, A.T., Gomes, A.M.O., and Coelho-Sampaio, T. Department of Biochemistry, Federal University of Rio de Janeiro, RJ 21941-590, Brazil

Although the replication of vesicular stomatitis virus (VSV) is a well known process, information on early stages of infection are still lacking. We have used a fluorescent conjugate of VSV with FL-Bodipy to follow in real time the kinetics of VSV disassembly inside intact cells. The fluorescence of Bodipy-VSV is insensitive to pH, allowing monitoring of fluorescence emission through the endocytic pathway. In addition, the highly subtituted Bodipy conjugate (10:1, mol of probe/mol of G protein) is highly self-quenched, allowing observation of large fluorescence increases upon G protein dissociation or proteolytic degradation. *In vitro* experiments showed that dissociation of G protein promoted a 4-fold fluorescence increase. Addition of various proteases promoted an additional 4-fold fluorescence increase. Dissociation was confirmed by fluorescence polarization of 2,5-DNS-labeled VSV and light-scattering measurements. In *in vivo* studies, Bodipy-VSV was incubated with macrophages for 10 min, washed and fluorescence observed by flow cytometry. In 2 hours a 12-fold fluorescence increase was observed in control cells while only a 3-fold increase occured in the simultaneous presence of ammonium chloride and a combination of protease inhibitors, when both proteolysis and pH acidification are inhibited. When the kinetics was followed only in the presence of protease inhibitors fluorescence increased 7-fold. Thus, our approach discriminates between dissociation and protein degradation and may be a powerful tool in studies of viral infection.

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THE MECHANISMS INVOLVED IN THE EXPRESSION CONTROL OF *BhB10*, A GENE AMPLIFIED DURING *Bradysia hygida* DEVELOPMENT, THAT CAN BE DISRUPTED BY CYCLOHEXIMIDE TREATMENT. Conacci, M.E., Valente, V. and Almeida, J.C. de Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brasil.

The *Bradysia hygida* salivary glands present three morphologically distinct regions: anterior (S1), granulosa (S2) and posterior (S3). Eight chromosomal sites form the more prominent DNA puffs, which expand in two different groups, during a 24 hours period, only in the S1 and S3 regions. *BhB10* is an amplified gene present in DNA puff B10. A *BhB10* DNA genomic fragment, isolated from a DNA puff B10 minilibrary, detects in Northern blots, prepared with total RNA from salivary glands, two RNA species: a 1.3 kb at age E7 and a 1.1 kb at the age E7+16 hours. At E7+8 hours a large spot, that probably includes both species is detected (Fontes et al., Brazilian J. Med. Biol. Res. 25:777, 1992). Our work hypothesis is that the 1.1 kb RNA is an inactive form produced by the shortening of one of the ends of the 1.3 kb mRNA, probably the poly A tail. Here we show, in a series of experiments, that the large spot detected in E7+8 hours corresponds to a molecular size gradient between the two RNA species. By inhibiting the transcription with actinomycin-D, at a critical age, we show by that a gradual shortening the 1.3 kb RNA originates the 1.1 kb species. It is also shown, using oligo-dT cellulose chromatography, that during the shortening process the RNA molecules still keep a small poly A tail during some time. The total loss of the tail probably leads to the complete RNA degradation. In previous communications, using total RNA from whole salivary glands, we have shown that a treatment with very low concentrations of cycloheximide (Chx), at the age E7, leads to a drastic change in the *BhB10* expression: the big spot containing both RNAs is maintained until E7+20 hours. Here we show that this effect is neither due to a delay nor to a prolongation of the transcriptional activity of the gene. However, in the case of the S2 region, were the DNA puff B10 does not form, the *BhB10* gene seems to be under a different control, presenting a shorter period of expression. Although the control mechanisms seem to be different for the S2 region, Chx has the same effect on the 1.3 kb RNA in this region. Therefore, we assume that the Chx treatment inhibits, to some extent, the synthesis of factor(s) involved in the shortening and inactivation of the 1.3 kb mRNA. In this way we

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HUMAN ERYTHROCYTES EXPRESS GLUT5 AND TRANSPORT FRUCTOSE. Concha, I.I., Velázquez, F.V.¶, Martínez, J.M., Angulo, C., Droppelmann, A., Golde, D.W.¶ and Vera, J.C.¶ Instituto de Bioquímica. Universidad Austral de Chile, Valdivia, Chile and ¶Memorial Sloan-Kettering Cancer Center, New York, USA.

Although erythrocytes readily metabolize fructose, it has not been known how this sugar gains entry to the red blood cell. We present evidence indicating that human erythrocytes express the fructose transporter GLUT5, which is the major means for transporting fructose into the cell. Immunoblotting and immunolocalization experiments identified the presence of GLUT1 and GLUT5 as the main facilitative hexose transporters expressed in human erythrocytes, with GLUT2 present in lower amounts. Functional studies allowed the identification of two transporters with different kinetic properties involved in the transport of fructose in human erythrocytes. The predominant transporter (GLUT5) showed an apparent Km for fructose of approximately 10 mM. Transport of low concentrations of fructose was not affected by 2-deoxy-D-glucose, a glucose analog that is transported by GLUT1 and GLUT2. Similarly, cytochalasin B, a potent inhibitor of the functional activity of GLUT1 and GLUT2, did not affect the transport of fructose in human erythrocytes. The functional properties of the fructose transporter present in human erythrocytes are consistent with a central role for GLUT5 as the physiological transporter of fructose in these cells.

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APOPTOSIS IS ACCOMPANIED BY EXPRESSION OF c-JUN IN THE DEVELOPING RETINA. Chiarini, L.B.; Freitas, F.G. and Linden, R. Instituto de Biofisica da UFRJ, Rio de Janeiro, Brasil.

During neural development over 50% of the neurons die by apoptosis, a form of programmed cell death characterized by chromatin condensation and internucleosomal DNA fragmentation. In the retina of newborn rats, apoptosis of ganglion cells (RGCs) depends on protein synthesis, while apoptosis of cells in the neuroblastic layer (NBL) is induced by inhibition of protein synthesis. The transcription factor c-Jun was described to modulate apoptosis in neurons. We investigated the association of c-Jun expression with apoptosis in the retina. Explants of newborn rat retinae were maintained *in vitro* for either 6 or 24 hours following various conditions of induction of apoptosis, and processed for immunocytochemistry using an antibody for c-Jun. Apoptotic cells were recognized by in situ nick end labeling (ISNEL) of fragmented DNA. In RGCs c-Jun was detected 6 hours after axotomy, preceding the appearance of ISNEL-positive cells, detected at 12 hours after axotomy. Apoptosis in the NBL was induced by anisomycin, cycloheximide, γ-irradiation, okadaic acid, thapsigargin, heat shock or 2-aminopurine. In all conditions we found strong immunoreactivity of c-Jun correlated with the induction of apoptosis in the explants. c-Jun immunoreactivity was also correlated with both naturally-occurring and lesion-induced apoptosis in the developing retina *in vivo*. These results are consistent with a role for c-Jun as a universal positive modulator of apoptosis of retinal cells.

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INTRACELLULAR IMMUNIZATION OF BHK-21 CELLS AGAINST THE FOOT-AND-MOUTH DISEASE VIRUS. de Almeida, C.J.G., Rodarte, R.S. and Tanuri, A., Instituto de Biofísica da UFRJ and Departamento de Genética da UFRJ.

Susceptible cells can become resistant to a virus by intracellular expression of viral proteins. This strategy is called intracellular immunization, and it was tested in this work as an approach against infection by the foot-and-mouth disease virus (FMDV). Two distinct expression vectors carrying the polimerase gene of FMDV in either native or mutant forms, were used to transfect BHK-21 cells, a strain of mammalian cells naturally susceptible to FMDV. Previously made site-directed mutations abolished the catalytic activity of the enzyme in *in vitro* assays. Transfected cells were selected with G418 and efficiency of transfection was about 42%. The presence of the cloned genes as well as the production of transcripts were confirmed with PCR techniques. No protein could be detected in either western blots or by immunoprecipitation techniques. Inhibition of infection was evaluated through titration with a virus from a serotype group different from that used for polymerase gene cloning. Resistance values reached about 98% in cells carrying either the mutant or the native genes from one of the chosen vectors. The results indicate that this approach can be useful as an alternative method for the prevention of foot-and-mouth disease.

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CALTRIN I PROTEIN INHIBITS ACROSIN ACTIVITY AND INDUCES SPERM-OOCYTE RECOGNITION. M. Andrea Dematteis, Ruben H. Ponce, Maria L. Novella, Daniel E. Winnica and Carlos E. Coronel. Cátedra de Química Biológica, Facultades de Ciencias Médicas y Odontología, Universidad Nacional de Córdoba, Argentina. E-mail: ccoronel@biomed.uncor.edu

Caltrin (calcium transport inhibitor), the small basic protein from seminal vesicle secretion (SVS), has two different molecular forms in the guinea pig and rat, designated caltrin I and II. Caltrin I binds to the sperm head, on the acrosome region, and inhibits Ca^{2+}-uptake and the exocytotic acrosome reaction. Caltrin II interacts specifically with the sperm plasma membrane on the tail and retards the Ca^{2+}-dependent hyperactivated motility (Mol.Reprod.Dev. 33: 74, 1992). Studies from our laboratory demonstrated that caltrin I inhibits trypsin activity and presents structural homology with a trypsin inhibitor protein (TIP) from SVS of the mouse and boar, and with other Kazal-type inhibitor proteins (Biol. Reprod. 52 (Supp. 1): 60, 1995). It has previously been reported that trypsin inhibitors block the fertilization in vitro. In this communication we present evidence indicating that rat caltrin I participates in the fertilization process inducing sperm-oocyte recognition and binding of the spermatozoa to the zona pellucida that surrounds the oocytes. Sperm-egg recognition is associate with acrosin inhibition by caltrin, while the extent of sperm-zona binding seems to be dependent on caltrin concentration. At high caltrin concentration (0.4 mg/10^6cells/ml) the spermatozoa were tightly bound and retained in the zona pellucida. On account of the inhibitor effect of caltrin I on Ca-uptake as well as on acrosin activity, we propose that it could operate as a regulator of the progesterone-sensitive calcium transport system of the spermatozoa.

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EXPRESSION OF HEXOSE TRANSPORTERS IN HUMAN SEMINOMA. Droppelmann,A., Norambuena, L.‡, Rivas, C.¶, Vera, J.C.¶ and Concha, I.I. Institutos de Bioquímica y de ‡Histología y Patología. Universidad Austral de Chile, Valdivia, Chile and ¶Memorial Sloan-Kettering Cancer Center, New York, USA.

One characteristic feature of human seminomas is that they contain cytoplasmic stores of glycogen. No information is available, however, on the expression of hexose transporters in these human tumors. We present here an analysis of the expression of facilitative hexose transporters in human seminomas. By using a panel of anti glucose transporter antibodies, we detected low to moderate expression of the transporters GLUT1, GLUT2, GLUT3 and GLUT5 in samples of stage I seminoma. GLUT1, GLUT2 and GLUT5 were present in all the samples analyzed, whereas GLUT3 expression was variable and was absent from some samples. GLUT4 was absent from the majority of the samples of stage I seminoma. Progression of seminomes to stage II was acompanied by a notable increase in the expression of the transporters GLUT1, GLUT2, GLUT3 and GLUT5, whereas no GLUT4 expression was observed. In situ hybridization studies confirmed the expression of mRNA for the transporters GLUT1, GLUT2, GLUT3 and GLUT5 in stage II seminomas, and the mRNA levels were well correlated to their respective protein levels. We also detected expression of the mRNA for GLUT4 in samples not expressing the respective protein. The data are compatible with the regulated expression of hexose transporters in human seminomas as the tumor progresses and suggest that GLUT1, GLUT2 and GLUT3 may be responsible for the uptake of glucose in these cells. The expression of GLUT2 and GLUT5 suggest that human seminomas may have a specialized need to metabolize fructose.

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ANTIOXIDANT ENZYMES IN EPIDERMAL TUMOR CELL LINES. Durán, H.A., Policastro, L., Fernández, M.L., Molinari de Rey, B. Comisión Nacional de Energía Atómica, Buenos Aires, Argentina.

There is evidence that the generation of reactive oxygen species is a critical event in the promotion of neoplastic transformation in mouse cells. The aim of this work was to correlate the degree of malignancy of tumor cells with the prooxidant state by evaluating the levels of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). Several epidermal tumor cell lines were used: PB, CH72-T4, AT5, PDV, PDVC57, whose tumoral phenotypes in terms of mutant *ras* expression, tumorigenicity, expression of simple epithelial cytokeratins, etc., have been previously characterized. Normal keratinocytes from SENCAR mouse were used as control. Both enzymes were assessed spectrophotometrically by measuring the disappearance of H_2O_2 at 240 nm for CAT and using the inhibition of NBT reduction technique for SOD. Our results showed that different epidermal cell lines and normal keratinocytes differed in their catalase activity, revealing decreasing values as a function of malignancy, ranging from 29 % (PB cell line) to 4 % (PDVC57 cell line) of the normal keratinocyte activity. SOD measurements revealed significant differences in this enzyme activity among these cell lines, which seem not to correlate clearly with malignancy.

We conclude that, in several transformed epidermal cell lines of diverse origins, a gradual decrease in catalase activity correlates with malignancy, irrespective of the origin of the cell line. Thus, a decrease in catalase levels during malignant transformation could favor the prooxidant state related with the induction of the tumor cell phenotype.

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ANGIOTENSIN CONVERTING ENZYME (ACE) EXPRESSION IN ADULT RAT CARDIOMYOCYTES IN CULTURE. Ebensperger R, Drexler H. Depto Bioquímica y Biología Molecular, Fac. Cs. Químicas y Farmacéuticas, U. Chile. Santiago,Chile y Universität Freiburg, Alemania .

Pressure-overload induced cardiac hypertrophy involves the participation of several trophic factors. An intracardiac Renin-Angiotensin System (RAS) plays an important role in the induction of this hypertrophic response. However, how the expression of the different RAS components are regulated in the heart remains unknown.

In this work we studied the ACE expression in adult ventricular cardiomyocytes (AVC) stimulated with Angiotensin II (AII). AVC were obtained from normotensive rat hearts by retrograde perfusion using collagenase. Isolated AVC were incubated in M-199 culture medium at 37°C and 5%CO2. ACE expression was determined by a quantitative RNA-PCR, using increasing amounts of a mutated competitor RNA, both in AVC incubated without (control) or with AII (1 μM) for 2 and 24h.

The results showed that ACE was expressed in adult ventricular cardiomyocytes in control conditions. However ACE expression did not show significant changes when AII (1μM) was present in culture medium during the study.

We concluded that adult cardiomyocytes, isolated from normotensive rats, express ACE and that AII, under our experimental conditions, does not regulate ACE expression.

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GAP JUNCTIONS FAVOR THE VASOPRESSIN-INDUCED GLYCOGENOLYSIS IN RAT HEPATOCYTES. Eugenín E., Sáez C.G. and Sáez J.C.. Depto. de Cs. Fisiol., Fac. de Cs. Biol. and Depto. de Hematol. y Oncol., Fac. de Med., Pontificia Univ. Católica de Chile, Stgo., Chile.

In rat liver acini, the neoglucogenic and glycogenolytic activities are high in periportal hepatocytes while receptors of hormones that activate these pathways are preferentially found in pericentral hepatocytes. Since cells of the hepatic acini are communicated by gap junctions (GJs), Ca^{2+} waves generated in cells bearing hormone receptors, such as vasopressin, propagate to cells metabolically competent in glucose generation. It remains unknown whether GJ-mediated cell signalling is involved in metabolic liver responses. Freshly dissociated adult rat hepatocytes plated on glass coverslips or tissue culture plates were used. Total glycogen content was measured at 490 nm after precipitation and reaction with phenol and H_2SO_4. Cell coupling mediated by GJs was tested by the spread of Lucifer yellow injected into one cell of cell pairs or cluster. Octanol (500 μM), a GJ blocker, inhibited (85%) the glycogenolytic response detected 3 h after a brief (15 min) vasopressin stimulation. Moreover, a synthetic peptide homologous to the extracellular loop 1 of Cx32, the main liver GJ protein, prevented formation of functional GJs during cell reaggregation and also inhibited the vasopressin-induced glycogenolysis (70%). Thus, cell signaling transferred via GJs is required to induce glycogenolysis in rat hepatocytes. FONDECYT 1960559 (J.C.S.).

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***IN VIVO* AUTOREGULATION OF MACROPHAGES. ANALYSIS OF SUPEROXIDE ANION PRODUCTION.** Fernández, M. L., Durán, H. A., Molinari de Rey, B. Comisión Nacional de Energía Atómica, Buenos Aires, Argentina.

We have reported that during the respiratory burst of peritoneal macrophages the superoxide anion production per cell shows an inverse relation with the cell density of the elicited macrophages. This phenomenon is known as autoregulation.

Phorbol esters (PMA) were used to induce the respiratory burst. Superoxide anion production was measured by evaluating the formazan intracellular deposits generated by NBT reduction.

Data on the superoxide production of the whole macrophage population was obtained spectrophotometrically at 572 nm.

In the present study the response of individual cells from various peritoneal populations of different cell densities was analyzed. The amount of formazan per cell and the correlation with cell-size changes which occur during the PMA-activation were evaluated employing an automatic image analyzer (Ibas-Kontron/LANAIS-MEF). This analysis revealed that the decrease in individual cell response at higher cell densities was due to a significant increase in the amount of non-reactive cells. Concomitantly, the compartment of reactive cells remained unchanged irrespective of the cell density of the population. A direct correlation between cell size and superoxide anion production was also demonstrated.

We conclude that variations in the non-responding cells compartment modulates the macrophage activation responses when excess cells are recruited to the peritoneum. Autoregulation of the macrophage population relies on the amount of non-reactive cells rather than on the differences in the reaction per cell.

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RESPECTIVE ROLES OF NITRIC OXIDE ($NO^{\cdot}$) AND SUPEROXIDE RADICAL ($O_2^{\cdot-}$) IN THE RESPIRATORY BURST ACTIVITY (RBA) OF RAT POLYMORPHONUCLEAR LEUKOCYTES (PMN) INDUCED BY HYPERTHYROIDISM. Fernández, V. Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Studies using zymosan-induced luminol-amplified integrated chemiluminescence (ICL) of isolated rat PMN have shown that the administration of L-3,3',5-triiodothyronine (T_3) induces an enhanced RBA, with increases in the NADPH oxidase ($O_2^{\cdot-}$ generator) and myeloperoxidase activities. Since activated PMN generate $NO^{\cdot}$ and peroxynitrite after reaction of $NO^{\cdot}$ with $O_2^{\cdot-}$, this work assesses the effect of T_3 administration on the generation of $NO^{\cdot}$, in relation to the production of $O_2^{\cdot-}$ and light emission, upon stimulation of these phagocytes. Isolated PMN from rats receiving 0.1 mg T_3/kg for 3 days, revealed a marked increase both in the ICL in the absence (2-fold) and presence (2.28-fold) of L-Arg, and in the rate of $O_2^{\cdot-}$ production (180%) over control values. L-Arg addition to PMN from control and T_3-treated rats increased the ICL (57 and 17% over values obtained in its absence, respectively), effect that was abolished by N^{ω}-nitro-L-Arg. However, the net L-Arg-dependent ICL was comparable in PMN from both experimental groups, and the respective rates of $NO^{\cdot}$ production were not significantly different, either in the absence or presence of nitro-L-Arg methyl ester. It is concluded that T_3-induced RBA of rat PMN is not dependent on changes in $NO^{\cdot}$ synthase activity, but rather on the adaptive increase in $O_2^{\cdot-}$ generation by NADPH oxidase. (Supported by FONDECYT 1940312).

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INTERCELLULAR GAP JUNCTIONAL COMMUNICATION BETWEEN ENDOTHELIAL CELLS OF HUMAN TONSIL IS REDUCED BY HISTAMINE. Figueroa X., Garcés G, Bono MR, Rosemblatt M and Sáez JC. Depto. de Cs. Fisiol., Fac. de Cs. Biol., Pontificia Univ. Católica de Chile and Depto. de Biol., Fac. de Cs., Univ. de Chile, Santiago, Chile.

Most vascular endothelial cells form a highly ordered monolayer. They play multiple roles in maintaining the hemostasis and regulating inflammatory processes. During diverse inflammatory responses, cell signals, such as histamine (HIS), are released and induce morphological and functional changes of the endothelium. Cell signals may also be exchanged via gap junctions (GJs) which allow cell-cell transfer of ions and small molecules. In this work, the expression of GJs and their regulation by HIS was studied in human tonsil endothelial cells (TEC). Primary cultures of TEC were used. Cell coupling was tested by the transfer to neighboring cells of Lucifer yellow (LY) injected into one cell. Levels and cellular distribution of GJ subunits, connexins (Cxs), were analyzed by immunoblot and immunofluorescent, respectively. TEC were well coupled and HIS (1μM) inhibited the cell transfer of LY within 1 h. The HIS effect was prevented with 10 μM loratidine, a H1 receptor blocker or by down regulation of protein kinase C (PKC). Cell coupling was not affected by PMA, a tumor promoter phorbol ester. Thus, PKC activation is required but not sufficient for the HIS-induced GJ inhibition. 8Br-cAMP (1 mM) increased coupling and reversed the HIS effect. HIS did not affect the levels of Cx43 but reduced the amount of Cx43 found at cell interphases. Inhibition of coupling between TEC may prevent propagation of signals from the inflammed area to normal regions of the endothelium. FONDECYT 1950686 (to M.R.), 1960876 (to M.R.B.) and 1960559 (to J.C.S.)

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POSTTRANSLATIONAL ARGINYLATION OF CYTOSOLIC PROTEINS FROM APOPTOTIC PC12 CELLS. S. Fissolo. G. Bongiovanni and M. Hallak. Dpto. Química Biológica. CIQUIBIC Fac. Ciencias Químicas Univ. Nac. de Córdoba. Córdoba, Argentina. mhallak@dqbfcq.uncor.edu

The posttranslational modification of proteins by covalent conjugation of arginine to glutamic or aspartic acid in the N-terminus position of a target protein is a tRNA dependent, ribosomal independent reaction. The addition of arginine to the N-terminus of specific test proteins is required for these proteins to be degraded by the ubiquitin pathway. This pathway represents the principal cytosolic mechanism in eucaryotic cells for the degradation.

The control of degradative mechanisms is probably a key regulatory step in apoptosis. It is reasonable to suspect that ubiquitin-dependent degradation is involved in programmed cell death since dramatic increases in the polyubiquitination of proteins has been observed in specific examples of programmed cell death.

In the present work, we studied the posttranslational arginylation of cytosolic proteins from apoptotic PC12 cells. Apoptosis was induced by serum deprivation for 12 hr. Briefly, PC12 cells cultured for 6 days were washed twice with medium and then incubated in medium with no added serum for 12 hr. Control cells were incubated in medium with serum. Cells were harvested and the proteins in the cytosolic fraction were arginylated in vitro with [^{14}C]-arginine. We found that the arginylation is 40% higher in apoptotic cells respect to control cells. Preliminary results show that other posttranslational modifications as tyrosination of tubulin are similar in apoptotic cells and in control cells. On the other hand, in 24 hr serum deprived cells, arginylation remains at similar levels after 12 hr of deprivation whereas in control cells almost no arginylation is observed. The possible consequence of this increase in protein modification activity in apoptotic cells will be discussed.

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DEPOLARIZATION INCREASES PROLIFERATION OF IMMATURE CEREBELLAR GRANULE CELLS. L. N. Borodinsky*, J.H. Neale# and M. L. Fiszman*. Centro de Investigaciones Médicas Albert Einstein*, Buenos Aires, Argentina and Biology Dept., Georgetown University#, Washington D.C., U.S.A.

The role of depolarization on *in vitro* neuronal survival and differentiation in the developing rat brain has extensively been studied. There are few evidences that suggest that neural activity can influence the proliferation of neuronal progenitors in vertebrates. The proliferation of these cells takes place during the first 36 hours after cells plating, after which they stop dividing and become differentiated phenotypes. We studied the effect of depolarizing concentrations of potassium on the proliferation of rat cerebellar granule cells (CGC). Cerebellum were excised from 6-8 day old animals and cell suspensions were plated for 24-48 hours in a chemically defined medium, in the abscence of serum, in resting (5mMKCl-5K) or depolarizing (25K) conditions at a density of 300,000 cells/multiwell (96 microplates). During the first 24 hours after plating, cells grown in 5K showed a high degree of ^{3}H-thymidine incorporation. During this period cells supplemented with 25K incorporate 34±4% (n:10) more ^{3}H-thymidine than those plated in 5K. The increase in cell proliferation was completely blocked by 10μM nifedipine and not by 1μM ω-conotoxin. There was a complete blockade by the addition of 10mM $MgCl_2$ which antagonizes the entry of extracellular Ca^{+2}. To elucidate whether the increase in cell proliferation was due to a trophic substance released into the extracellular space, some cells were plated in a conditioned medium obtained from cells grown in 25K for 24 hours. Under this condition, cell proliferation was of the same magnitude to that obtained in fresh 25K medium, and it was also blocked by nifedipine and $MgCl_2$. We conclude that the proliferation of CGC progenitors is increased by a rise in intracellular Ca^{+2} due to the activation of voltage gated Ca^{+2} channels of the L but not the N-subtype. This effect seems to be directly triggered by this mechanism and it is not due to the release of any endogenous substance.

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CD31 EXPRESSION IN ENDOTHELIAL CELLS DURING MURINE MAMMARY TUMOR DEVELOPMENT. Gazzaniga, S.[1], Vanzulli, S.[2], Vecchi, A.[3], Mantovani, A.[3], Wainstok, R.[1]. [1]Dpto. Q. Biológica, FCEN, Universidad de Buenos Aires.[2]Centro de Estudios Oncológicos (FM) Acad. Nac. de Medicina, Bs. As., Argentina. [3]Istituto Mario Negri, Milan, Italy.

Several studies have shown that tumor growth, spread and metastasis are dependent on tumor-associated angiogenesis. MEC 13.3 mAb reacts with CD31 antigen and specifically detects mice endothelial cells (EC). This prove to be a useful tool to quantify tumor neo-vascularization and to isolate mice EC. Frozen sections (6-8 μm thick) of a mice mammary tumor line were immunohistochemically stained using MEC 13.3 and the peroxidase-antiperoxidase technique. Stained microvessels within the tumor tissue were counted at 400X field at the most intense areas of tumor neovascularization (the «hot spots»). The earlier stages of tumor development (7 days post-transplantation) showed many isolated intratumoral cells or cell clusters and few vessels. The cell and microvessel counts were 8.5±3.1 and 4.5±1 respectively. In the latter stages (50 days post transplantation) MEC 13.3 showed predominance of developed microvessels, as it was suggested by their count: 15±2.71, whereas the number of single detected cells decreased: 0.5±0.71. The knowledge of MEC 13.3 reactivity pattern in the development of this tumor indicates that the latter stages are more convenient to truthfully isolate vascular EC, since the single MEC13.3-positive cells could be tumor ones that switch to the angiogenic phenotype as it was previously reported.

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ESTIMATION OF UBIQUITIN mRNA CONTENT IN MOUSE LIVER DURING PROTEIN DEPLETION AND REFEEDING.
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Mouse liver protein content decreases during protein depletion whereas a fast restoration of the lost protein can be induced by refeeding with a complete diet. We have previously observed that these changes are correlated with high and low rates of protein breakdown, respectively. We have also found in depleted and refed livers significant amounts of ubiquitin as tested by western blot or radioimmunoassay analysis. However, little is known about ubiquitin mRNA transcription. In the present report we used RNA blot hybridization with ^{32}P-labeled ubiquitin cDNA to estimate both, the amount and size of ubiquitin mRNA transcripts in mouse subjected to the following nutritional conditions: - control: fed *ad libitum* with a complete diet; - depleted: fed *ad libitum* during 5 days with a protein free diet; - refed: fed *ad libitum* during 5 days with a protein free diet and refed for 16 hours with a complete diet

Two ubiquitin specific probes from hamster: CHUB 1 (2.3 Kb) and CHUB 2 (5.0 Kb) were used.

For all the nutritional conditions studied, three ubiquitin transcripts with sizes of 2.7, 2.1 and 1.5 Kb were observed. Their content decreased with depletion. However, while both the 2.7 and 1.5 Kb transcripts content did not return to normal values, the 2.1 Kb transcript content was completely restored after refeeding. These results suggest that the ubiquitin system does not play a main role in the intracellular proteolysis elicited by dietary protein depletion.

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CHANGES IN INTERCELLULAR GAP JUNCTIONAL COMMUNICATION IN RAT LIVER DURING ENDOTOXIN-INDUCED INFLAMMATION.
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In the liver, gap junctions (GJs) allow for metabolic cooperativity. In various pathological conditions, including the endotoxin-induced multiple organ failure, a reduced intercellular gap junctional communication may contribute to hepatic disfunction. This work was undertaken to analyze Cx32 and Cx43, two gap junctional proteins in rat liver during endotoxin-induced inflammation. Male adult Sprague-Dawley rats were injected (i.v.) with E. coli lipopolysaccharide (LPS; 2 mg/kg) and sacrificed at different periods of time. Sterile saline (1 ml/kg) was injected to sham rats. In liver samples, Cxs were analyzed by immunocytochemistry and Western blot analysis. In LPS-treated rats liver damage and inflammation was evident after 8 h, characterized by hepatocyte swelling and necrosis, and polymorphonuclear (PMN) infiltration. A significant decrease in Cx32, the main liver GJ protein, was detected after 6 h of endotoxemia and persisted up to 30 h. In contrast, a progressive increase in levels of the unphosphorylated (NP) and phosphorylated (P2) of Cx43 was found. The latter was paralleled with an increase in the number of monocytes and macrophages detected with the ED1 monoclonal antibody. Although macrophage infiltration persisted after 30 h of LPS-treatment, Cx43 decreased after 24 h. During LPS-induced inflammation reduced levels of Cx32 might contribute to liver disfunction. The transient expression of Cx43 by inflammatory cells suggest that it may be necessary for leukocyte migration or inter-leukocyte communication. PG-12/96 CIM (H.G.) & FONDECYT 1960559 (J.C.S.).

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REGULATION OF THE MUSCARINIC DEPENDENT CONTRACTION IN THE AIRWAY SMOOTH MUSCLE.
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We investigate the temporary relationship between muscarinic agonists and the levels of the second messengers Adenosine 3'5'cyclic monophosphate (cAMP) and Guanosine 3'5'cyclic monophosphate (cGMP) on the airway smooth muscle contraction. Levels of cAMP was measured by radioanalysis and cGMP was measured by radioinmunoassay (Amersham). The M3 muscarinic receptor activation, induced by carbachol leads to significant increments in the levels of cAMP, cGMP. However, the kinetic of curve response was different, the cGMP showed a bifasic curve; a first peak at 20 sec coincides with the only peak of cAMP and a second peak at 60 sec. when the smooth muscle contraction reached a plateu. This later peak seems to be sensitive to NO-scavengers as methylene blue, suggesting the soluble form of the Guanylylciclase (GC) is the responsible for the second peak. Additional experiments using 1-H-[1,2,4] Oxadiazolo [4,3-a] quinoxalin-1one (ODQ) a selective inhibitor of NO-sensitive soluble GC showed a more complicated mechanisms, suggesting that the activation mechanism of soluble GC for muscarinic agents are unrelated to nitric oxide. This proyect was support by No. CDCH-UCV 09-33-3683/96 (L. Guerra).

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INHIBITION OF POLY(ADP-RIBOSYLATION) DELAYS SEA URCHIN EARLY DEVELOPMENT BLOCKING LARVAL MORPHOGENESIS.
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The requirement of poly(ADP-ribosylation) in early stages of sea urchin development was analyzed by comparing normal embryos and those cultured in the presence of 20 mM 3 aminobenzamide (3ABA), an inhibitor of the poly(ADP-ribose) synthetase. The incorporation of 3H-thymidine into DNA was determined and chromatin organization was investigated by analyzing the nucleoprotein particles generated by micrococcal nuclease digestion. It was observed that cleavage divisions were delayed until 180 min post insemination (p.i.) in the 3ABA-treated embryos, while the normal embryos had reached the 8 blastomeres stage. Then, the 3 ABA-treated embryos started to divide more slowly than normal embryos. Changes in chromatin structure were not observed. These results indicate that embryonic DNA replication is dependent on proper poly(ADP-ribosylation), whereas the transition G2-M is not. When the embryos were treated with 3ABA from 3 min p.i., their development was blocked at hatching. When this compound was added to the cultures at morula stage, hatching occurred normally, but the embryos were unable to perform the gastrula-prism transition. These results indicate that poly(ADP-ribosylation) is a requirement for the onset of the first S phase as well as for correct S phase during cleavage divisions and also fundamental for the larval differentiation of sea urchin embryos.

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***IN VITRO* ACTIONS OF THIRTEEN COUMARINS ON THE GROWTH OF THE 5ab1 MURINE MELANOMA CELL LINE.** Jiménez, F.A., Molina, J.A., and Mandoki, J. J. Depto. Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México. México,D.F., C.P. 04510. Apdo. Postal 70-297. email:jamg@servidor.unam.mx

Coumarins inhibit production of metastases and increase survival times of patients with several types of cancer, including melanomas. It has not been defined whether the beneficial effects result from cytostatic or cytotoxic actions on tumour cells, or from indirect actions, *e.g.*, stimulation of the immune system. Coumarin, is hydroxylated in mammals in all positions, and undergoes further transformations. We have obtained and tested the 3-, 4-, 6- , and 7- monohydroxylated coumarins, their acetates and methyl ethers. $1x10^4$ of melanoma cells/well, were exposed for 24h, 48h, and 72h, to concentrations of coumarins ranging from 10μg/ml to 160μg/ml. At the end of each period, the number of cells was estimated by the MTT reduction method.. The % inhibitory effects on growth with respect to its solvent-treated control was calculated of each compound for each concentration and exposure time. Coumarin and all the monosubstited coumarins had growth inhibitory effects. They varied however with respect to minimal inhibitory concentration, duration of inhibtory action, and magnitude of effect. Most had significant inhibitory effects only at higher concentrations (80μg/ml or 160μg/ml).

Grants: D.G.A.P.A.- U.N.A.M. IN210294 and IN211396.

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FORMATION OF SOMATIC HYBRID CELLS DURING SPONTANEOUS FUSION BETWEEN HUMAN LIMPHOCYTES AND CHINESE HAMSTER FIBROBLASTS

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We generated somatic hybrid cells by a new method taking advantage of the ability of lymphocytes to rapidly penetrate into target cells. In the revelant experiments human lymphocytes were cocultivated with Chinese hamster fibroblasts without the aid of fusogenic agents. Human lymphocytes were isolated from peripheral blood. We present the results of EM analysis of the consecutive steps during the formation of hibrids. Based on the EM data obtained the two following ways of formation of hybrids may be envisaged: the hybrid was formed as a result of the rapid passage of the lymphocyte into the nucleus of the fibroblast; the chromatin of the lymphocytes nucleus, residing in the fibroblast cytoplasm after 15-20 h of cocultivation, would fall apart into chromatin blocks. Each block seems to be outlined by a double membrane. In this case the hybrid would be formed at the time point of mitosis, when separate membrane-outlined fragments may be integrated into the assembling hybrid nucleus. The data obtained demonstrate the facility of high frequency (10^{-4} - 10^{-5}) production of somatic cell hybrids with human lymphocytes and hamster fibroblast without the aid of fusogenic agents. We investigated the karyotypes of the five hybrids clones with anomalies of the structure of metaphase chromosomos. This anomalies correlate to arrangements of the structure of the mitotic spindles of hybrid cells.

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Abstract withdrawn

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THE QUALITY CONTROL OF GLYCOPROTEIN FOLDING IN TRYPANOSOMATIDS. Labriola, C., Cazzulo, J. J. and Parodi, A. Fundación Campomar, Buenos Aires, Argentina.

The main components of the quality control of glycoprotein folding in the endoplasmic reticulum (ER) are chaperones (calnexin and/or calreticulin) that have a lectin-like activity that recognizes monoglucosylated oligosaccharides, the UDP-Glc:glyco-protein glucosyltransferase (GT) that is a sensor of the glycoprotein conformation and only glucosylates misfolded glycoproteins and glucosidase II (GII) that removes the tag (a glucose unit) introduced by GT. Trypanosomatids, at variance with all other eukaryotic cells, have a single pathway leading to the formation of monoglucosylated compounds, that catalyzed by GT. We have checked that *Trypanosoma cruzi* has an operative mechanism of quality control of glycoprotein folding as: a) the activities of both GT and GII were detected in cell-free assays; b) a protein having a high homology with human calreticulin was found to be encoded in the parasite's genome and c) in accordance with the proposed control model, exit of a glycoprotein (cruzipain, a lysosomal proteinase) from the ER was delayed upon inhibition of GII. Supported by TDR (WHO), NIH (USA), SAREC (Sweden) and UBA (Argentina).

Membranes, Lipids, Receptors - Hormones and Growth Factors, Signal Transduction, Cell Biology - Molecular Aspects

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EXPRESSION OF THE PROTEOGLYCAN SYNDECAN-1 DURING MYOGENESIS. Larrain, J.,. Cizmeci-Smith, G*., Carey, D.J.* and Brandan, E. Dept. Biologia Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica, Santiago, Chile and *Geisinger Clinic, Danville, PA 17822, USA.

Syndecan-1 (Syn-1) is an integral membrane proteoglycan that take part in the regulation of cell differentiation. In adults it is mainly expressed in epithelia but during development it is temporarily present at high levels in proliferating and condensing mesenchymas. To understand its unique developmental expression the promoter sequences of syn-1 gene have been intensively studied. It contains sequences for the binding of different transcription factors: Sp1, NF-Kβ, myogenin (E-boxes). The direct regulation of syn-1 expression by myogenin, a specific transcription factor which is up-regulated during myogenesis, it has not been demonstrated. We present evidence that mRNA level for syn-1 are higher in myoblasts compared with myotubes. Similar results were found at the protein level. To analyze if this downregulation during differentiation is consequence of myogenin expression, we prepared a CAT reporter containing a 667 bp of the rat syn-1 promoter region containing two E boxes (syn-1-CAT). Transient expression experiments on myoblasts indicate that this region of the promoter is enough for the observed downregulation of syn-1 reporter during skeletal muscle cell differentiation. Experiments designed to evaluate the direct participation of myogenin in the regulation of syn-1 are in progress. These results indicate that during skeletal muscle cell differentiation the expression of syn-1 is down regulated, and suggest that the region contained in the 667 bp promoter of syn-1 is in part responsible of this effect.

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SUPPRESSION OF SPARC EXPRESSION BY ANTISENSE RNA ABROGATED THE TUMORIGENICITY OF HUMAN MELANOMA CELLS. M.F.Ledda, S.Adris, C.Kairiyama, A.Bravo, L.Bover, Y.Chernajovsky, J.Mordoh and O. Podhajcer.Inst.de Inv.Bioquimicas Fundación Campomar, Hospital E.Peron, BsAs, Argentina.The Kennedy Inst.of Rheumatology, UK.

We have recently shown that SPARC is expressed by human melanoma cells and that this expression is associated with melanoma development. In order to study the rol of SPARC in melanoma progression one melanoma cell line was transfected with SPARC antisense cDNA.IIB-MEL-LES cell line, established in our laboratory was selected for transfection with human SPARC antisense cDNA. Isolated clones were analyzed for SPARC mRNA expression. Cells transfected with the vector lacking an insert (named LES-CMV) were used as a control.Three cell clones (named LES-IG, LES-IE and LES ID) exhibithing 12%, 53% and 88% decrease in SPARC mRNA expression were selected. The in vitro growth on plastic surface of the different clones were not affected. However when the different clones were analyzed for their capacity to invade matrigel we found a strong reduction in thier invasive capacity, related to LES CMV cells, wich correlate with a reduced expression of gelatinase A mRNA. When nude mice were inyected with the different cell clones we observed that all mice inyected with LES-CMV (7/7) demonstrate tumor formation while, after six month, mice inyected with cells obtained from different clones showed no sign of tumor formation (0/21). Hystologic analyses of MEL-1D inyection site demonstrated an extensive cellular infiltrate consisting predominantely of segmented neuthrophils, compared with LES CMV injection site.This is the first evidence that SPARC can play a critical role in the development of the invasive phenotipe of human malignant cell.

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ARACHIDONIC ACID-INDUCED CELL UNCOUPLING IN RAT ASTROCYTES IS NOT ASSOCIATED TO CHANGES IN THE STATE OF PHOSPHORYLATION OF CONNEXIN43. Martinez A. and Sáez J.C.. Depto. de Cs. Fisiol., Fac. de Cs Biol., Pontificia Univ. Católica de Chile, Santiago, Chile.

One of the most accepted functions of astrocyte networks is the spatial buffering of extracellular potassium during which the ion flows through the cytosol of one astrocyte into that of neighboring astrocytes via gap junctions (GJs). GJs are aggregates of intercellular channels permeable to ions and small molecules. Each channel is composed of proteins termed connexins (Cxs) and in rat astrocytes they are formed by Cx43. Previously, we found that Cx43 is internalized during arachidonic acid (AA)-induced inhibition of astrocyte GJs. In this work, the state of phosphorylation of Cx43 was evaluated during AA-induced cell uncoupling. Confluent cultures of astrocytes were prepared from brain cortex of 19 day old rat embryos. The functional state of GJs was tested by the transfer to neighboring cells of Lucifer yellow injected into one cell. The state of phosphorylation of Cx43 was evaluated by Western blot analysis and ^{32}P-incorporation. Cells were treated with various AA concentrations for 30 min at 15-18°C. Western blot analysis revealed slight changes in levels of phosphorylated (P1 and P2) and unphosphorylated (NP) forms of Cx43. Moreover, no significant changes in ^{32}P-incorporation into P1 and P2 were detected. Thus, the AA-induced cell uncoupling and internalization of Cx43 are not related to changes in the state of phosphorylation of the protein.

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MOLECULAR CHARACTERIZATION OF RAT TUBULIN TYROSINE LIGASE. EXPRESSION IN MUSCULAR AND NERVOUS TISSUE. Carlos R. Mas, Carlos O. Arregui, Carlos E. Argaraña, Héctor S. Barra. CIQUIBIC (UNC-CONICET). Dpto. de Química Biológica. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. 5016-Córdoba. Argentina.

Tubulin tyrosine ligase catalyzes, in a posttranslational reaction, the addition of tyrosine at the C-terminal of α-tubulin. In the present work we describe the cloning and the cDNA nucleotide sequence of ligase from rat olfactory bulb. In addition, the comparative analysis of the aminoacids sequence, and the developmental expression of the protein and mRNA in muscular and nervous tissues are shown. The aminoacid sequence of rat ligase diverges 8.2% from the pig ligase and 8.5% from the bovine one. The most divergent region is found in the last 15 aminoacids of the COOH-terminal where a deletion of two proline residues exists in bovine and rat in comparison with pig. In rat, ligase protein and mRNA as determined by western blot and in situ hybridization, respectively, are expressed prominently at early stages of the muscular development and become undetectable in rat mature muscle. In nervous tissue from adult animal, mRNA is restricted to certain population of cells like the Purkinje neurons. Preliminary studies of in situ hybridization show that in 13 and 16-day-old rat embryos, the ligase mRNA expression is essentially restricted to neuronal and muscular tissues. These results indicate that ligase is a highly conserved enzyme in the evolution and suggest a preponderant rol at early stages of developing muscular and nervous tissues.

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IN VITRO EVALUATION OF VIOLACEIN TOXICITY IN DIFFERENT CELL LINES

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Violacein is a pigment produced by *Chromobacterium violaceum* which was reported as potential drug for Chagas disease chemotherapy. The activities of violacein on bacteria and herpes virus cultures were previously reported. Now we present the results of the cytotoxicity of violacein on Chinese hamster lung fibroblasts (V-79), AIDS-related lymphoma (ARL) and 54 human tumor cell lines. Evaluation of IC_{50} through cell viability assays: DNA synthesis inhibition, formation tetrazolium/formazan (MTT) and neutral red incorporation (NR) on V-79 were carried out. Using cell viability tests in standard conditions, IC_{50} was 5, 7 and 12 μM for DNA synthesis inhibition, MTT, and NR, respectively. Five human lymphoma cell line (2 established from AIDS patients) and one leukemia cell line (CCRF-CEM) were used for ARL screening for antitumor capability. Viability and toxicity test using fluorescent dye propidium iodide were done in tumor cells. The most sensitive cells were: a non-small cell lung cancer NCI-H460, a leukemia MOLT-4 and colon cancer KM12 cell line with IC_{50} values of 0.03, 0.05 and 0.06 μM, respectively. Data obtained suggest that violacein is an antitumor drug with intermediate potency.

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BACTERIAL MODULATION OF HUMAN NATURAL KILLER CELLS: ROLE OF LIPOPOLYSACCHARIDE

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NK cells are defined as CD3-, CD16-56+; large granular lymphocytes that have the ability to lyse targets without previous sensitization. Bacteria and bacterial products enhance NK activity of human lymphocytes against cell targets; however the mechanisms involved are not well understood. In the present work we have studied the effect of bacteria and LPS, in particular the intracellular signaling mediated by LPS on a highly purified NK cell fraction (CNK).

NK cells were purified from human peripheral blood lymphocytes (PBL) by immunomagnetic methods. The effect of LPS (E.coli, serotype 0127:B8; 0.5-10.0 μg/mL; 24-72 h culture) and fixed *Salmonella typhi* was studied on cytolytic activity, phenotye and protein phosphorylation. Cytolytic activity was determined by the 51-Cr release assay, using K-562 and U937 cells lines as target cells; immunophenotype by flow cytometry and protein phosphorylation by anti-phosphotyrosine immunoblots.

According to our results, LPS and fixed bacteria stimulate cytolytic activity of PBL without change in the phenotype; however inhibits the activity of the CNK fraction. LPS also induces in the CNK fraction the tyrosine phosphorylation of several proteins with molecular masses (MM) between 50 and 70 kD suggesting the participation of protein tyrosine kinases in this action. In summary, LPS inhibits NK cells but the mechanisms involved are unknown.

Proyecto Fondecyt 193-0991

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INCREASED mdr2 GENE EXPRESSION IS ASSOCIATED TO PEROXISOME PROLIFERATION IN THE MOUSE LIVER.

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We have previously shown that fibrates induce mdr2 gene expression, its encoded P-glycoprotein in the canalicular domain of hepatocytes and increase biliary phospholipid output. (Biochem J. 1996, 314: 781). We now tested the hypothesis that mdr2 gene induction is not restricted to fibrates but is a common property of peroxisome proliferators.

Male CFI mice were fed on a diet supplemented with several peroxisome proliferator agents. Increased levels of mdr2 mRNAs were observed in the liver of mice treated with **phenoxyacetic acid herbicides: 2,4,5-T**: 570 ± 133%, **2,4-D**: 233 ± 54% compared to control group ($p< 0.005$); **plasticizers: DEHP**: 282 ± 78%, **DINP** 163 ± 40% and **PH**: 225 ± 48% ($p< 0.01$) and **partially hydrogenated fish oil: 15% PHFO**: 372 ± 138% ($p<0.005$). There was no correlation between the levels of mdr2 mRNA and the fatty acyl-CoA oxidase (FAO) or carnitine acyl-CoA transferase (CAT) enzymatic activities, both markers associated to peroxisome proliferation. Although at least on of these activities was increased for each treatment ($p<0.05$). This finding suggests that peroxisome proliferators may be acting through divergent pathways to regulate the expression of mdr2 gene and β-oxidation genes. P-glycoprotein content, as well as biliary phospholipid output, was increased in mice treated with the herbicide 2,4,5-T and with PHFO diet supplementation; both known as active peroxisome proliferators.

Our findings demonstrate that chemically and dietary induced mdr2 gene is associated to the peroxisome proliferation phenomenon and that mdr2-P-glycoprotein plays a crucial role on biliary phospholipid secretion in mice. At present, we are developing an in-vitro model to study the molecular mechanism of mdr2 gene induction

(Fondecyt 19610629)

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CELL ELISA ASSAY FOR SELECTIVE ESTIMATION OF SUBPOPULATIONS AMONG SOLID-PHASE-ADHERENT CELLS.

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With current methods, estimation of the number of adherent cells of a single type among a mixed population, requires its previous isolation, isotopic labelling, use of expensive equipment, and involves health hazards. Colorimetric methods do not distinguish between cell subsets.Our method allows estimation of a subset of cells among a heterogeneous population of adhesive cells using cell surface markers. Thyoglicollate elicited peritoneal macrophages exhibit the Mac-2 activation antigen. Macrophages from Balb/c mice were added to a microtiterplate, non treated for tissue culture, containing type II porcine collagen. The plate was centrifuged (400Xg for 5 min.), and incubated at 37° C for 30 min. The cells were fixed with glutaraldehyde 0.05% in PBS. After adding rat monoclonal antibodies (Boehringer) to Mac-2 antigens, they were incubated with a sheep $(Fab)_2$ fragment biotin-labeled anti rat IgG antibody, followed with peroxidase-labelled extravidin. Optical density was proportional to the number of adhered cells. Non elicited macrophages were used as controls.

Grants: D.G.A.P.A.- U.N.A.M. IN210294 and IN211396.

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IDENTIFICATION OF THE SECRETORY COMPOUNDS OF THE BOVINE FLOOR PLATE (FP) REACTING WITH ANTIBODIES AGAINST THE SECRETION OF THE BOVINE SUBCOMMISSURAL ORGAN (SCO). Muñoz, R.S., Montecinos, H. and Rodríguez, E.M. Instituto de Histología y Patología, Universidad Austral de Chile, Valdivia, Chile.

The FP extends along the midventral line of the embryonic spinal cord and hindbrain. The rostralmost region of the FP located in the region of the pontine flexure is known as the flexural organ (FO). Both, the SCO and the FO develops very early in ontogeny and display all features characterizing very active glands. The FP cells of the rat immunoreact with a polyclonal antiobody (AFRU) raised agaisnt the glycoproteins secreted by the bovine SCO. In these cells the AFRU-positive material is located within secretory granules mostly concentrated in protrusions projecting into the ventricle, and in basal processes. The bovine gestational period is 9 months. Four month bovine embryos display a SCO and a FO strongly reactive with AFRU. At late developmental atages only the SCO remains immunoreactive, whereas at early stages only the FO is AFRU-reactive. SDS-PAGE of extracts of bovine FP, followed by immunoblotting using AFRU has revealed two positive bands. These bands are being used to raise antibodies. This study, using a large FP, as that of the bovine, is allowing a biochemical analysis of the FP secretory compounds that present a partial homology with the secretion of the SCO.

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THE IRON RESPONSIVE PROTEIN (IRP)/IRON REGULATORY ELEMENT (IRE) SYSTEM IS ACTIVE IN CULTURED INTESTINAL EPITHELIA CELLS. Arredondo,M[1]., and Núñez, M.T[2]. [1]Unidad de Hematología, INTA, Universidad de Chile y [2]Departamento de Biología, Facultad de Ciencias, Univ. de Chile.

Body Fe levels are regulated by absorption through the intestinal epithelia, while cellular levels of Fe are tightly regulated by the translational regulation of genes products involved in cellular Fe metabolism such as ferritin and the transferrin (Tf) receptor (the IRP/IRE system). As both processes are regulated by intracellular Fe levels, we decided to investigate if intestinal epithelia cells have an active IRP/IRE system, and if this system is involved in the regulation of Fe absorption.

Caco-2 cells were cultured to equilibrium with varied concentrations of Fe as to obtain cell with varied intracellular Fe levels. In these cells we determined transcellular Fe fluxes, levels of Tf receptors, ferritin and IRP activity. We found that increasing levels of intracellular Fe in the range 20-200 μM produced: a) a decreased apical-to-basal Fe transport, b) increased levels of ferritin, c) decreased levels of Tf receptors, and d) decreased mRNA binding IRP activity. Increasing the intracellular Fe concentration beyond 200 μM elicited little further response in the parameters tested. Immunodetection of IRP revealed that increasing intracellular [Fe] produced a decrease in the mass of both IRP1 and IRP2. The results suggest that the IRP/IRE system is active in cultured intestinal cells, and that intestinal Fe absorption could be regulated by it.

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EXPRESSION OF THE HEXOSE TRANSPORTERS GLUT1 AND GLUT2 DURING THE EARLY DEVELOPMENT OF THE HUMAN BRAIN Nualart, F., Reinicke, K., Coloma, L., Muñoz, E., Vera, J.C[♦]. Departamento de Histología y Embriología, Fac. de Ciencias Biológicas, Univ.de Concepción. [♦]Memorial Sloan-Kattering Cancer Center, New York, USA.

The pattern of expression of the facilitative hexose transporters in the human fetal brain has not been established. We used immunohistochemistry with anti-hesoxe transporter to document the presence of different GLUT isoforms in fetal brain 10 to 21 weeks old.

In all stages investigated, GLUT1 was present in brain microcapillares, pineal gland, the epithelial cells of the choroid plexus and neurons. High expression of GLUT2 was observed in cerebellum of brains 21 weeks old, but GLUT2 was absent at earlier stages. GLUT3 and GLUT4 were absent at all stages studied. GLUT5 immunoreactivity was evident only in the cerebellar region of 21 week old fetal brains.

It is concluded that GLUT1 plays a fundamental role during early brain development. The data showing that fetal brain expresses GLUT2 and GLUT5 suggest that the cerebellum of the developing brain has the capacity to transport fructose, a substrate that has not been identified as a source of metabolic energy in the adult human brain.

Dir. Investigación Univ. de Concepción.

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CHANGES OF SOLUBLE PROTEINS INDUCED BY EXPOSITION TO HEAVY METALS IN *CORBICULA FLUMINEA*. Oliver C., Chiavellini J., Ermácora M., Porta A. , Departamento de Ciencia y Tecnología, Universidad de Quilmes, Argentina.

The study of the biological effect of environmental contaminants is important to understand detoxification mechanisms and provides useful tools to monitor the quality of the environment. Molecular and cellular alterations in response to contaminants usually precede whole organism changes and therefore are preferred markers of damage. In addition, the evaluation of biomolecules as markers is more informative than the direct chemical evaluation of contaminants because the biological effects are mediated by bioavailability. Thus, environmental damage in many cases is not a simple function of contaminant concentration.

This work is a preliminary account of the changes in the pattern of cytosolic proteins of the mollusc bivalve *Corbicula fluminea* exposed to heavy metals (Cu^{2+}, Cd^{2+}). The specimens were obtained from the coastal zone of Magdalena (Río de La Plata) in February-July 1996. We analyzed four mollusc tissues: gill, digestive gland, foot and mantle. The selected tissues were homogenized in Tris-sacarore-PMSF-DTT (pH 8.6). Cells and particulate debris were eliminated by centrifugation (40 000 g, 2 hours, 4º C) . Soluble proteins were precipitated with 10% trichloroacetic acid, dissolved in SDS-PAGE sample buffer, and separated by electrophoresis. Among a number of changes in the protein pattern of exposed tissues compared with unexposed controls, we observed the increase of two protein bands corresponding to 10 and 22 kDa. The former may correspond to metallothionein, a known scavenger of heavy metals. The second may be a uncharacterized stress protein. Further studies to clarify this subject are in progress.

We also observed the specific disappearance of protein bands. The complex changes observed in the soluble protein expression pattern provide a number of markers that may be useful to monitor metal exposure. The dose-response of these protein changes is being evaluated, but most of them occur at very low doses compatible with frequently occurring contamination events and even at concentrations considered safe in many legislations.

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ACTIVATED LYMPHOCYTES FORM GAP JUNCTIONS THAT MODULATE THEIR PROLIFERATIVE RESPONSE.
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Organization of lymphocytes into clusters occurres early after treatment with mitogens, such as concanavalin A (Con-A). During lymphocyte proliferation, cell-cell contact, secretion and response to cytokines, such as interleukin-2 (IL-2), play a key role. Cell contact mediated by cell adhesion molecules (CAM) is required for normal immune response. CAM also allow for gap junction (GJ) formation. The evidence of GJ presence between lymphocytes is limited and their function remain unknown. Lymphocytes from lymph nodes or peripheral blood were obtained from male Balb/c mice and normal human volunteers, respectively. Cells stimulated with 10 µg/ml Con-A were cultured for 24 h. During the following 24 h, cell proliferation was tested by ELISA, using the 5-bromo-2'-deoxiuridine (BrdU) colorimetric immune assay. Functional GJs were detected by the cell-cell transfer of Lucifer yelow (LY) microinjected into one cell of a cluster. After Con-A stimulation (1 h) transfer of LY ocurred and was reversible blocked with 500 µM octanol. Moreover, GJ formation was prevented with a synthetic peptide (Lab-1) homologous to the extracellular loop 1 of Cx32, a GJ subunit. Lab-1 also induced a 25-46% reduction in BrdU incorporation. Thus, activated lymphocytes form GJs that may allow for transfer of cell signals that control cell proliferation, such as those induced by IL-2.
PG-13/96 CIM (F.P.) & FONDECYT 1960559 (J.C.S.).

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ANTIBIOTIC LIKE PEPTIDE MW 5 kDa PRODUCTION BY A431 AND M-HeLa CELLS TRANSFECTED WITH TRANSFORMING GROWTH FACTOR TYPE α GENE.
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The metabolic changes in autocrine stimulated A431/1522-4 and M-HeLa/1522-25 cells expressing TGFα under the control of metallothionein promoter were investigated. Cells in confluent were incubated during 48 hours without serum in presence of 1.5 µM $CdCl_2$. Peptides from the conditioned medium were fractionised and tested. As a result the peptide Mm~5kDa (liquid chromatography and SDS-PAGE) with antimicrobial and hemolytic activity was isolated.

The hemolytic concentration for human erythrocytes appeared to be nearly 10^{-6}M. The cytolytic effect on bacteria was shown in concentration range 10^{-7}-10^{-6}M for E.coli K 12, B. mesentericus, S. aureus 209, and P. aeruginosa. Added to culture cells $2x10^{-6}$M 5 kDa peptide led to cell lysis, and the lower concentrations caused dose dependent changes of membrane associated protein kinases activity.

5 kDa bioactive peptide production was detected both in A431/1522-4 and M-HeLa/1522-25 sublines, while in wild A431 cells it was not found, and in M-HeLa cells the rate of expression turned out to be some times lower.

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ORNITHINE DECARBOXYLASE AND [^{3}H] THYMIDINE INCORPORATION INDUCED BY RETINOL PRE-TREATMENT IN CULTIVATED SERTOLI CELLS WERE DECREASED BY UV IRRADIATION.
Ribeiro,N.C; Dal Pizzol,F.; Sarmento, C.F.J.; Castro,M.A.; Moreira,J.C.F. and Bernard,E.A.Departamento de Bioquímica, Inst. de Biociências, UFRGS, Porto Alegre -RS, Brasil.

Our previous results suggested that retinol treatment induced large changes in chromatin conformation observed by changes in nuclear proteins (histones an HMGs) phosphorylation patterns and increased sensitivity to DNAse I digestion. Both effects were time dependent and were reverted by pretreatmente with 1,10 phenanthroline (an iron chelator). We also observed that ODC activity was changed by retinol treatment. To elucidate the role of retinol (7µM) pretreatment in ODC activity of Sertoli cells from 15 days old Wistar rats, we pretreated Sertoli cells with retinol for 24 h and submitted the cells to UV irradiation 254 nm ($5J/m^2$). The activity of ODC, the incorporation of thymidine to DNA and the survival rate were determined (30 min, 24h and 48h after UV irradiation). We had observed that pretreatment with retinol induced an improvement in ODC activity and in thymidine incorporation, both increased rates observed (ODC activity and [^{3}H]thymidine incorporation) were decreased by UV irradiation (30 min after UV irradiation). Retinol pretreated Sertoli cells were more sensitive to UV irradiation than non treated cells We believe that in pretreated cells ODC induction system and DNA synthesis are more highly sensitive to UV irradiation. Pretreated Sertoli cells presented a lower survival rate when compared with control cells in a lower culture density.
(supported by CNPq, PROPESP/UFRGS and FINEP)

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PHOSPHOLIPASE A_2 ACTIVITY DURING THE INDUCTION OF LIPOCYTE PHENOTYPE IN A LIVER CONNECTIVE TISSUE CELL: RESPONSIVENESS TO CALCIUM IONOPHORE A23187 AND PHORBOL ESTERS.
Rosa, T. Gomes; Borojevic, R.; Guaragna, R.M. Departamento de Bioquímica, Instituto de Biociências, UFRGS, Porto Alegre, Brasil.

Liver connective tissue cells have been caracterized as perissinusoidal myofibroblasts and hepatic lipocytes (Ito-cells). A concept of a single mesenchymal cell population that may be modulated between these two phenotypes has been postulated. The molecular controls of this switch were studied in the murine GRX cell line, representative of liver connective tissue. This cell line may be induced *in vitro* to convert into lipocytes by insulin-indomethacin. Analysis by GC of GRX cell line lipids revealed araquidonic acid (AA) only in phosphatidylethanolamine, both myofibroblasts and lipocytes. Activation of AA release is currently considered the principal mechanism for stimulation of eicosanoid synthesis. The phospholipase A_2 (PLA_2) plays a central role in provinding AA for subsequent metabolism. PLA_2 activities were measured in GRX cell line. The cells were suspendend in 50mM Tris -HCl (pH 8.5), sonicated 30s and centrifugated at 100.000xg for 1h at 4°C. PLA_2 activities were measured in the soluble and particulated fractions by the free [^{14}C] arachydonic acid released from 1-acyl-2-[1-^{14}C]arachidonoyl-sn-glicerol-3- phosphoethanol-amine.Cytosolic and membrane associated PLA_2 activity decreased correlated with the growth curve of GRX cells. Lipocytes (12 days of induction) restored the greatest cytosolic PLA_2 activity when incubated 2h with A23187 (1µM). Membrane associated PLA_2 activity in the lipocytes, was stimulated 50% with PMA (10nM) and A23187 (1µM). The inibition of prostaglandins synthesis, with a possible acumulation of the AA in the cell membranes was the proposed mechanisms for indomethacin in the induction of the lipocyte phenotype.
FINEP/CNPq/CAPES/ FAPERGS.

Membranes, Lipids, Receptors - Hormones and Growth Factors, Signal Transduction, Cell Biology - Molecular Aspects

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2D-PAGE MAPPING OF MOUSE LIVER PROTEIN CHANGES INDUCED BY DIETARY PROTEIN DEPLETION. Sanllorenti, P., Rosenfeld, J.*, García-Mata, R. and Conde, R. Instituto de Investigaciones Biológicas, Universidad Nacional de Mar del Plata, Mar del Plata, ARGENTINA, and *Laboratoire de Biochimie des Protéines, Sanofi-Elf Bio Recherches, Labège Innopole, FRANCE.

Dietary protein depletion for five days changes the proportion and synthesis rate of several mouse liver cytosolic proteins and decreases total protein content by 50%. The aim of this work was to use high resolution 2D-PAGE combined with computer-assisted densitometry for the characterization of the changes elicited by protein depletion in the proportions of GSTs and other mouse liver cytosolic proteins. Because their high isoelectric points, GSTs were not resolved by conventional 2D-PAGE. Then, for their separation and quantification, a non equilibrium pH gel electrophoresis (NEPHGE) protocol was designed. The results obtained allowed the preparation of a data base containing the comparative analysis of 384 2D-PAGE and 305 NEPHGE quantitated protein spots (in total, more than 500 quantitated protein spots). The optical densities of protein depleted cytosol spots were related to those from normal fed control. The following distribution percentages were found :

	a) 2D-PAGE	b) NEPHGE
Increased more than 2 times	13.3 %	17.6 %
Increased up to 2 times	16.8 %	21.2 %
Unmodified	34.1 %	37.2 %
Decreased up to 2 times	20.8 %	16.1 %
Decreased more than 2 times	14.1 %	7.9 %

Affinity purified GSTs led us to identificate and quantitate GST subunits by NEPHGE gels. The mu-class Yb1 and the pi-class Yf subunits proportion increased while the alpha-class Yc proportion decreased, indicating a GST subunits reorganization after protein depletion.

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ENZYME ACTIVITY AND ULTRASTRUCTURE OF TESTES AND OVARIES OF ADULT *DIPETALOGASTER MAXIMUS*. P.Y.Scaraffia, S. Remedi, C. Maldonado*, A. Aoki* and N. M. Gerez de Burgos. Cátedra de Química Biológica and *Centro de Microscopía Electrónica. Facultad de Cs. Médicas. Univ. Nacional de Córdoba, C.C. 35, Suc. 16, 5016 Córdoba, Argentina. E-mail :pscaraffia@biomed. uncor.edu.

D. maximus is a triatomine insect, vector of *Trypanosoma cruzi* in Mexico (southernmost Baja California Sur). Knowledge of functional aspects of organs involved in reproduction of this species may be useful for the design of control methods. We have determined activity of several enzymes related to carbohydrate and amino acid metabolism in adult testes and ovaries: hexoquinase (HK), glucose-6-phosphate dehydrogenase (G6PDH), fructose-6-phosphate kinase (F6PK), glutamate dehydrogenase (GlDH), aspartate aminotransferase (AAT), malate dehydrogenase (MDH) and glycerol-3-phosphate dehydrogenase (GPDH).

Activities, expressed in U per mg of protein, were: HK 0.015; F6PK 0.034; G6PDH 0.009; GlDH 0.016; AAT 0.073; MDH 0.922; GPDH 0.024 for testes and HK 0.012; F6PK 0.022; G6PDH 0.024; GlDH 0.030; AAT 0.049; MDH 0.582; GPDH 0.035 for ovaries. On the basis of these results it could be assumed that: 1.Glycolysis is active in both tissues, while the pentose pathway is relatively more important in ovaries than in testes. The abundant lipid droplets in follicular cells and oocytes indicate that the synthesis of fatty acids and the need for NADPH must be important. 2. Glycogen particles were observed in follicular cells. 3.Although GlDH is present in both tissues, its activity in ovary indicate a higher capacity to metabolize amino acids in this tissue than in testes. 4. Values of AAT and MDH are higher than that of GPDH in both tissues, suggesting that the malate/ aspartate shuttle would be relatively more active than that of the glycerophosphate, at variance with the findings in muscles from the same insect.

Mitochondria change strikingly in shape, size and distribution along the spermatogenic cell line. In follicular cells and oocytes, mitochondria are small and thin. The abundance of these organelles in cells from testes and ovary indicate that an intense respiratory activity occurs in both organs.

Rough endoplasmic reticulum and free ribosomes are conspicuous in spermatocytes and follicular cells. Free ribosomes predominate in oocytes.

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MASS DNA BREAKS DURING IN VITRO DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

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Studies of sister chromatid exchanges (SCE) and recombination rate of certain minisatellite DNA have demonstrated that their levels are considerably higher during the preimplantation than at later developmental stages of embryos. It appeared likely that single-stranded DNA breaks (SSB) may be relevant to both events during early development.We used in vitro retinoic acid-induced and spontaneous differentiation of murine embryonic stem cells to analyse the time course of changes in the SSB level. Using the methods of nucleoid sedimentation and the single-cell DNA electrophoresis, we observed a dramatic increase in the SSB during the first 1-3 mitosis after the beginning of differentiation of embryonic cells, followed by a gradual return to the basal level characteristic of undifferentiated ones. According to our estimation, not less than half of the genomic DNA was nicked at the early steps of differentiation. The decrease in SSB level was observed in spite of progressing cell differentiation, as judged by embryonic antigens and morphological criteria. Also, the increase in the SCE level was coincident with that of SSB, possibly being its consequence. The scheduled "surge' of SSB in DNA may be the earlist event in commecing differentiation at steps without its phenotypic manifestation.

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***IN VITRO* ANTITUMOR EFFICACY OF TENIPOSIDE (VM-26) *VERSUS* VINBLASTINE (VBL) AGAINST RENAL CELL CARCINOMA (RCC), AND EFFECTS ON DRUG CYTOTOXICITY OF SCHEDULE AND CHEMO-MODULATION.** D.H. Faria, M. A. Sohne, D.R.A. Mans, A.B. da Rocha, G. Schwartsmann, South-American Office for Anticancer Drug Development (SOAD), Porto Alegre, RS, Brazil.

Aim. To reevaluate VM-26 for improved efficacy in RCC with respect to Vbl, applying various schedules and chemomodulators.

Methods. Cultures of RXF-393 human RCC cells were incubated with serial dilutions of VM-26 or Vbl in the absence or presence of the DNA polymerase α inhibitor aphidicolin glycinate (AG; 0.2 μM) or the ribonucleotide reductase inhibitor hydroxyurea (HU; 200 μM), following 24-hours pretreatment. Next, the cultures were assessed for growth inhibition, DNA damage, and cell cycle phase distribution, using SRB staining, a fluorescence enhancement DNA damage assay, and fluorescence-activated cell sorting, respectively.

Results. Unlike Vbl cytotoxicity (IC_{50} of 0.004 ± 0.002 μM), that of VM-26 was schedule-dependent, improving about 10-fold upon drug exposure for 4 days continuously instead of 2 hours (IC_{50}s of 0.04 ± 0.01 μM and 0.6 ± 0.4 μM, respectively). AG- or HU-treatment potentiated VM-26 cytotoxicity further about 2- to 3-fold, respectively. The latter results could be due to inhibition by AG of repair of the VM-26-induced DNA strandbreaks, and to synchronization by HU of the cultures in the S phase of the cell cycle, where VM-26 has optimum activity. No modulation of Vbl cytotoxicity was observed, probably because of the agent targeting at the microtubuli, having thus optimum activity in the M phase.

Conclusion. Proper scheduling and chemomodulation may improve the antitumor efficacy of VM-26 significantly, making it a candidate for clinical reevaluation in RCC.

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GENETIC ADAPTATION OF MITOCHONDRIA TO OXIDATIVE STRESS. Soto, U*., Guerrero, J.*, Weindruch, R#. and Leighton, F*. * Facultad de Ciencias Biológicas. P. Universidad Católica de Chile, Casilla 114-D, Santiago, Chile. # Institute on Aging, University of Wisconsin.

The mitochondrial oxidative phosphorylation system generates most of the free radicals in the cell. We propose that in the presence of an extramitochondrial generated oxidative stress, mitochondria will reduce free radical generation as an adaptive response. This adaptive response would be the consequence of a decrease in the expression of mitochondrial and nuclear genes involved in the biogenesis of the mitochondrial electron transport chain.

Oxidative stress was induced in rats with menadione (50 mg/kg, i.g) for 7 days. Afterwards we studied, by northern blot, the expression of three genes from heart involved in the biogenesis of the electron transport chain. These genes were: **COX III**, subunit III of the cytochrome c oxidase; **mtTFA**, mitochondrial transcription factor, and **NRF-1**, nuclear transcription factor that regulate the expression of mtTFA. In addition, we studied the expression of those three genes in the heart of aged rats (33 months) since they represent a model of chronic oxidative stress.

The expression of COX III was reduced by menadione, but no change was observed in old rats compared to young animals. The expression of mtTFA and NRF-1 are under evaluation. In plasma, the treatment with menadione led to a 40% increase in the levels of total gluthation, without modification of vitamine E and C.

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INHIBITION OF THE FACILITATIVE HEXOSE TRANSPORTER GLUT1 BY FLAVONES AND ISOFLAVONES. Strobel[¶], P., Iribarren[¶], R., Nuñez-Alarcón[$], J., Slebe[¶], J.C. and Reyes[¶]A.M. Instituto de Bioquímica[¶] and Instituto de Química[$], Universidad Austral de Chile, Valdivia, Chile.

GLUT1 is a hexose transporter protein which is expressed ubiquitously in cells and is responsible for the basal uptake of glucose and ascorbic acid. We showed recently that the isoflavone genistein, a dietary-derived product often used to inhibit the activity of protein-tyrosine kinases, is a potent inhibitor of this protein. To test if this effect is shared by analogous compounds, we explore the action of a set of isoflavones and flavones on the transporter activity of GLUT1.

As human erythrocytes contain high levels of GLUT1, we measured the uptake of deoxyglucose, methylglucose· and dehydroascorbic acid on these cells as a functional test for GLUT1. The isoflavone biochanin A, and the flavones myricetin, quercetin, rhamnetin, isorhamnetin, and morin were efficient negative effectors of GLUT1 in the micromolar range. The inhibitory action of these compounds was instantaneous. Transport was not affected by the isoflavone daidzein and the flavones chrysin, and apigenin. Furthermore, binding studies showed that myricetin and quercetin inhibited glucose-displaceable binding of cytochalasin B to erythrocyte membranes in a competitive way. The data indicates that these compounds inhibit GLUT1 by directly interacting with the protein, as genistein does. These results emphasize the ability of GLUT1 to interact with molecules structurally unrelated to its transported substrates. (Supported by grants from FONDECYT 1951215, and DID-UACH, S-94-10, S-95-41, S-96-02).

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ECTO-ATP-DIPHOSPHOHYDROLASE ACTIVITY IN MDCK CELLS. Valenzuela, M.A., Cuevas, F., Collados, L., Kettlun, A. M., Marzolo, M.P*. and González, A*. Depto Bioquímica y Biología Molecular, Fac. Ciencias Químicas y Farmacéuticas, Universidad de Chile. *Depto Biologia Celular, PUC.

The ATP-diphosphohydrolase activity (apyrase) has been found present in rat kidney microvilli and vascularization. This enzyme has the following features: broad nucleotide specificity, dependence on millimolar concentrations of Ca2+ or Mg2+, insensitivity towards sulphydryl and hydroxyl reagents and to inhibitors of ATPases and phosphatase. These characteristics clearly differenciate apyrase from transport ATPases. The function of this enzyme has been related to the extracellular metabolism of nucleotides.
The purpose of the present work was to confirm the existence of this enzyme in the cellular line of renal cells (MDCK) with an ecto-localization. The release of Pi from cells incubated with TBS was followed; the integrity of cells was checked by the lack of LDH activity or Pi liberation in the absence of nucleotide. These cells were non-specific towards different nucleotides and the activity was insensitive to oligomycin, ouabain, levamisole and Ap5A. Only fluoride and DES were inhibitors of apyrase activity. The activity is absolutely dependent on Ca2+ or Mg2+. Nucleotide analogs inhibited both ATPase/ADPase activity. These results together with the inhibitory effect on nucleotide hydrolysis produced by antibodies against potato tuber apyrase point out to the existence of apyrase activity in MDCK cells.
Financed by Grant Fondecyt 193-1003.

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MODULATION OF RETINAL APOPTOSIS BY cAMP-DEPENDENT PROTEIN KINASE. Varella, M.H.and Linden, R., Instituto de Biofisica da UFRJ, Rio de Janeiro, Brasil.

In the retina of newborn rats there is evidence for two mechanisms of programmed cell death. Apoptosis of ganglion cells (RGCs) following axotomy depends on protein synthesis. In contrast, inhibition of protein synthesis leads to apoptosis in the neuroblastic layer (NBL). The fact that apoptosis in the explants can occur while protein synthesis is inhibited, suggests that post-translational modifications of apoptosis-associated proteins may be crucial to the cell death programs in the developing retina. Cyclic nucleotides have been implicated in apoptosis in various cell types. We investigated the possible role of cAMP and cGMP upon apoptosis in retinal explants *in vitro*. An increase in the intracellular concentration of cAMP produced by either the adenylyl-cyclase activator forskolin (10 μM) or 8-Br-cAMP (1 mM), prevented apoptosis induced in the NBL by inhibition of protein synthesis, but had no effect upon RGC death. In contrast, neither 8-Br-cGMP (1 mM) nor the specific cGMP-phosphodiesterase inhibitor zaprinast (10-100 μM) had any significant effect on apoptosis in the retina. The cAMP-phosphodiesterase inhibitors IBMX (0.1-1 mM) and Ro-201724 (50-200 μM) also prevented apoptosis in the NBL. The isoquinolinesulfonamide H89 (20 μM), a specific PKA inhibitor, partially reverted the protective effect of either forskolin or IBMX. We suggest that a specific increase in the intracellular concentration of cAMP prevents cell death induced in the NBL, through protein phosphorilation mediated by a cAMP-dependent protein kinase.
(Supported by CNPq, FINEP, CEPG-UFRJ, FAPERJ)

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OXIDATIVE STRESS PRODUCED BY MALATHION AND EXOGENOUS SPERMIDINE METABOLIZATION IN TOAD LARVAE. Venturino, A.[1], Gauna, L.[1], Cocca, C.[2], Bergoc, R.M.[2] and Pechen, A.M.[1] LIBIQUIMA, U.N.Comahue[1] and Fac. Fcia. y Bioq. UBA [2], Argentina. aventu@uncoma.edu.ar

We have previously reported that polyamines (PAs) enhance malathion (Mtn) toxicity in toad larvae. In the present work we evaluate possible interactions between the metabolic routes of both compounds: alterations of normal levels of PAs may cause severe cellular stress and toxicosis; PAs are able to modify membrane fluidity and thus change Mtn diffusion; both Mtn and PAs consume GSH as a result of degradative metabolism, thus the antioxidant defense may be seriously affected. We measured spermidine (Spd) uptake and endogenous PAs by TLC and spectrofluorometry of the dansyl derivatives. The effect of Spd on Mtn uptake and biotransformation was traced with the ^{14}C-compound in media and larvae. GSH pool was evaluated by Ellman's reaction with dithio-bis-nitrobenzoate.

We observed a rapid uptake of Spd by the larvae, with a concomitant metabolization to putrescine and in a lesser extent to spermine, which were first excreted to the media and then entirely removed at 48 h. Mtn and controls did not cause PA excretion. Endogenous PAs were not modified by Spd or Mtn treatments. In turn, 0.5 mM Spd altered Mtn uptake and bioavailability increasing the concentration of the xenobiotic in larvae. GSH pool was depleted by both compounds as a result of their metabolization, and a potentiated depletion was dose-dependently observed.

We conclude that oxidative degradation of PAs affects GSH pool and in consequence interferes Mtn detoxication enhancing its bioavailability, leading in this way to a potentiation of toxicity.

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INFLUENCE OF HYPERTHYROIDISM ON KUPFFER CELL (KC) FUNCTION. Videla,L.A.,[1] Tapia,G.[2] and Smok,G.[3] Departamentos de [1]Bioquímica, [2]Biología, and [3]Anatomía Patológica, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Hyperthyroidism in mammals involves an enhancement in their metabolic rate and acceleration of hepatic respiration, comprising higher rates of $O_2^{\cdot-}$ and/or H_2O_2 production at microsomal, mitochondrial and peroxisomal sites. In addition, KC hyperplasia is present in the liver of hyperthyroid rats in the form of focal aggregates within the lobule, cells known to produce $O_2^{\cdot-}$ and $NO^{\cdot}$ upon stimulation. The influence of hyperthyroidism (single dose of 0.1 mg T_3/kg) on KC function was studied in the isolated perfused rat liver by colloidal carbon (CC) infusion, which is taken up exclusively by non-parenchymal cells. In the concentration range of 0.2 to 2 mg CC/ml, livers from euthyroid rats exhibited a sigmoidal-type kinetics of CC uptake, with Vmax = 4.8 mg/g liver/min and $K_{0.5}$ = 0.82 mg/ml. CC-induced O_2 uptake presented a hyperbolic-type kinetics, with Vmax = 4.57 µmol/g liver and Km = 0.74 mg/ml. Infusion of 0.25 to 0.75 mg CC/ml did not modify liver LDH efflux in control rats, while it was enhanced in T_3-treated animals, together with higher rates of CC uptake and the associated O_2 consumption. These effects were abolished by pretreatment of the rats with gadolinium chloride ($GdCl_3$), which selectively destroys KC. It is concluded that hyperthyroidism enhances KC function, an effect that correlates with the increased number of liver macrophages observed histologically. T_3-induced $GdCl_3$-sensitive liver O_2 uptake may reflect an enhanced respiratory burst activity of KC, thus representing an alternate source of reactive oxygen species to that induced in parenchymal cells (Supported by FONDECYT 1940312).

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N-TERMINAL MUTATIONS ON MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE AFFECTS ITS *IN VIVO* FOLDING. Yáñez A.J., Iriarte* A., and Martinez-Carrion M., Instituto de Bioquímica, Universidad Austral de Chile. (*) School of Biological Sciences, University of Missouri-Kansas City, USA.

The amino terminus of the precursor for mitochondrial aspartate aminotransferase (pmAspAT) contains two highly conserved tryptophans at positions 5 and 6. These residues serve to anchor one subunit to the other by specifically interacting with a hydrophobic pocket on the surface of the neighboring subunit. To study the relative importance of these residues in the folding of the enzyme, a family of single and double mutants was prepared by site-directed mutagenesis. We followed the folding of the pmAspAT mutants in a cell free translation system (RRL). The extent of folding of one group of mutants, typified by W5F/W6F is similar to that of native enzyme, while another group, typified by W5A/W6A, was unable to fold. The results suggest that these two residues are critical for the folding of this dimeric enzyme synthesized in RRL. However, the folding yield of mutant W5V is only slightly lower than of wild type, whereas replacement of Trp-6 by Val (W6V) markedly decrease the yield of folded protein. Similar results were obtained for other pairs of single mutants, like W5A or W6A, and the double mutants W5I/W6F - W5F/W6I. Then, the presence of the Trp at position 6 is more critical than in position 5 for the folding of the enzyme. The only substitution at position 6 which seems to be acceptable for proper folding of pmAspAT is the changing the Trp to Phe. These results support the requirement for a hydrophobic amino acid at this position. (Supported by NIH Grants HL-38412 and GM-38341, and by CONICYT)

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HUMAN MONOCYTE CHEMOTACTIC PROTEIN-1 (MCP-1) IS HIGHLY EXPRESSED IN MELANOMAS. **Wainstok, R.[1], Bravo, A.I.[2], Bover, L.[3], Mantovani, A.[4] and Mordoh, J.[3] [1]Dept.Qca.Biológica, FCEN,UBA; [2]Htal.Eva Perón, San Martín; [3]Instituto Invest.Bioq. "Fundación Campomar", Buenos Aires, Argentina. [4]Instituto "Mario Negri", Milan, Italy.**

MCP-1 has been implicated as an important factor in mediating monocyte infiltration of tissues in a wide variety of inflammatory diseases as well as in macrophage infiltration of tumors, including melanomas. The aim of this study was to determine the expression of MCP-1 in human melanoma metastases, and to consider its relationship with macrophages infiltration and tumor angiogenesis. The following murine MAbs directed against human antigens were used in immunohistochemistry determinations: 5D3-F7 (MCP-1), HAM56 (CD68); QBEND10 (CD34). Briefly, paraffin-embedded tissue blocks of 5 µm thick, were incubated with the above mentioned MAbs. An avidin-biotin bridge was used, and the reaction was developed with diaminobenzidine (0.02%). MCP-1 expression was detected in 50 (100%) human metastatic melanomas, 100% of dysplastic and intradermal nevi, as well as in squamous superficial ephitelium. Although every melanoma tissue expressed MCP-1, the percentage of positive malignant cells differed. In 85.7 % of evaluated melanomas could be observed that more than 75 % of malignant cells expressed MCP-1. A relationship between this reactivity and the neovascularization grade could be established. The presence of peri- and intratumoral macrophages was also observed. These results suggest that macrophages recruited by MCP-1 to the tumor site could be responsible of angiogenesis since they coincide with vessels in areas of inflammatory infiltrates. Experiments are being performed to confirm this suggestion.

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Poster Session 3

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THE ANALYSIS OF 16S rRNA GENE FOR THE CHARACTERIZATION OF THE RHIZOBIAL POPULATION SYMBIOTICALLY ASSOCIATED TO WILD BEAN AND TO OTHER WILD LEGUMES IN THE NORTHWEST OF ARGENTINA. López, V. and O.M.Aquilar.

Phaseolus vuglaris L.is native to the Americas. Domestication of wild bean took place independentely in the Mesoamerica center of origin and in the Andean center in South America that includes our Northwestern area (NOA). *Rhizobium* strains that induce nodule formation in the host *Phaseolus vulgaris* (bean) are known to be highly diverse, however the phylogenetic nature of the native population of rhizobia found associated to wild bean in the NOA is still unknown. In our laboratory, we had established a rhizobial collection of isolates from nodules of wild bean.and other wild legumes that share the habitat in the Nortwest of Argentina, and had initiated its characterization by using a combination of molecular markers.

We had used a pair of primers to specifically amplify a nif sequence from type I strains and found that all of the members isolated from wild bean nodules belong to the type I. In addition, these isolates showed a restricted host range as they produced nodules in bean but not in leucaena unlike type II strains. In addition, we have used RFLP analysis of the PCR-amplified 16S rRNA gene to demonstrate the presence of species *R.etli* and *R.leguminoarum* bv.phaseoli in our type I bean rhizobial collection.

The rhizobia isolated from nodules of other wild legumes such as *P.augusti*, *Crotalaria*, *Desmodium*, *Eriosema* were assessed for the ability to induce nodules in *P.vulgaris*. It was found that all of them produced small nodules on bean and the internal aspect of the nodules was white (lack o' leghemoglobin). This collection of unclassified microorganisms was furthe examined by the analysis of the 16S rRNA sequences. A DNA regior corresponding to an internal sequence of the *E.coli* 16S rRNA gene wa amplified from these isolates with primers Y1 and Y2 (Young e al.J.Bact.1991, 173:2271), and analyzed by DNA sequencing.

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THE CCAAT-BINDING PROTEINS CP1 AND NF-I COOPERATE WITH ATF-2 IN THE TRANSCRIPTION OF THE FIBRONECTIN GENE. Claudio R. Alonso, C. Gustavo Pesce and Alberto R. Kornblihtt*. INGEBI and Dept. Biol. Sci., Facultad de Ciencias Exactas y Naturales, UBA, Obligado 2490, Buenos Aires, 1428, Argentina. E-mail: ark@proteus.dna.uba.ar

Our lab has previously proposed a molecular interaction between the liver factors that bind to the CRE and CCAAT sites of the fibronectin (FN) gene. After several studies we proved that ATF-2 and NF-1/ CP-1 are the factors involved in the occupation of the CRE and CCAAT elements of the promoter respectively (Srebrow et al (1993) ***FEBS Lett*** 327, 25-28; Alonso et al (1996), ***J. Biol. Chem.*** in press). Nevertheless we were not able to show our interaction hypothesis by standard co-immunoprecipitation, presumably as a consequence of the small amounts of the relevant factors present in the protein extracts used as protein source. In the search of a more sensitive and non disruptive assay to prove the above commented interaction we developed a new method that combines UV-crosslinking and immunoprecipitation. in brief this method consist of a specific immunoprecipitation of a complex composed by a oligonucleotide probe together with the protein factors that interact with it, where the radiolabeled oligo allows to trace the identity of the factors involved in the assembly of the complex. However this method had to be conveniently validated by several experiments to rule out any possible artifact. These controls involved studies with different probes and different oligonucleotide competitors to test the specificity of the assay, western blots of the nuclear extracts to confirm the existence of the interacting proteins in the protein source as well as band-shift and PAGE-SDS analysis of the UV crosslinked products confirming the quality of the radiolabeled species involved in the interaction. By this novel approach, we show that antibodies specific to ATF-2 are able to specifically precipitate protein-protein-DNA complexes containing NF-I and CP1 validating the proposed protein-protein interaction between the transcription factors that bind the CRE and CCAAT elements in the FN promoter (supported by grants of the University of Buenos Aires, CONICET, Fundación Antorchas and ICGEB).

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TWO NEW STRAINS OF SWEET POTATO FEATHERY MOTTLE POTYVIRUS: PRELIMINARY RESULTS. Alvarez, V., S.F Nome y D.A. Ducasse. Instituto de Fitopatología y Fisiologia Vegetal (IFFIVE-INTA), Córdoba, Argentina.

Sweet potato cultivar Morada INTA is affected by a complex viral disease called "sweet potato chlorotic dwarf" (SPCD). In an attempt to identify the possible causal agents of the SPCD disease a cDNA library was synthesized from viral genomic RNA purified from sweet potato plants showing typical SPCD symptoms. Dot-blot hybridization analyses were made. Several clones that reacted only to sweet potato feathery mottle potyvirus (SPFMV) were selected from those that recognized SPCD infected sweet potato plants. After their reciprocal southern-blot hybridization analyses, two of the SPFMV clones selected showed a very low hybridization signal under high stringency conditions. Sequencing data from these two clones, named FMV5 and FMV6, showed that both spanned the viral "nuclear inclusion body b" (NIb) coding region. Moreover, it was found that while FMV5 also had the complete coding region for the coat protein and the 3' non coding region, FMV6 only presented one third of the coat protein coding region. Sequence alignments of the N-terminal region of their coat proteins showed a high degree of diversity, thus corroborating the hybridization data. Their restriction enzyme pattern also differed although they both conserved the "nuclear inclusion body a" (NIa) protease recognition site and the DAG box characteristic of potyviruses. These data suggests that FMV5 and FMV6 may represent two new strains of SPFMV. Further studies are pursue to confirm this assumption.

This work was supported by INTA (Instituto Nacional de Tecnología Agropecuaria).

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RESISTANCE MECHANISMS IN *Lactobacillus delbrueckii* subsp. *lactis* STRAINS AGAINST THE LYTIC DEVELOPMENT OF THE TEMPERATE PHAGE *lb539*

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Lactobacillus delbrueckii subsp. *lactis* and *bulgaricus* are extensively used in the manufacture of cheese and yogurt. Gene transfer systems have not been defined for this species. Analysis and characterization of its phages and its restriction-modification (R/M) systems might contribute to the genetic studies of *Lactobacillus delbrueckii*. *L. bulgaricus* CRL539 harbors a temperate phage, *lb539*, which is inducible by mitomycin C (MC). MC-induced phage *lb539* plaques on the sensitive cells LKT, CNRZ326 and CRL934, with an efficiency of plaquing (e.o.p.) of 1, 10^{-3} y 10^{-2}, respectively. Inhibition of *lb539* adsorption to CNRZ326 and CRL934 was discarded, since phage particles adsorve efficiently to them (94 to 99%). A R/M mechanism was showed in CNRZ326. Phage DNA of *lb539*, modified in CNRZ326, resulted in phage *lb539.326*, which plaqued on LKT and CNRZ326 with an e.o.p. of 1. The passage of *lb539.326* phage through CRL934 rendered phage *lb539.326.934*, which, similar to phage *lb539*, plaques on CNRZ326 with an e.o.p. of 10^{-3}. The resistance to *lb539*, observed in CRL934, seems to operate by an abortive-infection or a "phage growth limitation-like" mechanism, similar to the one described in *Streptomyces coelicolor* A2 cells for phage *c31*. CNRZ326 and CRL934 do not have any plasmid suggesting that the resistance mechanism observed in these cells are chromosomally located.

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EFFECT OF SODIUM NITROPRUSSIDE ON PATHOGENESIS-INDUCED TRANSCRIPT LEVELS IN POTATO LEAVES. Beligni*, V., Laxalt*, A., and Lamattina, L., Inst. Inv. Biológicas, UNMdP, CC 1245, 7600 Mar del Plata, Argentina. *Both authors have contributed equally to this work.

Nitric oxide (NO) is a short-lived messenger molecule involved in many biological and physiological processes, one of which is its citotoxicity against microorganisms in cells of the immune response, thus preventing infection. Several evidences support the presence of a NO-sensitive transduction pathway in plants. Previous results obtained in our lab. showed the ability of SNP, a NO donor, to maintain the levels of chlorophyll in potato leaves infected with the pathogenic fungus *Phytophthora infestans*. In this work, we analyzed the ability of SNP to modulate the mRNA levels of three pathogenesis-induced proteins in potato leaves: phenylalanine ammonia-lyase (PAL), β-1,3-glucanase and glycer aldehyde-3,P-dehydrogenase (GAPDH).

Potato leaf sections were floated in petri dishes containing water or different concentrations of SNP (10^{-9} to 10^{-4} M). Treatments were done for 0.5, 1, 3 and 6 h. The relative amount of messenger RNA levels varied between 0.5 and 2.5 in SNP-treated leaf sections, related to water-treated ones. These variations were dependent on SNP concentration and time of treatments, showing a sinusoidal behaviour. These results demonstrate that NO could be able to directly or indirectly regulate mRNA levels, in a short period of time and at concentrations as lower as 10^{-9} M, not only in animals, but also in plants. This type of regulation of NO action on some plant mRNA levels resembles the sinusoidal mode of response of certain targets to stimulation by neurotransmitters.

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TEMPORAL AND SPATIAL ANALYSIS OF GLICER ALDEHYDE-3,P-DEHYDROGENASE mRNA LEVELS IN POTATO LEAVES INFECTED WITH *PHYTOPHTHORA INFESTANS*. Beligni, V., Laxalt, A., Madrid, E. and Lamattina, L., Instituto de Investigaciones Biológicas, UNMdP, CC 1245, 7600 Mar del Plata, Argentina.

Gliceraldehyde-3,P-dehydrogenase (GAPDH) is a glycolytic enzyme that has been proved to be regulated under various stress situations. Results obtained in our lab. showed that GAPDH mRNA levels increased in potato leaves and tubers when plants were infected with the pathogenic fungus *Phytophthora infestans* (P.i.) (1). This work was directed to study the temporal and spatial induction of GAPDH transcripts in this patho-system.

Detached potato leaves were inoculated with 10^5 sporangia/ml of P.i. at specific points (local, L) for GAPDH mRNA analysis. At different times after inoculation, L areas were cut and the rest of each leaflet was considered as systemic "short distance"(SSD). The non-inoculated terminal leaflet was considered as systemic "long distance" (SLD). Control leaves were water inoculated.

Water treatment showed a 3-fold increase of GAPDH mRNA in L area at 12 h and then reached its basal level at 48 hs. When infected, the maximum induction (4 fold over the control leaves) of GAPDH mRNA levels was in SSD area at 12 h, then it decreased gradually reaching its basal level at 48 h. The GAPDH mRNA levels were nearly invariable in L and SLD areas when compared to water treatment. These results indicate that tissues surrounding infected areas are responsible for the average modulation of GAPDH transcript level observed in total potato leaf.

1.- Laxalt *et al* (1996) Plant Mol Biol 30, 961-972.

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IDENTIFICATION OF A NOVEL GARLIC-MITE BORNE VIRUS PRESENT IN ARGENTINA. Helguera M.[1,2], Bravo-Almonacid F.[1], Kobayashi K.[1], Rabinowicz P.[1], Conci V.[2] and Mentaberry A.[1] [1]INGEBI (CONICET/FCEyN-UBA) and [2]IFFIVE-INTA. Argentina. E-mail: amenta@proteus.dna.uba.ar

In garlic, as in most crops which are vegetatively propagated, viruses produce significative yield looses. Disease is induced by an infective complex including a variable number of viral members that are difficult to isolate and purify due to their relatively restricted host-range. This often introduces confusion when traditional plant virology criteria, such as morphology and serology, are used to identify individual viruses. A cDNA library was obtained using as template RNA extracted from a mix of garlic viruses. At random sequencing of several clones allowed the identification of a genomic sequence (pCC6) that was 91% homologous to garlic virus A (GarA), a novel filamentous virus that was also included among the garlic mite-borne viruses (MbFV-G). This sequence contained the complete viral coat protein gene. This viral gene was amplified by PCR and the DNA fragment obtained was cloned in the plasmid vector pBSSK. The cloned insert was sequenced and subsequently subcloned in the expression vector pRSETa. *E. coli* cells transformed with this plasmid yielded a 31 kDa polypeptide that was recognised by anti-MbFV-G antibodies, but not by other antisera directed against different poty- and carlaviruses commonly infecting garlic. The 31 kDa polypeptide was then purified and used as antigen to produce polyclonal antibodies. In a survey performed with infected garlic plants, the anti-31 kDa antiserum reacted only with extracts that were negative to OYDV, LYSV or CLV antibodies. This antiserum is currently being assayed for use in immunosquash an DAS-ELISA tests as a diagnostic tool.

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DEVELOPMENT OF A mRNA AMPLIFICATION SYSTEM BASED ON THE PVX VIRAL REPLICASE Rattner B., Calamante G., Cabral S., Mentaberry A. and Bravo-Almonacid F. INGEBI (CONICET and FCEyN-UBA) Bs. As, Argentina. E-mail: fbravo@proteus.dna.uba.ar

Potato virus X (PVX) is a single-stranded plus sense RNA virus. In addition to the genomic RNA, at least two subgenomic RNAs (sgRNA) are synthesized during PVX infections. sgRNAs are produced from internal subgenomic promoters and are a means of selecting and amplifying certain viral ORFs to achieve differential expression. Two of them, the 0.9 and 2.1 kb sgRNAs, are transcribed from internal subgenomic promoters and mediate expression of the viral coat protein and a cell-to-cell movement protein, respectively. The aim of this work is to develop an RNA amplification system based on the activity of the PVX viral replicase. Two sets of genetic constructions containing the GUS reporter gene flanked by different viral sequences putatively involved in replication were obtained. These viral sequences are the 5' and 3' non-coding genomic regions and two sequences containing the subgenomic promoters of the 0.9 and 2.1 sgRNAs. Cloning of different combinations of these sequences in the plasmid vector pUC allowed the generation of plasmids p5TBGUS3, p5GUS3, p5CPGUS3 and pCP-GUS3. These constructions were *in vitro* transcribed and are currently being tested in co-inoculation experiments with PVX in tobacco plants. A second set of constructions was obtained by subcloning these sequences in the plant expression vector pBI121. The resulting plasmids were used to transform tobacco plants via the *Agrobacterium*-mediated system. Transgenic plants carrying these constructions are being characterized and will be used to test the expression level of the reporter gene before and after PVX infection.

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ADENOVIRUS 7H RESULTED FROM SEVERAL RECOMBINANTION EVENTS BETWEEN ADENOVIRUSES 3 AND 7.

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Adenovirus (ADV) 7h is the principal viral agent in Chile responsible for severe acute respiratory infections in infants with consequence of death or permanent lung damage. Previously we designed a diagnosis method to detect ADV 7h in nasopharyngeal aspirates by PCR amplification of its fiber gene. As a result of those studies we found out that different strains of ADV 7h have a fiber similar to ADV 3 and not 7. These was later confirmed by sequentiation of the E3 and fiber region of the ADV 7h prototype strain, revealing that there was a 7/3 recombination event inside the E3 region (Kajon, A and Wadell, G., Virology 215(2):190-196, 1996). This recombinant have a deletion of 56 bp and a mutation of the start codon of the corresponding E3-7.7K gene of ADV 7p and E3-9K gene of ADV3p. We designed primers that flanked the recombination region, to study clinical samples by PCR. There were 4 products of different sizes between the 56 samples analized, which were later sequenced. It was revealed that there was ADV 7h produced by at least 4 different events of 7/3 recombination during the time of the study (1988-1995). 82% of the samples showed the 7h-1 pattern, 10% were 7h-2, 4% were 7h-3 and 4% were 7h-4.

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***TRYPANOSOMA RANGELI* NEURAMINIDASE: ITS RELATIONSHIP WITH *T. CRUZI* TRANS-SIALIDASE**

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Trypanosoma rangeli is an American trypanosome which, at difference with *T. cruzi*, is unable to invade mammalian cells. We have previously shown that *T. rangeli* has a gene (S5) whose sequence is 85% identical to the *T. cruzi* virulence factor trans-sialidase (TS), which works as a neuraminidase (NA) in the absence of an adequate syalic acid acceptor substrate. However, the product of S5 does not have TS or NA activity. Since a NA has already been described in *T. rangeli*, it was interesting to know if it is related to *T. cruzi*'s TS. The NA of *T. rangeli* was purified from culture media supernatants and its N-terminus sequenced, revealing 100% identity with the TS. Genomic DNA of *T. rangeli* was digested with restriction enzymes, blotted and hybridized with a probe derived from TS observing from one to ten bands. After constructing a genomic library, we cloned twelve inserts and sequenced the 5' end coding regions. At least seven different genes which can be divided in three groups according to sequence similarity, were identified. These inserts were subcloned to the expression vector pTrcHis, by PCR, and three of them, which are currently being sequenced, code for a fully active NA. We conclude NA is homologous with TS and similar in its genomic organization, belonging to a gene family with "active" and "inactive" members. Its characterization might serve as a model in studies of TS function in host cell invasion and adhesion.

We acknowledge the financial support from TDR-WHO, Universidad de Buenos Aires and CONICET.

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Comamonas testosteroni β-HYDROXYSTEROID DEHYDROGENASE (β HSD): DETERMINATION OF THE TRANSCRIPTION START POINT AND ANALYSIS OF THE PROMOTER/REGULATORY REGION. Cabrera, J., and Genti-Raimondi S. Departamento de Bioquímica Clínica. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Córdoba. Argentina. E-mail: jcabrera@fcq.uncor.edu.

C. testosteroni can grow on a variety of steroid compounds as the sole carbon and energy source. In a previous work we have cloned and sequenced the β-HSD gene from *C. testosteroni* (Gene, 1991, 105:43). The β-HSD enzyme is produced in response to several steroids. However, the regulation of its activity at the molecular level is poorly understood. In the present study we have carried out Northern blot analysis, indicating the presence of a 1 kb transcript inducible with testosterone. Primer extension analysis has been used to identify the transcription start point from β-HSD promoter. Two major bands were observed as reverse transcription products of RNA from induced *C. testosteroni* cells. The smallest-sized product was the most abundant. This expression pattern might be due to the presence of a promoter which is of the -24/-12 class wich is recognized by the RNA polymerase-σ^{54}. We show by EMSA that protein extracts from induced *C. testosteroni* cells were able to interact specifically with a DNA fragment carrying β-HSD promoter. Additionally, southwestern blot analysis demonstrated the presence of two different 54 kDa testosterone induced proteins. Taken together, our results suggest that the DNA binding proteins and their target sequences may be involved in the regulation of β HSD mediated by testosterone.

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DEVELOPMENT OF PLANT VIRAL VECTORS FOR TRANSIENT EXPRESSION IN PLANTS. Calamante G., Bravo-Almonacid, F. and Mentaberry A. INGEBI (CONICET/UBA). Obligado 2490 (1428), Buenos. Aires. Argentina.
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In the last few years, infectious full-length clones of several plant viral genomes have been used as vectors to transiently express foreign genes in plants. A complete cDNA copy of the PVX genome (strain CP) was cloned into a plasmid vector allowing transcription of infectious viral RNA. In an attempt to develop expression vectors that can be safely contained, a trans-complementation system based on deleted viral versions and transgenic plants will be assayed.

A PVX version in which the coat protein gene was substituted by the β-glucuronidase gene was firstly developed. Due to the considerable size of the viral genome, an intermediate plasmid was used to introduce the GUS gene, replacing the coat protein gene and placing its sequence downstream of the 0.9 kb subgenomic promoter. The resulting plasmid was named pPVX-ΔCP-GUS. *In vitro* transcripts derived from pPVX-ΔCP-GUS will be inoculated onto transgenic tobacco plants expressing the viral coat protein and co-inoculated with PVX onto non-transformed plants.

A second version of PVX, including both the β-glucuronidase gene and the viral coat protein, is been currently under construction. In this case, an additional subgenomic promoter will be added to the sequence of the reporter gene. This version, in which all viral functions are provided in *cis*, will be used as a control for expression of the reporter gene.

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CHIMERIC-ANTIGEN CODING PLASMIDS ENGENEERING FOR DNA-VACCINES. Capozzo,A.V.*;García Briones,M.; Periolo,O.; La Torre,J.L. and Grigera,P. CEVAN.CONICET. Argentina. *Docent (Biología - CBC) and fellow, Universidad de Buenos Aires. UBA. Argentina.

We have been able to induce an excellent immune response against whole foot and mouth disease (FMDV) particles in mice injected with a baculovirus-expressed chimeric VSV glicoprotein (pG) containing a short aminoacid sequence of the antigenic site A of FMDV VP1 capsid protein.

Here we report the engineering of DNA vectors containing either the sequence of chimeric or native VSV pG and on their potential as virus-free immunogens able to elicit specific serological response in polynucleotide vaccinated hosts. The immunogen coding sequences were cloned in pcDNA3 vectors under control of CMV and T7 promoters and their expression verified in transfected CV1 cells expressing the T7 polymerase, by Western blot analysis. Intracellular location of the expressed products was also studied by immunofluorescent staining of the transfected monolayers.

The analysis of the serum specific activity of mice inoculated by intramuscular injection (in the quadriceps) with three 50 ug doses of purified plasmids coding for the FMDV-pG hybrid shows that constructions under CMV promoter elicited anti FMDV ELISA titres almost three orders of magnitude higher than the background levels obtained from gP sequence containing plasmids. We are currently exploring the use of antigen coding plasmids like those presented here to characterize qualitative and quantitatively the immune response against FMDV and VSV in larger animals (i.e:cattle).

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CHARACTERIZATION OF A PROMOTER SEQUENCE FROM *STAPHYLOCOCCUS AUREUS* BACTERIOPHAGE. Daniela Carbonelli, María Elisa Pavan and Jorge Zorzópulos. Instituto de Investigaciones Biomédicas Fundación Pablo Cassará - Buenos Aires- Argentina

Eventhought many bacterial and phage promoter sequences are known, the rules that govern its efficiency are not well understood. In order to investigate this problem, we have constructed a plasmid vector containing the β- lactamase gene without its promoter which was replaced by a multiple cloning site. Additional features of this plasmid are replication origins for Gram positive and Gram negative bacteria and antibiotic resistance genes for selection. Using this vector, we have isolated several promoters from *S. Aureus* bacteriophages. In this report, we described the structure of one of these promoters, named X8 which is very strong, according to biochemical assays.

First, we analysed the sequence of the X8 fragment (400 pb) in order to find consensus sequences of prokariotic promoters (Pribnow box and -35 element). We found a 80 bp. region rich in A/T in with possible Pribnow box sequence and a region 20 nucleotides upstream of this, similar to an UP element described as a third responsible element for the promoter efficiency in rRNA operons. This 80 bp fragment was synthetized and used in binding and gel shift experiments. It was found that, this region bind very efficiently to the RNA polymerase. On the other hand, binding and gel shift experiments using the complete X8 fragment were not successful and footprint experiments demonstrated only a weak protection of the 80 bp region. Moreover, upstream of the 80 bp region, we observed the presence of stop points during PCR amplification that indicated the presence of a DNA region with abnormal structure. These results suggested that the strength of the X8 promoter may be related to structural features rather than to the composition of the Pribnow box and -35 regions.

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A DOUBLE-STRANDED RNA MYCOVIRUS IN *Botrytis cinerea*. Castillo, A., Vilches, S. and Obreque, J. Departamento de Ciencias Biológicas, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile.

The mycoviruses are intracellular particles found in both yeasts and filamentous fungi. They are designated virus-like particles (VLPs) because their different properties compared with animal, plant and bacterial viruses. Most of the reported VLPs have double-stranded RNA (dsRNA) as genetic material. In some species such as *Saccharomyces cerevisiae* and *Cryphonectria parasitica* it has been found the presence of killer and hypovirulent phenotypes associated with these elements.

In wild-type strains of *B. cinerea* we have detected the presence of extrachromosomal genetic elements corresponding to molecules of dsRNA. These molecules have been designated L, M_1 and M_2 with molecular sizes of 8.3, 2.0 and 1.4 kilobase pairs, respectively.

The visualization of mycelia ultrathin sections by electron microscopy showed the presence of isometric VLPs (38 nm in diameter). A linear sucrose gradient centrifugation of mycelia-free extracts was done to determine if the dsRNAs were associated with VLPs. The gradient profile at 260 nm revealed a major peak that was analyzed by agarose-gel electrophoresis and electron microscopy. The results obtained revealed the presence of only the L-dsRNA molecule and isometric VLPs of similar morphology and size of those detected in the mycelia sections, suggesting that only the L-dsRNA would be encapsidated.

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TISSUE-SPECIFIC EXPRESSION OF BOVINE ß-CASEIN PROMOTER SEQUENCES IN THE MAMMARY GLAND OF TRANSGENIC MICE. Cerdan, M., Young, J., Zino, E., Falzone, T., Torres, H., and Rubinstein M. INGEBI (Universidad de Buenos Aires - CONICET) y Depto. Química Biológica, FCEyN (Universidad de Buenos Aires), Buenos Aires, Argentina

ß-casein is one of the major proteins in rodent milk and is expressed in the mammary gland of pregnant and lactating rodents. To determine ß-casein gene sequences responsible for its specific expression in the mammary gland, we produced three independent transgenic mouse pedigrees through the microinjection of 3.8 kb of the bovine ß-casein gene promoter coupled to the human growth hormone (hGH) gene. Milk samples of lactating females (day 11) from lines #3 and #45 were analyzed by Western blot using a specific anti-hGH serum. In line #3, a major band comigrated with a hGH standard (22 kD) whereas no bands were detected in milk from line #45 or from non-transgenic females. Radioimmunoassays (RIA) evidenced high levels (350 to 500 µg/ml) of hGH in the milk of line #3 and low levels (100 to 125 ng/ml) in line #45. A Northen blot analysis using an hGH probe revealed the presence of trangenic transcripts only in the mammary gland whereas all other tissues examined were silent even at long-time exposures indicating a faithful regulation of transgenic expression. However, the detection of low levels (10 ng/ml) of hGH in serum samples of all transgenic mice suggests the possibility of a transcriptional leakage of the 3.8 kb promoter. A temporal expression analysis of mRNA followed by Northen blot during pregnancy and lactation revealed the presence of hGH mRNA only at gestational day 17 reaching plateau on lactation day 1 maintaining high levels until weaning in close agreement with mouse ß-casein expression. *In situ* hybridization performed on mammary gland slices from lactating females showed that hGH expression is homogenous in all epithelial cells expressing ß-casein. A third pedigree is currently being analyzed to further asses the targeting strength of this 3.8 kb promoter.

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CLONING, SEQUENCING AND EXPRESSION IN MAMMALIAN CELLS OF THE ORNITHINE DECARBOXYLASE GENE FROM *CRITHIDIA FASCICULATA*. Ceriani[1], C., Svensson[2], F., Heby[2], O., Algranati[1], I.D. and Persson[2], L. [1]Instituto de Investigaciones Bioquímicas "Fundación Campomar", Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (Argentina) and [2]Department of Physiology and Neuroscience, Lund University (Sweden).

Mammalian ornithine decarboxylase (ODC) is among the most labile of cellular proteins (half-life of 30 minutes). Truncations and mutations in the C-terminus of mammalian ODC have been shown to prevent the rapid turnover of the enzyme indicating the presence of a degradation signal in this region. Moreover, ODC from the trypanosomatid parasites *Trypanosoma brucei* and *Leishmania donovani*, which lack this C-terminal domain, are metabolically stable (half-life higher than 8 hours), and recombination of the *T. brucei* ODC with the C-terminus mammalian ODC confers a short half-life to the fusion protein. We have cloned and sequenced the ODC gene from *Crithidia fasciculata*. This is the first protozoan shown to have an ODC with a rapid turnover. The sequence analysis revealed an open reading frame of 2,163 bp corresponding to a 721 amino acid protein with a high homology with *L. donovani* ODC, despite the difference in stability. *C. fasciculata* ODC lacks the C-terminal degradation domain of mammalian ODC. However, the *Crithidia* enzyme has a very rapid turnover even when expressed in mammalian cells. Our findings indicate that *C. fasciculata* ODC contains unique signals, targeting the enzyme for rapid degradation by a proteolytic pathway present also in mammalian cells.

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DNA SEQUENCE AND ANALYSIS OF THE GENES INVOLVED IN MICROCIN J25 PRODUCTION AND IMMUNITY. Solbiati, J.O[1]., Ciaccio, M.[1], Chiuchiolo, M.J.[1], Farías, R.N.[1], Moreno, F.[2], and Salomón, R.A.[1] Dpto. Bioquímica de la Nutrición, INSIBIO (CONICET-UNT), Chacabuco 461, 4000 Tucumán, Argentina[1], and Unidad de Genética Molecular, Hospital Ramón y Cajal, Madrid, España[2].

In previous communications we have reported the cloning and partial sequencing of a 5,2-kb fragment containing the determinants for production of and immunity to Microcin J25 (MccJ25), an antibiotic peptide produced by an *Escherichia coli* strain isolated from human feces.This fragment was now entirely sequenced. It contains four open reading frames, which were shown to be true genes by different complementary approaches. Three genes, *mcjABC*, are required to produce microcin. The fourth gene, *mcjD*, provides immunity. *mcjBCD* are transcribed in the same direction, and probably constitute an operon transcribed from a promoter located upstream of *mcjB*. *mcjA* is transcribed in the opposite direction and seems to encode a 58-amino-acid precursor of MccJ25. A comparison of predicted polypeptide products from *mcjABC* with proteins included in databases did not show any significant homology. The hydrophobicity analysis of McjD suggests that it is an integral membrane protein. On the other hand, McjD is highly homologous to several members of the ABC transporters superfamily. A model for MccJ25 biosynthesis, export and immunity is proposed.

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STABILITY OF THE HALOAROMATIC DEGRADING- AND Hg RESISTANT- PLASMID pJP4. P.Clément*[1], V. Meriggio[1], D. Springael[2] and B. González[1]. [1]Lab de Microbiología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Chile. [2]Lab. of Genetics and Biotechnology, SCK/CEN-VITO, Belgium.

Plasmid pJP4 confers to *Alcaligenes eutrophus* JMP134 the ability to grow on 2,4-dichlorophenoxyacetic acid (2,4-D) and 3-chlorobenzoate (3-CBA), and to resist mercurial compounds. We have reported that this strain also grow on 2,4,6-trichlorophenol (2,4,6-TCP), through chromosomal genes. In this work, plasmid stability and simultaneous expression of catabolic and resistance abilities were studied. The strain grew on 2,4-D, 3-CBA, 2,4,6-TCP, phenol, and LB rich medium, in the presence of $HgCl_2$ or merbromin. pJP4 was stable after 50 generations of growth on 2,4,6-TCP or LB medium, with or without merbromin. It has been suggested that pJP4 rearrange when it is transferred to new host strains. Three transconjugants (B12, B26, and T2) were selected from matings between strain JMP134 and *E. coli* XL1. Plasmids from B12, B26 and T2 were smaller than pJP4. SphI and EcoRI restriction patterns of B26 and B12 plasmids were identical, but different from pJP4. E-B fragment had a reduced size while E-D, S2, S3, S5 S7 and S8 fragments were absent. In turn, the restriction pattern of T2 plasmid indicated that E-A had a reduced size, S2, S5 and S7 were absent and a new SphI fragment appeared. Hybridization with *tfd*A-E probes (coding for 2,4-D degradation) showed that the deletion is not in catabolic genes. Transference properties are probably in the deleted portion, because transfer back the deleted plasmid from *E.coli* to *A.eutrophus* was not possible, unless a helper strain containing pRK2 *tra* functions was provided.

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CONSTRUCTION OF A SYNTHETIC GENE ENCODING APIDAECIN, A BACTERICIDAL PEPTIDE FROM BEES, AND EXPRESSION STUDIES IN *E. coli*. Mardones, G. and Venegas, A. Laboratorio de Bioquímica. Facultad de Ciencias Biológicas. P. Universidad Católica de Chile. Alameda 340, Santiago, Chile.

Apidaecin is a bactericidal peptide found in the haemolymph of honeybees (*Apis mellifera*) after injury or bacterial infection. It is 18 amino acid long and rich in proline (33%), being stable after heating at 100°C or incubation at pH 2. The main goal of this work was to clone the gene in a regulated vector and try to express it in bacteria in spite of its toxicity.

The nucleotide coding region was chemically synthesized as 2 complementary 65mer and ligated into the NcoI site of pET-11d vector, under T7 promoter control. *E. coli* BL21(DE3) cells were transformed with this plasmid to allow expression after IPTG induction of T7 RNA polymerase gene carried by this strain. From several isolated clones, 4 were sequenced and one showed the right sequence. In order to analyze expression, cells in exponential growth phase were induced by IPTG which produced an immediately interruption of bacterial growth but no peptide was detected in gels. Apidaecin has been reported as a bacteriostatic peptide acting on Gram negative preferently. Using a plate assay that we have developed to test bacteriolytic compounds, we have demonstrated that apidaecin induces cell lysis under the described conditions.

It is concluded that in spite of the repeated failed attempts to detect the peptide directly in transformed cells by PAGE-SDS analysis, the expression of this peptide was clearly noticed by its drastic inhibitory effect on growing cells.

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THE POLYMERASE CHAIN REACTION (PCR) IN THE DIAGNOSIS OF GASTRIC MALIGNANT LYMPHOMA. Corvalan.A., Perez,J., Vargas,A., Martinez,C., and Pisano,R. Laboratorio de Patologia-Instituto Chileno Japones de Enfermedades Digestivas, Santiago-CHILE.

Gastric Malignant Lymphoma (GTML) is the most common extranodal type of Lymphoma. Most of these tumors arise from hiperplastic gastric lymphoid tissue (Gastric Lymphoid Hyperplasia, GTLH). The diferential diagnosis between GTML and GTLH by histology is difficult, especially when has to be made from minute endoscopic biopsies. Recently, a PCR based assay has been developed for lymphocytic clonality. This assay is based on the polimorphic rearrangement of the immunoglobulin heavy chain-third complementary determining region (IgH-CDR3) in hiperplastic-lymphocytes, but clonal rearrangement in Lymphoma-lymphocytes. We apply the PCR-IgH-CDR3 assay to endoscopic biopsies from GTML cases. First, we analyze DNA from 20 surgically resected cases of GTML by PCR-IgH-CDR3. We found clonal bands in 15/20 cases (75%). Next, we analyze DNA from the endoscopic biopsies from 8 of these 20 GTML, including 2 GTLH cases. Clonal bands were found in 6/8 of biopsies (75%). Both 2 GTLH showed clonal bands. These bands have the same migration pattern on electrophoresis that those of surgically resected specimen. This data strongly suggest the utility of PCR-IgH-CDR3 assay to cases were the differential diagnosis between GTML and GTLH has to be made.

Proyecto Cancer Organos Digestivos,JICA-Japan

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EFFECT OF DIFFERENT RNA POLYMERASE II PROMOTERS ON ALTERNATIVE SPLICING. Paula Cramer and Alberto Kornblihtt. INGEBI and Dept. Biol. Sci., Facultad de Ciencias Exactas y Naturales, UBA, Obligado 2490, Buenos Aires, 1428, Argentina. E-mail: ark@proteus.dna.uba.ar

Transcriptional activity by RNA polymerase II has been shown to occur in 20-50 discrete regions within the cell nucleus, known as nuclear speckles or "transcript domains". The fact that these clustered domains not only concentrate poly (A)$^+$-RNAs, but also molecules involved in RNA splicing such as small nuclear RNPs and the non snRNP splicing factor SC35, suggested that transcription and splicing might not be independent events, but on the contrary, highly coordinated processes both at the functional and structural levels. In order to test the existence of a close coordination between the processes of transcription and alternative splicing, we chose the following experimental model. Different RNA polymerase II promoters, including human alpha globin, human alpha 1-antitrypsin, human cytomegalovirus immediate early promoter (CMV), human fibronectin, and Mouse mammary tumor virus (MMTV), were cloned upstream of a chimerical gene called alpha globin/fibronectin minigene. This gene contains the EDI human fibronectin exon embedded into the third globin exon. EDI codes for a type III unit of fibronectin and its presence in the protein varies depending on the tissue, stage of development, age, and proliferating status. A single fibronectin gene codes for EDI$^+$ or EDI$^-$ mRNAs by alternative splicing. After transfecting hepatic and non hepatic cell lines with the battery of constructions above mentioned, the relative abundance of the two mRNA species (EDI$^+$ and EDI$^-$) was assessed in each case by Northern blotting and RT-PCR analysis. The results obtained so far show different and reproducible alternative splicing patterns for each construction, suggesting a relationship between transcription and alternative splicing (supported by grants of the University of Buenos Aires, CONICET, Fundación Antorchas and ICGEB).

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ACTIVE AND INACTIVE MEMBERS OF *TRYPANOSOMA CRUZI* TRANS-SIALIDASE FAMILY. Maria Laura Cremona, Daniel O. Sánchez and Alberto C. C. Frasch. Instituto de Investigaciones Bioquímicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

The trans-sialidase (TS) of *Trypanosoma cruzi* is encoded by a family of several genes, some of them rendering inactive products. They contain a conserved region in the sequence coding for the catalytic domain of the enzyme. A natural mutation Tyr^{342} to His was demonstrated to occur in inactive proteins and is responsible for the total loss of TS activity (Cremona et al, GENE 160:123-128, 1995). This mutation is encoded by a codon with a single nucleotide change T to C in a highly conserved region between the members already sequenced. In order to address the question of the association between the active and inactive members and the levels of expression of these groups of genes, two antisense primers were designed: 5'CGGAGCTGTAGGCGGAAT3' to identify the active and 5'CGGAGCTGTGGGCGGAAT3' to identify the inactive ones. Hybridizations in the presence of TMAC (tetramethylammonium chloride) were performed on dot-blots to quantify the numbers of active and inactive genes present in the parasite's genome. Approximately 50 and 150 genes code for the active and inactive proteins, respectively, per haploid genome. On Southern blots, the probes detected nine differential bands ranging from 2 to 9 kbp. We are currently investigating the level of expression of active and inactive TS. The existence of mRNA from the numerous genes coding for inactive proteins might indicate a role for these proteins in some stage of the parasite's life cycle.

We acknowledge the financial support from TDR-WHO, Universidad de Buenos Aires and CONICET.

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MOLECULAR CHAPERONES AS MULTIFUNCTIONAL ENZYMES. STRUCTURAL IMPLICATIONS OF CHAPERONE FUNCTION. Csermely, P. Department of Medical Chemistry, Semmelweis University, Budapest, Hungary

Molecular chaperones are highly conserved, abundant proteins, which help to reach and maintain the conformational stability of other proteins in the cell. In recent years we have established several classical enzyme activities of the 90 kDa heat shock protein (hsp90), a mostly cytoplasmic molecular chaperone. Our studies indicated that under various conditions the protein behaves as a protein kinase, ATP/GTP-ase, protease, topoisomerase, helicase and may also have nuclease and redox properties. This multifunctionality is a general feature of molecular chaperones. The high variety of enzymatic activities may pose chaperones as "omnipotent" enzymes. In fact, chaperones have a rather poor catalytic activity in all the above enzyme actions. This is in agreement with the high heat stability of these proteins, which indicates that their three dimensional structure may be too rigid to accomodate the high flexibility for efficient catalysis. Thus, chaperones may be "omni-impotent", archaic enzymes which kept their functions as an inefficient, but vast reserve under extreme environmental stress. The high number of active sites may also help chaperones to bind to their protein targets. This suggests, that chaperones "embrace" the folding proteins by a variety of hydrophobic and ionic interactions, "pull them away", and by creating a relative "vacuum" in the inner, hydrophobic core of the target, help the formation of the tightly packed inner structure of proteins in the native state.

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HIGH VARIABILITY OF THE MOLECULAR STRUCTURE WITHIN THE C4/CYP21 LOCUS IN FAMILIES WITH THE CLASSICAL FORM OF CONGENITAL ADRENAL HYPERPLASIA

De Mello, M. P.: Araujo, M.; Sanches, M.R.; Paulino, L.C.; Lau, I.F.; Guerra Jr., G.; Marini, S.H.V.L..Centro de Biologia Molecular e Engenharia Genética, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, SP, Brazil

We report the molecular studies on the genetic defect in families with one or more affected offspring diagnosed as having the classical form of congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. DNAs from patients and their relatives were initially analyzed with three restriction enzymes. Hybridization with 21-hydroxylase cDNA probe and the 5' end of a C4 genomic probe disclosed gene deletion in 9.5% (4/42) of the disease-related chromosomes. The rate of large gene conversion was 19.0% (8/42), no abnormality in the hybridization pattern was observed in 62.0% (26/42) of the disease alleles, and 9.5% have either duplication or deletion of C4 and CYP21A units. Densitometry of the autoradiographs was used to determine the ratio of the copy-number of the 21-hydroxylase gene (CYP21B) to the copy-number of its pseudogene (CYP21A). An anomalous 5.4 kb Taq I fragment was detected in the hybridization with 21-OH probe in one family, and an anomalous 3.7 kb Taq I fragment was obtained in the hybridization with C4 probe in the other. In addition, the restriction pattern analysis of a patient showed the presence of both C4 and CYP21 fusion genes. This C4/CYP21 haplotype has never being observed in humans. To the present, 23 families were genotyped for point mutations. Four mutations in 35 disease alleles were analyzed: Sp, a frame-shift mutation in intron 2 that causes abnormal RNA splicing; I172, a missense mutation at residue 172 in exon 4; Hig, a cluster of three missense mutations in exon 6 and R356, a single substitution in exon 8. One of the four tested specific mutations was found in about 50 % of the disease alleles. Differences in phenotype-genotype relationship and the low frequency of gene deletion compared to the frequency of gene conversion suggest that 21-hydroxylase deficiency in Brazil may involve different molecular rearrangements.

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CitR, A NEW PROTEIN INVOLVED IN THE REGULATION OF CITRATE METABOLISM IN *Lactococcus Lactis* biovar diacetilactis. Martin, M.[1], Magni, Ch.[1], García, N.[2], López, P.[2] and de Mendoza, D.[1] [1] PROMUBIE (CONICET), Dpto. de Microbiología, Fac. de Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina and [2] CIB, Madrid, España.

The transport of citrate in *Lactococcus Lactis* biovar diacetilactis is mediated by the citrate permease P (CitP), encoded by the *cit*P gene carried in an 8.3 Kbp plasmid. Two partially overlapping open reading frames, named *cit*Q and *cit*R were identified upstream the *cit*P gene. These three genes constitute the *cit* cluster.

It was found that CitP is the only essential requirement for the transport of citrate in this bacterium and CitR regulates negatively the expression of citP at a post transcriptional level.

By means of a translational fusion between *cit*P and a *cat* reporter as well as transport assays, we show that translation of *cit*P is pH dependent with maximum CAT and transport activity observed at lower pH. This process seems to involve CitR.

The *cit*R gene was overexpressed in *E. coli* and the amino terminal sequence was determined. Antibodies have been obtained that reacts specifically against CitR. In Western blot assays employing these antibodies, CitR homodimers and homotrimers were detected, even in denaturing conditions.

Finally, *cit*R and *cit*P genes were found in *Leuconostoc lactis* strains using PCR and Southern blot techniques

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SEQUENCE AND EXPRESSION OF A *Leptospirillum ferrooxidans* GENE CODING FOR A CHEMOTACTIC RECEPTOR-LIKE PROTEIN. Delgado, M., Toledo, H. and C.A. Jerez. Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Escherichia coli controls its motility by means of a regulated chemotactic system which includes four closely related trans-membrane chemotactic receptors: Tar, Tsr, Trg and Tap. We have previously shown that *Leptospirillum ferrooxidans*, a highly motile chemolithotrophic acidophilic bioleaching bacterium, is chemo-tactically attracted by ferrous ions. To study chemotactic receptors in this microorganism, we cloned and sequenced a *L. ferrooxidans* DNA fragment that hybridized with a probe containing part of the *E. coli tar* receptor gene. The sequence revealed two promotor regions: one recognized by the sigma-28 factor, specific for chemotaxis and motility genes, and the other recognized by sigma-70. We found a 1,731 nucleotide open reading frame preceded by a characteristic Shine-Dalgarno element. We named LcrI the predicted polypeptide, which possessed two regions with properties of membrane spanning segments, suggesting a typical chemotactic receptor organization: an N-terminal trans-membrane region, a periplasmic domain, a second transmembrane region and a C-terminal cytoplasmic domain. The LcrI cytoplasmic domain included the highly conserved region that is present in all known chemotactic receptors. The *in vitro* expression of the recombinant plasmid resulted in the synthesis of a protein cross-reacting with a polyclonal antibody against the *E. coli* Tar receptor, strongly suggesting that *lcrI* is a *L. ferrooxidans* gene homologous to the *E. coli tar* gene, and that the LcrI protein is a chemotactic receptor.

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MUTAGENESIS IN *ZYMOMONAS MOBILIS* USING CHEMICAL AGENTS, PBR322 AND THE F'PLASMID.

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Zymomonas mobilis is an anaerobic Gram - negative bacterium capable of producing almost theoretical yields of ethanol from glucose and has been shown to be a promising alternative to yeast for the industrial production of ethanol reaching high ethanol concentrations. Moreover, *Z. mobilis* produces antimicrobial agents and it was also tested as a host for the expression of heterologous proteins. The genetics of *Z. mobilis* remains largely unknown. Genome organization, sexual exchanges, recombination and DNA repair mechanism are poorly understood as yet, however several research reports inform about the resistance of *Zymomonas* to mutagenesis and that many of the mutants obtained exhibit a marked phenotypic instability. In this context we transformed *Z. mobilis* ATCC 10988 Tetracycline sensitive (Tc^s) using pBR322, by electroporation and the calcium chloride procedure. Stable auxotrophic mutants (Tc^R) for *ser, gly, pro, cys*, etc. were isolated. DNA isolated from the mutants was digested with BglI and hybridized with pBR322 as probe. The pattern revealed different size bands in the different processed DNAs. Using N-methyl-N-nitroguanidine and stabilizing with caffeine a 4-fold increase in the number of mutants was obtained, including 13 different stable aminoacid auxotrophs. F'(Tc^R) was transferred from Escherichia coli to *Z. mobilis*, obtaining resistant strains. These methods allowed us to obtain a set of mutants which will be used for the definition of the genomic map of *Zymomonas*.

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HIGH DIVERSITY IN MUCIN GENES AND MUCIN MOLECULES IN Trypanosoma cruzi. Di Noia, J.M.[¶], Pollevick, G.D.[¶],Xavier, M.T.[*], Previato, J.O.[*], Mendoça-Previato, L.[*], Sánchez, D.O.[¶] and Frasch, A.C.C.[¶,¶] *[¶]Inst. Inv. Bioquímicas "Fund. Campomar", Buenos Aires, Argentina; [*]Depto. de Microb. Geral, U.F.R.J., Rio de Janeiro, Brasil.*

T. cruzi has mucin-type glycoproteins that play a relevant role in the interaction with, and invasion of, host cells. We have previously reported a family of mucin-like genes in *T. cruzi* whose structure resemble that of mammalian mucin genes. We have now analyzed their diversity and proved the relationship between these genes and mucin proteins. A mAb specific for a *T. cruzi* mucin carbohydrate epitope, and a polyclonal serum directed to a recombinant apo-mucin detected identical bands in the same *T. cruzi* strains. Both sera reacted with equivalent bands in epimastigote and metacyclic forms and did not react with the cell-derived trypomastigote form. Mucins immunoprecipitated with the mAb are recognized by the anti-recombinant serum. When expressed in an eukaryotic cell, the product of one of these genes is post-translationally modified, most likely, through O-glycosylation. Genes isolated from different strains, showed new features, like variation in the number and characteristics of the described repeats that conform the central region of this proteins, extending the diversity of the family. Compositional analysis and molecular mass determinations of mucin core proteins also demonstrated the large heterogeneity of the family of *T. cruzi* mucins.

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AN ANTISENSE RNA STRATEGY TO INVESTIGATE THE ROLE OF BASAL EXPRESSION OF THE *Trichoderma reesei* CELLULASE SYSTEM IN INDUCTION OF ITS OWN TRANSCRIPTS. Escobar Vera, J., Carle Urioste, J.C., Henrique-Silva, F., El-Gogary, S., Torigoi, E., Matheucci Jr., E., Crivellaro, O and El-Dorry, H. Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brasil

In *Trichoderma reesei*, the cellulase system is induced at least 1100-fold by cellulose. The members of this system include at least two cellobiohydrolases, CBHI and CBHII and two major endoglucanases, EGI and EGII that act synergistically in the hydrolysis of cellulose to oligosaccharide. We have previously proposed that *T. reesei* expresses low, constitutive levels of cellulase system and that the activity of these enzymes on cellulose produces a soluble disaccharide which can enter the cell and effect induction. We designed an antisense strategy to establish convincingly that the inductive mechanism of the cellulase mRNA by cellulose in *T. reesei* requires basal expression of the cellulase transcripts. Therefore, we decided to examine the effect of the expression of an antisense RNA against, CBHII, EGI and EGII mRNAs, on the induction of *cbh1* transcript. Our results shows that cellulose-induced expression of the *cbh1* transcript was suppressed when antisense RNA against the three members of the cellulase system was expressed *in vivo*. More critical is the fact that expression of the antisense RNA have not effect if a soluble inducer of the cellulase system was used. The results reveal a critical requirement for basal expression of the cellulase system in induction of its own transcripts by cellulose.

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BACTERIAL COMMUNITY IN A COPPER COMMERCIAL-SCALE BIOLEACHING CONDITION DESCRIBED BY DIRECT DNA ANALYSIS. Espejo, R.T and Romero, J. SMP Tecnología S.A., Santiago, Chile.

Most copper bioleaching plants operate with a high concentration of sulfate salts due to the continuous addition of sulfuric acid and the recycling of the leaching solution. Since the bacteria involved in bioleaching have been generally isolated at low sulfate concentration, the population in ores leached with the high sulfate solution (1.25 M) employed in a copper production plant was investigated. The complexity of the original population was assessed by the length pattern of the spacer regions between the 16S and 23S rRNA genes, observed after PCR amplification of the DNA extracted from the leached ore. Six main spacers were distinguished by electrophoretic migration but they could be further resolved into eight by nucleotide sequence homology. The degree of homology was inferred from the electrophoretic migration of the heteroduplexes formed after hybridization. One of the spacers was indistinguishable from that found in *Thiobacillus thiooxidans*, four could be related to *Thiobacillus ferrooxidans* and three to *Leptospirillum ferrooxidans*. Only five of the spacers in the original sample could be recovered after culturing in media containing different inorganic energy source. Altogether the results indicated that the bacteria in the leached ore conformed a community composed by at least three species; a fairly homogeneous population of *T. thiooxidans* and two heterogeneous populations of *T. ferrooxidans* and *L. ferrooxidans*.

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MOLECULAR CHARACTERIZATION OF INSECT VIRUSES (BACULOVIRIDAE). P. D. Ghiringhelli[1,2], A. Parola[1,2], D.M. Posik[1,2], A. Sciocco de Cap[3] y V. Romanowski[1,2]. *[1]Dto. de Ciencia y Tecnología, CEI, Universidad Nacional de Quilmes, Bernal. [2]IBBM, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata. [3]IMYZA, CICA-INTA, Castelar, Argentina.*

The baculovirus family comprises some 600 viruses that are pathogenic for arthropods. Most of them are known to infect lepidopteran insects, and exhibit a narrow host range. Their genomes consist of circular supercoiled DNA ranging in size between 88.000 and 200.000 base pairs. An outstanding structural feature of baculoviruses is that virions are occluded in a cristalline protein matrix. According to the type of occlusion bodies, the baculoviruses may be grouped in two genera, *i.e.* nuclear polyhedrosis viruses (NPV) characterized by polyhedral cristal structures (1 - 15 μm) accumulating in the nuclei of infected cells, and granulosis viruses (GV) with smaller cytoplasmic elipsoidal inclusion bodies. These inclusion bodies consist almost exclusively of a single protein of *ca.* 30 kDa, polyhedrin and granulin in NPV and GV, respectively.

We have used the limited genome sequence information for four GVs and seventeen NPVs to design a pair of consensus oligonucleotides complementary to the 5´ and 3´ termini of the polyhedrin and granulin genes. These oligonucleotides were used to PCR amplify a 600-800 nucleotides region of three viral isolates from *Epinotia aporema*, *Erinnyis ello* and *Trichoplusia ni* larvae (agricultural pests of legume crops).

The DNA for these studies was isolated from inclusion bodies obtained from infected larvae, fractionated by a sequential homogeneization, diferential centrifugation, proteinase treatment, phenol extraction and ethanol precipitation. The DNAs were characterized by restriction enzyme analysis and the hybridization of the PCR amplified sequences. The genome sizes were also assessed from the sum of restriction enzyme fragments. Further studies on the genome organization will be instrumental for genetic enhancement of the bioinsecticidal potential of these insect viruses.

We acknowledge the support by Universidad Nacional de Quilmes, CABBIO, CIC BA and SECYT-CONICET (Subprograma de Innovación Tecnológica)

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AUXIN AND DEVELOPMENTAL REGULATION OF A PLANT eEF-1β GENE. [1]Manuel Gidekel and [2]Luis Herrera-Estrella. Departamento de Producción Vegetal, INIA - Carillanca, Temuco, Chile. [2]Departamento de Ingeniería Genética de Plantas, Centro de Investigación y de Estudios Avanzados del I.P.N., Unidad Irapuato, 36500 Irapuato, Gto., México

Regulation of protein synthesis occurs at several levels, including transcriptional and posttranslational mechanisms. Regulatory mechanisms also operate on the components of the translation apparatus, such as the elongation factors (EFs). These proteins regulate the specific interaction of aminoacyl-tRNA with the ribosome during the elongation phase of translation. The eukaryotic EF (eEF) is a pentameric complex consistent of four different subunits: 2α:1β:1γ:1δ. Recently, the elongation process has been suggested to be a key step in the regulation of the overall translation process. In order to examine some the factors affecting the expression of genes encoding elongation factors in plants, we conducted an analysis of the expression of an *Arabidopsis* eEF-1β gene promoter by fusing it to the GUS reporter gene. This promoter is expressed in tissues undergoing active growth, including apical and vegetative meristems, reproductive and vascular tissue. Since massive amounts of proteins, and hence of high levels of protein synthesis are required to sustain such growth, these results are in agreement with what would be expected from genes whose products are involved in protein synthesis. We also report that auxins is involved in the regulation of the eEF-1β gene. A model in which these growth regulators may modulate the expression of this gene in the context of the control of protein synthesis in different developmental stages, or as a result of environmental changes (i.e., light) is discussed.

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PROTEIN KINASES RELATED TO CELL DIVISION IN *Trypanosoma cruzi*. Gómez, E. B., Kornblihtt, A. R. & Tellez-Iñón, M. T. INGEBI and FCEyN, UBA, Buenos Aires, Argentina.

In eukaryotic organisms G1/S and G2/M cell cycle transitions are controlled by the activity of cyclin-dependent protein kinases (CDK). The prototype of this enzyme is p34, the product of the cdc2 gene. In protozoans such as *T. brucei, T. congolense, Leishmania* and *Crithidia fasciculata*, cdc2-related protein kinases (CRK) have been described.

In this study 2 genes named tcrk1 and tcrk2, corresponding to CRKs from epimastigote forms of *T. cruzi* were cloned using PCR. The sequences of these kinases showed very high identity with CRKs of other trypanosomatidae. Southern blot assays indicated that tcrk1 is encoded in the epimastigote genome by a single copy gene while tcrk2 is present as a two copy gene. RT-PCR assays showed that the mRNAs for these two kinases are expressed in epimastigotes (replicative form), as well as in trypomastigotes (non replicative form). Tcrk2 was expressed as a fusion protein to a hemagglutinin (HA) tag. Cos-7 cells were transfected with this construction and the TCRK2 protein was immunoprecipitated (IP) with anti-HA antibodies. This IP showed histone H1 and retinoblastoma (Rb) kinase activities demonstrating that tcrk2 belongs to the CDK family. Lysates of transfected cos-7 cells were IP with different anti-cyclin antibodies and immunoblotted with anti HA antibodies, indicating that TCRK2 associates primarily with cyclin E. These results show that *T. cruzi* possesses protein kinases involved in the regulation of the cell cycle, similar to those described in other eukaryotes

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LOW EXPRESSION IN *E. COLI* OF ATTACIN, A BACTERICIDAL PROTEIN FROM BY GIANT SILK WORM. DIFFICULTIES FOUND FOR CLONING AND BACTERIAL EXPRESSION. Gómez, I., Pezzoli, M., Mardones, G., Martínez, M.T., Bruce, E. and Venegas. A. Laboratorio de Bioquímica. Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Alameda 340, Santiago, Chile.

Attacin is a bactericidal protein detected in the haemolymph of the silk worm *Hyalophora cecropia* after injury or bacterial infection. The main goal of our research is to develop a strain capable to produce a bactericidal agent that could be used as biological control of bacterial pathogens.

We have tried different cloning approaches in order to express the bactericidal protein attacin in *E. coli* and we have partially succedeed but with a rather modest level of expression.

Initially we attempted to clone attacin cDNA from *H. cecropia* in different expression systems such as pPL-lambda/N99cI+ cells (thermo inducible), pOSEX/BL21(DE3) (IPTG inducible) and pTrxFus/GI724 with a thioredoxin plasmid (Trp-inducible), the last two being protein fusion systems and also pYES2 vector in INVSc1 yeast cells (sucrose inducible) with no success. In most of these cases no transformant was isolated but sometimes a few ones were isolated with bizarre deletions that precluded any putative expression of this gene.

Finally, using the pET/BL21(DE3) systems which included tightly regulatable IPTG-inducible plasmids (pET11d and pET21d), we were able to detect biological activity of the protein which immediately stopped bacterial growth after induction.

In order to enhace this low level of expression we are currently testing a new approach that involves to construct a hybrid protein with protease B from *Erwinia chrysanthemi* to secrete this protein to the external medium diluting its natural toxic effect.

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ISOLATION OF GENES DIFFERENTIALLY EXPRESSED DURING THE MALE DEVELOPMENTAL PROGRAM IN THE DIOECIOUS PLANT *Melandrium album*. **González, E.[1], Zuñiga, J.[1], Verdugo, I.[1], Theoduloz, C.[1], Ruiz-Lara, S.[1] and Mouras, A.[2] . (1) Programa de Biología Vegetal, Universidad de Talca, (2) Laboratoire de Biologie et Biotechnologie Vegetale, Université de Bordeaux II.**

The transition between vegetative and sexual reproductive growth in plants is the result of a well coordinated program of differential gene expression. When studying this proccess, the dioecious plant *Melandrium album* constitutes a suitable model. Due to the existence of heteromorphic sex chromosomes, it shows a clear sexual dimorphism, thus allowing the dissection of male and female developmental program.

In order to analyze the genes involved in the male flower development, we have utilized the substractive hybridization and the differential display methods to identify mRNAs specifically transcribed during the different stages of this proccess. After genetic expression studies by Northen and *in situ* hybridization, several cDNA clones, corresponding to genes expressed during the early events of male differentiation, has been identified. The screening of a *M. album* genomic library allowed the isolation of the respective genes, which are under molecular characterization.

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The influence of *ilv*C mutations on *nod* expression is strain dependent in *R.meliloti*. Grasso. D.H. López, J.C., Riccillo, P., and Aguilar, O.M.. Instituto de Bioquímica y Biología Molecular, Facultad de Cs. Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

Our previous studies have indicated that a functional rizobial *ilv*C gene is required for a successful symbiotic association. We have studied a *R.meliloti* auxotrophic mutant for isoleucine and valine (strain *Rme* 1028) who is unable to infect the alfalfa roots. In this mutant, the *ilv*C mutation completely abolish the luteolin-inducing activity of the *common nod* genes (Aguilar, O.M., and D.H.Grasso.1991. J. Bacteriol. 173:7756-7764). However, the pleiotropic effect of a mutation in a metabolic gene does not rule it out the possibility that the Nod$^-$ phenotype might be a consequence of several alterations in addition to an absence in the *common nod* gene expression. It has been reported the construction of a *nod*D hybrid between the *R.meliloti* and *R.leg.*bv trifolii *nod*D genes which confers a FITA phenotype (Flavonoid Independent Transcriptional Activity) in *R.meliloti*. Here we will show that the introduction in *Rme* 1028 of this hybrid *nod*D does not restore the symbiotic capacity. Furthermore, we have isolated another *R.meliloti ilv*C mutant derived from a different parent wild type strain (L5-30), that showed a Nod$^-$ phenotype but contrarily to the mutant 1028, the levels of *nod* gen activation was similar to the wild type strain. In this mutant (as in *Rme* 1028) the introduction of the *R.meliloti ilv*C gene restores both the prototrophy and the Nod$^+$ phenotype. This results suggest the needs of additional steps between *nod* expression and the step in which plant response is detected.

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GENETIC STUDIES ON THE ATCC 33020 *Thiobacillus ferrooxidans* STRAIN. Guiliani, N., Bengrine, A., Borne, F., Chippaux, M., Bonnefoy, V. Institut de Biologie Structurale de Marseille. Laboratoire de Chimie Bacterienne. Marseille, France.

T. ferrooxidans is one of the main microorganisms involved in bioleaching, that is solubilization of the metals from minerals. Ferrous iron oxidation involves different proteins in which the rusticyanin, a blue copper protein, is one of the most intriguing. To study the physiological role of these proteins, we started a reverse genetics program. For this, we decided to look for the *rus* gene, coding the rusticyanin, and to develop the electro-transformation procedure to introduce DNA into *T. ferrooxidans*. With a first approach using degenerated primers to screen a genomic bank, we cloned the *recA* gene coding the RecA protein, the *recX* gene coding the RecX protein and the *alaS* gene coding Alanyl-tRNA synthetase. These *T. ferrooxidans* genes showed the same genomic organization of the *E. coli* ones. The *AlaS* gene was a monocistronic unit and his promotor was not recognized by the *E. coli* RNA polymerase. With a second approach, using degenerated primers in PCR experiments, we have cloned the *rus* gene. The DNA sequence analysis of the *rus* gene encoding and flancking regions revealed the presence of many stem/loop structures. Only one copy of the *rus* gene was present on the genomic DNA and it was also a monocistronic unit. Regarding the genetic transfer method, we have demonstrated that the ATCC 33020 strain is electropermeabilizable.

Supported by l'Agence de l'Environnement et de la Maîtrise de l'Energie (A.D.E.M.E.), le Bureau de Recherche Géologique et Minière (B.R.G.M.), la Compagnie Générale des Matériaux (CO.GE.MA.) and le Centre National de Recherche Scientifique (C.N.R.S.).

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MOLECULAR INVESTIGATIONS INTO THE HIGH RATE OF GENETIC MUTATION OF THIOBACILLUS FERROOXIDANS: IMPLICATIONS FOR INDUSTRIAL APLICATIONS.

Eugenia Jedlicki, Maria Eugenia Cabrejos, Osmán Vásquez, and David Holmes. Depto. Bioquímica, Facultad de Medicina, Universidad de Chile and Depto. Ciencias Biológicas, USACH, Santiago, Chile.

T. ferrooxidans is a microorganism that is involved in the commercial recovery of copper. It oxidizes iron and sulfur compounds in the environment, releasing copper which can then be recovered using chemical engineering procedures.

We have observed that *T. ferrooxidans* undergoes rapid phenotypic and genetic changes in the laboratory. Detailed investigation of one of these changes demonstrates that it is caused by the reversible insertion of an insertion sequence, termed IST1, into an ORF that we postulate encodes a protein involved in iron oxidation.

In this poster we present the DNA sequence of IST1 and of the region of its integration into the genome. We present an analysis of the evidence for the reversible insertion of IST1 and we propose a model to explain the resulting phenotypic changes. We discuss how this type of extraordinarily rapid mutational event might be important in the production of strains of *T. ferrooxidans* for industrial applications.

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MODIFIED OLIGONUCLEOTIDES WITH POTENTIAL APPLICATION IN GENE THERAPY

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The synthesis of modified oligonucleotides has received a great deal of attention during the last few years, due to the very important applications that this class of compounds has displayed as antisense fragments. Several different modifications have been proposed in order to obtain molecules that are stable to the action of nucleases and that can recognize complementary sequences.

Very recently, a new strategy has been developed based on the use of ribozymes as antisense molecules. The improvement of this approach is the generation of a catalytic activity.

In the design of both chemically modified antisense oligonucleotides and ribozymes several steric requirements must be taken into account.

The results obtained so far regarding the synthesis, biological properties and hybridization characteristics of a new class of antisense probes, namely (2'S)-2'-deoxy-2'-C-methyloligo nucleotides, as well as the recent advances in the design of a chemically modified ribozyme that carries the essential 2'-hydroxyl group will be shown in this poster.

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CNS' MITOCHONDRIAL ELECTRON TRANSPORT FROM GENERATIONAL "ACETALDEHIZADED" RATS & ROL INTRAMITOCHONDRIAL Ca^{2+}.
Egaña, E., Velásquez, P., Jara, J. & Osorio.C. University of Chile - Faculty of Medicine;Institute of Experimental Medicine; Lab. of Neurochemistry, Santiago 7 - Chile.
There is strong evidence that the primary oxidation product of the EtOH is AcCHO, reaction which occurres in liver, CNS, neuroendocrine systems, etc. The deleterious effect of drinking EtOH has probably simultaneous effects: (i) The EtOH effect per sé and (ii) AcCHO properly. We have been interested to know which is the AcCHO own deleterious action on some CNS' areas. We reported (1986) results on the "in vitro" action to CNS' electron transfer and proton movement of different low AcCHO dose on CNS' mitochondrial areas. This result suggested us to study the AcCHO deleterious effect "in vivo" and so we created the "acetaldehizaded" rats whose received during life (intra & extrauterine) dose of AcCHO permissible.At present we have 26th generation. Adult "Acetaldehizaded" ♂ & ♀ rats (17th, 18th & 19th generations) received dayly AcCHO i.p. 200 mg/Kg rat/ 24 h. Five CNS areas: brain cortex, hypothalamus, hippocampus, cerebellum and midbrain (normal vs "Acetaldehizaded"); mitochondria obtained through differential centrifugation.
Two sites of the e Site I piruvate-malate and Site II succinate. Electrodes O_2 & Ca^{2+} this last to study the incorporation intra mitochondrial (Ca^{2+}).
The Site more affected was I NADH dependent; ADP/O was affected only in ♂; the velocity of Ca^{2+} mitochondrial incorporation shows deleterious effect in ♂ not occurring in ♀.
Generational AcCHO show an inhibition effect on mitochondrial electron transfer in CNS' studied Site I and Site II in 5 CNS' areas above mentioned.

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EXPRESSION OF TRANSCRIPTIONAL FACTOR PIT-1 AND GROWTH HORMONE DURING THE ACCLIMATIZATION OF THE CARP. Kausel, G., Figueroa, J., Hernandez, J., San Martin R. and Krauskopf, M. Instituto de Bioquímica, Universidad Austral de Chile, Valdivia, Chile.

Seasonal changes of environmental factors cause a complex compensatory response in the eurythermal teleost *Cyprinus carpio* entailing, according to our working hypothesis, coordination by the hypothalamus-hypophyseal axis. In this context, we undertook the study of the tissue specific transcription factor Pit-1 and the regulatory sequences of one of its target genes: the growth hormone gene.

Screening of a carp genomic library with a carp POUs-specific probe, generated by PCR using oligonucleotides, derived from the highly conserved POU-domain of Pit-1, led to the isolation of two genomic Pit-1 clones, GP1 and GP5. The sequence of the subclone $pGP5_{1700}$ reveals four coding regions corresponding to exons III, IV, V and VI as estimated by the genomic organization of the Pit-1 gene in mammals. Additional genomic clones, GP6 and GP7, were isolated by screening with an exon III specific probe and are currently being characterized. Concurrently, we have isolated recombinants containing the 5'-upstream region of the carp growth hormone gene.

Expression of Pit-1 as estimated by *in situ* hybridization reveals clear differences between winter- and summer-acclimatized fish, whereas growth hormone levels do not exhibit such notorious seasonal differences. A more quantitative evaluation of seasonal Pit-1 and growth hormone expression is being now approached by RT-PCR.

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CHARACTERISTICS OF DNA STRUCTURE IN THE 3' UNTRANSLATED REGION OF MAMMALIAN GENES. Kleiman, F.E. and Argaraña, C.E. Departamento de Química Biológica, CIQUIBIC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.

Previously, we found that a 310bp fragment corresponding to the 3' untranslated region (UTR) of the human β-Hexosaminidase (HEXB) gene has an anomalous low mobility on polyacrylamide gel electrophoresis (PAGE). In addition to this property, the fragment showed a nucleotide composition and sequence arrangement similar to those described for bent DNA.

A "TG" deletion located 7bp from the polyadenylation signal partially eliminated the anomalous migration of this fragment. This dinucleotide is part of a consensus sequence present in many mammalian genes. In this work we determined that a bent structure was also present in the 3'UTRs of other genes, suggesting that this structure is likely to occur in many mammalian genes.

We subcloned the bent fragments (+TG and ΔTG version) in pBS and analyzed their effect on some properties of the close circular and linear plasmid constructions. It was found that both plasmid constructions had a different degree of supercoiling. Both constructions were linearized with restriction enzymes at different positions and the gel mobility was analyzed on PAGE. Only the linear plasmids containing any of both fragments at the end of the construction showed an anomalous mobility.

The presence of a bent DNA structure in the 3'UTRs of some mammalian genes and its effect on surrounding sequences suggest a possible functional role for this structure.

These studies were granted by CONICET, CONICOR, SECYT (UNC), and TWAS.

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IDENTIFICATION OF A NOVEL GARLIC-MITE BORNE VIRUS PRESENT IN ARGENTINA. Helguera M.[1,2], Bravo-Almonacid F.[1], Kobayashi K.[1], Rabinowicz P.[1], Conci V.[2] and Mentaberry A.[1] [1]INGEBI (CONICET/FCEyN-UBA) and [2]IFFIVE-INTA. Argentina. E-mail: amenta@proteus.dna.uba.ar

In garlic, as in most crops which are vegetatively propagated, viruses produce significative yield looses. Disease is induced by an infective complex including a variable number of viral members that are difficult to isolate and purify due to their relatively restricted host-range. This often introduces confusion when traditional plant virology criteria, such as morphology and serology, are used to identify individual viruses. A cDNA library was obtained using as template RNA extracted from a mix of garlic viruses. At random sequencing of several clones allowed the identification of a genomic sequence (pCC6) that was 91% homologous to garlic virus A (GarA), a novel filamentous virus that was also included among the garlic mite-borne viruses (MbFV-G). This sequence contained the complete viral coat protein gene. This viral gene was amplified by PCR and the DNA fragment obtained was cloned in the plasmid vector pBSSK. The cloned insert was sequenced and subsequently subcloned in the expression vector pRSETa. *E. coli* cells transformed with this plasmid yielded a 31 kDa polypeptide that was recognised by anti-MbFV-G antibodies, but not by other antisera directed against different poty- and carlaviruses commonly infecting garlic. The 31 kDa polypeptide was then purified and used as antigen to produce polyclonal antibodies. In a survey performed with infected garlic plants, the anti-31 kDa antiserum reacted only with extracts that were negative to OYDV, LYSV or CLV antibodies. This antiserum is currently being assayed for use in immunosquash an DAS-ELISA tests as a diagnostic tool.

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CHARACTERIZATION OF AN ARGENTINE STRAIN COLLECTION OF ACID TOLERANT ALFALFA NODULATING RHIZOBIA. Del Papa, M. F (1), Balagué, L. J. (1), Aguilar, O. M. (1), Castro, S. (2), Martínez-Drets, G. (2), Martínez Abarca, F.(3), N. Toro (3), Niehaus, K. (4), Pühler, A. (4) and Lagares, A. (1).

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The alfalfa fields of Argentina cover more than 4.10^6 ha being a significant proportion under acid soil conditions (soil pH between 6.0-5.0). The nitrogen fixing symbiosis established between *Rhizobium meliloti* and alfalfa becomes seriously altered in acid soils environments accounting for a significant reduction in the yields and persistence of cultures. Current research supports that low pH affects both symbionts, the plant and the bacteria. In our laboratory we begun a project to investigate basic mechanisms of acid tolerance in alfalfa nodulating rhizobia (ANR). In a first stage of the project a collection of more than 300 ANR was established (LPU collection) and screened for the presence of acid tolerant isolates. Growing tests in laboratory conditions revealed 13 isolates having the ability to grow at pH 5.0. These rhizobia were characterized in their growing kinetics (SAIB, 1995), melanin production ability, temperature tolerance (39°C), and lipopolysaccharide profile in SDS-PAGE. All isolates had a Nod^+ phenotype in alfalfa and showed a positive signal in a PCR assay designed to detect an internal fragment of the *nifH* gene. Plasmid analysis in agarose gels by an *in situ* bacterial lysis procedure, and genomic fingerprinting PCR (ERIC, MboREP, BOXC) indicated that most acid tolerant isolates share a closely related genetic background, and similar to that of strain *Rhizobium* spp. OR191 previously described as an acid tolerant rhizobia that nodulate both alfalfa and common beans (Bertrand et al., Appl. Environ. Microbiol. **58**:1809-1815, 1992). Preliminary plant tests indicated that OR191 and the acid tolerant LPU strains are also Nod^+ on *Leucaena leucocephala* showing a broad host-range for nodulation of legumes. However, all strains presented a weakly Fix+ phenotype in alfalfa. We started to carefully characterize the symbiotic properties of the strains and the genetic basis of the acid tolerant phenotype.

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A PRACTICAL APPROACH FOR BACTERIAL DNA WALKING. Petrucelli, S. (2), Pich Otero A. (1), Wynne, M.E. (1), Hozbor, D (1) and Lagares, A. (1).

(1) Instituto de Bioquímica y Biología Molecular (IBBM), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina.

(2) Centro de Investigaciones y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata - CONICET, Argentina.

Experimental strategies to get partially overlapping genomic clones (DNA walking) are usually based on library screening procedures involving tedious hybridization steps. A different approach for bacterial DNA walking based on homologous recombination and simple cloning steps is described here. The rationale of the strategy underlies on the following steps: 1) Cloning of a bacterial DNA fragment from which the chromosome walking is desire to start. The construction has to keep at least one restriction site (i.e. recognized by enzyme A) beside the insert. The orientation of the fragment regarding this restriction site will define the direction of the walking in the genome. 2) Transfer of the cloned fragment to the target bacteria containing the genome to be walked. The vector should not replicate in this host. 3) Selection of clones in which the vector has been integrated by a single site specific recombination event (one crossover), and 4) Recovery of the original plasmid carrying an extended host DNA sequence by digestion of total DNA with enzyme A, ligation, and transformation of *E. coli* electrocompetent cells. The recovered construction will include the original plasmid sequence plus a piece of DNA that extends up to the next recognition site. In our laboratory we have successfully used this method to make progress in the cloning of a *Rhizobium meliloti* transcription factor. The method presented here can be easily extended to other gram-negative and gram-positive bacteria in which site specific homologous recombination has already been used as a genetic tool.

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IDENTIFICATION AND MOBILIZATION OF CRYPTIC PLASMIDS FROM ARGENTINE SOIL ISOLATES OF *Rhizobium meliloti*. Pistorio, M., Aguilar, O. M., and Lagares, A.

IBBM, Fac. Cs. Exactas, Univ. Nac. La Plata, Argentina.

Transfer of genetic information among bacteria is well a documented phenomena. In particular, the frequency of plasmids transfer between soil bacteria is currently under active investigation, as plasmids can actually exchange genetic material with the chromosome, Therefore, transmissible plasmids should also be considered as important carrier for chromosomal gene transfer among bacteria.

In our laboratory, we are interested in the characterization of plasmid transfer in soil conditions. Using *Rhizobium meliloti* (*Rme*) as a model system, we devised a strategy to search for indigenous mobilizable plasmids within a collection of isolates from the central area of Argentina. Initially, each member of this collection was examined for the plasmid profile by using an *in situ* bacterial lysis procedure. Results showed at least 22 different banding profiles. Most isolates (85%) presented more than one plasmid band. The most frequent plasmid pattern presented four bands (25%). The characteristic two symbiotic plasmids from *Rme*, pSym a and pSym b, were not resolved in our experimental conditions. All plasmid profiles and their relative frequency will be shown.

It was observed that isolates from a same soil sample presented a very similar plasmid profile, but some of them shown an additional plasmid (designed as the "differential" plasmid). In one of these strains, LPU88, we investigated if this additional plasmid was naturally transmissible. LPU88 was mutagenized randomly with Tn*5* (Nm^r), and all the Tn*5*-mutants were used *in mass* in a mating as potential donors of the neomycin marker to another recipient rhizobia (*Rm* 2011, Sp^r). *Rm* 2011 Sp^r -Nm^r transconjugants were analyzed for the presence of any extra plasmid that has been detected in the original donor strain. By using this procedure we could isolate (only) the "differential" plasmid from strain LPU88. We do not know at the moment if the rescued plasmid is self transmissible or requires helper functions. Now, we will investigate the mobilization properties of this indigenous transmissible plasmid in the laboratory and in soil conditions.

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STUDIES ON THE SPECIFICITY OF tRNA REMOVAL BY THE RNase H DOMAIN OF HIV-1 REVERSE TRANSCRIPTASE. [1] Smith, C., [2] Leon, O. and [1] Roth, M.

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Previous studies have shown that an isolated domain of HIV-1 RNase H recognizes the cognate $tRNA^{lys,3}$/DNA viral intermediate of reverse transcription (Smith and Roth, J. Virol., **67**, 4037 (1993)).

In order to define the substrate determinants for tRNA removal, studies involving truncation of the RNA portion of this substrate, have been performed. The results indicate that the terminal 8 ribonucleotides are sufficient for specific cleavage by the isolated RNAse H domain. Further truncation showed that substrates containing the terminal 6 ribonucleotides are also specifically cleaved, although at lower efficiency than the wild type substrate.

Mismatches and BrdU residues were also introduced at specific sites of the substrate as a first approach to determine the positions important for recognition. The effect of these mutations on the rate and specificity of cleavage is presented. The results indicate that the determinants for specific tRNA removal by the HIV-1 RNase H domain lie within the 6 ribonucleotides of the acceptor stem of $tRNA^{lys,3}$.

The isolated domain of HIV-1 RNase H is unable to cleave the MLV's cognate substrate $tRNA^{pro}$. However, this substrate can be efficiently cleaved after introducing specific base changes within the RNA portion.

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A METHOD FOR SITE-DIRECTED MUTAGENESIS USING ALTERED SPECIFICITY OF ß-LACTAMASE FOR SELECTION.
Lesley, S. A. and Vavra, S. Promega Corp., 2800 Woods Hollow Rd., Madison, WI 53711-5399 USA

Site-directed mutagenesis techniques typically involve a selection step to enrich for the desired mutation. Current methods are limited due to inefficiency of the selection step, secondary mutations from low fidelity polymerases, or special strain or vector requirements. We have developed a novel method for site-directed mutagenesis which uses antibiotic selection as an enrichment for desired mutations. The principal requirement of the method is a functional ß-lactamase gene in the desired target. This gene is common to virtually all cloning vectors and provides resistance to ampicillin typically used for plasmid selection. The method can be used with single- or double-stranded DNA and requires no specialized strains or vectors. High fidelity T4 DNA polymerase is used keeping unwanted secondary mutations to a minimum.

Mutations in the active site of ß-lactamase can confer resistance to a broad spectrum of ß-lactam antibiotics. This change in substrate specificity of the ß-lactamase enzyme is coupled to the desired mutation resulting in mutagenesis efficiency as high as 100%. The extended spectrum ß-lactamase retains resistance to ampicillin allowing subsequent selection on this antibiotic. Mutations at positions 69, 104, 164, 238, 240, 241, and 244 in the active site of ß-lactamase were constructed. The resistance of these mutations to a number of ß-lactam antibiotics was determined to identify conditions for selection.

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MOLECULAR CLONING OF MANGANESE PEROXIDASE AND LACCASE FROM THE LIGNINOLYTIC FUNGUS *Ceriporiopsis subvermispora*. Lobos, S., Karahanian, E., Corsini, G., Larrondo, L.F., Salas, L. and Vicuña, R. Laboratorio de Bioquímica, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile.

C. subvermispora produces two families of ligninolytic enzymes, namely manganese-dependent peroxidase (MnPs) and laccases. Four different clones were isolated from a cDNA library using an heterologous probe for MnP from *P. chrysosporium*. The complete sequence of MnP13-1 clone consists of 1,285 nucleotides, excluding the poly(A) tail. The deduced mature protein contains 364 amino acids, which are preceded by a 24 amino acid leader sequence. Consistent with the general mechanism of peroxidases, proximal His, distal His and distal Arg are all conserved in MnP, while regions flanking these residues display homology with other peroxidases. Like MnP from *P. chrysosporium*, the manganese binding sites E^{35} and E^{38} are conserved. A four amino acid motif, L/V/I-P-X-P, designed as an aromatic binding site, shares high homology with other peroxidases, although it is less hydrophilic. This suggests that MnP13-1 from this fungus is able to bind and oxidaze aromatic compounds in the absence of Mn(II), which is in agreement with recent observations in our laboratory.

A gene coding for MnP from *C. subvermispora* was cloned by PCR amplification using upstream and downstream primers designed from the MnP13-1 clone. Seven shorts intervening sequences as well as consensus intron/exon splicing sequences have been identified within the *mnp* gene. In turn, to clone a genomic laccase gene from *C. subvermispora* by PCR, two degenerate primers were designed using the codon usage frequence obtained from cDNA MnP clone. The upstream primer derived from the N-terminal sequence, whereas the downstream primer corresponded to an amino acid sequence located aproximately 60 residues before the C-terminal, which is conserved among five laccases from different fungi.

Financed by FONDECYT 0649/94

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ENDOGENOUS RETINOIC ACID REGULATES SONIC HEDGEHOG EXPRESSION AND ANTERIOR-POSTERIOR (A-P) PATTERNING IN *Xenopus laevis* EMBRYOS. López, S., Paganelli, A., and Carrasco, A. Instituto de Biologia Celular y Neurociencias. Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires. Argentina. (e-mail: rqcarras@criba.edu.ar).

Retinoic acid (RA) treatments of vertebrate embryos produce anterior shifts of Hox genes domains within the hindbrain. A graded distribution of endogenous retinoid activity regulating the patterning of the primary A-P axis was proposed, with a caudally located highpoint. To study whether endogenous retinoids actually regulate A-P patterning, we treated *X. laevis* embryos with the retinoid antagonist Ro 41-5253 (Ro) during gastrulation. We observed a dramatic caudal expansion of *Krox-20* domains demarcating rhombomeres 3 and 5 and a down-regulation of *Hoxc-6* expression in the spinal cord. These results, together with the previously reported down regulation and caudal shift of *Hoxb-7* protein in *X. laevis* and chicken embryos (López et al., 1995) are opposite to RA-induced phenotypes and indicate that endogenous retinoids are actively involved in A-P patterning of the main body axis. Treatment with 10 µM RA at the beginning of gastrulation increases the expression of X-*shh* in the posterior notochord and reduces X-*Shh* expression in the anterior floor plate. In contrast, 7.5 µM Ro decreases X-*shh* expression in the posterior notochord. These results together indicate that at least two regulatory domains of *X-shh* expression can be observed in the notochord and that endogenous retinoids regulate the spatial expression of *shh* in the caudal domain. In complete exogastrulae, where vertical transmitted signals from the axial mesoderm to the overlying ectoderm during gastrulation are absent, X-*shh* transcripts were present in the notochord. In contrast, we did not detect expression in the ectodermal sac. Therefore, planar signals are not enough to induce X-*shh* in the ectodermal sac of exogastrulae. In conclusion, the expression of X-*shh* by floor plate cells may depend on inductive signals from the underlying mesoderm during normal development. RA could not mimic the vertical signal that induces *shh* in the ectodermal sac of exogastrulae, suggesting that RA may be one of the factors involved in the activation of *shh* in the notochord, but not in the neural ectoderm. This research was made possible by grants from the E.E.C. (EU CT93-0017) and Fundacion Antorchas (A-13359/1).

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Characterization and screening of a genomic YAC library for the *Trypanosoma cruzi* Genome Project.

Lorenzi, H.*; Medrano, C.*; Ferrari, I.*; Santos, M.♣; Cano, I.♣; Schijman, A.*; da Silveira, F.♣; Levin, M.J*. INGEBI-CONICET, Buenos Aires, Argentina*; EPM, Sao Paulo, Brazil♣.

As part of the *Trypanosoma cruzi* Genome Project a *T.cruzi* YAC library was constructed at CEPH (Paris, France). The DNA chosen for the library construction derived from the *T.cruzi* CL strain (clone CL-Brener). Its PFGE karyotype reveals 20 chromosomal bands: 12 ranging from 1 to 3.5 Mb, and 8 between 0,45 and 1 Mb. The library contains 3000 YACs with a mean size of 365 Kb representing more than 10 genome equivalents. A portion of the library has been characterized to test its representativity and stability. PCR screening protocols of pooled arrays of YACs have been used to identify YACs containing house-keeping genes, genes encoding relevant antigens, as well as repetitive elements, such as SIRE. Stability assays demonstrated that YACs were stable through several replications.

The screening of the YAC library was also performed with labeled *T.cruzi* derived DNA probes, such as JL7, which hybridize with the chromosomal band XVI of 2,3Mb, ribosomal P1 protein gene, that hybridized with chromosomal bands XIX, XX, and SZ5 sequence that maps to chromosomal band III and VII. Several positive clones were isolated, among them, a 400 kb YAC that hybridized with JL7 probe was further characterized. Its restriction pattern was similar to the one obtained by genomic Southern hybridization with JL7 probe, indicating the lack of cloning artifacts such as chimeras and deletions. Interestingly, when this YAC was probed to CL-Brener electrokaryotypes, only the chromosomal band XVI was revealed, indicating the lack of repetitive sequences. This feature suggest that repetitive sequences could not be uniformly distributed along the genome.

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MOLECULAR IDENTIFICATION OF NOVEL ARENAVIRUSES.
Lozano, M.E.[1,2], Posik, D.[1,2], Albariño, C.G.[1,2], Ghiringhelli, P.D.[1,2], Roberts, L.T.[2], Schujman, G.[2], Calderón, G.[3], Sabattini M.[3] & Romanowski, V[1,2]. *1:Dto. de Ciencia y Tecnología, CEI, Univ. Nac. de Quilmes, Bernal; 2: IBBM, Dto. Cs. Biológicas, Fac. Ciencias Exactas, Univ. Nacional de La Plata, La Plata; 3: Instituto Nacional de Estudios sobre Virosis Humanas, Pergamino, Argentina.*

Arenaviruses are enveloped viruses with a genome composed of two ssRNA species. Both RNAs, designated L (large, 7kb) and S (small, 3,4 kb), have an ambisense coding strategy. The arenaviruses were divided in two major groups (Old World and New World), based on serological properties and genetic data, as well as the geographic distribution.

We used an RT-PCR based assay to detect and molecularly characterize novel arenaviruses. A 19-nucleotide long sequence at the 3' end is characteristic of all sequenced arenavirus S RNAs. This sequence is also conserved in the L RNAs and is complementary to the 5' termini of both genomic RNAs. We did a sequence alignement analysis on all reported of arenavirus S RNAs and identified 17 additional conserved regions. The consensus sequences of these regions were used to design generalized amplification primers to RT-PCR amplify a set of overlapping cDNA fragments comprising the complete arenavirus S RNA. A restriction analysis (RFLP) was designed to rapidly typify the amplified fragments. This RT-PCR/RFLP approach was tested with Old World (LCM) and New World (Junin and Tacaribe) arenaviruses. Furthermore, using this procedure the whole S RNA of two novel arenaviruses, isolated from rodents trapped in central Argentina, were amplified and characterized. The partial nucleotide sequence data were used for phylogenetic analyses that showed the relationships between these novel arenaviruses and the rest of the members of the family. This relatively simple methodology will be useful both in basic studies and epidemiological survey programs.

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CHARACTERIZATION OF ORGANELAR DNA IN THE GREEN ALGAE *DUNALIELLA SALINA*

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Molecular tools are being used to study phylogenetic relationships between plant and algal organelles. There is a significant amount of information regarding brown algae (Phaeophyta), red algae (Rhodophyta) and land plants, but only little is known about green algae (Chlorophyta). In an effort to obtain more information on this topic, we have started the characterization of organelar DNA in the green algae *Dunaliella salina* (Chlorophyta, Chlorophyceae, Dunaliellaceae).

Organelar DNA has been prepared from liquid cultures of *Dunaliella salina* and purified using CsCl-bisbenzimide density gradients. Conserved primers for the large subunit of Rubisco (*rbcL*) were synthetized and used for PCR amplification, yielding the expected 1.2 kb product. DNA sequencing confirmed the *rbcL* identity and demonstrated the presence of chloroplastic DNA in our preparations. Similar attempts to amplify a mitochondrial DNA fragment by PCR and Southern analysis using mitochondrial probes were unsuccesful, suggesting the absence of this organelar DNA in our preparations. Digestion of this DNA with various restriction enzymes shows a characteristic pattern that allowed us to estimate the size of the chloroplast genome (~134 kb).

An organelle genomic library was constructed in Bluescribe and a 5 kb EcoRI clone containing *rbcL* was isolated using the 1.2 kb PCR product as a probe. This clone had been characterized by restriction mapping and sequence determination, and the *rbcL* sequence is being used to perform phylogenetic analysis.

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TOWARD THE IDENTIFICATION OF PUTATIVE RECEPTORS INVOLVED IN SPECIFIC POTATO-*PHYTOPHTHORA INFESTANS* INTERACTION.
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The initial infection process involving adhesion/recognition events between plants and fungal pathogens is essential for the establishment of pathogenesis. The basis of the specificity of potato-*Phytophthora infestans* (P.i.) could lie on the recognition between Extracellular Matrix-Plasma membrane (ECM-PM) components of fungal zoospores and host cells during earlier stages of infection.

The main purpose of this work was to identify the ECM-PM components that could be involved in the specific potato P.i. interaction. We have used the anti-Idiotypic (*anti-IT*) antibodies as a tool to detect ECM components from potato. The *anti-ITs* were generated as follow: 1) injecting rabbit with zoospores or cysts of P.i. (*AZ* and *AC*, respectively); 2) purifying rabbit IgGs (idiotypes *AZ* and *AC*) and injecting them in mouse in order to produce *anti-ITs*. These *anti-ITs* (called *a-AZ* and *a-AC*) were able to elicit plant defense responses commonly observed in potato infected tissues.

Western blot experiments were performed on tuber and leaf components. *Anti-IT a-AC* detected two bands of approximately 25 and 22 kDa from tuber ECM. These bands were absent when *a-PI* (anti-preimmune idiotype) was used. From leaf-component preparations, *a-AC* detected bands of approximately 55 and 25 kDa in total proteins from epidermal extractions, while bands of approximately 35 kDa were revealed from ECM fractions. Screening of expression libraries with *anti-ITs* are in progress to isolate cDNA clones corresponding to recognized bands.

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DETECTION AND TYPING OF MYCOBACTERIA BY PCR.
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Tuberculosis is the principal cause of dead worldwide from a single infectious disease. Emergence of multidrug resistant strains and high incidence of tuberculosis in AIDS patients worsen this situation. Furthermore, the presence of atipic forms complicates the clinical diagnosis. Most diagnostic methodologies, with different degrees of specificity and sensitivity, make identification of the etiologic agent slow and tedious. In this work we used PCR for the detection of mycobacteria tha were further the characterized by PCR fingerprinting. We used primers designed against repetitive genetic elements from different bacteria (*ERIC* y *REP* from Gram negative, *BOX* from Gram positive and *IS* from mycobacteria) to classify micobacteria and typify *M. tuberculosis*. The method was optimized with DNA extracted from isolated cultures of mycobacteria. The fingerprint patterns obtained in PCR with primers *ERIC*, *REP* and *BOX* allowed differentiation among almost all members of Mycobacterium genera. In these assays, only *M. tuberculosis* and *M. bovis* yielded similar patterns. On the contrary, the fingerprint patterns obtained in PCR with *IS* primers gave distinct patterns for both species and differentiated strains of *M. tuberculosis*. Combining these primers with other primers designed in our laboratory it was possible to maintain the discriminative power of the fingerprinting technique while adding an internal control of specific PCR. The target for one of these primer pairs (*MYC*) was part of the 16 S rRNA gene of all mycobacteria, and the other (*SOD*) was specific for *M. tuberculosis* superoxide dismutase gene. Finally, the results correlated well with epidemiological data and the assay is simple enough to be used as a rapid method for *Mycobacteria* typing.

This work has been supported by CEI, UNQ, Argentina.

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HEAT SHOCK PROTEINS REVEAL TWO METABOLIC STATES AND DISTINGUISH TWO MAIN REGIONS IN *Echinococcus granulosus* PROTOSCOLECES.

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As an approach to understand the molecular mechanisms of parasitic adaptation, we have studied the stress response revealed by heat shock, in the plathyhelminth *E. granulosus*. Protoscoleces (PS), larval form of the parasite, preincubated at 24°C, were subjected to temperature shifts to 37°, 39° and 42°C, and synthesised protein profiles were analysed. We found that PS, obtained from different intermediary hosts (ovine, bovine, porcine and human) can be in two distinct metabolic states, as evidenced by protein synthesis. In the first state, parasites show a typical heat shock response; the main heat shock proteins (HSPs) have been identified by one and two dimensional gel electrophoresis analyses, and some of them were further characterized by Western blotting. Their apparent molecular weights were 93, 76, 69 and 60 kDa. In the other state, PS isolates show an overexpression of Hsp69 and Hsp93, a reduction of the general protein pattern and do not exhibit further response when subjected to temperature shifts. When kept at 4°C for several days, PS acquire the ability of synthesise the general protein profile.

Preliminary studies on Hsp70 localization by immuno-histochemistry on whole mount, showed strong staining during early developmental stages of PS. In fully developed PS, at 24°C, Hsp70s signals appear in the apical regions of invaginated PS whereas at 42°C, a differential expression between the rostelar and the body regions of evaginated PS is detected.

These results show that PS obtained from different hosts can be under two main metabolic states, not previously described, which could be related to the biology of this parasite. Hsp70s expression could be an indicator of developmental and metabolic activities in PS.

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AMPLIFICATION, CLONING AND NUCLEOTIDE SEQUENCE OF A FRAGMENT OF CHICK PROTEIN KINASE C.

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Protein kinase C, a phospholipid-dependent serine-threonine kinase, is thought to play a key role in signal transduction of many hormones. Crucial roles have been assigned to members of the PKC family such as in the regulation of the cell cycle and modulation of ion channels. PKC consists of a family of related proteins. Although they have many structurally similar elements, differ from one another in cellular and tissue distribution. While the conventional PKCs (PKCα, βI, βII y γ) require Ca^{2+} for their activation, the novel PKCs (δ, ε, η and θ), lack the calcium-binding domain and can be activated without Ca^{2+}. All these isoforms can be activated by phorbol esters in the absence of Ca^{2+}, phospholipids or diacylglycerol. A third group, the atypical PKCs (λ, ζ and μ) require only phosphatidylserine for activation and do not bind to phorbol esters. Previous studies of our laboratory have demostrated that PKC mediates both rapid-non genomic and genomic effects of $1,25(OH)_2D_3$. In addition, we have obtained immunological evidence on the presence of different PKC isoforms in cultured chick muscle cells. In order to study the participation of individual PKC isoenzymes in $1,25(OH)_2D_3$ actions we have planned to design specific antisense oligonucleotides. This requires to know the nucleotide sequence of chick PKC isoforms. The objective of the present work was to amplify a fragment of chicken cDNA by PCR using degenerated primers corresponding to highly conserved protein regions (C4) in mammals. A fragment of 320 bp was obtained. The specificity was corroborated by Southern Blot with an heterologous probe from human PKCα. The fragment was cloned in a pGem-T vector system and amplified in E.coli DH5aF. After purification by Wizard Minipreps, the nucleotide sequence was determined using a *fmol* kit (Promega) and [α^{32}P] dATP. Gels were exposed to X-ray film. Computational analysis of the nucleotide sequence showed a 53% of homology and the protein sequence analysis showed a 64% of homology with mammal PKC isoforms. These data are the first partial nucleotide sequence of chick PKC isoenzymes. Future experiments of specific hybridization, cloning and sequencing are necesary to obtain longer fragments to identify individual PKC isoforms present in chick muscle cells.

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MOLECULAR CHARACTERIZATION OF CELLULASE NEGATIVE MUTANTS OF *T. REESEI*

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Cellulose-degrading enzymes of the filamentous fungus *Trichoderma reesei* are among the best characterized; this cellulolytic system consists of three major classes of enzymes: cellobiohydrolases (CBH), which cleave cellobiosyl units from the nonreducing end of cellulose chains; endoglucanases (EG), which cleave internal glucosidic bonds; and β-glucosidases, which cleave cellooligosaccharides to produce glucose. Four mutants of *Trichoderma reesei* defective in cellulose utilization were characterized at the molecular level. Genomic analysis of the cellulase genes and transcript induction using two well established inducers of the cellulase system - the insoluble polymer, cellulose and the soluble inducer, sophorose - revealed that these mutants are defective in the transcription of the cellulase genes. The results also indicate that the cellulase genes are coordinately expressed and most probably are regulated by the same mechanism. Using a heterologous gene construct in which the hygromycin-B-resistance-encoding gene was placed under the control of the promoter of the major cellulase gene, *cbh1*, the results showed that the mutants are defective in the inductive but not the basal expression of the cellulase genes.

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PHYLOGENY IN YEAST GENUS *Metschnikowia* INFERRED FROM RDNA RESTRICTION ANALYSIS

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The yeast genus Metschnikowia was originally described by Metschnikoff in 1884. Actually, this genus have ten species based mainly on morphology of ascospores: *M. reukaufii, M. bicuspidata, M. gruessii, M. hawaiiensis, M. lunata, M. zobellii, M. pulcherrima, M. agavae, M. krissii and M. Australis.* This is one of the most studied genus of yeast, at the ecological and taxonomic level, but few studies were made at the molecular level. Mendonça-Hagler (1985), construted a philogeny based on partial sequencing of 17S and 25S rRNA genes, concluding that is a great variability, in spite of phenotipic homogeneity, suggesting that exist the possibility of more than one genus.In this work, we present data on restriction analysis of ribosomal DNA(rDNA) and the philogeny of the group inferred from these results.We have isolated genomic DNA from above species, then were made digestions with restriction endonucleases: *Eco*R I, *Bam* HI, *Hpa* I, *Apa* I, *Hinc* II, *Sca* I, *Hind* III, *Pst* I, *Bgl* II and *Xba* I. Digestions were electrophoresed in agarose gels, transferred to nylon membranes and hybridized with specific probes. A data matrix constructed by scoring for the presence (=1) or absence (=0) of bands from restriction patterns was used as input for the SIMQUAL module of the NTSYS-pc software. Clustering tree construction were performed with the SAHN/UPGMA feature of the program. The genus *Debaromyces* was used as outgroup and data obtained were according to literature.

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CLONING AND EXPRESSION OF A DELTA-ENDOTOXIN FROM A NATIVE *Bacillus thuringiensis* STRAIN WITH A HIGH RELATIVE POTENCY AGAINST *Scrobipalpuloides absoluta* (south american tomato moth). **Meza-Basso, L., Theoduloz, C., Padilla, C., Vásquez, C., Salazar, M. and Bravo, J.. Programa Biología Vegetal, Facultad Recursos Naturales, Universidad de Talca, Talca, Chile.**

The larva of *S. absoluta* is the most devastating insect pest of tomato production in Chile. The potential for using bacterial insecticides was studied, analysing the relative toxicity of native *B. thuringiensis* (BT) isolates collected from soil samples.

PCR technique was used to facilitate the screening. Specific primers homologous to regions within *cryI* genes were employed. Due to its insecticidal efficacy, one strain (121e) was selected. Purified protoxin from native strain was characterized by SDS-PAGE, Western Blot and bioassays on *S. absoluta* larvae.

The native isolate seems to harbour a single gene of *cry IA* subclass. Gene isolation was performed, amplifying the complete gene by PCR using appropriate primers. The cloning and expression of the 3,4 kbp gene, was carried out in Pinpoint XA-1 T-vector. The protoxin protein was expressed as a fusion product, being recognized by AB against protoxins of BT *kurstaki*. The gene was characterized by restriction analysis and sequencing.

The results would allow to conclude that the native gene displays some differences compared to those classified as *cry IA(b)*. **Supported: FNC 1941173; DIAT, U. de Talca.**

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REQUIREMENT OF DISTAL AND PROXIMAL PROMOTER SEQUENCES FOR CHROMATIN ORGANIZATION OF THE OSTEOCALCIN GENE IN BONE-DERIVED CELLS. *Montecino, M., Frenkel, B., Lian, J., Stein, J., Stein, G. *Departamento de Biologia Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile, and Department of Cell Biology, University of Massachusetts Medical Center, Worcester, MA, USA.

The osteocalcin gene (OC) encodes a 10 KDa bone-specific protein which is expressed with the onset of mineralization during differentiation of normal diploid osteoblasts. We have previously reported that transcriptional activation of this gene is accompanied by the presence of two DNase 1 hypersensitive sites, both located in the promoter region spanning key basal (promoter site, -170 to -70) and steroid-dependent enhancer (distal site, -600 to -400) elements. Here, we have examined stably transfected ROS 17/2.8 cell lines, carrying OC promoter-reporter transgenes which contain series of 5'deletions, and determined the effect of these truncations on the chromatin organization. It has been found that, 1) DNase 1 hypersensitivity at -600 is not a requirement for vitamin D-dependent transcriptional upregulation; 2) basal transcriptional activity and proximal nuclease hypersensitivity depend exclusively on protein-DNA interactions ocurring within the proximal promoter region, and; 3) within the chromatin context, the proximal 100 bp promoter fragment containing essential elements such as the OC box (-99 to -76) and TATA box (-44 to -31) is insufficient to support formation of the proximal nuclease hypersensitive site and transcriptional activity.

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Cloning of a fragment of a heat shock gene in *P. chilensis*. **Ortiz, C., Cardemil, L. y Nieto, J. Departamento de Biología , Facultad de Ciencias, Universidad de Chile e Instituto de Biotecnología, UNAM, México.**

Using maize hsp98 as an heterologous probe, we isolated a clone containing a fragment of an homologous gene in *P. chilensis*. By comparison of the 900-1200 bp region between hsp101 soja gene and maize hsp98 we obtained two primers for PCR reaction. Using genomic DNA of *P. chilensis* seedlings as a template, we isolated a PCR product corresponding to a fragment of a *P. chilensis* hsp98 gene. The PCR product was isolated, purified and cloned into a pBs KS+ vector. Electrompetent *E. coli* cells were transformed by electroporation and selected in an appropiated media. White colonies were isolated and a second selection was performed. A screening of white colonies shown twelve positives . These colonies were grown and minipreparations of plasmid DNA was done. Restriction analysis shown the presence of the fragment in seven colonies . Southern blots of plasmid DNA were performed to demonstrate the presence of the fragment corresponding to hsp98. The fragment was isolated and purified to be used as an homologous probe.

The presence of a heat shock gene homologous to maize hsp98 could be part of the strategy of *P. chilensis* to resist high environmental temperatures. This strategy could be related to thermotolerance events.

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TRANSLOCATION INTO CHLOROPLASTS OF AN ACTIVE PRECURSOR CONTAINING NONCOVALENTLY BOUND FAD Ottado, J., Krapp, A., Carrillo, N. and Ceccarelli, E., PROMUBIE, Biochemistry Faculty. University of Rosario, Suipacha 531. 2000 Rosario, ARGENTINA. email: cecca@unrobi.edu.ar

The cytosolic precursor of the chloroplast flavoprotein ferredoxin-$NADP^+$ reductase (preFNR) was expressed in *Escherichia coli*, rendering a soluble protein that contained bound FAD. The recombinant precursor displayed extensive packing of its polypeptide chain, including the 3-D motif required to accommodate FAD. The mechanism of plastid translocation was studied under defined conditions using this precursor holoprotein and intact pea chloroplasts. The first step in the import pathway, namely, binding of preFNR to chloroplasts, was saturable at ~2000 molecules/plastid, and showed a high affinity interaction with a K_D of 5-10 nM. Binding was not affected by the addition of soluble leaf extracts or by previous denaturation of preFNR with urea. Initial import rates at different preFNR concentrations indicated the existence of a single translocation system. Inclusion of leaf extracts in the assay resulted in a 30-fold increase of the import efficiency. Comparison of K_M and K_D values under various conditions suggests that the binding step is largely irreversible, favouring import and processing. In the absence of extract, an urea-denatured precursor proved to be a better substrate for import than the holoprotein. Introduction of a 10- or 30-residues spacer between the transit peptide and the mature region of preFNR did not affect the properties or the transport-competence of the precursor. Stimulation by leaf extracts is probably related to preFNR unfolding prior to or during translocation.

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cDNA Cloning and Immunological characterization of a ribosomal P2β-like protein from *Leishmania (Viannia) braziliensis* (Lbb P2β-G).

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A 750 pb cDNA recombinant was isolated from a λgt11 *L. (V.) braziliensis* library using as DNA probe, a *L. infantum* P2alfa coding sequence. Sequence analysis showed an open reading frame of 264 bp encoding 87 residues of the globular and C-terminal domains of a ribosomal P protein, and the remaining 486 bp corresponded to the polyadenylated 3'non-coding region. Aminoacidic sequence comparison showed a high degree of homology with the P2β counterparts of *L.(V.) peruviana* (72%) and *L. infantum* (69%). A detailed analysis showed several characteristic features: 1) a unique globular motif RPTMPIIFAR not found in other parasite P2 proteins; 2) a longer alanine rich hinge, and 3) the C-terminal acidic motif harbored a Gly residue instead of the canonical Leishmanial Ala residues; consequently it was named LbbP2β-G protein. The anti-LbbP2β-G humoral response of patients with Mucocutaneous Leishmaniasis was characterized by ELISA using GST-LbbP2β-G fusion protein as antigenic reagent. ELISA measurements were carried out with Mucocutaneous (ML), Andean and Sylvatic Cutaneous (CL) Leishmaniasis sera , Chagas disease (CH) and Systhemic Lupus Erythematosus (SLE) sera. Four out of 35 tested ML sera gave a positive reaction with LbbP2β-G (11%), while none of the other sera reacted with it. Inhibition were performed with the homologous protein and R-13, H-13 and A-13 peptides, corresponding to the 13 C-terminal residues epitope from *T.cruzi*, human and *Leishmania* P2 proteins respectively. Although, the recombinant P protein abolished the reaction of positive ML sera, none of the synthetic peptides did, suggesting that the Leishmanial P protein epitope is not located within the 13 C-terminal region, known as target of the humoral response in CH and SLE.

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SONIC HEDGEHOG REGULATES ANTERIOR DEVELOPMENT IN *Xenopus laevis* EMBRYOS. Paganelli, A., López, S., Krivokapich, S., Franco, P., and Carrasco, A. Instituto de Biología Celular y Neurociencias, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina. (e-mail: rqcarras@criba.edu.ar)

We have isolated sonic hedgehog (*X-shh*) from a *X. laevis* cDNA library of neurula stage. Frog *shh* transcripts were visualized by whole mount in situ hybridization with a digoxigenin labeled riboprobe. At early gastrula the transcripts are restricted to the dorsal blastopore lip. At tailbud stages, they are seen in the the notochord, foregut, floor plate and ventral regions of the brain, except in the infundibulum. On the other hand, the Rathke's pouch (prospective ectodermal part of the pituitary gland) shows *shh* transcripts. In the pharyngeal arches, expression is restricted to the posterior-ventral region, suggesting a possible role of *shh* in polarizing this tissue, resembling the activity of the segment polarity gene hedgehog in the parasegments of *Drosophila* embryos. An antibody directed against *X-shh* found high levels of the endogenous protein in the notochord and the cement gland. The latter is devoid of *shh* trancripts, suggesting that the protein has migrated from a different site and found specific receptors on the cells within the gland. We injected synthetic chicken *shh (c-shh)* mRNA into two-cells frog embryos. Overexpression of *shh* produced anterior defects including microcephaly, reduction of cement glands and reduction of the eyes. A subpopulation of these embryos also showed posterior defects such as elongation deficiencies and spina bifida. The phenotype was associated with a reduction of the expression pattern of the cephalic marker *Xotx2* in the forebrain and the retina. This phenotype is opposite to that obtained after overexpression of another member of this multigenic family, banded hedgehog (*bhh*; Lai et al., 1995). *X-bhh* is expressed in a non-overlapping complementary pattern in comparison to *X-shh* (Ekker et al., 1995). We conclude that *shh* may be antagonizing *bhh* by interfering the interaction between *bhh* and a putative common receptor. Alternatively, the target cells may be expressing a combination of specific receptors for *shh* and *bhh* that trigger opposite downstream pathways. Normal anterior development might require a balance between these antagonic pathways. Overexpression of *shh* or *bhh* disrupts this balance, leading to opposite phenotypes. This research was made possible by grants from the E.E.C. (EU CT93-0017) and Fundacion Antorchas (A-13359/1)

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THE ROLE OF rRNA EXPRESSION IN SEASONAL ACCLIMATIZATION. ISOLATION OF CARP rRNA AND snoU3 RNA GENES. Ríos, M., de la Fuente, E., Quezada, C., and Vera, M.I. Instituto de Bioquímica, Universidad Austral de Chile, Valdivia, Chile.

The acclimatization process of eurythermal fish, involves differential gene expression. In winter-acclimatized carp, the transcription and processing of pre-rRNA is scarce compared to the summer-adapted carp. At the ultrastructural level this condition yields, in winter, the segregation of the nucleolar components, which morphologically indicates the temporary inactivation of the ribosomal RNA gene expression. As is rRNA, the cellular content of small nucleolar U3 RNA (snoU3 RNA) is also notably diminished during the cold season. U3 RNA is the most abundant snoRNA and it is essential for the early processing events in the maturation of pre-rRNA molecules. On the contrary, during summer, the transcription and maturation of pre-rRNA, and the cellular content of snoU3 RNA are remarkably higher compared to that observed in winter.

To understand the molecular mechanisms underlying the seasonal environmental regulation of the expression of both genes, we searched genomic clones containing rRNA regulatory and coding sequences. We have isolated several recombinants containing sequences of carp rDNA and sno U3RNA. One subclone of 4815 bp contains 18S, 5.8S and 28S rRNA sequences. We are currently analyzing other rRNA and sno U3RNA clones to map the upstream elements located in the regulatory regions. The identification of *cis* control elements of rDNA genes should allow the study of carp RNA pol I transcription factors.

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THE *rpl5-rps14-cob* GENE ARRANGEMENT IN THE *Solanum tuberosum* MITOCHONDRIAL GENOME

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We have previously characterized the potato gene for apocytochrome b (cob) and found it in two different mitochondrial genomic contexts. We have now found that these contexts diverge in a 5' distant region. In one of these contexts, we identified by sequence analysis the L5 ribosomal protein gene (*rpl5*) and a S14 ribosomal protein pseudogene, both located upstream of cob. The *rpl5-Ψrps14-cob* arrangement is similar to that found in the *A. thaliana* mitochondrial genome. However, the mutation in the potato pseudogene is different from that in the *Arabidopsis* gene

rpl5 and *Ψrps14* are cotranscribed, and editing of eight C residues into U in the *rpl5* coding region results in 8 amino acid changes that increase the homology between potato and other RPL5 polypeptides. Interestingly, these transcripts are not edited in the altered *rps14* reading frame, suggesting that after gene inactivation the ability to edit its transcripts was lost.

Our results and data from other groups indicate that, during the course of evolution, the organization of ribosomal protein genes has undergone drastic changes in the mitochondrial genomes of angiosperms. These changes are probably the result of genomic recombination events in the mtDNA and gene transfers to the nucleus.

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THE USE OF Tn5-lux FOR THE ISOLATION OF R.tropici MUTANTS SENSITIVE TO THE ENVIRONMENTAL STRESSES TEMPERATURE AND ACIDITY. Riccillo, P. and O.M.Aguilar. Instituto de Bioquímica y Biología Molecular, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina.

High soil temperatures in the bean producing area from the Northwest of Argentina are a major problem for biological nitrogen fixation by the symbiotic association bean-Rhizobium. It was shown that bean rizobial strains that belong to the type II (R.tropici A and B) are able to nodulate beans and fix nitrogen under a regime of high temperaturas as well as under other environmental stresses such as acidity and high level of aluminium.

In order to gain better insight into the bases of the tolerance to environmental stresses we had random mutagenized R.tropici, and selected mutants sensitive to high temperatures and low pH for further studies. The Tn5-lux transposon carried into plasmid pRL1063 was used for triparental mating to the wild type strain R.tropici. About 6000 Sm^R Nm^R transconjugants were screened for the unability to grow on minimal medium incubated at 40°C, and on minimal medium buffered to pH 5.0, respectively. The rate of auxotroph mutants was about 0.7%, and 55 T^S and acid sensitive mutants were isolated. Some of the mutants were sensitive to both high temperature and acidity. After an initial heat shock period of about 45-60 minutes the mutants shown different response to grow in liquid media that permitted further grouping. lux expression (light emission) was detected in some of the mutants after the cells were shifted from neutral to acid media.

The physical analysis of the genomes of the different mutants was perfomed by Southern using a probe for the transposon. It was found that a significant number of mutants showed more than the expected single EcoRI band per genome. This suggests that these mutants may undergo some kind of genomic rearangement.

The symbiotic phenotype of the mutants was assessed by plant inoculation experiments under normal conditions (neutral pH and temperature ranging 20-30 °C). All the mutants but one nodulated bean and fixed atmospheric nitrogen. Finally, we performed analysis by SDS-PAGE to investigate for mutants affected in the accumulation of heat shock proteins.

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STUDIES ON THE MOLECULAR MECHANISM OF ACTION OF HEXACHLOROBENZENE ON MALIC ENZYME GENE EXPRESSION. A. Loaiza[1,3], D.L. Kleiman de Pisarev[2], A. Randi[2], H.A. Sancovich[1], A.M. Ferramola de Sancovich[1] and P. Santisteban[3]. Química Biológica, (1) Facultad de Ciencias Exactas y Naturales, (2) Facultad de Medicina, UBA, CONICET, Buenos Aires, Argentina, (3) Instituto de Investigaciones Biomédicas (CSIC), Madrid, Spain.

Hexachlorobenzene (HCB) is a widespread environmental pollutant. Chronic exposure of laboratory animals to HCB elicits a number of effects such as triggering porphyria, liver microsomal enzymes induction, low levels of serum T_4 and normal levels of serum T_3. Our previous data have indicated that the treatment of Wistar rats with HCB (100 mg / 100 g b.w.) increases the activity of cytosolic hepatic enzymes such malic enzyme (ME), glucose 6 phosphate dehydrogenase and 6 phosphogluconate dehydrogenase. Since ME activity is regulated by thyroid hormones, we have studied firstly (i) whether HCB could modulate T_3 binding properties and (ii) mRNA levels of T_3 nuclear receptor (iii) the potential role of HCB on ME gene expression. The results obtained show that HCB did not modify T_3 binding to its nuclear receptors neither at 15 days nor at 30 days of intoxication. mRNA levels of T_3 nuclear receptors measured by Rnase protection assay was not modified by HCB treatment. The ME mRNA in liver increases just from day 9 to 15 of HCB treatment. Futhermore mRNA levels from Glyceraldehyde Phosphate Dehydrogenase, another T_3 regulated enzyme, was not affected in treated rats. The above data together with the low T_4 and normal T_3 levels in serum indicate that the increase of malic enzyme mRNA is not directly mediated through the binding of T_3 to its receptor, but may take place at the transcriptional level.

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PCR IDENTIFICATION AND CLONING OF OUTER MEMBRANE OMP1 FROM A NON SUBTYPABLE CHILEAN STRAIN OF *NEISSERIA MENINGITIDIS*. [1]Real, C., [2]Sein, J., [2]Jaureguiberry, B. and [1]Venegas, A. [1]Laboratorio de Bioquímica, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Alameda 340, Santiago and [2]Centro de Investigaciones y Desarrollo, ISP, Marathon 1000, Santiago, Chile.

OMP1 from *N. meningitidis* has been useful for subtype classification which is based on antigenic loops 1 and 4 of this protein. In addition, OMP1 stands as candidate to develop a new vaccine because of its immunogenicity and induced bactericidal response in rat models. In Chile, the major strain is P1.3 but nonsubtypable (NT) strains have increased since 1993.

The major goals of this work were: to identify an NT chilean strain by comparison of loop 1 and 4 PCR amplified sequences respect to P1.3 and other strains, evaluate OMP1 immune response in rabbit and human sera, and clone the porA gene encoding OMP1 of 6 NT strains. PAGE-SDS electrophoretic profile of outer membrane proteins from NT strains revealed that OMP1 was not present in 1 NT strain. Western blot studies indicated that humoral response of human and rabbit sera against NT OMP1 proteins were quite remarkable. Sequences of loop 1 and 4 of a NT strain matched almost perfectly with subtype P1.5b,10 except for a missing codon in loop 1 and 3 deleted bases in loop 4. Expression studies were attempted by cloning NT porA gene in pCRII plasmid under control of T7 promoter in *E. coli BL21(DE3)* cells. After IPTG induction, cells showed a delay during growth, but no protein was seen in gels.

Regarding classification of NT strains we concluded PCR approach is preferable, since not all of them did showed OMP1 serum reactivity, however all of them carried the porA gene as detected by loop 1 and 4 PCR amplification.

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EARLY COMPARATIVE STUDIES OF UROPORPHYRINOGEN DECARBOXYLASE RNAs FROM LIVER OF NORMAL AND PORPHYRIC RATS. Guidi, S., *Paveto, C., San Martín de Viale, L. and Ríos de Molina, M.C. Facultad de Ciencias Exactas y Naturales. UBA. *INGEBI. CONICET. Argentina.

Uroporphyrinogen decarboxylase (UroD) is an enzyme involved in the biosynthesis of heme. It catalyzes the removal of the four carboxyl-groups of uro- to yield copro-porphyrinogen. Abnormal UroD activity has been associated with porphyria cutanea tarda (PCT). There are two PCT: a familiar form that is inherited as an autosomal dominant defect, characterized by at least a 50%decrease in the UroD activity in all tissues investigated and a sporadic form where the UroD is only decreased in liver, and its reduction is suspected to be associated with a genetically determined predisposition. May be liver UroD enzyme is under a genetic control different from the other ones. To study the UroD gene, we inserted a cDNA encoding human erythrocytes UroD in the plasmid pGEM7zf+. The resulting hybrid molecules were used to transform *E. coli* JM109 cells by heat shock method. Recombinant clones were used to obtain plasmidic DNA by the boiling method or using Quiagem minicolumns. The identity of the clones was confirmed by plasmid digestions with different restriction endonucleases. A 1300 bp fragment was purified by polyacrylamide gel electrophoresis, random primed and used to probe rat genomic DNA or RNA. The RNA arising from normal and porphyric rats were isolated and electrophoresed, and then transferred onto a nylon filter and hybridized to a nick-translated ^{32} P-labeled DNA probe. The results suggest that there is a high homology between the RNA of both rat groups and the cDNA used.

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IDENTIFICATION OF SEQUENCES HOMOLOGOUS TO Ty1-*copia* LTR RETROTRANSPOSONS IN *Licopersicon chilense*. Ruiz-Lara, S.[1], Verdugo, I.[1], Yañez, M.[1] and Prat, S.[2]. (1) Departamento de Ciencias Biológicas, Universidad de Talca, (2) CID-CSIC Barcelona.

Two major groups of LTR retrotransposons , Ty1-*copia* and Ty3-*gypsy*, have been identified, according to its sequence homology and gene order, These elements are common features of plant genome, representing potential tools for genetic analysis and manipulation.

We have used the PCR technique to analyze the presence of Ty1-*copia* retrotransposons in *Lycopersicon chilense* genome. An amplification product of roughly 300 bp was obtained when degenerate oligonucleotides, corresponding to highly conserved domains in *copia* retrotransposons, were used as primers. This DNA fragment has been cloned and its nucleotide sequence was determined. The deduced aminoacidic sequence, shows a high similitude with that reported for Ty1-*copia* encoded reverse transcriptase protein.

Southern hybridization experiments with the amplified fragment as molecular probe, confirms the above results and suggests a limited copy number for this element.

The screening of a *Lycopersicon chilense* genomic library constructed in λGEM-12 vector, allowed the isolation of 5 clones which are under characterization.

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Localization of functional sites in *Bst*VI restriction endonuclease. Saavedra, C., Loyola, C, González, E.* and Vásquez, C (Departamento de Ciencias Biológicas, Facultad de Química y Biología, Universidad de Santiago de Chile y * Departamento de Ciencias Biológicas, Facultad de Recursos Naturales, Universidad de Talca).

*Bst*VI restriction endonuclease belongs to the restriction-modification system of the facultative thermophile *Bacillus stearothermophilus* V. The structural gene for the enzyme has been cloned in *Escherichia coli*, and the recombinant protein has been purified and characterized. It consists of a 224 aminoacid polypeptide chain which recognizes the palindromic DNA sequence 5'- C*TCGAG-3' cleaving it at the position indicated by the asterisk. The result is then the generation of cohesive protruding 5' ends of four bases.The enzyme showed a broad range of optimal temperature and had no apparent requirements for sulphydril reagents for activity. Besides heat, it also showed resistance to other protein-denaturing agents. As most nucleases, it is highy dependent upon divalent cations, mostly Mg^{2+}. Conserved aminoacid sequences which would play a role in the binding of this metal have been described for other restriction endonucleases. Since these are present at equivalent locations in the *bstVIR gene*, several studies were carried out to test if the conserved residues play a similar role in the *Bst*VI enzyme. The experimental approach was to perform site-directed mutagenesis using the polymerase chain reaction to change the pertinent residues involved in both, magnesium ion binding and disulphide bond formation.

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TOWARDS THE MAPPING OF THE *TRYPANOSOMA CRUZI* GENOME; ANALYSES OF A REPRESENTATIVE COSMID LIBRARY AND CONSTRUCTION OF CHROMOSOME-SPECIFIC CONTIGS. [1]Sánchez, D.O., [2]Hanke, J., , [3]Henriksson, J., [3]Åslund, L., [3]Pettersson, U., [1]Frasch, A.C.C. & [2]Hoheisel, J.D. [1] Fundación Campomar, Buenos Aires, Argentina. [2]Molecular-Genetic Genome Analysis Group, Deutsches Krebsforschungszentrum, Heidelberg, Germany. [3]Department of Medical Genetics, Biomedical Center, University of Uppsala, Sweden

With the aim of generating a contiguous cosmid coverage of the genome of the protozoan parasite *Trypanosoma cruzi* as a preparation for a large scale sequence analysis, a cosmid library of 36,864 individual, primary clones was generated from total genomic DNA of the reference strain CL Brener. For cloning, a modified cosmid vector was used that simplifies various aspects of analysis. The library's representation is about 25 genome equivalents, assuming a size of 55 Mb per haploid genome. No chimerism of clones inserts could be detected by a stringent assay. The co-linearity between cosmid inserts and genomic DNA was verified and hybridisations of clones towards the organism's karyotype were carried out as a quality check. Gridded onto two nylon filters, the library was analysed with a variety of probes, indicating its usefulness for a mapping approach based on hybridisation. Apart from being used for a combined physical and transcriptional mapping of the genome, library filters and clones are made available to interested parties. At present we are mapping chromosomes #1, #2, #3 and #4 using specific sub-libraries and sampling without replacement hybridization.

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INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) MORPHOGENESIS IN CHSE-214 CELLS. Villanueva R.A. and Sandino A.M. Laboratorio de Virología, Departamento de Ciencias Biológicas, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile.

The infectious necrosis pancreatic virus (IPNV), member of the *Birnaviridae* family, possesses an icosahedral capsid of 60 nm of diameter and two ds RNA segments as genome with a VPg linked to each 5' extreme. IPNV has economic importance, given the high mortalities that produces in salmonides.

The segment A of 3100 pb, codifies for a polyprotein of 100 kDa, which is processed giving rise to three structural proteins, the pre VP2, VP3 and VP4; and segment B of 2990 pb codifies for the polypeptides VP1, which has been suggested would correspond to the viral RNA polymerase and VPg. IPNV replicates in the citoplasm of salmon embryo Chinook cells, CHSE-214, in which the replicative cycle takes 24 hours at 18°C, aproximately. However, the particles that are produces during the infective cycle they have not been identified at present. This work consisted of isolating isotopically labeled particles, obtained at different times during the viral cycle and analize their polypeptide and nucleic acid composition. For this purpose the particles were partially purified from celular extract through simple sedimentation and sedimentation in sacharose cushion. The different intermediaries of IPNV were analized acording to their electrophoretic mobility in agarose Tris-glicine gels. The results shown that the apparition of the viral particles is totally dependent on the synthesis of genomic RNA and during the IPNV infective cycle only two kinds of particles are produced. Apparently, it take place a structural rearrangement of the particle during virus maduration.

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BINDING OF N PROTEIN TO JUNIN VIRUS S RNA INTERGENIC REGION. M.A. Tortorici[1], P.D. Ghiringhelli[1,2], S. Tasso[1] y V. Romanowski[1,2]. [1] *Instituto de Bioquímica y Biología Molecular (IBBM), Dto. de Ciencias Biológicas, Facultad Ciencias Exactas, Universidad Nacional de La Plata, La Plata;* [2]*Dto. de Ciencia y Tecnología, CEI, Universidad Nacional de Quilmes, Bernal, Argentina.*

Junín virus, the etiologic agent of Argentine hemorrhagic fever, is a member of the *Arenaviridae* family. All the family members are enveloped viruses with a genome composed by two ssRNA species, designated L (large, 7 kb) and S (small, 3.4 kb). Both RNAs have an ambisense coding strategy. The L RNA codes for the Z (or p11) and L (RNA polymerase) proteins, and the S RNA codes for the GPC (glycoprotein precursor) and N (nucleocapsid) proteins. We demonstrated that the N protein participates in the transcription-replication regulation of viral S RNA acting as antiterminator, in addition to the structural role. Computational analyses of arenaviruses N protein sequences were used to identify two potential sites of interaction with RNA: an arginine-rich motif, located at the N-terminal portion of the protein and a zinc finger like structure in the C-terminal region. In order to define the RNA binding domains and their relationship with the antiterminator function, we generated truncated variants of the N protein. The N protein and truncated variants were expressed, alternatively, in baculovirus and *E. coli* systems, and their identities were confirmed by Western blot analysis. The RNA binding assays were performed ih dot blot, with immobilized proteins and ^{32}P labeled *in vitro* RNA transcript corresponding to the intergenic region of Junín S RNA as probe. The interaction has been also tested by a band shift assay. The results suggest that the zinc finger like structure has an important role in the RNA binding. In order to assess the specificity of interaction, competition experiments, in presence or absence of Zn^{+2}, with homologous and heterologous probes are in progress. These results will be considered in the design of *in vivo* experiments aimed at testing the transcription antitermination properties of N.

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R1 RETROTRANSPOSONS IN *Drosophila arizonae*

Tovar, F.J., Menezes, R., Klann, E., Magela, C., Paulo de Campos, D. And Leoncini, O. Departamento de Genética, Inst. de Biologia, CCS, Universidade Federal do Rio de Janeiro. Rio de Janeiro, Brasil.

Retrotransposons with specific integration sites in rDNA (ribosomal DNA) have been observed in many insects. R1 is a non LTR retrotransposon, 5.3 kb in length that is present in some rDNA repeating units, in a specific region of 28Sβ gene. It has been completely sequenced in various species (genus *Drosophila* and *Bombyx*). From these sequences becomes evident the presence of two ORFs coding for two putative proteins, one of 471 aa and the other with 1021aa. The second ORF have a region homologous to retrotranscritase.

In these work, we were studing the rDNA structure in *D. arizonae* , a member of the Cluster *Mojavensis* (Mulleri Complex). We constructed a genomic library in λEMBL-4 and isolated recombinant phages with homology to rDNA probes. The rDNA fragments were subcloned into plasmids, maped and partially sequenced. Some of these clones revealed the presence of parts of retrotransposons. We have partially sequenced portions of these clones in order to identify the point of insertion. Using this criterion, we identified these element as a R1 type. We present here these data (maps, sequences and genomics "southerns") that show the presence of these elements in flies of the so called Mulleri Complex (Repleta Group).

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MOLECULAR PHYLOGENY OF A NEW ARCHAE DISCOVERED IN A COPPER COMMERCIAL-SCALE BIOLEACHING PLANT CONDITION.

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Some commercial-scale bioleaching copper plants operate with leaching solutions containing up to 1.5 M sulfate ions. When copper sulfide was bioleached using a high sulfate solution from a commercial production plant, archae were detected by PCR specific amplification of the 16S rDNA in the DNA extracted from the leached ore. These archae grew in batch culture of the leaching solution employing ferrous iron, elemental sulfur or chalcopyrite as energy source. The potential genetic heterogeneity of the amplified 16S rDNA and its relation with other archael 16S rDNA was determined by a simple procedure based on the reduced electrophoretic mobility of heteroduplexes formed between 16S rDNAs. This assay may become a simple general procedure to asses both population heterogeneity and phylogenetic relationships. The 16S rDNA amplification product contained a single nucleotide sequence as determined by the absence of heteroduplex formation after denaturation and renaturation. It formed with *Sulfolobus acidocaldarius* 16S rDNA, the other described autotrophic acidophilic archae, an heteroduplex of very low mobility indicative of a large divergence. Nucleotide sequencing confirmed these observations; the sequence was very different to that of *S. acidocaldarius* and other described archae, suggesting that it might belong to a novel genus.

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Organization and Structure of SIRE, a short interspersed repetitive element of *Trypanosoma cruzi*.

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We have previously described a Short Interspersed Repetitive element (SIRE) present in the TcP2β-H1.8 locus and distributed throughout the entire *Trypanosoma cruzi* genome (1). SIRE may be important in the transcription or stability of transcripts of TcP2β-H1.8 locus since this element , located in front of the gene, introduces a new functional 3' SL acceptor site, consequently 38 bases of the TcP2β 5'untranslated region are directly transcribed from SIRE.

The characterization of SIRE elements was extended by cloning 20 different loci containing SIRE sequences. From this analysis, we define the target insertion site of SIRE, a poly dT sequence, and a structure with three regions. It was confirmed that SIRE is widely distributed in the parasite genome, present in all the chromosomes and seemed not to interrupt gene coding sequences. Moreover, SIRE proved to be an important tool for the analysis of *Trypanosoma cruzi* nuclear genome and provides the basis to obtain a novel type of STS markers, SAS (SIRE Associatted Sites) that are relevant in the construction of "ready to sequence" physical map of the parasite.

(1) Vázquez et al. Mol. Biochem. Parasitol. 64, 327-336, 1994.

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A NOVEL MITOCHONDRIAL RNA CONTAINING THE 16 S rRNA IS PRESENT IN MOUSE SPERM CELLS. Villegas J., Zárraga, A.M., Montecinos L., Brito, M., Meneses, A. M., Werner, E., Zamora, P and Burzio, L.O. Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia.

Northern blot analysis of testis and sperm RNA probed with clone λ-MS134 isolated from a mouse cDNA library, indicated that the corresponding RNA was enriched in the sperm. The sequence of this clone shows the presence of an invert repeat of 121 bp at the 5' end of the 16 S mtRNA. Amplification between tRNA Val and 16 S RNA produced a single fragment of 342 bp from mtDNA of mouse testis, sperm, liver and blood, indicating that the RNA of clone 134 is a post-transcription product. RT-PCR of total sperm heads RNA suggested that the 16 S rRNA was localized in the nucleus of mouse, rat and human sperm. The nuclear localization was confirmed by *In Situ* hybridization (ISH) assays. The antisense oligo labeled with digoxigenin hybridized with the sperm nucleus and midpiece, indicating the unexpected translocation of a mtRNA to the nucleus. A working hypothesis is that this novel RNA may play a role in cell proliferation and differentiation. Human HL-60 cells provided an adequate system to test this possibility. ISH localized the 16 S mtRNA in the nucleus and cytoplasm of HL-60, suggesting also a translocation of a mtRNA to the nucleus. Treatment of these cells with a phorbol ester (TPA) induced, both, differentiation to macrophage and, a sharp decrease in the content of 16 s mtRNA. Moreover, the nuclear staining after ISH decreased markedly. In conclusion, this novel RNA appears to be involved in cell proliferation and differentiation (Grant 1960492 and 2960062 of FONDECYT, Chile, and Grant S-94-14 DID, UACH).

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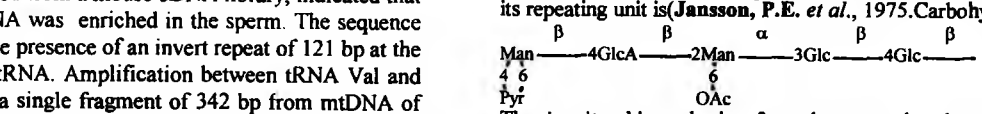
CHARACTERIZATION OF DEFICIENT EXOPOLISACCHARIDE MUTANT *X. campestris.* 8004::Tn5$_{70}$ Voinov, A., Zorreguieta, A., Bassi, D., Salibe, M. and Dankert, M. Instituto de Investigaciones Bioquímicas Fundación Campomar. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

Xanthomonas campestris is a plant pathogen causing the disease called black rot. In addition it produces an extracellular polysaccharide (EPS), of great industrial importance, called xanthan. The structure of its repeating unit is(**Jansson, P.E.** *et al.*, 1975.Carbohydr.Res.**45**:274).



The *in vitro* biosynthesis of xanthan gum has been studied in our laboratory in detail (**Ielpi** *et al.*, 1993. J. Bacteriol., **175**: 2490). It occurs in at least two stages. In the first, the repeating unit is sequentially assembled linked to a polyprenol trough a diphosphate bridge. In a second stage, the repeating units are polymerized and liberated into the growth medium. It has been reported that the genes involved in xanthan biosynthesis are located in a cluster (**Barrère** *et al.*, 1986. Int. J. Biol. Macromol, **8**:372). This cluster (12 genes) was found to have about 16 Kb and some of the genes involved in the sugar transfer were identified (**Vanderslice** *et al.*, 1990. Synergen, Inc., USA) but the location of the genes responsible for the polymerization and export process is until unclear. Here we reported an EPS defective strains, *X. campestris* 8004::Tn5$_{70}$, that turned out to be able to synthesize *in vitro* the lipid linked pentasaccharide repeating unit, from the three sugar donors: UDP-Glc, GDP-Man and UDP-GlcA one of them ^{14}C-labeling, but unable to polymerize it. Southern blot experiment showed that the Tn5 was located within a 1,9 *Eco*RI fragment, this fragment was isolated and the insertion site was sequenced. The Tn5 is placed 15 bp upstream of the initiation site of *gumB* gene (first gene of the cluster). It suggests that *gumB* is involved in the polimerization process. The expression of other genes located downstream of *gumB* could be due to other promoter activities.

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NEURAL-SPECIFIC EXPRESSION OF THE PROOPIO-MELANOCORTIN (POMC) GENE IN TRANSGENIC MICE

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The POMC gene is expressed mainly in pituitary melanotrophs and corticotrophs and in a limited subset of arcuate hypothalamic neurons. Expression in each of these cellular groups is believed to be drived by different *cis*-acting regulatory sequences. Results from studies using transgenic mice have demonstrated that 5'flanking promoter sequences of the POMC gene confers pituitary specific expression but fails to direct proper specific expression in the hypothalamus. In order to study the differential transcriptional regulation of the POMC gene in endocrine cells and neurons, we produced transgenic mice carrying POMC27*, a 27 kb genomic fragment harboring sequences of the mouse POMC allele. POMC27* contains the entire transcriptional unit, 13 kb and 8 kb of 5' amd 3' flanking sequences and it is tagged whith a heterologous 30 bp oligonucleotide in the third exon. *In situ* hybridization of transgenic mice showed a restricted and cell specific pattern of expression of the transgene in the pituitary and in the arcuate neurons of the hypothalamus. Immunocytochemistry with a β-endorphin antiserum followed by *in situ* hybridization of transgenic mRNA demonstrated the colocalization of the transgenic transcript in POMC producing cells. Hypotahlamic POMC27* levels of expression were compared to those of the endogenous mRNA using an RNAse protection assay resulting in a calculated ratio of 1. Ontogeny of the trangenic transcript analyzed by *in situ* hybridization revealed that POMC27* followed an identical spatial and temporal pattern of expression as the endogenous POMC gene. These results suggest the existence of, at least, one specific *cis*- acting element of POMC capable of targeting its neuronal expression. To localize and identify this sequence, we generated two deletional transgenes which are being analyzed.

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REGULATION OF FIBRONECTIN GENE EXPRESSION IN TWO MAMMARY ADENOCARCINOMAS WITH DIFFERENT METASTATIC POTENTIAL. Santiago E. Werbaih*, Alejandro J. Urtreger#, Elisa Bal de Kier Joffé#, Lydia Puricelli# and Alberto Kornblihtt*. *INGEBI and Department of Biological Sciences, Facultad de Ciencias Exactas y Naturales, UBA, Obligado 2490, Buenos Aires, 1428, Argentina, and #Instituto de Oncología Angel Roffo.
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Fibronectin (FN) is a high molecular weight glycoprotein present in the extracellular matrix (ECM) and plasma which can promote cell migration, attachment and spreading, and is related to fundamental processes such as embryogenesis, hemostasis, malignant transformation, wound healing, host defense and maintenance of tissue integrity. The capacity of tumor cells to metastasize is linked to their ability to synthesize and secrete FN. We found that primary cultures from murine adenocarcinoma tumors of moderate metastasizing ability, M3, exhibit a conspicuous fibrillar pattern of FN, while a highly metastasizing variant MM3, is immunohistochemiccally negative. Northern blots show that in M3 cells FN mRNA is abundant while it is undetectable in MM3 cells. Southern blot assays indicate that this difference is not due to a significant deletion of FN gene in MM3 cells. This suggests that the absence of FN mRNA in the MM3 cells could be due to a major mRNA instability or to a specific transcriptional inhibition. M3 and MM3 primary cultures were transiently transfected with three different constructs carrying the CAT reporter gene under the control of 1.6 kb, 0.5 kb and 0.22 kb segments of the human FN promoter. MM3 cells showed no CAT activity with all three constructs, while M3 cells expressed the CAT enzyme with an intensity proportional to the promoter length. These results indicate that the 220 bp proximal promoter region is involved in a negative control of transcription. Cloning and single strand conformation analysis (SSCA) of this region from the murine cells suggest that there are no differences in sequence between M3 and MM3 FN promoters. Footprinting and gel shift experiments are being done in order to characterize the factors involved in the observed negative regulation of transcription. (supported by grants of the University of Buenos Aires, CONICET, Fundación Antorchas and ICGEB).

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STUDIES ON THE ORGANIZATION AND EXPRESSION OF THE POTATO MITOCHONDRIAL GENOME. Zanlungo, S., Quiñones, V. and Jordana, X. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago, Chile.

We have reported for the first time in angiosperm mitochondria the presence of a gene encoding for ribosomal protein S10. This gene (rps10) and its expression have now been characterized. The RPS10 polypeptide of 129 amino acids is encoded by two exons of 307 and 80 bp respectively, which are separated by a 774 bp class II intron.

It is known that RNA editing is required for maturation of mRNA in plant mitochondria. Editing of complete rps10 coding region was studied by sequence analysis of rps10 cDNAs. Editing creates a putative translational initiation codon, a new stop codon and produces two additional amino acid alterations. One editing event observed in the intron sequence improves the secondary structure of the intron and may thus be required for the splicing reaction.

To determine if the RPS10 polypeptide is present in potato mitochondria a fusion protein was produced in *E. coli* and used to raise specific antibodies. Antibodies against RPS10 fused to a 6xHis affinity tag recognize a 16 KDa protein in a mitochondrial fraction enriched in ribosomes. We are now characterizing the proteins of this fraction by two dimensional gel electroforesis .

Our results indicate that in potato mitochondria the rps10 gene is transcribed, its transcripts are edited, spliced and translated, and the RPS10 polypeptide is assembled in the ribosome.

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CHARACTERIZATION OF A ß-(1,3)-GLUCANASE GENE OF BARLEY INVOLVED IN FUNGAL DEFENSE MECHANISM. Zanor M.I., Valle E.M.*, Vallejos R.H. Centro de Estudios Fotosintéticos y Bioquímicos, *Programa Multidisciplinario de Biologia Experimental, CONICET, Fac. Cs. Bioq. y Farm., UNR, Suipacha 531, 2000 Rosario, Argentina.

The ß-(1,3)-glucanase genes (*gnl*) of plants are often considered defense-related genes because the substrate of the genes products, glucan, is a major cell wall component of microbial phytopatogenes. This hydrolytic activity of ß-(1,3)-glucanase may inhibit the pathogen growth in plants and the glucan fragments may act as signals in activating genes involved in plant defense. In barley, the ß-(1,3)-glucanase gene family encodes at least six isoenzymes, which are designated GI to GVI. The isoenzyme GII is induced upon fungal invasion. We isolated a ß-(1-3)-glucanase gene (*gnl II*) using a PCR strategy, based on the nucleotide sequence of the BGL32 cDNA, on genomic DNA of barley. The gene consisted of 1.3 kb and contained a 165 bp intron. Like 80% of monocot introns (Simpson et al. 1996 Plant J 9:369) four YUNAN sequences were found, 21bp upstream of the 3´-splicing site. This intron had the typical monocot splicing sequences and a motif (TGCGCCGC) which is known to enhance the transient gene expression in maiz. Several approaches toward the study of the expression of *gnl II* will be described. The intron motif effect on the *gnl II* expression was studied by transient gene expression assay, measuring *gnl II* mRNA level as well as glucanase activity. We are also attempting to isolate the *gnl II* promoter by inverse PCR, in order to characterize its funcional elements by fusion to *gus* as reporter gene.

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EVALUATION OF SEVEN AMARANTHUS SPECIES THROUGH PROTEIN ELECTROPHORETIC PATTERNS. Amao, I.[1]; Rivas, R. and Pastor, A. [1]Centro de Investigación de Bioquímica y Nutrición. Facultad de Medicina. UNMSM. Facultad de Química. PUCP. Lima-Perú.

Amaranth (***Amaranthus caudatus***) has a good potential as a food and/or feed crop with high levels of protein quantity and quality.

This study is to evaluate the protein electrophoretic analysis of seeds from seven ecotypes of different American geographical regions. They have been cultivated in the Andean highlands under the same agronomical conditions in order to be able to make a comparison of the intrinsic responses of these species.

Here, we used amaranth seed protein of Noel Vietmeyer, Copaca Accha, Caopaca Paruro (Perú), Cahuayuna tar. (Bolivia), INIAP Ataco (Ecuador) and Revancha and Panoja roja (Mexico). They were analyzed by SDS-PAGE according to the method of Laemmli with a 5-20% acrylamide gradient.

The SDS-PAGE of total protein in the varieties mentioned revelead above revealed differences in the quality of the polypeptides, mainly in the regions of high molecular weight (30-70 kDa). The electrophoretic patterns of albumins and globulins are similar. However, the main differences observed in this study were in the polypeptides of high molecular weight from the glutelins fraction and in minor grade in prolamins fractions.

The significance of these results for a posible taxonomic classificarion will be discussed.

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THE PATTERN OF WHEAT LEAF EXTRACELLULAR MATRIX ENDOPROTEINASES DEPEND ON THE CULTIVAR TYPE. Avilés L., Pinedo M. and Conde R.D.
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The study of Extracellular Matrix (EM) function is a subject of interest in several fields of cell biology like growth regulation, wound repair and resistance to pathogen invasion in both plants and animals.

In plants, part or the components of EM can be isolated when leaves are buffer infiltrated and centrifugated yielding a fraction named intercellular washing fluid (IWF) which contains several hydrolytic enzymes like glucanases, chitinases and proteases.

We have previously described the occurrence of at least two serin proteinases in the IWF of wheat leaves (cv. S.A). In addition, we found that infection with the phytopatogenic fungus *Septoria tritici* either increases or decreases these proteolytic activities in either resistant or susceptible cultivars, respectively.

The aim of the present work was to determine the occurrence of variations in the IWF endoproteinases from 10 comercially important wheat cultivars.

The IWFs obtained from fully expanded first leaves were submitted to SDS-PAGE with and without substrate (0.1 % gelatin). The 70 kDa proteinase was identified in all the cultivars tested. However, cultivars SA, Nan, Fed, Gran, and Pal. displayed two enzymes of 100 and 110 kDa while Pi, IV, Cat, Oas and Poncho displayed either a 100 or 110 kDa activity. When total IWF proteolytic activity against casein was measured, SA, Pal and IV displayed estimates in terms of unists/gr of FW twice higher than the other cultivars. Then, the total IWF proteolytic activity seems not to depend on the number of isozymes exhibited. The differences observed between the examined cultivars could be related to their susceptibility to different stress conditions.

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POLYAMINE METABOLISM IN *Helianthus annuus* L. DURING GERMINATION UNDER SALT STRESS. Benavides MP. Aizencang G, Buldain G and Tomaro ML. Dto. Química Biológica. Facultad de Farmacia y Bioquímica, UBA. Argentina.

Polyamines are nitrogen compounds involved in growth and differentiation processes. The levels of putrescine (Put), spermidine (Spd) and spermine (Spm), the most common polyamines, are influenced by abiotic and environmental stress. We tested the variation of the main polyamines and two of the enzymes related to their synthesis, arginine decarboxilase (ADC) and ornithine decarboxilase (ODC) in sunflower plants subjected to salt stress.

Sunflower seeds were germinated in Petri dishes with distilled water, 50 mM and 150 mM NaCl. The treatment lasted 72 h. Samples were collected each day to determine polyamines, ADC and ODC.

During the first 24 h, no polyamines were detected. Put increased up to 72 h while Spd reached the highest value 48 h after the onset of germination. ADC increased after 72 h of germination, but ODC activity remained undetectable along this time. Salt stress affected both polyamines and enzymes activity. ADC decreased 56% respect to the control with 50 mM NaCl and 89% with 150 mM NaCl. Put was 66% and 85% lower than the control with 50 mM and 150 mM respectively. Spd was little affected by NaCl and Spm was neither detected in normal nor in treated sunflower plants.

Polyamines are affected by different types of environmental stress. The present study showed that salt stress affected the normal polyamine metabolism, inducing a decay in Put and ADC levels, and almost not changed Spd content. ODC seemed not to be involved in the normal Put biosynthesis and also was not influenced by the saline treatment.

We are studying if a direct relationship between polyamines levels and germinaton under stress conditions actually exist.

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Phytochrome and photomorphogenesis in gymnosperms

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Phytochrome is a red/far-red light photoreceptor that in dicots y monocots presents several isoforms whose apoproteins are encoded by divergent genes most of our knowledge of the function of different members of the phytochrome family comes from studies with the small weed Arabidopsis. Phytochrome genes cloned from gymnosperms show relatively poor homology to angiosperms phytochromes. Thus, we decide to investigate whether functions typical of phytochrome A in angiosperms have a counterpart in gymnosperms. Dark-grown seedlings of *Pinus elliottii* and *Pseudotsuga menziesi* showed a phytochrome pool recognized by a monoclonal antibody against phytochrome A, the most abundant phytochrome in dark-grown angiosperms. As in angiosperms, this phytochrome was below detection in light-grown tissues. The decrease in phytochrome levels observed immunologically after dark to red light transition was significantly slower than in angiosperms, particularly in *P. elliottii*. Two isoforms with slightly different mobility in SDS-PAGE gels were observed in *P. menziesi* which show different light lability. When exposed to continuos far-red light *P. elliottii* seedlings show enhanced cotyledon growth and *P. menziesi* seedlings anthocyanin synthesis. Pulses of far-red light were not effective in inducing the latter responses. Although phytochrome A is not present in gymnosperms a pool of phytochrome found in dark-grown seedlings is able to perform rudimentary aspects of a function that in angiosperms is exclusively assigned to phytochrome A.

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THE 26S PROTEASOME FROM THE HIGHER PLANT *Lemna minor*. Caeiro[1], A.S. Ferreira[1,2], R.B., Santos[1], C.N., and Teixeira[2], A.R.. [1]Inst. Tec. Quím. Biol., Oeiras, Portugal and [2]Ins. Sup. Agronomia, Lisboa, Portugal.

The ubiquitin-dependent proteolytic pathway is, in all eukaryotic cells, responsible for the selective degradation of both abnormal and short-lived proteins. In this pathway, multiple ubiquitin molecules are enzymatically ligated to proteins destined for catabolism. These high molecular mass conjugates are subsequently recognized by a large ATP-dependent proteolytic complex (the 26S proteasome) wich degrades the target protein and releases the intact ubiquitin molecules (Hershko and Ciechanover, 1992, *Ann. Rev. Biochem.*, vol. 61, pp. 761-807). The 26S proteasome has been detected and studied in animal cells (reviewed by Peters, 1994, *TIBS*, vol.19, pp. 377-382). A single study has been published on this proteolytic complex from plants cells (Fujinami *et al.*, 1994, *J. Biol. Chem.*, vol. 269, pp. 25905-25910).

We have detected a large, ATP-dependent protease in *Lemna minor* L. cells. This enzyme was purified by isopycnic glycerol density gradient ultracentrifugation, by anion exchange chromatography on the FPLC Mono Q colunn and by gel filtration on the FPLC Superose 6 colunn. The purified protease exhibits chymotrypsin-like activity (measured with *N*-Succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin), trypsin-like activity (measured with *N*-t-Boc-Leu-Ser-Thr-Arg-7-amido-4-methylcoumarin) and peptidyl-glutamyl hydrolysing activity (measured with *N*-Cbz-Leu-Leu-Glu-β-naphthylamide).When analysed by SDS-PAGE, its polypeptide composition resembles closely that of animal origin. The catalytic activity is stable at high temperatures, exhibiting a maximun at 60°C.

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HISTOLOGICAL LOCATION AND BIOCHEMICAL CHARACTERIZATION OF LATEX PROTEASES IN *MORRENIA BRACHYSTEPHANA* GRISEB. (ASCLEPIADACEAE). Bettiol, M.[1], Conforti, P.[1], Arribére, M.C.[1], Cortadi, A.[2], Priolo, N.[1], Gattuso, M.[2] and Caffini, N.[1]. (1) LIPROVE, Depto. de Cs. Biológicas, Fac. de Cs. Exactas, Univ. Nac. de La Plata, Argentina; (2) Cátedra de Botánica, Area Biología Vegetal, Depto.de Cs. Biológicas, Fac. de Cs. Bioqcas. y Farmacéuticas, Univ. Nac. de Rosario, Argentina.

Ramified, non articulated laticifers containing proteolytic enzymes were observed in stem macerates of *Morrenia brachystephana* Griseb. (Asclepiadaceae). The enzymes were detected *in situ* by histochemical assays made in longitudinal sections of fresh stems, according to Denker's film-substrate method.

Latex obtained by superficial incisions of stems was gathered on 0.1 M phosphate buffer (pH 6.5) containing 5 mM EDTA and cysteine and centrifuged at 16.000g for 30 minutes. This crude extract shows remarkable proteolytic activity on caseine: maximum activity was reached at pH 8-10 with 12 mM cysteine. The enzyme preparation was strongly and irreversibly inhibited by very low concentrations of iodoacetate (0.01 mM) and Cl_2Hg (0.1 mM), but not fully and reversibly inhibited by 10 mM PMSF, suggesting that the proteases belong to the cysteine proteases family.

The crude extract was purified by fractioned acetone precipitation followed by catioinc exchange chromatography (CM Sepharose CL-6B; elution buffer cítric acid-sodium phosphate pH 6.4; 0.3-0.8 M sodium chloride gradient). Two basic (pI higher than 9.3, isoelectric focusing) proteolytictic active fractions were obtained, both homogeneus by SDS-PAGE and with similar molecular masses (about 24 kDa).

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EFFECT OF COLD AND FREEZING ON *in vivo* FLUORESCENCE IN *Deschampsia antarctica Desv.* Casanova[1], A., Corcuera[2], L. J., Zúñiga[3] G.E. and Alberdi[1], M. [1]Instituto de Botánica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia; [2]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago; [3]Facultad de Química y Biología, Universidad de Santiago de Chile.

Low temperatures (cold and frost) usually induce alterations of the photosynthetic apparatus that may be reflected in increased fluorescence (F) and a decrease in the photosynthetic efficiency (PE). *Deschampsia antarctica* is one of the two angiosperms that grow in the Antarctic. We postulate that the survival capacity of *D. antarctica* in this cold and harsh environment may be related to the capacity of maintaining its PE under low temperature conditions.

PE (PE=Fv/Fm, variable and maximal fluorescence) and initial fluorescence (Fo) in *D. antarctica* under chilling (2± 1,5°C), frost (-5 a -25± 0,1 °C) and at optimum temperature (13± 1,5°C, control) were determined. Chlorophyll contents were also determined.

PE of control plants and plants growing under chilling conditions did not change during treatment. Chlorophyll contents of chilling plants decrease about 50%. Plants subjected to frost showed a decrease in PE only at -15° C, determined by a 50 % downfall of Fm. Fo remained constant. These values were similar to those obtained with plants growing in the Antarctic. This photosynthetic efficiency was ratified by the high CO_2 fixation rates observed in the field.

Maintaining the PE over a wide range of temperature could help *D. antarctica* to survive in the Antarctic. The decrease in chlorophyll could be a mechanism for decreasing the light harvesting capacity under low temperature.

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POSSIBLE PHYSIOLOGICAL ROLE OF NADP-MALIC ENZYME FROM WHEAT. P. Casati. C. P. Spampinato and C. S. Andreo. Centro de Estudios Fotosintéticos y Bioquímicos CEFOBI (CONICET, F.M. Lillo). Suipacha 531. 2000 Rosario. Argentina.

The NADP-malic enzyme (NADP-ME) catalyses the oxidative decarboxylation of L-malate to yield pyruvate, CO_2, and NADPH and requires a bivalent metal ion as an essential cofactor. In C_4 and CAM plants, an abundant isoform releases CO_2 to be used in carbon fixation by ribulose-1,5-bisphosphate carboxylase. However the apparently universal presence of NADP-ME in plants suggests that it has a function broader than these specialized purposes. In this way, the possible role of the enzyme in wheat (C_3 plant) was studied. Thus a variety of compounds was tested as possible inductors of the enzyme. Detached leaves of wheat were incubated at 20 °C under a 14 h photoperiod in the absence or presence of different compounds. The cellulase treatment (0.5 mg/ml) resulted in the strongest activation of the enzyme after 48 h-incubation period (2.9 fold increase). Macerozyme (0.5 mg/ml) and glutathione (10 mM) were another inducers, giving a 1.7- and 1.8-fold increase in NADP-ME activity under the same conditions. Additional compounds were found to be ineffective in inducing the enzyme activity. NO_3^- (0.8 and 40 mM), abscisic acid (100 µM), H_2O_2 (300 mM) and ascorbic acid (10 mM) did not affect NADP-ME activity. In this way, since cellulase and macerozyme of wounded wheat leaves were reported to cause induction of lignification and glutathione stimulated several enzymes of lignin biosynthesis, we suggest that malic enzyme of wheat could be implicated in defense-related deposition of lignin.

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POSTHARVEST HEAT TREATMENT AND STRAWBERRY FRUIT RIPENING. Civello. P.M., Martínez, G.A., Chaves, A.R., Añón, M.C. CIDCA (UNLP-CONICET). La Plata, Argentina.

Application of heat treatments at high temperatures during short times may be an alternative physical method to delay ripening and improve fruit conservation. Strawberries have a short postharvest life so the use of such treatments could be useful. In the present work, heat treatments and their effects were analized in strawberries. Fruits of commercial maturity (100% Red) were treated at temperatures ranging from 39°C up to 50°C during 1, 2, 3, 4 and 6 hours. Afterward, fruits were placed at 20°C during 3 days and fruit decay, color development and fungal growth were evaluated. It was observed that treatments at 42°C and 48°C during 3 h showed less level of decay and fungal development. Both treatments were chosen to analyze the evolution of ripening parameters. For this purpose, fruits with lesser ripening stage (50-75% Red) were utilized. Heat treated strawberries showed lower external color development, which was evaluated by the a/b rate, and greater brightness, which was evaluated by L parameter. Moreover, anthocyanin level and PAL (phenylalanine ammonia-lyase) activity of treated fruits were lower than those of control fruits. Analysis performed by SDS-PAGE showed an increase of 18.4 and 69.8 kDa polypeptides and lesser accumulation of 40.3 kDa polypeptide in treated fruits. Incorporation of S^{35}-methionine into proteins was also evaluated and it was observed that heat treatments caused a decrease in fruit protein synthesis. It concludes that heat treatments on strawberry fruits reduce fungal growth and delay ripening. Therefore, these treatments could be useful to prolong the storage life of strawberries.

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INITIAL STUDIES ON TREHALASE ACTIVITY FROM PEANUT RHIZOBIA.-Dardanelli M., González P., Bueno M., and Ghittoni N.-Depto. Biología Molecular, Univ. Nac. Río Cuarto, Argentina.- Email: NGhittoni@UNRCCC.EDU.AR

Recently (Ghittoni & Bueno 1996) we reported trehalose accumulation in four peanut rhizobia cultured under hypersalinity, and we have also communicated (SAIB '95) increased activity of trehalose-P-synthetase in those bacteria. The activity of the catabolic enzime trehalase was only detected in some *Rhizobium* strains (Hoelzle & Streeter 1990). In the present work, we studied trehalase activity from peanut inoculants recommended by international collections. Bacteria were cultured as described elsewhere (Ghittoni & Bueno 1995). Trehalase activity was determined by the method of Müller et al (1992) with some modifications. After subjecting the cells to chloroform shock (Ames et el 1984) the released activity of trehalase was 9-fold greater than the cellular activity before shock, result indicative of a periplasmic localization. Crude periplasmic trehalase manifested a pH optimum between 6.4 and 7.0, and a temperature optimum at approximately 60°C. During 24 hs of bacterial growth in basal medium, changes in trehalase activity were not detected. On the other hand, in bacteria grown under hypersalinity for 24 hs, trehalase activity diminished to 47 % ($p<0.001$) of control values. This preliminary result suggests that decreased degradation of trehalose could be one of the responsible factors for the disaccharide accumulation in peanut rhizobia under hypersaline stress.

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TRANSFORMATION OF TOBACCO AND ARABIDOPSIS WITH THE MOVEMENT PROTEIN OF TWO TOBAMOVIRUSES.

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Most plant RNA viruses encodes a movement protein(MP). This MP tobacco mosaic virus (TMV) is crucial for the virus cell to cell movement in plants. TMV strain U1 infects both *N. tabacum* Xanthi NN and Xanthi nn, and also infects *A. thalinana* when is artificialy inoculated in high concentration. TMV strain Cg infects *A. thalinana* (Col 0 and C24 ecotypes) and *N. tabacum* (both cultivars). In infected plants TMV Cg and TMV U1 cause virus accumulation in systemic leaves. Our mayor goal is to study the role of MP in the virus spread. In order to do that we will evaluate the complementation of mutant virus deleted in MP (TMV-Cg ΔMP and TMV-U1 ΔMP) in transgenic tobacco and Arabidopsis that express MP of U1 or Cg strains. We have transformed *N. tabacum* and *A. thaliana* with TMV-Cg MP or TMV-U1 MP, using nptII as a marker gene, and we are currently evaluating the plants. ELISA shows high expression level of NPTII in some of this plants (similar to our transgenic control plants). Besides, transgenic plants are being evaluated by complementation with TMV-ΔMP infection and also by PCR analysis of MP gene. We hope that some of the 30 tobacco transgenic lines and 8 Arabidopsis lines could virus movement function.

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PROTEIN CONTENT OF FIFTY PERUVIAN VARIETIES OF TROPAOELUM TUBEROSUM (MASHUA). **Espinoza E., Sofía. Centro de Investigación de Bioquímica y Nutrición. Facultad de Medicina. Facultad de Odontología, UNMSM, Lima, Perú.**

The andean tuber Tropaeolum tuberosum (Mashua) is an underexploited plant. It's domestication occurred 5000 years ago and it's use was related with it's antiaphrodisiac properties in men and it's fertilizing properties in women. Mashua is know for it's high nutritional value in carbohydrates, and Vitamins C and A. However no studies are known of the protein content of this tuber. This work reports the protein nitrogen content by Kjeldahl method coupled to a colorimetric method (Nessler's Reaction) in 50 varietes of Mashua from Junín, provided by CIP-PERU. The range of protein content for the samples (N factor x 6,25) varied between 1,2 to 5,2 grams %. We have identified 4 varieties with a protein content greater than 4-5 grams % of edible portions, which will be characterized biochemically and genetically.

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IDENTIFICATION OF 20 S PROTEASOME FROM WHEAT LEAVES Fernández Murray P., Giordano C. V. and Barneix A., Cátedra de Microbiología, Facultad de Agronomía, Universidad de Buenos Aires and CIBYF (CONICET), Buenos Aires, Argentina.

The 20 S proteasome, also known as multicatalytic proteinase (MCP) is an essential protease found in all eukaryotic organisms and is responsible as the degradative core of the ubiquitin-proteasome pathway of the highly specific degradation of most cytosolic proteins. Recent accumulated evidence indicate that the protein degradation via this pathway is essential in the basic regulation of the cellular metabolism. In order to initiate the study of the role of this proteolytic system in the wheat leaf senescence process, the 20S proteasome from this source was purified to near homogeneity by DEAE-Sephacel, Q-Sepharose, Sepharose and hydroxilapatite column chromatography. Substrate specificity and inhibition/activation studies as well as structural characterization of the enzyme (native and SDS-PAGE; 2D electrophoresis, electronic microscopy) show that the wheat leaf 20 S proteasome possess the canonical features of this conserved proteinase.

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ANTIPHYTOPATHOGENIC ACTIVITY OF STRAWBERRY (*Fragaria ananassa*) LEAF PROTEINS. Filippone, P., Diaz Ricci, J., Mamaní de Marchese, A., Ontivero, M., Farías, R. and Castagnaro, A. Dept. Bioquímica de la Nutrición. INSIBIO (CONICET-UNT). Chacabuco 461, 4000 Tucumán, Argentina.

Plants have different mechanisms of defense against pathogens that can be constitutive or inducible. This work, which is part of a project that aims the characterization of proteins involved in plant defense, started with the extraction of soluble proteins from strawberry leaves (cv. Chandler). Proteins were precipitated with ammonium sulfate (80% sat.) and dialyzed through a benzoylated membrane. Biological activity of this extract was tested with a reference bacterium (*Clavibacter michiganensis*) by observing growth inhibition halos. The extract, that displayed inhibitory activity, was filtered through a Sephadex G75 column and two peaks with apparent molecular weight of 40 and 3.5 Kda were obtained. Although both fractions showed antibiotic activity, the one with lower molecular weight showed higher inhibition. The latter protein fraction was subjected to FPLC (C1/C8 column, Pharmacia) with acetonitrile gradient (0-100%) in Tris (5mM) / EDTA (0.5mM) buffer (pH 7.5) and two peaks were detected at 0% and 80%, respectively. SDS-PAGE (15%) analysis of both fractions showed one electrophoretically pure band of 4.5 Kda for the first peak, and four bands of about 3, 7, 11 and 14 Kda, respectively, for the second one. The 4.5Kda protein showed high antibiotic activity against not only with *C.michiganensis* but also with *Colletotrichum fragarie* - the fungus causing Anthracnose, a major strawberry disease in tropical and subtropical agroecosystems.

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AMINO- AND IMINOPEPTIDASE FROM AMARANTHUS SEEDS. Franzoni, L*. Troiani, R.M.de**, Martina, R.*& Isola, M. C.*(*)Departamento de Biología Molecular, Universidad Nacional de Río Cuarto,Río Cuarto, Argentina.(**)Cátedra de Química Agrícola.Facultad de Agronomía.Universidad Nacional de La Pampa, La Pampa, Argentina. (*)@ UNRCCC.edu.ar

It has been generally accepted that the small peptides produced by the joint action of endo- and carboxypeptidases on the proteins are hydrolized by amino- and dipeptidases to free amino acids. From the amaranth seeds *(Amaranthus mantegazzianus* Pass.cv. Don Juan) we have separated by PAGE one aminopeptidase which among the aminoacyl β-naphtylamides tested as substrates, would preferably attack those of ala and arg, and another specific for the pro derivative (iminopeptidase). We have analyzed in detail the latter enzyme in a preparation partially purified by ionic exchange chromatography and gel filtration. This iminopeptidase hydrolyzes specifically pro-β-naphtylamide and pro-p-nitroanilide to a optimal pH of 8. The Km for pro-p-nitroanilide was 0.1 mM. The metal chelating agent 1,10-phenanthroline had no effect on the activity, suggesting that the enzime is not a metalloenzyme. This is sensitive to leupeptin and p-hidroximercuribenzoate, inhibitors of cysteine-proteinases, suggesting the presence of a thiol group essential for the enzymatic activity.The enzyme, however, is not activated by dithioerythritol . Pepstatin, an inhibitor of aspartil-proteinases, it also inhibits the iminopeptidase of amaranth, a feature that differentiates it from other similar enzymes, although the explanation thereof is still pending.

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REGULATION OF STARCH BIOSYNTHESIS IN AMYLOPLASTS. STUDIES ON ADPGLUCOSE PYROPHOSPHORYLASE FROM WHEAT AND BARLEY ENDOSPERM. Gómez Casati, D.F. and Iglesias, A.A. Instituto Tecnológico de Chascomús. INTECH. Casilla de Correo 164. Chascomús. 7130. Argentina.

Starch synthesis in higher plants is a plastidic event that occurs *via* ADPglucose (ADPG), being its formation (catalyzed by ADPG pyrophosphorylase, EC 2.7.7.27; namely ADPG PPase) the regulatory step of the metabolic pathway. Although the structural, kinetic and regulatory properties of ADPG PPase from chloroplasts (photosynthetic cells) were well characterized, the enzyme from amyloplasts (reserve tissues) was less studied. Moreover, the entire understanding of carbon metabolism and partitioning in storage cells is far from complete. We determined that ADPG PPase from barley and wheat endosperm is inhibited by Pi ($I_{0.5}$= 0.8 mM) and this effect reversed by 3P-glycerate (3PGA). Studies carried out with the purified enzyme show that amyloplastic ADPG PPase is also inhibited by ADP and fructose-1,6-bisP and that 3PGA, but also fructose-6P are effective to reverse the inhibitory effects. Interestingly, neither 3PGA nor fructose-6P have effect on ADPG PPase activity when assayed in the absence of inhibitory compounds. Kinetic parameters for one specific effector were swayed by the presence of other modulator(s) of the enzyme activity. Results suggest that, in the amyloplast, ADPG PPase activity is determined not only by the 3PGA:Pi ratio, but also by levels of different compounds playing key role in carbohydrates metabolism.

Supported by grants from Fundación Antorchas (Argentina).

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OXIDATIVE STRESS CAUSED BY METAL IONS IN SUNFLOWER LEAVES. Susana M. Gallego. María P. Benavides and María L. Tomaro. Dpto de Química Biológica, Fac. de Farmacia y Bioquímica, U.B.A. ARGENTINA E-mail ptomaro@ffyb.uba.ar

Uptake of toxic quantities of heavy metals by higher plants can result in inhibition and/or induction of several enzymes. In many areas of biology, highly reactive free radicals have been implicated directly in the molecular damage associated with exposure to a wide range of pollutants. The antioxidant system in plants act as an important stress tolerance mechanism by protecting the cell against damage caused by the toxic oxygen species. The relationship between heavy metal ions toxicity and oxidative stress in plant cells was studied. Leaf discs (12 mm in diameter, 0.3 g) from 14 day-old *Helianthus annuus* L seedling were incubated under fluorescent light in solutions containing 0.5 mM of Fe(II), Cu(II), or Cd(II) ions for 12h. Controls were incubated in demineralized water. When the effects of scavenger of free radicals were investigated, the segments were floated on a 0.5 mM solution of metal ions that contained 10 mM sodium benzoate or 10 mM mannitol. After the incubation period, leaf discs were washed with distilled water and extracted for the differents determinations. All metal ions studied produced a chlorophyll and GSH contents decrease as well as lipid peroxidation and lipoxygenase activity increase. Scavengers of free radicals, such as sodium benzoate and mannitol, prevented either decrease in chlorophyll and GSH contents or lipid peroxidation increase and lipoxygenase increase. While Fe(II) and Cd(II) ions caused decrease in superoxide dismutase activity, Cu(II) ions produced increase one. However, the three metal ions caused decrease in the other antioxidant enzymatic defenses (catalase, ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase). Scavengers of free radicals protected these enzymes against inactivation. Not effect of these scavengers was obserbed on superoxide dismutase activity. These results indicate that excess of Fe(II), Cu(II), and Cd(II) ions produce oxidative damage in plant leaves, as can be demonstrated by increase of reactive oxygen species (measured as lipid peroxidation and lipoxygenase activity) and the decrease in both, soluble and enzymatic antioxidant plant defenses.

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PEROXIDASE AND IAA OXIDASE ACTIVITIES IN SUNFLOWER PLANTS. M.D.Groppa. M.L.Tomaro and M.E.Fernández. Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

Peroxidases are hemoproteins involved in a great variety of processes, including lignification and stress response. They are also related with plant development in a complex manner. Due to the occurrence of multiple isozymes, it is not easy to study their in vivo mechanisms of control. Indoleacetic acid (IAA) catabolism is also attributed to peroxidases, although there is still controversy on this matter. We have begun to study the expression of peroxidases during plant development in order to shed light on these subjects. We worked with sunflower plants processed 7, 14 and 28 days after sowing. Different parts of the plants were homogenized and the soluble extracts were studied. Peroxidase activity was found in every sample. A peptidic activator that was found in seeds associated to membranes, was not detected in plants. Nevertheless soluble plant extracts were activated up to 200% by the seeds activator. Plants samples were analyzed by native PAGE followed by specific peroxidase staining. When cathodic gels were performed, 4 bands were revealed in seeds but no bands were detected in plants, except in cotyledons where a band coincident with one of the 4 seeds isoforms appeared. Several isoforms were detected in anodic gels for all plant fractions, but no anodic bands were present in seeds. IAA oxidase activity was not detected in any of the plant fractions although we could find it in seeds. We could stablish that in plants there is a soluble factor that functions as an inhibitor of seeds IAA oxidase. The physiological implication of this control mechanism is apparent: once the growing process has begun IAA oxidase activity is inhibited to control auxin catabolism.

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INVOLVEMENT OF MAGNESIUM AND ATP IN THE REGULATION OF NITRATE REDUCTASE ACTIVITY IN SINAPIS ALBA. Herrera, R.. Johnson, C.B.(1). Departamento de Ciencias Biológicas, Facultad de Recursos Naturales, Universidad de Talca, Talca, Chile. (1) Department of Botany, Plant Science Laboratories, The University of Reading, Whiteknights P.O. Box 221, Reading RG6 6AS, UK.

NADH:nitrate reductase (NR, EC 1.6.6.1) is the key enzyme involved in the first step of nitrate reduction to nitrite. In plants, both phytochrome and a phosphorylation mechanism have been reported to play important roles in the regulation of NR activity.

NR from mustard (*Sinapis alba* L. cv. Sultons) seedlings and one month old plant extracts were 40% inhibited by 5 mM Mg^{2+} and 4 mM Ca^{2+}. Mg^{2+} and ATP (1 mM) also inhibited NR activity. The crude and desalted extracts from light treated plants and seedlings were 60 and 66% inhibited, respectively.

Two isoforms of NR have been separated using an anion-exchange chromatography. Both isoforms extracted from light treated plant/seedling were inhibited by Mg^{2+}/ATP, but isoforms extracted from dark treated material were insensitive to inhibition. The more negatively charged isoform of NR (NR_2) was highly sensitive, and strongly inhibited by Mg^{2+}/ATP.

The activity of dark treated material is comparable to the remaining activity of light treated material previously incubated with Mg^{2+} and ATP. NR_2 is the light inducible form and, according to current evidence, the target for activity modulation.

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THE *IN SITU* ACTIVITY OF VACUOLAR INVERTASE FROM *Solanum tuberosum* TUBERS. Isla, M.I., Ordóñez, R.M., Vattuone, M.A., Sampietro, A.R.; Cátedra de Fitoquímica, Instituto de Estudios Vegetales. Facultad de Bioquímica, Química y Farmacia. UNIVERSIDAD NACIONAL DE TUCUMAN. Ayacucho 461. 4000 - San Miguel de Tucumán. ARGENTINA.
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In higher plant cells sucrose is stored essentially in the vacuoles. It may be movilized from this compartment according with the metabolic needs. Little is known about a possible hydrolysis of sucrose before or after the sortening of sucrose from the vacuole. We demonstrated the vacuolar localization of acid soluble invertase from *Solanum tuberosum* tubers. This paper shows by the first time, the hydrolysis of sucrose *in situ* (into the vacuole). For this purpose vacuoles were isolated and purified from *Solanum tuberosum* tubers var. Kenebec, their diameter was measured (171.7 ±33 µm, n=200) and their volume was calculated ($0.26x10^{-2}$±0.05 µl). In vacuoles isolated and maintained at 30°C and 37°C β-fructofuranosidase activity was determined as time function by measuring the residual sucrose content into the vacuole. Intra and extravacuolar concentrations of glucose, fructose and sucrose were determined. Glucose and fructose concentrations raised during 45 minutes of incubation, while sucrose diminished. The reaction was spontaneously stopped when fructose concentration was 0.0654 µmole/µl. The same results were found with purified invertase in *in vitro* assays and in identical experimental conditions. The vacuolar pH measured with molecular probes was about 5.2±0.2, n=10. *In vitro* assays show that spontaneous hydrolysis of sucrose does not occurs at this pH.

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SOY PROTEIN CONCENTRATES TREATED WITH PROTEASES FROM NATIVE PLANTS. A. Enzyme treatment and electrophoretic analysis of the hydrolysates. López, L.M.I.*, Brullo, A., Vairo Cavalli, S., Natalucci, C.L.**, Caffini, N.O.** (LIPROVE), Wagner, J.R.*, and Sorgentini, D.A. (CIDCA), Depto. Cs. Biológicas, Fac. Cs. Exactas, Univ. Nac. de La Plata, 47 y 115, (1900) La Plata, Argentina.

Soy protein concentrates (70% protein content) were prepared by alcoholic extraction of defatted soy protein flour and hydrolyzed with native plant proteases isolated at our laboratory (LIPROVE): hieronymin and macrodontin, two cysteine proteases present in fruits of *Bromelia hieronymi* Mez and *Pseudananas macrodontes* (Morr.) Harms, respectively, and pomiferin, a serine protease from the latex of *Maclura pomifera* (Raf.) Schneid. A microbial commercial protease (alcalase) was also assayed for comparative purposes.

Soy concentrate water dispersions (8%) were incubated (3 h) at 45°C and optimum pH conditions for each enzyme and the degree of hydrolysis was determined according to the Adler-Nissen procedure. Inactivation of enzymes was achieved by heating the samples (75°C) at low pH (4.2) for ten minutes. The soluble products were analyzed by SDS-PAGE and native and denaturing bidimensional electrophoresis. SDS-PAGE showed a characteristic proteolytic pattern for each enzyme:. the 7S-α' polypeptide was fully degraded, the 7S-β and the 11S-B polypeptides were only partially degraded, and the 7S-α and the 11S-A polypeptides were detected solely in pomiferin hydrolysates. Bidimensional electrophoresis suggests the existence of peptide association. The electrophoretical behavior of the hydrolysates correlates with the modification of their functional properties (see Part B).

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SHORT-TERM TREATMENT WITH HIGH SUCROSE STIMULATED CDPK ACTIVITY IN *Solanum tuberosum*.

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Calcium-dependent protein kinase activity (CDPK) was characterized in potato plants. This enzyme activity increased during the early stages of tuber development. Our goal is to identify the effect of sucrose, a tuber inductor, on CDPK activity.

Adult potato plants were cultured in short-term treatment during three days under continuous light with 8% sucrose, water or sorbitol. In addition, potato explants were grown during 45 days, long-term treatment, in MS with 8% sucrose or water, under a 16 hours light photoperiod. A 60% increase of the CDPK activity was observed in the soluble fraction of sucrose treated plants, after the short-term treatment. This fractions were partially purified by a Mono-Q column. The CDPK activity that eluted at 0.4 M NaCl was 4-fold higher in the sucrose induced plants than in the controls with water or sorbitol. In the plants subjected to the long-term treatment the CDPK activity, in the crude fractions, was 50% lower in the sucrose induced plants compared to the controls with water. Phosphatase inhibitors added to the kinase assay mixture restored CDPK activity to the control levels. Sucrose addition in the kinase assay, did not modified the CDPK activity, indicating that the enzyme is not the direct receptor of the sugar.

These results suggest that this sucrose-stimulated CDPK activity might regulate various cellular activities involved in tuber development.

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THE POTATO γ-SUBUNIT mRNA OF MITOCHONDRIAL *F1-ATPase* IS UP-REGULATED DURING INFECTION PROCESSES AND ELICITOR TREATMENTS. Madrid E., Laxalt A., Beligni V. and Lamattina L., Inst. Inv. Biol., UNMDP, CC 1245, 7600 Mar del Plata, Argentina.

Mitochondrial F_1F_o-ATP synthases contain in dicotyledoneous plants six different subunits named α, β, γ, δ, δ' and ε in decreasing order of their molecular weights. Except for the α-subunit, which is synthesized in mitochondria, all other subunits are encoded by nuclear genes. A key subunit in the coupling mechanism is the γ-subunit, which appears as a single copy in the complex interacting with the α and β subunits that contain the catalytic sites.

Since an increase of respiration rate and metabolic activity of mitochondria have been observed during infection in plants, we examined the expression of the γ-subunit of F1-ATPase during biological stress conditions generated by the infection of potato plants with the pathogenic fungus *Phytophthora infestans* (P.i.). We amplified by RT-PCR and cloned a DNA fragment corresponding to the 3'-end of potato γ-subunit cDNA. The comparison of partial nucleotide sequence of potato γ-subunit showed 84 % homology with the sweet potato counterpart. The comparison of aminoacid sequence of potato γ-subunit showed 83 % identity and 96 % similarity with the sweet potato sequence. Northern blot experiments revealed that potato γ-subunit transcript level increased 2-fold in leaves after 72 h of infection. Fungal elicitors like Eicosapentaenoic Acid (EPA) and glucans from P.i.-cell wall were used in induction experiments on potato tuber. The γ-subunit mRNA level were respectively increased by 1.8, 1.4 and 2.2-fold in EPA, glucans and EPA + glucans treatments.

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EFFECT OF WATER STRESS ON SUCROSE METABOLISM IN WHEAT (*Triticum aestivum*) AND RICE (*Oryza sativa*). Martín M.L., Puebla A. and Pontis H.G. Centro de Investigaciones biológicas, FIBA, PROBIOP-CONICET, Mar del Plata, Argentina.

Water is the compound more abundant in plants and one of external factors that strongly affect their growth and development.

Water deficit elicit a complex of responses beginning with stress perception, which initiate a signal transduction pathway and is manifested in changes at cellular, physiological, and developmental levels. Among these responses we can cite changes in enzyme levels and activities, a variation in the protein pattern and a decrease of photosynthetic rate.

We have studied sucrose metabolism during water deficit in wheat (*Triticum aestivum*) and rice (*Oryza sativa*). Water deficit has been induced by increasing the concentration of polyethylene glycol 8,000 (5% to 20 %) during 8 days. Water status of plants during treatments was measured by relative water content (RWC). Sucrose levels of controls and stressed plants were measured and enzymes of sucrose metabolism were assayed: sucrose-phosphate synthase (SPS), sucrose synthase (SS) and acid invertase (INV). Sucrose content in shoots and roots wa increased under water stress conditions in both species. SS and SPS activities showed concomitant increases in the sucrose synthesis direction, while INV did not show any change in its activity in response to drought. Our results suggest the possible participation of sucrose metabolism in the response of gramineae to water deficit.

This work was supported by a Rockefeller Foundation Grant.

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'IN VITRO' DEGRADATION OF CHLOROPHYLLS BY PEROXIDASE FROM STRAWBERRY FRUIT. Martínez, G.A., Civello, P.M., Chaves, A.R., Añón, M.C. CIDCA (UNLP-CONICET). La Plata, Argentina.

Little is known about chlorophylls degradation during fruit ripening. In strawberries, particularly, a great decrease of the chlorophyll content occurs at the beginning of the ripening. It has been reported that peroxidase could be involved in chlorophyll catabolism, so we decided to study the action of this enzyme obtained from strawberry fruit. An acetone powder was obtained from green fruits and enzyme extraction performed. Peroxidase was then precipitated with $(NH_4)_2SO_4$ up to 85% saturation and redissolved in phosphate buffer pH=6.0. The degradatory activity was evaluated by mixing chlorophyll and H_2O_2 with the enzyme and following the decrease of absorbance at 669 nm. The enzyme needed the presence of resorcinol as cofactor. It was found that the optimal concentration of resorcinol was 3 mM. The effect of H_2O_2 concentration was also analyzed. The enzyme showed maximum activity at 1 mM H_2O_2, but greater concentrations than 5 mM caused an inhibitory effect. The optimum temperature reaction was 35°C, though it was observed an activity near to 25% of maximum activity at 55°C. Reaction mixtures prepared at different pH showed two maximum activity peaks, the principal peak at 4.5 and a secondary peak at 7.8. In conclusion, peroxidase extracted from strawberry fruit can degrade chorophylls 'in vitro' and could be involved in chlorophyll catabolism during strawberry fruit ripening.

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DIFFERENTIAL EFFECT OF NITROGEN ON THE EXPRESSION OF NADP-MALIC ENZYME ISOFORMS IN MAIZE TISSUES. Maurino V. G., Drincovich M. F., Estavillo E. G. and Andreo C. S. Centro de Estudios Fotosintéticos y Bioquímicos (CONICET, F. M. Lillo). Suipacha 531. 2000 Rosario, Argentina.

C_4 maize plants grown at high NO_3^- concentrations (5-20 mM) have higher CO_2 fixation rate and photoasimilates synthesis than that grown at low concentrations (0.8-2 mM). This increment in the photosynthetic activity is closely related with higher levels of key enzymes. It has been shown that RuBisCO, PEPCasa and PPdK content increase two times when inceasing NO_3^- concentration from 2 to 15 mM. At least two isoforms of NADP-ME have been characterized in maize tissues. One, implicated in the C_4 metabolism and the other, apparently constitutive, with anaplerotic roles. The aim of the present work was to relate the content and activity of NADP-ME isoforms with the growth of maize plantelets at different NO_3^- concentrations. Maize seedlings were germinated in absence and presence of 0.8, 5 and 20 mM NO_3^-. An increase in NADP-ME activity and enzyme content detected by Western blots analysis using a specific antibody was determined in green leaves extracts. Northern blot analysis using a specific probe against the green leaf isoform showed an accumulation of a 2.2 kb transcript, indicating a possible transcriptional control. On the other hand, a significant increase in NADP-ME activity, protein content and RNA accumulation was not observed for the root isoform, suggesting that the expression of the root isoform is not regulated by the nutritional levels of NO_3^-.

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ETHYLENE BIOSYNTHESIS BY CLIMACTERIC FRUITS. PROPERTIES AND ESTIMATION OF ACC OXIDASE DURING RIPENING. Moya León, M.A. and John, P[1]. Departamento de Ciencias Biológicas, Facultad de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile. [1]Plant Science Laboratories, The University of Reading, Reading RG6 6AS, United Kingdom.

The plant hormone ethylene induces and regulates the ripening process of climacteric fruits. The last step of its biosynthesis is catalysed by the enzyme ACC (1-aminocyclo-propane-1-carboxylic acid) oxidase, which transforms ACC into ethylene.

ACC oxidase enzyme from apple fruit (*Malus domestica* Borkh. cv Granny Smith) was purified to homogeneity in 3 chromatographic steps (FPLC system): anion exchange (Mono Q), chromatofocusing (Mono P), and gel filtration (Superdex-75). A 34-fold purification was achieved. The purified enzyme is active as a monomer of 38 kDa, with a pI of 4.6. The enzyme requires ascorbate and iron during the *in vitro* assay and its activity is stimulated by CO_2. A Km_{ACC} of 36μM was determined. The apple enzyme shares immunogenic epitopes with ACC oxidases from other fruits.

During ripening, the ethylene production by apple fruit increased dramatically. We have found that the increase in ethylene production is highly correlated with an increment of both ACC oxidase activity and ACC oxidase protein content. ACC oxidase protein content was estimated by densitometric analysis of immunoblots. These results strongly suggest an increase in the expression of ACC oxidase gene during ripening.

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PROTEOLYTIC ACTIVITY INVOLVED IN POTATO-*FUSARIUM* INTERACTION.

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We have previously reported the increase of proteolytic activity in the intercellular washing fluids (IWFs) from infected tubers with *Fusarium solani* f.sp. *eumartii* (pathogen isolate 3122). In contrast, not such activity was detected in control tubers. Otherwise, when *F. eumartii* 3122 was grown in liquid medium, proteolytic activity was significantly detected in the liquid filtrate. Both protease activities showed similar behavior against protease inhibitors, resulting included in the serin group. On the other hand, when potato tubers were infected with non-pathogen *Fusarium solani*, isolate 1402, proteolytic activity was not detected in IWFs.

In addition, the level of potato chitinase activity of IWFs infected with either 3122 or 1402 isolates showed similar temporal pattern . Nevertheless, the activity was slightly higher for pathogen than non-pathogen isolate. In order to test if extracellular chitinases from non-infected tubers are possible substrates of fungal and IWF proteases, we analysed their effects by western blot. using anti-chitinase antibody. Potato chitinase of molecular weigth approximately 30 kDa was partially digested during the treatment with either fungal or infected IWF proteases.

Little is know about the role(s) of these proteolytic activities and their relationship with other/s protein/s. Further studies of these activities could help to elucidate molecular aspects of potato-*Fusarium* interaction.

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AN ACID SOLUBLE INVERTASE FROM RIPE FRUITS OF *Cyphomandra betacea* SENDT. Ordóñez, R.M., Isla, M.I., Vattuone, M.A., Sampietro, A.R.; Cátedra de Fitoquímica. Instituto de Estudios Vegetales. Facultad de Bioquímica, Química y Farmacia. UNIVERSIDAD NACIONAL DE TUCUMAN. Ayacucho 461. 4000 - San Miguel de Tucumán. ARGENTINA.
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An acid soluble invertase and a glycoprotein with agglutinating activity were isolated from ripe fruits of *Cyphomandra betacea* Sendt., a native plant from South America with edible fruits. A polypeptide, M_r 19 kDa was isolated from the cell wall fraction.

The purpose of this work was the study of the invertase regulatory mechanism at the activity level. The invertase purification was made through the application of saline fractionation, affinity chromatography (Con A Sepharose 4B) and gel filtration (Sephadex G-100). Rechromatography on Sephadex G-100 showed an M_r of 63 kDa. Its optimum pH is 4.5 and its E_a is 6.4 kcal/mol. Studies on substrate specificity showed that the enzyme is a β-fructofuranosidase (EC 3.2.1.26) with a K_m=1.6x10^{-2} M and also attacks the oligosaccharides of the raffinose family with a K_m=2.5x10^{-2} M for raffinose and a K_m=4x10^{-2} M for stachyose. The Con A affinity for invertase demonstrates the glycoprotein nature of the enzyme. Activity inhibition studies showed that fructose is a classical competitive inhibitor. Proteins do not suppress the inhibitory effect of fructose. Otherwise, the lectin purified from the same origin was an enzyme activator as other proteins and glycoproteins. The polypeptide isolated and purified from the cell wall fraction showed to be a non-competitive inhibitor of the enzyme activity with a K_i=3.3x10^{-2} M. Exogenous proteins with known action on invertase activities from other organisms were assayed.

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TOPOGRAPHY AND FUNCTION OF GOLGI UDPASE. RELATIONSHIP TO PRIMARY CELL WALL POLYSACCHARIDE BIOSYNTHESIS. Orellana,A., Neckelmann,G. and Norambuena,L. Departamento de Biología, Facultad de Ciencias Universidad de Chile.

Hemicelluloses and Pectin are synthesized in the Golgi apparatus of plant cell by Glycosyltranferases. These enzymes use nucleotide sugar as donor for sugar polymerization, and the products of the transfer reaction are, a polymer with one additional unit, and the nucleoside diphosphate (NDP). What is the fate of NDP? Little is known in plant Golgi apparatus about the metabolism of the nucleotide moiety, although, it is known that it contains a UDPase activity. Therefore, it is possible that this enzyme is involved in transformation of UDP generated by substrates such as UDP-glucose, thus, connecting UDPase activity to polysaccharide biosynthesis. To test this hypothesis, we decided to analyze the topography and actual function of Golgi UDPase in pea stems Golgi vesicles. In a first step, we identified and separated Golgi UDPase from other UDPase activity located in pea stems, by linear sucrose gradients, and detection of activity on native polyacrylamide gels. We found that Golgi UDPase is a membrane bound protein, based on partition on Triton X-114. Treatment with Proteinase K of intact or permeabilized vesicles suggested that its active site faces the lumen of Golgi vesicles. Expression of activity through the stem of pea seedlings suggested that Golgi UDPase is highly expressed on the elongating region,correlating with UDP-glucose incorporation for polysaccharide biosynthesis in Golgi vesicles. Analysis of the metabolism of [β^{32}P]UDP-glucose in Golgi vesicles, suggested that [β^{32}P]UDP is quickly hydrolyzed to $^{32}P_i$ + UMP, showing a coupling between the Glucosyltransferase and UDPase reactions. This coupling would drive the glucose polymerization reaction, making it more effective on those places where cell growth and elongation are taking place. Supported by : Fondecyt 1940571 and Fundación Andes

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DIFFERENTIAL ACTION OF EFFECTORS OF SPSs FROM ETIOLATED AND GREEN RICE TISSUES. Pagnussat, G.C. and Salerno, G.L. Centro de Investigaciones Biológicas, F.I.B.A., PROBIOP-CONICET, C.C. 1348, 7600, Mar del Plata, Argentina.

Sucrose-phosphate synthase (SPS) catalyzes the formation of sucrose-phosphate from UDPG and fructose-6P and has a key function in the regulation of sucrose synthesis. This enzyme has important regulatory properties, including both allosteric regulation by glucose-6P (activator) and Pi (inhibitor), and covalent modification of the enzyme in response to light as demostrated in spinach leaves. The aim of this study was to characterize the two SPS forms isolated from etiolated rice seedlings and to compare their behavior in the presence of the described effectors (Pi and glucose-6P). SPS-1 and SPS-2 have similar native molecular masses and polypeptide composition (in Western blot analysis).SPS-1 has shown similar properties than those of SPS isolated from green leaves harvested during the dark period. On the contrary, SPS-2 was less sensitive to Pi and was differently activated by glucose-6P in the presence of the inhibitor. These results led us to suggest that SPS-1 could be the same enzyme form expresed in photosynthetic tissues, while SPS-2 could be related to the one present in white tissues as the embryo.

This work was supported by Rockefeller Foundation Grant.

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STUDIES ON SUCROSE-PHOSPHATE SYNTHASE/SUCROSE-PHOSPHATE PHOSPHATASE INTERACTION. Paris, G. and Salerno, G.L. Centro de Investigaciones Biológicas, F.I.B.A., PROBIOP-CONICET, C.C. 1348, 7600, Mar del Plata, Argentina.

Recent studies on sucrose-phosphate synthase (SPS, EC 2.4.1.14) have shown the presence of a proteinaceous activating factor (SAF). Further investigations strongly suggest that SAF and sucrose-phosphate phosphatase (SPP, EC 3.1.3.24) were identical. The fact that SPP activated SPS might indicate that there could be an interaction between both proteins. Later studies have shown that an intermediate channeling occurred. The aim of this study is to add new evidences to prove the physical interaction between SPS and SPP. Both enzymes were highly purified from rice (*Oryza sativa*) leaves. SPS alone and SPS + SPP were electrophoresed in native polyacrylamide gels and enzyme products were measured in each lane. A peak of SPS activity was overlapped by SPP when both enzymes were present. Similar results were obtained when the enzymes were electrophocused in a slab system. Kinetic parameters of SPP were determined in the presence and absence of SPS. No modification of SPP affinity for its substrate (sucrose-6 phosphate) was reached at. Additionally the effect of metabolites related to SPS regulation (Fru-6-P, Glu-6-P, UDP-Glc and UDP) on SPP activity was investigated. The data presented support the hypothesis that a metabolic channeling is contributing to increase the net sucrose synthesis in leaves.

This work was supported by a Rockefeller Foundation Grant.

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PROPERTIES, CHARACTERIZATION AND DEVELOPMENTAL PROFILE OF ACTIVITY AND CONCENTRATION OF THE NADP-MALIC ENZYME (NADP-ME) FROM COTYLEDONS OF *RICINUS COMMUNIS*.

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In germinating seeds of *Ricinus communis,* the conversion of sucrose to triacylglycerols is a major metabolic activity. The purpose of this work was to reveal possible relationships between the activity of NADP-ME and fatty acid synthesis in *Ricinus* cotyledons. The NADP-ME catalyses the conversion of L-malate to pyruvate and NADPH, potential substrates for fatty acid synthesis. NADP-ME activity and immunoreactive protein levels were monitored during germination, up to 20 days post-imbibition. The developmental profile shows a peak in activity (6 times of the basal value) at day 7. The immunoblot analysis (using anti-maize NADP-ME) revealed analogous results, evidencing a band of around 72 kDa. The enzyme was partially purified (8 times) and characterized. The optimum pH was around 7.1. The *Km* values for L-malate and NADP were 0.68 mM and 8.2 µM, respectively. The enzyme used Mg^{2+} or Mn^{2+} as cofactors. Several metabolites were assayed as potential enzyme modulators. Succinate, CoA, acetyl-CoA and palmytoil-CoA were activators of NADP-ME, at saturating or subsaturating substrate concentrations. The *Ka* for CoA and derivative compounds were in the micromolar range (*i.e.* 0.8 µM for CoA). No remarkable effects were obtained with Krebs cycle intermediates and aminoacids (*i.e.* α-ketoglutarate, glutamate, glutamine, fumarate). Activity was 29 times higher in the forward direction. Results suggest that cotyledon NADP-ME behaves as a regulatory enzyme in *Ricinus*. Its activity is responsive to precursors of the fatty acid synthesis pathway, and thus a role in this metabolism is possible.

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COMPARATIVE STUDY ON SUCROSE METABOLISM ENZYMES IN THREE SPECIES OF CYANOBACTERIA.

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Recently we have presented the first clear evidence for sucrose synthesis in a prokaryotic organism: the filamentous cyanobacterium *Anabaena 7119*. We have shown that there are two forms of sucrose-phosphate synthase named SPS-I and SPS-II which have remarkable differences with respect to those found in higher plants. Also we have demonstrated the presence of two sucrose synthases. As the role of these enzymes are still unclear, in the present study we investigated sucrose metabolism in cyanobacteria with different cellular organization. Proteins extracts were prepared from *Anabaena variabilis*, a filamentous heterocystic organism and the unicellular *Synechococcus*. The comparison of the patterns of the partilally purified enzymes and their biochemical properties with those of *Anabaena 7119*, suggest that the different enzyme forms may not be related neither to the filamentous state nor to the presence of heterocysts.

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A THERMOSTABLE α-GALACTOSIDASE FROM *LENZITES ELEGANS*. Quiroga, E.N., Rojo, H.P.,., Vattuone, M.A., Sampietro, A.R.; Cátedra de Fitoquímica. Instituto de Estudios Vegetales. Facultad de Bioquímica, Química y Farmacia. UNIVERSIDAD NACIONAL DE TUCUMAN. Ayacucho 461. 4000 - San Miguel de Tucumán. ARGENTINA. E-mail: postmaster@untmre.edu.ar.

Lenzites elegans, a wood decaying fungi, possesses many hydrolytic enzymes which were used in our laboratory for the production of plant protoplasts. The purpose of this work is the isolation and characterization of an α-galactosidase produced by this fungus.

L. elegans was grown in liquid media containing sucrose, xylane or pectin as sole carbon source. When the last nutrient was used the enzyme production was the highest. The enzyme was partially purified by the application of saline fractionation and gel filtration. Native PAGE produced only one activity band and two protein bands. The enzyme activity was measured by the p-nitro-phenol release from p-nitrophenyl α-D-galactopyranoside used as substrate. Using this substrate the K_m was 5 mM and the optimal pH was 4.5. The protein is stable from pH 3.0 to 7.5. Other sugars are also attacked by the enzyme preparation (raffinose, stachyose, melibiose, polygalacturonic acid and agarose). It is strongly inhibited by Hg^{2+} and Ag^{1+}. Its M_r, calculated by gel filtration is 125 kDa and its E_a is 17,1 kcal/mol. The optimum temperature for the enzyme activity is about 80°C (5 min at pH 4.5). The enzyme preparation incubated at 80°C for 30 min retained 53 % of its activity. The comparison with α-galactosidases from other fungi (*Penicilium purpureum* and *Mortierella vinaceae*) indicates a higher heat stability. Consequently, the α-galactosidase from *L. elegans* is a thermostable enzyme.

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REDUCTIVE MODULATION OF COLZA/CANOLA CHLOROPLAST FRUCTOSE-1,6-BISPHOSPHATASE: SITE-DIRECTED MUTAGENESIS OF CISTEINE RESIDUES.

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In higher plants Fructose-1,6-bisphosphatase (CFBPase) catalyzes the irreversible dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate in the CO_2 fixation cycle (Benson-Calvin Cycle). Its activity is thightly regulated by light *via* bivalent cations, metabolites and the Ferredoxin-Thioredoxin system. We had previously constructed a cDNA library of colza/canola (*Brassica napus*) leaves, sequenced a full-lenght clone coding for the CFBPase precursor, and then expressed in *E.coli* the mature form of the enzyme. Homogeneous preparations of the recombinant enzyme were catalytically active and the structural and kinetic characteristics analyzed did not reveal significant differences with the native form of spinach leaves. Of particular interest is the role of Cys174 and Cys179 residues in the light-dependent modulation of CFBPase. To adress this question we replaced, by site-directed mutagenesis, these amino acid residues by serine. After purification of both variants of CFBPase, only the C174S mutant appeared constitutively activated in the absence of reductants. The C179S mutant showed a low level of activity, resembling the wild type enzyme behaviour. Thus, it is strongly suggested that Cys174 is effectively involved in the reductive regulation but, accordingly to the working model, it remains to unravel wich other Cys residue does form, together with the C174S, the regulatory disulfide bridge. This results undoubtely strenght, for the CFBPase, the reductive activation scheme in which the reduction of (at least) one disulfide bridge, present in the inactive form, leads to the subsequent activation of the enzyme.

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KINETIC STUDY OF THE UBIQUITIN CONJUGATION SYSTEM FROM *Lemna minor*. Rangel, M.[1], Santos,C.[1,2], Ferreira,R.[1,2], Teixeira,A.[2]. [1]Inst. Tec. Quím. Biol., Oeiras, [2]Inst. Sup. de Agronomia, Lisboa, Portugal.

The ubiquitin-dependent proteolytic pathway is, in all eukaryotic cells, responsable for the selective degradation of both abnormal and short-lived proteins. The latter including many important regulatory proteins, (Finley and Varschavsky, 1985, *TIBS*, vol. 10, pp 342-347). In this ATP dependent proteolytic system, multiple ubiquitin molecules are enzimatically ligated to proteins and then released in a free, functional form during the degradation of the target proteins (Ciechanover, 1994, *Cell*, vol. 79, pp 13-21). The synthesis and isolation of ubiquitin-protein conjugates are essential prerequisites for the study *in vitro* of ubiquitin-mediated proteolytic pathway, (Hershko and Ciechonover, 1992, *Ann. Rev. Biochem.*, vol. 61, pp 761-807). Recently we have developed a simply and rapid methodology for the synthesis of high molecular mass ubiquitin-protein conjugates using crude extracts of the higher plant *Lemna minor* L. The conjugates were formed *in vitro* by the covalent ligation of exogenously added ^{125}I-ubiquitin to endogenous proteins (Ramos *et al*, 1996, *J.Exp.Bot.*, vol. 47, pp 569-575) and detected by autorradiography.

We have analysed some parameters that affect the kinetics of this plant ubiquitin conjugation system, namely, temperature, incubation time, ubiquitin concentration, and other factors that affect the catalytic activity of this system, such as the concentration of the ATP-regeneration system. Maximum activity of the conjugation of the conjugation system was achieved for a 30 minute incubation at 25°C. This activity decreased sharply for temperatures above 30°C. It was also observed a saturation kinetics in relation to ubiquitin concentration.

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EFFECT OF CADMIUM AND ARSENITE ON THE UBIQUITIN MEDIATED PROTEOLYTIC PATHWAY FROM *Lemna minor*

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Heavy metals are potent poisons for living cells. The reason for their toxicity *in vivo* remains uncertain but is well known that they contribute to the accumulation of aberrant proteins (Jungmann *et al.*, 1993, *Nature*, vol. 361, pp. 369-371; Chen and Piper, 1995, *BBA*, vol. 1268, pp.59-64). The synthesis of heat shock proteins (hsp), a universal response of cells to heat-shock, is also activated by treatments with heavy metals (Edelman *et al.*, 1988, *Plant Physiol.*, vol. 86, pp. 1048-1056) and recently the resistance to cadmium has been related with the ubiquitin proteolytic pathway (Jungmann *et al.*, 1993, *Nature*, vol. 361, pp. 369-371). In this pathway, multiple ubiquitin molecules are enzymatically ligated to proteins destined for catabolism in an ATP-dependent process. The high molecular mass ubiquitin-protein conjugates generated are then degraded by a specific, ATP-dependent protease complex, releasing amino acids and intact ubiquitin (Hershko, 1988, *J.Biol. Chem.*, vol. 263, pp 15237-15240).

In our work we followed the changes in both free ubiquitin and ubiquitin-protein conjugates during the incubation of *Lemna minor* fronds in the presence of cadmium and arsenite concentrations that confers thermotolerance to the plants. The observed increased levels of ubiquitin conjugates, detected by immunoblotting using anti-ubiquitin antibodies, suggests an involvement of the ubiquitin-mediated proteolytic pathway during arsenite treatment.

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CHANGES IN PEROXIDES LEVEL, PROTEOLYTIC AND CHITINOLYTIC ACTIVITIES IN STRESSED WHEAT LEAVES. Segarra, C.I., Casalongué, C.A. and Conde. R.D. Instituto de Investigaciones Biológicas, FCEyN, Universidad Nacional de Mar del Plata CC 1245 Mar del Plata (7600) Argentina. e-mail: segarra@uni-mdp.edu.ar

We have previously reported that inoculation of cultivar Pigüé wheat plants with conidiospores of the fungus *Septoria tritici* increases the proteolytic activities of extracellular matrix in leaf. That cultivar is fairly resistant to septoriosis, the disease produced by the fungus.

Whether the inoculation with conidiospores of *Septoria titici* also elicit plant responses commonly associated with the defense against pathogens is unknown. Then, both the leaf level of peroxides and chitinase activity were tested; the results indicated 300% and 500% increases over control respectively.

The effect of salicilic acid (SA), autoclaved spores and spore wall extracts was also studied. In every case peroxides increased 300%, but neither proteolytic nor chitinase activity increased.

The present results suggest that the increase of both proteolytic and chitinolytic activities requires the action of viable conidiospores and that widespread used elicitors such as SA not always induce the same response that a pathogen.

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CHARACTERIZATION OF AN INDUCIBLE APOPLASTIC ß-1,3 GLUCANASE IN COTTON. Sesma, J.I. and Vallejos, R.H. CEFOBI, Suipacha 531, 2000 Rosario, Argentina.

Plants have developed a variety of constitutive and inducible mechanisms to resist colonization by potencial pathogens. ß-1,3 glucanases are capables of hydrolysing fungal cell walls and may serve as antimicrobial defense system in plants. Unlike most species, where there are several glucanase isoforms, we found only a single soluble ß-1,3 glucanase in leaves and stems of cotton, although several were detected in calli cultures. The enzyme is induced by 50 mM salicilic acid after three days of treatment. Leaf protein extracts and intercellular washing fluids were analyzed by isoelectrofocusing and native PAGE resulting in a single acidic band of ß-1,3 glucanase activity. The enzyme has an isoelectric point of 4.7 and it was found in the apoplast. SDS PAGE and silver staining indicated that this enzyme had an estimated molecular mass of about 43 kDa.

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COMPARISON OF STRUCTURAL, THERMAL BEHAVIOR AND SURFACE PROPERTIES OF SOY WHEY PROTEINS AND SOY PROTEIN ISOLATES.

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The composition, surface hydrophobicity, thermal behavior and surface properties of soy whey proteins (SWP) were studied and compared those of the native soy isolate (SI) at different ionic strengths (μ=0-1M). By SDS-PAGE, we verified the presence of trypsin inhibitors, agglutinins and enzymes in the SWP, and the constitutives polypeptides of glycinin and β-conglycinin in the SI. By differential scanning calorimetry (DSC), it was observed that , in absence of NaCl, both samples presents two thermal transitions (peaks I and II: 75.0±1.0 °C and 89.4±0.5°C). As μ increases, the temperature of peaks I and II of SI (denaturation of β-conglycinin and glycinin, respectively) increase markedly, while those of SWP vary in a lesse extent. The lower thermal stability of SWP (lower ΔH values) could be caused by its low surface hydrophobicity (S_o) (measured by fluorometry), hardly modified by μ. The differences in composition, molecular size and hydrophobicity between proteins of SWP and SI are reflected by their surface properties. Unlike the SI, the SWP form low-stability emulsions and show high foam forming capacity (even at low μ), with low stability. This would be explained by their low molecular size and their inability to form a resistant protein lamella. The SI (higher molecular size and hydrophobicity) have difficulties in forming the foam but it shows higher stability.

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A REDUCTASE-BINDING PROTEIN COMPLEX IN ROOT PLASTIDS IS INDUCED BY NITRATE. Sosa, G.M., and Vallejos, R.H. CEFOBI, Suipacha 531, 2000 Rosario, Argentina.

Spinach root plastids diaphorase activity is membrane-bound and seems to be associated with a smaller polypeptide similar to the chloroplast reductase binding protein. A complex of these two polypeptides was purified from maize root plastids and chloroplasts. These polypeptides were recognized by antibodies against spinach thylakoid reductase and binding protein. However the MW of the reductases was different, 36 kDa in thylakoids and 34.1 kDa in plastids while the electrophoretic mobility of the smaller polypeptide was identical. Both the diaphorase and its binding protein were induced in maize and spinach root plastids by nitrate.

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DIFFERENTIAL EFFECT OF MODIFIED ATMOSPHERES ON ETHYLENE PRODUCTION, CAROTENOID BIOSYNTHESIS AND β-GALACTOSIDASE ACTIVITY IN TOMATO FRUIT RIPENING.

Trinchero, G.D., Sozzi, G.O. and Fraschina, A.A. Cátedra de Bioquímica, Facultad de Agronomía, Universidad de Buenos Aires, Argentina.

Modified atmosphere packaging during tomato fruit ripening reduces weight loss and spoilage and delays changes in acidity, sugar content, texture and polygalacturonase activity. The precise mode of action of low O_2 and/or high CO_2 on fruit ripening is not well understood. The objective of this study was to determine the effects of two different types of films on the evolution of ethylene, β-carotene (β-Car), lycopene and β-galactosidase (β-Gal) activity in two different tomato ripening stages (turning and light red).Greenhouse-grown tomatoes (cv. Ivan BHN-USA) were placed in small trays and sealed with 2 different films: (T_1) a polyolefin film and (T_2) a polyvinyl chloride film. Non-sealed fruit served as controls (T_0). Measurements were performed at least in duplicate once a week during 28 days. Ethylene production was monitored using GC; lycopene and β-Car contents were determined by means of spectrophotometric assays at 478 and 505 nm respectively, β-Gal activity was assayed using *p*-nitrophenyl-β-D-galactopiranoside as specific substrate and measuring the released *p*-nitrophenol at 400 nm.

The results showed: 1) a dramatic inhibition of ethylene production in treatments T_1 and T_2; 2) a marked delay in the synthesis of β-Car and lycopene in turning tomatoes (fruit in T_1 showed a two-week delay in comparison with T_0); 3) depressed activity levels for β-Gal, in particular in light-red fruit. It is speculated that β-Gal plays a key part in fruit softening. We can conclude that modified gaseous conditions with low O_2 and high CO_2 regulate many biochemical and physiological processes in ripening fruit, among them ethylene biosynthetic pathway. We are grateful to Universidad de Buenos Aires for financial support (UBACyT, grant AG060, resol. C.S. 1679/95).

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ANTIOXIDANT DEFENSES AGAINST TOXIC OXYGEN SPECIES IN SOYBEAN NODULES SUBJECTED TO SALT STRESS.

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Symbiotic nitrogen fixation in legumes is reduced in saline soils. Because of the high potential of nodules to produce activated oxygen species through the breakdown of leghemoglobin, the alteration of the nodule surroundings by salt stress could be the way to modify the balance between production and scavenging of these toxic species. Two soybean cultivars, one relatively tolerant (377) and one more sensitive (411) to salt stress were watered with a nutrient solution containing 150 mM NaCl. Nodules were excised to measure superoxide dismutase (SOD), ascorbate peroxidase (AP), glutathione reductase (GR), catalase (CAT), glutathione (GSH), nitrogenase determined by C_2H_2 reduction (ARA), leghemoglobin and malondialdehyde (MDA).

In cultivar 377, SOD increased 84%, catalase decreased 17%, GSH was reduced to 75%, GR declined to 68%, AP was 12% lower and MDA was 30% higher than the control. In cultivar 411, SOD increased 65%, CAT decreased 10%, GSH decreased to 88%, GR decreased to 81%, AP increased 4% and MDA increased 24% over the control. Nitrogenase decreased to 73% in 377 and to 56% in 411. Leghemoglobin was 12% larger than the control in 377 cultivar and decreased 16% respect to the control in 411.

Salt stress induced oxidative stress in soybean nodules by causing an overproduction of $O_2^{\cdot}$ and H_2O_2 both in tolerant and sensitive plants which is partially counterbalanced by SOD and AP. The lower level in AP observed in the most tolerant cultivar could be explained as a consequence of a rapid inhibition of AP by the higher H_2O_2 levels in 377. The detoxification system did not prevent the increased lipid peroxidation in the most tolerant cultivar, which probably implies that the molecular mechanism of salt tolerance involves the concurrence of several components added to the nodules detoxifying system.

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POTATO DEFENSE REACTIONS AND THEIR CONTRIBUTION TO VERTICAL AND HORIZONTAL RESISTANCE TO *Phytophthora infestans* (P.i)

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Genetically controlled disease resistance to plant pathogens can be classified as either vertical (VR) or Horizontal resistance (HR).In the VR a single R-gene is sufficient to protect the plant complefely against particular races of pathogen. The HR,or field resistance is assumed to be multiple gene based. Breeding efforts are therefore aimed to introduce durable field resistance into commercial potato cultivars.We describe here the increase in chitinases and glucanases activities, and phytoalexins production in two potato cultivars infected with two races of P.i. Disks of potato tuber of the Kennebec cultivar, multiple regarding VR and with a low level of HR, and of the Huinkul cultivar, resistant (VR) and with a high level or HR, were infected with either race "0"(lacking avirulence genes) or "C"(complex, with fine avirulence genes) of the fungus. Homogenates of tuber tissue (0-7 days) were prepared.Extracts from the Kennebec cultivar infected with race "0"showed a strong increase in chitinases and glucanases activities when compared to infection with race "C". These resullts would indicate that race "C is able to block or suppress reactions involved in the defense response. No significant difference were observed when the Huinkul cultivar was infected with both races. When the production of phytoalexins was measured similar results as with chitinases and glucanases were obtained. The relative contribution of these components to VR is poorly understood. However, their contribution to HR is unknow, and our results would not support a conclusive role for them in the interaction.

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CRYOPROTECTIVE PROTEINS IN *Deschampsia antarctica* Desv.

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D. Antarctica, the only gramineous plant that has colonized the Maritime Antarctic, is characterized by a high frost tolerance. This species must have mechanisms that allow the maintenance of metabolism during the Antarctic summer and survival during winter. One of them, could be related with changes in the concentration of proteins with cryoprotective capacity. In order to elucidate this hypothesis, we determined the total protein concentration and studied the cryoprotective capacity of proteins in plants growing in two habitats in the Antarctic (Robert and King George Islands) and of plants collected from these habitats but cultivated at 13± 1.5 °C and also cold acclimated at 2± 1.5°C in the laboratory. Oxygen evolution by chloroplasts from barley (*Hordeum vulgare*) was used as cryoprotection assay.

The SDS-PAGE protein patterns from control plants, cold acclimated plants in the laboratory, and plants grown in the Antarctic were very similar. Total protein extracts from plants growing in their natural habitat had a greater cryoprotective capacity (52-93%) than those growing at 13°C or 2°C (26-52%). Electroeluted bands of the most expressed proteins differed in their cryoprotective activity (17-89%) in extracts from Antarctic collected plants with respect to extracts of those grown in the laboratory.

Since Antarctic grown plants reduced their cryoprotective activity when cultivated at low temperature in the laboratory, factors other than just temperature may be involved in the development of the cryoprotective activity of protein extracts of *D. antarctica*.

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REGULATION OF AMINO ACIDS COMPOSITION OF DEVELOPING AND RIPENING FRUITS OF TOMATO. Valle E.M., Marro M.L., Palatnik J.F., Boggio S.B. Programa Multidisciplinario de Biología Experimental (PROMUBIE), CONICET, Facultad Cs. Bioquímicas y Farmacéuticas, UNR, Suipacha 531, 2000 Rosario, Argentina.

Developing fruits are very dependent on the photoassimilate import from the leaves until ripening. During maturation fruits undergo a complex series of physiological and biochemical events involving changes in colour, taste, aroma and texture. Amino acids (AA) contribute to the flavor of ripe fruits, but little is known about their import and assimilation in tomato fruits. We studied the long distance transport of AA and the capacity of developing and ripening fruits to metabolize free AA. Phloem sap was obtained by using a combination of the aphid technique and the leaf exudate. Gln and Glu were the main species translocated by the sieve-tube sap. The Gln content of immature fruits was low and its content raised during fruit growth. In these fruits a notable high content of GABA was observed, which could be explained by a resistance of young fruits to pathogenic agents. In all madure fruits Gln was the most abundant free AA showing the highest content just before ripening. Glu, which contribute to taste perception in foods, increased 10-times when ripening. To understand the regulation of Gln synthesis in tomato fruits we isolated a cDNA encoding Gln synthetase 1 (*gs1*) by RT-PCR. The gene was more highly expressed in madure green than in ripening fruits. The protein level showed a similar pattern indicating that Gln synthesis in ripening fruits is impaired.

This work has been supported by the Volkswagen Foundation

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CONSTITUTIVE PRESENCE OF A CHITINASE AND A THAUMATIN-LIKE PROTEIN IN THE APOPLAST OF HEALTHY *Lupinus albus* ORGANS.

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The plant cell walls constitute an extracellular matrix which is continuous with a system of intercellular air spaces and with the xylem. As a whole, this space is termed the apoplast, now recognized to be both the site at which signals originate to elicit defense reponses and where many defense-related products accumulate. In this work we are studying the soluble or ionically bound proteins from the apoplast of *L.albus* leaves. *L.albus* is an important grain legume crop considered to have some resistance to pathogen attack. We sequenced the amino termini of the four most prominent polypeptides from the apoplast of healthy *L.albus* leaves and surprinsingly found that two of them have been described as PR (pathogenesis-related)-proteins: a thaumatin and a chitinase. Using antibodies against each of the proteins we have shown that they are present in leaves, stems and roots and like other PR proteins can be extracted from whole tissues at low pH. Although the ubiquitous presence of these proteins in healthy, nonstressed tissues of *L.albus* cannot be explained at the moment, it is possible that the plant has recruited PR proteins for use as a constitutive preformed defense against fungal pathogenes.

This project is supported by PRAXIS XXI (grant nº BM/452/94))

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SOY PROTEIN CONCENTRATES TREATED WITH PROTEASES FROM NATIVE PLANTS. B. Structural and functional properties of the modified concentrates. Wagner, J.R.*, Sorgentini, D.A.; Gara Martorell, P.D. (CIDCA); Caffini, N.**; Natalucci, C.** and López, L.* (LIPROVE). Depto. Cs. Biológicas, Fac. Cs. Exactas, Univ. Nac. de La Plata, 47 y 115 (1900) La Plata, Argentina.

Soy protein concentrates prepared by alcoholic extraction of defatted soy protein flour exhibit very low water solubility (<10% w/w), a fact that restricts their utilization in food products.

Soy concentrate proteins were hydrolyzed with different proteases (hieronymin, pomiferin, macrodontin) isolated from native plants (see Part A). By this procedure, protein solubility was increased both at pH 7 and 4.5 (isoelectric pH), and the surface properties improved, these results being comparable to those produced by a commercial protease (alcalase).

The lower thermal stability (DSC) of the hydrolyzed 11S protein (which in its native form is a storage soybean protein) correlates with the decrease of hydrophobicity (fluorometry) in the order flour>concentrate>hydrolysate. This may indicate that the peptides released fold and hide their hydrophobic zones to form soluble aggregates, which would explain the stability increase of dispersions of the hydrolysates and the decrease of their water imbibing capacity. If compared to the original cocentrate at neutral or isoelectric pH, all hydrolysates show better foam forming and stabilizing capacity as well as improved emulsifying capacity (not modified by the ionic strength). Such functional behavior of the hydrolysates obtained would allow them to be used in those liquid foods that require stable dispersed particles, emulsions or foams formation at low pH.

(*) CONICET (**) CIC

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CARBOHYDRATE CONTENTS IN *Deschampsia antarctica* DESV. FROM SOUTH SHETLANDS, MARITIME ANTARCTIC.

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Deschampsia antarctica (Desv.) is the only gramineous plant that has colonized the Maritime Antarctic. This region has a cold, moist maritime climate with mean monthly temperature near to 0 °C. During this period, when irradiation is sufficient to allow photosynthesis, temperatures often fall below 0 °C, exposing to *D. antarctica* to diurnal cycles of freezing and thawing. The mechanisms that allow to *D. antarctica* to tolerate the adverse Antarctic climatic conditions are unknown. In this work we report the content of carbohydrates (glucose, fructose, sucrose and fructans) in leaves and roots of *D. antarctica* growing under summer field conditions.

Plants were collected in Robert Island, South Shetland, and were extracted in 80 % ethanol. Total soluble sugars were analyzed in leaves and roots by colorimetric and HPLC techniques. Compared with other gramineae, the levels of sucrose and fructans were higher. These substances reached their maximum levels by the end of summer. We suggest that the unusually high accumulation of sucrose and fructans may be one of the protective mechanisms against low temperature that have allowed *D.antarctica* to grow in the Maritime Antarctic.

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AROMATIC COMPOUNDS AND LIGNOSULFONATE AS INDUCERS OF POLYPHENOL OXIDASES BY *BOTRYOSPHAERIA* SP. Barbosa, A.M. *, Dekker, R.F.H. and Sargent, K. BES, Bitechnology Program, Murdoch University, Perth, Australia and *Depto. de Bioquímica, Universidade Estadual de Londrina, Londrina-PR, Brazil.

The ascomycete, *Botryosphaeria* sp., isolated from screening 40 wood-decay fungi, was found to produce 2 polyphenol oxidases (PPO-I and PPO-II) constitutively when grown in submerged culture on glucose. PPO-I was active towards ABTS, and showed optimal activity at pH 3 and 50 °C, while PPO-II was optimally active at pH 6.5 and 45 °C when assayed against 2,6-dimethoxyphenol (DMP). Both enzymes were induced to higher titres when the fungus was grown for 96 h in the presence of 40 mM veratryl alcohol (VA). Aeration of submerged cultures using baffled flasks increased 4-5 fold both enzyme titres when *Botryosphaeria* was grown on glucose plus VA, and 500-fold (PPO-I) and 18-fold (PPO-II) when compared to cultures grown on glucose alone using standard flasks. PPO was also produced when the fungus was grown on glucose in the presence of several aromatic compounds including abietic acid; phenols (catechol, 4-chlorophenol, DMP and guaiacol); benzoic acids (benzoic, 2,6-dimethoxybenzoic, syringic, vanillic, veratric); alcohols (vanillyl, veratryl), veratrylaldehyde; and lignin precursors (coumaric and ferulic acids), at various concentrations. Lignolsulfonates (a waste product arising from the chemical pulping of softwood) at concentrations of up to 8 % (w/v) also induced PPO. *AMB acknowledges CNPq Brazil for a Research Fellowship.

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EVALUATION OF PRODUCTION OF RECOMBINANT HUMAN ERYTHROPOIETIN IN A PERFUSED STIRRED TANK BIOREACTOR. Beccaria, A., Etcheverrigaray, M. and Kratje, R. Instituto de Tecnología Biológica (INTEBIO), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. E-mail: rkratje@fbcb.unl.edu.ar

Erythropoietin (EPO) is a glycosylated protein and thus, a totally functional molecule can only be produced by mammalian cells. Optimization of the hormone production process mainly depends on the growth cell type: suspension or monolayer culture. Stable clons, with a productivity of at least 1 µg EPO per ml of culture medium were selected and adapted to grow in spinner flasks in a low serum content media in order to simplify the product purification step and in a suspension mode as this method presents several advantages during process scale-up. Following culture parameters were determined: total cell number by nuclei staining method, proportion of dead cells by trypan blue exclusion, glucose and lactate concentrations by an enzymatic method, ammonia concentration by a colorimetric method, protein content by Bradford and hormone concentration by ELISA. Preculture was performed in spinner flasks with BHK medium. Culture conditions in the bioreactor were: temperature 37°C, pO_2 40% air saturation (2,8 mg O_2/l), pH 6,8, stirrer 80 rpm, spin-sieve stirrer 25 rpm, mass flow 0,5 l_N/min. The perfusion rate was adjusted according to glucose consumption rate and reached values up to 1,2 reactor volume/d. Final cell densities on the order of 1,5 x 10^7 ml^{-1} (viability 95%) were reached while the erythropoietin production rate was 38,4 mg/d. Maximal cell densities and product yield could be mantained even when protein-free medium was perfused.

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PUMP-PROBE STIMULATED-EMISSION SPECTROSCOPY AND MICROSCOPY. Buehler, Ch., Dong, C.Y., So, P.T. and Gratton, E. Laboratory for Fluorescence Dynamics, Department of Physics, University of Illinois at Urbana-Champaign, 1110 West Green Street, Urbana, IL 61801

Time-resolved stimulated-emission fluorescence spectroscopy and microscopy allow both super-diffraction limited spatial resolution and sub-nanosecond time resolution by using a multi-photon process. In this novel technique, two high repetition rate, pulsed lasers, called the pump and the probe, are simultaneously focused onto a common volume of the fluorescent sample. The wavelengths of the two lasers are chosen such that the pump excites the sample and the probe induces stimulated emission. By slightly offsetting the lasers repetition rates, the high frequency information of the fluorescence decay is heterodyned to the cross-correlation frequency regime of the two lasers. By observing the fluorescence at these beating frequencies, a 3-D sectional effect comparable to confocal or two-photon microscopy can be obtained since the stimulated emission process occurs primarily at the overlapping volume between the pump and the probe beam. Further, due to the high harmonic content of the two pulsed lasers, the frequency-domain heterodyning method samples the complete fluorescence dynamics simultaneously, and the achievable time resolution is ultimately limited by the pulse widths of the two lasers. Using this methodology, we present the characterization of the point-spread function, determine the fluorescence saturation power for both the pump and probe, and show lifetime-resolved images of latex spheres.

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KILLER CHARACTER IN CONVENTIONAL AND NON-CONVENTIONAL WILD YEASTS. Caballero, A., Brizzio, S., Lavalle, L., Ruffini, A., Zajonkowsky, I., Assadourian, M. and van Broock, M. Laboratorios de Microbiología y Biotecnología, Facultad de Ingeniería (Neuquén) y Centro Regional Universitario Bariloche. Universidad Nacional del Comahue Argentina.

Killer phenomenon is an important factor for competence in naturally occurring communities of yeasts as well as among yeasts during fermentation processes. It also provides a useful model for protein processing and secretion, toxin sensitive cell interaction and expression of eukaryotic viruses. To assess the killer profile of yeasts isolated from wild berries, nectarine flowers, grapes and wine grape musts from Comahue Region (Argentina), twelve killer type strains (K_1-K_{11}) and two sensitive tester strains were used. Killer screening method was as described by Starmer et al. (1987). Yeasts isolated from grapes, wild berries and nectarine flowers showed a low incidence of killer activity (5-10%) while neutral and sensitive strains accounted for 20-25% and 70-75% respectively. Grape musts accounted for 20-25% of killer active yeasts.
In agreement with other authors we conclude that when selective environmental conditions appear (grape must) killer factor is expressed.

Ref. Starmer et al. (1987) Can. J. Microbiol. **33**:783-796.
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ENZYMATIC PRETREATMENT OF KRAFT CELLULOSE PULP WITH FERULOYL ESTERASE AND XYLANOLYTIC COMPLEX. [1] Aguirre, C., [1]Castillo, C., [1]Rojas, V., [2] Durán, N. and [1]Curotto E. [1]Laboratorio de Bioquímica, Instituto de Química, Universidad Católica de Valparaíso. Casilla 4059. Valparaíso. Chile. [2]Instituto de Química, Universidad Estatal de Campinas, Brasil.

The pulp and paper industry are interested in the application of biotechnology in order to substitute the traditional pulp bleaching processes, at least partially, by a xylanolytic complex (X) treatment. β-Xylanase, arabinofuranosidase, β-xylosidase, acetyl esterase, and also feruloylesterase could facilitate the elimination of lignin from cellulose pulps by enzymatic pretreatment. *Aspergillus cervinus* 2M1 and *Penicillium canescens* CP1 strains produce the xylanolytic complex and feruloylesterase (FE). Both enzymatic systems were characterized and degrade xylan to xylose, xylobiose, xylotriose and fenolic derivatives. Enzymatic pretreatment was carried out with enzymatic extract of CP1 strain following a bleaching sequence; enzyme, 80% chlorine dioxide, alkaline extraction and hydrogen peroxide (XD_{80} EP). In the pretreated pulp and the corresponding controls, the kappa index, brightness, bleaching and viscosity were evaluated. Physical's properties of the paper were also determined, showing no differences with a paper obtained in a cellulose plant by Kraft method. The AOX determined in the effluents showed a 40 % decrease in comparison with untreated pulps. X and FE produced by 2M1 strains were tested in a (FE,X)ZEP, sequence with ozone and free of chlorine. The results show synergism between these enzymes, with an improvement in the prebleaching indexes compared to corresponding controls.

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PHENOLIC COMPOUNDS BIODEGRADATION IN AEROBIC/ANAEROBIC SERIAL SYSTEM. Vidal, G.; Diez, M.C.; Inostroza, L. Universidad de La Frontera, Dpto. Ing. Qca. Casilla 54 - D. Fono/Fax: 253177. E-mail: gvidal@werken.ufro.cl. Temuco.

The biodegradation of phenolic compounds arising from fiber board wastewaters in aerobic/anaerobic sequence system has been evaluated.

Aerobic (4 L) and anaerobic (2 L) systems were operated in continuous systems. The following control parameters have been measurement for each system: pH, SVI (sludge volume solid), for the aerobic one and pH, rate alkalinity for the anaerobic one. On the other hand, the percentage of phenolic compounds, color, biological oxygen demand (BOD) and chemical oxygen demand (COD) removals have been evaluated. Moreover, the total solid removal in aerobic biodegradation systems have also been evaluated. Finally, the molecular weight distribution and the spectro have been studied too.

The wastewaters characteristics indicate high phenolic (0.65 mgphenol/L), color (22240 UPtCo), BOD (8.1 g/L) and COD (22.2 g/L) concentrations.

The results show that the aerobic/anaerobic systems can remove the total solid (50 until 80%), COD (70 until 92%) and phenolic compounds (around 40%). However the FPLC (Fast Performance Liquid Chromatography) analysis shows that the effluent has two recalcitrant fractions corresponding to the phenolic (5000 Da) or to the high molecular weight (43000 Da) (polimerized phenol) compounds.

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FORMALDEHYDE RESISTANCE GENE OF *Kluyveromyces marxianus* CBS6556. Escóbar[1], M.E., Barbieri[1], A., Brendel[2], M., Henriques[1] J.A.P. and Ayub[1], M.A.Z. [1]Biotechnology Center of The Federal University of Rio Grande do Sul State, Av. Bento Gonçalves, 9500, CEP 91501-000, Porto Alegre, RS, Brazil. [2]Institut für Mikrobiologie der J. W. Goethe Universität, theodor Stern-Kar 7, Haus 75,600 Franfurt am Maim, Federal Republic of Germany.

Kluyveromyces marxianus is an industrially attractive yeast due to its short generation time and its ability to grow at higher temperature and in several carbon sources other than those which are metabolized by *Saccharomyces cerevisiae*. *SFA*1 gene contained in the multicopy vector YFRP1 (Yeast Formaldehyde Resistance plasmid) confers hyperresistance (HYR) to formaldehyde (FA). Neither stable auxotrophic markers in recipient cells nor defined synthetic media are needed when YFRP1 is employed for yeast transformation (Wehner et al. 1993). *S. cerevisiae* YFRP1 transformants acquired stability to the vector when propagated in complex media supplemented with 3 to 5 mM FA. Our experiments demonstrated that *K. marxianus* CBS 6556 transformed with YFRP1 show two fold higher hyperresistance to FA than *S. cerevisiae* transformants. In order to exploit the industrially advantageous *K. marxianus* yeast, we developed a project to clone *SFA*1 gene of this microorganism. A gene sub-bank from *K. marxianus* was constructed in YEP352 plasmid using 2-5kb of *Eco*RI and 4-7kb fragments of a *Bam*HI digestion of total DNA of this yeast which hybridized with the *SFA*1 gene of *S. cerevisiae*. We isolated the *SFA* homologue gene of *K. marxianus* by ligating *Eco*RI digested genomic *K. marxianus* gene bank for FA-HYR in *S. cerevisiae*, showing an effective expression. When *S. cerevisiae* MKPoα *SFA*::Δ strain is transformed with YEP-E36 - containing the *SFA*1 gene of *K. marxianus* - it shows a very high resistance towards formaldehyde.

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GENOTYPING A BOVINE MILK PROTEIN USING ALLELE DISCRIMINATION BY PCR TECHNOLOGY. Felmer R. and Butendieck N. Departamento de Producción Animal, Centro Regional de Investigación INIA-Carillanca, Temuco, Chile.

Caseins are a family of milk proteins that exist in several molecular forms and are the main proteins that are present in the bovine milk. Genetic variants of these proteins have been associated with the quality and quantity of cheese derived from milk. Thus, cheese produced from κ-casein BB milk have a higher protein content, higher yield and better quality than those produced from κ-casein AA.

The purpose of the present study is to set up a technique to be used in order to speed up the increase of the frequency of the desired casein alleles in the dairy cattle population and thus reduce the time required with traditional livestock improvement methods. A 350 bp fragment of the genomic bovine κ-casein gene was amplified by PCR. Two Hinf I sites are found in the amplified fragment of allele A, one at position 134 and a second at position 266; only the latter site is present in allele B. Thus, digests of alleles A yielded 84 bp and 132/134 bp bands and digests of alleles B resulted in 84 bp and 266 bp bands. These bands, and the corresponding genotypes AA, AB and BB, were identified by agarose gel electrophoresis and ethidium-bromide staining. This technique is being used to determine the κ-casein allelic frequency in our Friesian dairy herd.

This molecular genetic technique allows the direct genotyping for milk κ-casein with certainty and accuracy in bulls and females used in different programs of bovine genetic improvement. Therefore the early and precise identification of milk protein genotypes will have a direct impact on dairy cattle breeding strategies.

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INTERACTION OF REACTIVE DYES WITH ASPARTYL-PROTEASES. Fernández-Lahore, H.M., Fraile, E.R., Biscoglio de Jiménez Bonino, M., and Cascone, O.
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Dye ligand chromatography is currently accepted as an important method for large-scale purification of proteins. Reactive dyes can bind proteins either by specific interactions at the active site or by a range of non-specific interactions. The affinity purification of some microbial aspartyl-proteases is hampered because they tends to bind so tigthly to Pepstatin A, a commonly used peptide ligand.
We report here the screening for dye ligands useful in the purification of recombinant bovine chymosin, *Mucor miehei* protease, and *Mucor spp.* acid proteases. Fungal enzymes were found to bind 5/9 dyes tested at pH 4.1 (in acetate buffer) but no binding occured at higher pH's. On the other hand, rbChymosin was found to bind 8/9 dyes even at pH 6.5 in phosphate-acetate buffer. Elution was shown to be dependent on the solid support used (particulate gels or polyethylene membranes) and no effect of divalent cations (2 mM buffer content) was noticed.
Dyes such as Blue R-HE, Orange R-HE, Red 141 and Red F-5B were selected as potential pseudo affinity ligands for aspartyl-proteases purification.

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FRACTIONATION OF HYPERIMMUNE HORSE PLASMA BY SALT-PROMOTED ADSORPTION METHODS. Kijak, G., Camperi, S.A., Fernández-Lahore, H.M. and Cascone, O.
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Most protocols for antivenom production are based on hyperimmune horse plasma coarse fractionation (e.g. caprilic acid or ammonium sulphate precipitation). However, their administration induces some unwanted allergic reactions, due in part to the presence of non-immunoglobulin plasma proteins not eliminated during such treatment. As a model, we report here the purification of a horse gammaglobulin with neutralizating activity against Vipera Russelli venom by adsorption column (or membrane) chromatography using two different thiophilic (or metal affinity immobilized) ligands. Antibody titers were monitored by an enzyme immunoassay using 5 µg/well crude venom as antigen. Process parameters were as follows:

Parameter	T-Gel	NT-Gel
Yied (%)	52	38
Protein content (mg/ml)	22	6
Dynamic capacity (mg/ml)	4.1	2.5
Turbidity (A_{310})	0,021	0,024

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A NEW METHOD FOR THE RAPID PURIFICATION OF PHOSPHOLIPASES. Guberman, A.[1], Florin-Christensen, J.[1], Caramelo, J.[1], Tiedtke, A.[2], Florin-Christensen, M.[1] [1]INEUCI, CONICET-UBA, Ciudad Universitaria, Pab.II, 4° Piso, RA-1428 Buenos Aires, Argentina; [2]Institute for General Zoology and Genetics, Schlossplatz 5, D-41849 Münster, Germany.
Phospholipase activities are involved in various cellular processes, such as signal transduction and exogenous or endogenous lipid metabolism. We here deviced a simple and economical method to purify extracellular phospholipase A_1 from the ciliate *Tetrahymena thermophila*. This method consists of the following steps: a) incubation of extracellular medium with 0.5 mg/ml of Asolectin, a soy bean phospholipid extract, in 50 mM sodium acetate, pH 4.75; b) precipitation of the phospholipid-phospholipase complexes with 10 mM $CaCl_2$ and centrifugation; c) elution of the phospholipase A_1 from the pellets by treatment with 40% dimethylformamide; the resulting suspension is centrifuged to remove the lipids and the enzyme is recovered in the supernatant, which is immediately diluted 4 fold with 10 mM Tris-HCl, pH 7.4. Using this protocol, a yield of 26 % of enzyme activity with a 360 fold purification was achieved. This method may be easily adapted to other phospholipase activities. For instance, $CaCl_2$ is required for the binding of several phospholipases A_2 to the lipid substrate and Ca++ chelators, instead of dimethylformamide, could be used in these cases for the elution of the enzymes. This exploitation of the selective separation of enzyme-substrate complexes is akin to affinity chromatographic procedures, but more economical due to the low cost of the materials involved. Variations of this procedure can be envisaged for the purification of other proteins of interest. *Supported by grant CI1*-CT94-0026 from the European Commission and CONICET.*

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DIRECT LACTOFERRIN SEPARATION FROM BOVINE WHEY BY DYE AFFINITY MEMBRANE CHROMATOGRAPHY . Grasselli, M., Navarro del Cañizo, A.A. and Cascone, O. Cátedra de Microbiología Industrial y Biotecnología. Facultad de Farmacia y Bioquímica. Junín 956, (1113) Buenos Aires, Argentina.

Lactoferrin (Lf) is an iron-binding glycoprotein with antimicrobial activity against bacteria and yeasts. It has been isolated from bovine whey by different chromatographic methods. We have demonstrated the direct Lf separation from bovine whey by using pseudo-specific dye affinity chromatography with soft gels, but an extensive clarification step as prechromatographic treatment was necessary.

Microporous affinity membranes with an attached ligand for affinity chromatography offer a better performance because of their high surface area, reduced diffusion distance and low operating pressure, thus resulting in high-volume throughput, high ligand utilization and low cost.

Red HE-3B was bound to hydrophilized polyethylene hollow-fiber microporous membranes and the adsorption behaviour of Lf was investigated. The performance of the dye membrane for direct extraction of Lf from clarified whey, skim-milk whey and crude whey was assessed.

No significant differences in dynamic capacity (around 300 mg Lf/0.5 m^2 cartridge) were found. While clarified and skim-milk whey were processed directly, crude whey had to be submitted to a rapid clarification step to prevent membrane fouling. However, the chromatographic advantages of membranes over soft gel columns were evident in all cases.

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PECTIC ENZYME FRACTIONATION BY IMMOBILIZED METAL ION AFFINITY CHROMATOGRAPHY ON MEMBRANES. Camperi, S.A., Grasselli, M., Navarro del Cañizo, A.A., Smolko, E.* and Cascone, O. Cátedra de Microbiología Industrial y Biotecnología. Facultad de Farmacia y Bioquímica. Junín 956, (1113) Buenos Aires, Argentina.
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Pectic enzyme preparations are widely used in the food industry to clarify fruit juices. They contain a mixture of different enzymes, mainly pectin lyase (PL), polygalacturonase (PG) and pectinesterase (PE). The use of PL alone instead of the combination of PG and PE prevents the reduction of juice stability due to the formation of de-esterified pectin - endogenous Ca^{2+} and the release of methanol in the juice thus constituting a potential health hazard.

We demonstrated that immobilized metal ion affinity chromatography in columns is able to separate PL from PE and PG. Affinity membrane is a kind of convective chromatography where the high porosity and minimal mass transfer resistance allow a high volumetric throughput, thus resulting in extremely short process times. In the present work we report the pectic enzyme fractionation using immobilized metal ion affinity membranes.

A novel technique of grafting polymerization was used to immobilize iminodiacetic acid onto membranes: hydrophilized polyethylene hollow fibre membranes (0.33 µm pore) were surface-modified by grafting glycidylmethacrylate in a ^{60}Co gamma radiation chamber. We obtained membranes with 0.17-1.3 mmol Cu^{2+}/ml fibre by using different grafting degrees and/or iminodiacetic acid reaction conditions.

A dynamic capacity of 78 PE units/µmol of chelated Cu^{2+} was obtained by using a home-made 0.1 ml-hollow fibre cartridge.

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EVOLUTION OF AN ANTARCTIC SOIL BACTERIAL POPULATION AFTER EXPOSURE TO GAS-OIL. Mac Cormack,W.P.[1] and Fraile,E.R.[2] 1:Instituto Antártico Argentino. 2:Cátedra de Microbiología Industrial y Biotecnología, Fac. Farmacia y Bioquímica,U.B.A, Argentina.

While the ecosystems contain an abundant microflora includding strains able to degrade anthropogenic compounds, sometimes,the adverse natural conditions make the activity of the indigenous microflora insufficient to reduce the environmental pollution. In this study, growth evolution of natural and seeded bacterial communities of hydrocarbon polluted Antarctic soils were compared under natural conditions. Fractions (250 g) of sieved (2 mm ø) soils were placed in 1 l flasks in 10 different conditions in order to analyse:I)The effect of the pH control. II)Effect of the presence of the hydrocarbon degrader strain *Acinetobacter* B-2-2, *Acinetobacter* ADH-1 or both. III)Effect of the addition of P and N as nutrient. Flasks were exposed to natural conditions during 51 days. Soil moisture (10%) was mantained along the study. No difference was observed with or without pH control.Autoctonous microflora raised from $2.2x10^6$ UFC/g to $1x10^7$ UFC/g in the first 28 days and decreased at the end of the study. In the presence of B-2-2 or ADH-1 highest values were reached early (14 days). In the presence of both strains, higher values were observed and continuously raised until the end of the study ($4.2x10^8$ UFC/g at 51 days). High nutrient content (C:N:P=100:12:3) determined an important decrease of the counts in all cases.These results showed that ADH-1 and B-2-2 grow more efficiently together than separately suggesting a sinergic interaction or an co-oxidation processes. Finally, P and N could act as growth inhibitors when added to the bioremediation processes at high concentrations.

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FUNGAL TREATMENT OF PULP AND PAPER INDUSTRY: KRAFT EFFLUENT-E_I AND FINAL EFFLUENT-E_F AFTER AEROBIC TREATMENT: Massai, L.R.,[@] Esposito, E.,[#] Haun, M.[*] and Durán, N.[#]
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Several methods - physical, chemical and biological - have attempted for reducing pollutants in KRAFT EFFLUENTS with main purposes: to reuse of water tap and reduce volumes to be discharge (Harris et al., 1992). Despite their low toxicity, pulp and paper discharges have a toxic impact on receiving waters because of the tremendous volumes discharged with color persisting and organic pollutants even in treated and partially treated effluent (Garric et al., 1993). Many compounds are formed during pulp bleaching are not removed in the secondary treatment leading to and pulp mills effluents content of hundred differents compounds (Rogers, 1973). In this study a comparative chemical analysis was made with samples of KRAFT EFFLUENT-E_I (First Extraction) a principal types of chlorine bleach plant wastewater and with the FINAL EFFLUENT-E_F after treatment in aerated lagoon with hydraulic detention time of 3 days. The samples were pH adjusted to 5,0 with H_2SO_4 1M and stored at 4°C in polyethylene bottles in volumes of 500mL to ensure that all assays will be done with the same stock. Fungal treatments (triplicates) were done with each different effluent samples (Esposito, 1994). After incubation with *Lentinus edodes UEC-2021* and filtration (Millipore 0,22µ) samples were conveniently prepared to analysis. It was obtained reduce of toxicity, a 47% of color removal in effluents and phenol content was reduced 60% in effluents. (Financial Support:CNPq, FINEP and FAPESP).

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A SEARCH FOR A RAPD MARKER FOR THE PARCHMENTLESS POD TRAIT IN PEA.

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This work was aimed to find a RAPD (Random Amplified Polymorphic DNA) marker for the parchmentless pod trait in pea (*Pisum sativum*). This character leads to indehiscent pods, which resist seed shattering when mature dry and permit to avoid losses during harvesting. The trait is being introduced into high-yielding, afila backgrounds by the pea improvement project at INIA-Carillanca.

Even when easily identifiable, the presence/absence of parchment is detectable after the pods are formed, once the opportunity to make hybridizations is over. A molecular marker would allow the detection of desirable individuals prior to flowering, in order to perform crosses, thus saving one generation.

Samples of plants from two reciprocal F_3 populations, 89-5017 and 89-5045, were used. After trying 212 primers and 261 combinations of two primers, 110 and 110 polymorphisms between the parents were detected, respectively. However, when the primers were applied to the progeny, no polymorphic band was associated to the parchmentless trait. This result was unexpected, since the number of polymorphisms detected should cover a significant portion of the pea genome. A total of 102 primers gave no polymorphic bands between the parents, as well as 151 combinations of two primers.

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SOYBEAN PEROXIDASE PURIFICATION FOR INDUSTRIAL APPLICATIONS. Miranda, M.V., Fernández Lahore, H.M. and Cascone, O. Cátedra de Microbiología Industrial y Biotecnología. Facultad de Farmacia y Bioquímica. Junín 956, (1113) Buenos Aires, Argentina.

Peroxidases are an ubiquitous class of enzymes whose primary function is to oxidize a variety of hydrogen donors at the expense of hydrogen peroxide. They are useful in many industrial applications, such as medical diagnostic reagent supplies (which require high purity enzyme in small quantities), in the removal of phenolic contaminants from wastewaters, and in the manufacture of specialty polymers and resins.

In this work we describe a method of harvesting and purification of peroxidase from the seed coats of *Glycine max*. Soybean hulls were extracted in the presence of 0.1 %, Triton X-100, an additive was introduced at a fixed concentration, and aqueous two-phase formation were induced by temperature (35° C). The enzyme was recovered in the upper, detergent-poor phase, with a 99 % yield and a purification factor of 6.0. This product is amenable for peroxidase-catalyzed removal of aromatic contaminants and the synthesis of phenolic resins. A further purification step using immobilized concanavalin A rendered an analytical grade enzyme (purification factor of 41), useful for the manufacture of medical diagnostic kits.

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SELECTION OF PROTEIN ANTIGENS FROM *HELICOBACTER PYLORI* USEFUL IN DIAGNOSTIC AND VACCINE DEVELOPMENT. Müller, I., Hevia, E., Opazo, P., Venegas, A.* and Yudelevich, A. Lab. de Biol. Mol., Bios Chile IGSA, Santiago and *Lab. de Bioquímica Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago, Chile.

Helicobacter pylori has been strongly implicated in the etiology of gastric and duodenal ulcera and as a significant risk factor for development of gastric adenocarcinoma. Strains associated to duodenal ulcera display higher virulence and produce an 87 kDa VacA cytotoxin and 120-130 kDa protein (CagA, associated to cytotoxin activity).

In order to extend these studies and search for new antigens with useful properties for *H. pylori* diagnostic and vaccine development we have amplified by PCR seven *H. pylori* described genes. We have used for this purpose a Chilean clinic isolate named CHCTX-1. We have expressed those genes in *E. coli* using the pET3d and 11d vectors. The expresion level varied between from 1 to 30% of the total bacterial protein, being urease A subunit, VacA, pA17 and 26 kDa protein the highest expressed antigens. Using Western blot analysis with patient sera we have selected antigens pA17 and VacA as the most suitable for *H. pylori* identification since they show a direct correlation with cytotoxicity and pathogenicity.

Finally, we have developed an ELISA assay with purified *H. pylori* proteins and have undertaken clinical studies to evaluate the assay performance by antibody reactivity against total extracts. We have used pA17/VacA and urease A/B pairs as well as FlaA antigen. Using pA17 and VacA antigens, we have detected pathogenic strains in over 90% of the sera derived from duodenal ulcera patients.

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EXPRESSION OF RECOMBINANT NUCLEOCAPSID PROTEIN FROM TOMATO SPOTTED WILT VIRUS IN *E. Coli*.[1] Ramos, Ma. L., S. F. Nome and D. A. Ducasse. IFFIVE-INTA, Cno. 60 cuadras Km 5 1/2 CP (5119) Córdoba, Argentina.

Due to the severe damage that tospoviruses cause to several crops in Argentina our laboratory has engaged in a research program that includes the production of tospovirus diagnosis reactants and tospovirus resistant transgenic plants. In that research context, a cDNA clone containing the nucleocapsid ("N") protein coding region from an Argentinean isolate of tomato spotted wilt (TSWV) was obtained by RT-PCR and was cloned into a pUC derived cloning vector. The fragment was sequenced and its identity was confirmed by nucleotide sequence comparisons.

In previous report we have shown that when a recombinant tospovirus "N" protein is used as antigen, the resulting antiserum is more specific than other antiserum raised against native viral "N" protein purified from infected plants, although none of the compared antisera presented reaction against healthy plant. This differential antiserum specificity may be due to the absolute abscence of spurious viral protein contaminations when a recombinant protein is used as antigen. Under the same rational, the produccion of a recombinant TSWV "N" protein was decided. The above described fragment was subcloned into the *E. coli* expression vector pET-15b, resulting the plasmid pET-TS. After transforming *E. coli* BL21 cells, the bacterial culture was induced by IPTG and total bacterial protein was analyzed by SDS-PAGE. A band of the expected molecular weight (29 Kd) could be observed only in the protein pattern from pET-TS transformed clones when the gel was stained by Coomasie blue. When western blot analyses were performed, the band was recognized by an antiserum raised against viral native "N" protein purified from plants infected by the same TSWV isolate. Next step will include the purification of the recombinant TSWV "N" protein and the production of an antiserum against it.

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RESTRICTION ENZYME STABILIZATION AT LOW HUMIDITIES AND GLASSY STATE. Rossi,S.; Buera,P.; Moreno;S. and Chirife,J. Dpto. Qca Biológica and Dpto. de Industrias, Facultad Ciencias Exactas y Naturales, UBA, Argentina.

Most proteins become denatured under stress conditions such as heating, freeze-thawing and drying. Many studies have demonstrated that certain disaccharides stabilize proteins against the deletereous effect of desiccation. The restriction endonuclease is one of the best model proteins becauses it recognizes only a certain nucleotide sequence, cleaves the substrate DNA strictly at that site and is known to be a labile protein. The stability of these enzymes has been shown to increase by addition of appropiate matrices such as disaccharides during desiccation. However, as the humidity conditions and the matrices' physical state has not been established, the mechanism by which they protect has not been explained. The aim of this work has been to sudy the importance of the matrix physical state in its protective effect. The restriction enzyme used was EcoRI. As protective agents we used the saccharides: sucrose (S) and trehalose (T) and the polymers maltodextrine (MD) and polyvinylpyrrolidone (PVP) which have a glass transition temperature (Tg) higher than saccharides. The enzyme was desiccated by vacuum-drying at room-temperature in the presence of the protector agent and stored under controlled conditions of relative humidity (HR) ((0 a 58%) and temperature (between 7 and 70 °C). The residual activity was measured by assaying the cleaving activity of the enzyme towards pTar , a plasmid derived form pBR322, and DNA fragments were analyzed by 1% agarose gel electrophoresis. When the enzyme solution containing trehalose or sucrose was vacuum-dried at different humidities, the enzyme remained stable for 10 days at 45°C for both matrices under conditions at which the saccharide crystallization was prevented. Trehalose needs two water molecules for crystallization and therefore retains its protective effect at higher humidities than sucrose. When the vacuum-drying was performed in the presence of MD or PVP, the enzyme lost its activity after three days at 7°C storage. The results suggest that a glassy state is not a condition for enzyme protection and that it is very probable that disaccharides protect through the formation of specific interactions with proteins, replacing water.

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THE PROCESSING OF LATEX FROM *Carica papaya*. Silva, L. G. , Lopes, M. T. P and Salas, C. E. Departamentos de Bioquímica & Imunologia and Departamento de Farmacologia, Instituto de Ciências Biológicas, Universidade Federal Minas Gerais, Belo Horizonte, MG, BRASIL.

The purpose of this study is to evaluate the role of proteolytic enzymes in the process of latex coagulation in *Carica papaya*.

To answer this question we looked for possible changes in latex protein composition following a superficial wound (2mm) of the unripe fruit, while attached to the plant. The fresh exudate was collected with dry-ice at several time intervals in the presence or absence of proteinase inhibitors and stored in the dark at -70°C. Fractions containing equivalent amounts of proteins (4µg) were analyzed by SDS-PAGE. After silver staining the protein profiles were evaluated by monodimensional laser densitometry. The results measured by the relative variation in contents of each peptide as a function of time (1-1,200s) were grouped into six categories: I) increase (600%) followed by decrease and further stabilization; II) accentuated (1,200%) decrease followed by further stabilization; III) increase up to 70% followed by stabilization; IV) a sustained decrease (300%) without reaching baseline during the interval studied; V) no significant variation in peptide content during the period studied and VI) the peptide variation follows a random change.

The sequential processing observed of peptides in latex suggest a coordinated participation of various proteolytic enzymes. Proteolytic activity of various fractions measured in situ after SDS-PAGE support this notion. Supported by FAPEMIG, CNPQ and PRPQ-UFMG.

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SEQUENCING OF IC-RT-PCR AMPLIFIED GENOME FRAGMENTS FROM A GRAPEVINE VIRUS

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The grapevine fanleaf virus (GFLV), an important virus responsable for crop disease, was immunocaptured from crude plant extracts using immobilised virus specific antibodies. The virus RNA genome was then used as template for cDNA synthesis using reverse transcriptase. The cDNA obtained was amplified by the PCR technique using suitable primers, targeting two different genome regions (coat protein and part of the polimerase genes) showing DNA products of expected length (500bp and 687 bp, respectively). The amplified DNA products were cloned into a plasmid and sequenced. The corresponding pol cDNA fragment revealed a nucleotide identity of 87% when compared with the sequences of the same genome region of a french isolate. The amplified product corresponding to the coat protein gene is now partially sequenced. The same procedure is being applied to other portions of the fanleaf grapevine virus genome and the sequencing of other cDNA fragments is now in progress. With this strategy we hope to identify specific DNA regions within the virus genome which will enable us to design sets of primers, as universal as possible, to devise a PCR based virus diagnostic Kit.

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ISOLATION OF A THERMOPHILIC FUNGUS WITH HIGH XYLANASE ACTIVITY. Steiner, J..[1] Mella, M.,[1] Díaz, M. C.,[2] Piontelli, E.[3] and Eyzaguirre J.[4] [1]Depto. de Bqca. y Biol. Molec. Fac. de Cs. Qcas. y Farmac. U. de Chile. [2] Depto. de Microb. Fac. de Medicina Oriente. U. de Chile. [3] Cátedra de Micología Escuela de Medicina. U de Valparaíso [4]Depto. de Bqca. Fac. de Cs. Biol. Pontifcia U. Católica. Stgo. Chile.

Xylanases are being increasingly used for cellulose pulp biobleaching. For this purpose alkaline and highly thermostable xylanases are required and new sources of enzymes are being studied. A thermophilic fungus producing extracellular xylanases was isolated from wheat straw compost samples in a medium containing oat spelts xylan as the only carbon source. The fungus was identified as *Humicola grisea* Traacn var *thermoidea* Cooney & Emerson and has an optimum growth temperature of 45°C.

Highest xylanase activity: 528 U/ mL, was obtained after eight days of culture on oat spelts xylan at 45°C and 150rpm. On birch wood xylan and wheat straw 426 U/mL and 60 U/mL of xylanase were obtained respectively. No ß-xylosidase or esterase activity were detected.

The enzyme shows a very high thermostability. When culture supernatants were incubated in the absence of substrate an 86% of activity was retained after 6 days at 45°C. A 64% and 51% of activity were retained after incubation at 50° and 60°C in the same period. When enzyme activity was assayed at different pHs, best activity was found at pH 7. These results suggest that these enzymes could have a great potential for industrial applications.

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IDENTIFICATION OF LACTIC ACID BACTERIA USING BOX-PCR. de Urraza P.J.[1,3], Lozano M.E.[2], Romanowski V.[2,3] and De Antoni G.L.[1]. 1.CIDCA, Facultad Ciencias Exactas, UNLP, 47 y 116, La Plata. 2.IBBM, Facultad Ciencias Exactas, UNLP, 50 y 115, La Plata. 3.Departamento de Ciencia y Tecnologia, Universidad Nacional de Quilmes, Roque Saenz Peña 180, Bernal.

Lactic acid bacteria are extensively used in fermented food industries. Industrial applications and taxonomic studies require reliable and simple identification methods. To this end we developed a PCR-based DNA fingerprint assay. We employed synthetic oligonucleotides representing the repetitve elements found in the genome of *Streptococcus pneumoniae i.e.* BOX sequences. These primers were used to generate differential PCR amplification patterns for differentiation between strains of *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. These PCR profiles were also compared with those obtained from other lactic acid bacteria. Seventeen strains of lactobacilli belonging to both subspecies and twenty one strains of thermophilic streptococci were used. Total DNAs obtained from 1.5 ml overnight culture, were purified DNA and quantified by absorbance at 260 nm. The PCR reaction contained 200 ng of template DNA, 1 µM BOX-C primer, 2.5 mM $MgCl_2$, and 0.25 units of Taq polymerase. PCR amplifications were done with initial denaturation (92°C 2 min), followed by 35 cycles (92°C 30 seg, 40°C 1 min, 72°C 2 min) and a final extension (72°C 5 min). BOX-PCR profiles of *Lactobacillus delbrueckii* subsps. *bulgaricus* and *lactis*, and of *Streptococcus salivarius* subsp. *thermophilus* clearly differentiated all three species. Moreover, differences observed among the profiles obtained with the strains analyzed in our study, distinguished some strains within the same subspecies.

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GENETIC ENGINEERING OF WHEAT HMW GLUTENIN FOR IMPROVING BREAD-MAKING QUALITY. Vallejos, R.H., Alvarez, M.L., Bacigaluppo, S., Halford, N.G.*, Heisterborg, C.M., Morata, M.M., Ravizzini, R.A. and Shewry, P.R.*. CEFOBI, Suipacha 531, 2000 Rosario, Argentina; * University of Bristol, IACR Long Ashton Research Station, Bristol BS18 9AF, U.K.

Spring wheat grown in Argentina usually contains 4-5 HMW subunits of glutenin which are considered related to bread-making quality. We have developed an efficient wheat transformation method that can be used to introduce a different or extra copies of genes for that proteins. Wheat varieties containing HMW glutenin subunits 1Ax1, 1Dx5, 1Dy10, 1Bx7 and 1By8 or 1By9 were transformed with the gene for subunit 1Ax1 under its own promoter. Immature embryos were bombarded using a home-made, helium-driven, gene gun, with gold microparticles carrying a mixture of pGL-2 containing the *hpt* gene and pHMW-1Ax1. Explants were cultures under the selective pressure of 25 ml/L of hygromycin and wheat plants were obtained and grown to maturity. Protein extracts from R_1 seeds were analyzed by SDS-PAGE revealing that the 1Ax1 gene was overexpressed in seeds of the transgenic plants, resulting in almost double the amount of 1Ax1 glutenin protein expressed. The transgenic half seeds were grown and R_2 seeds obtained which also expressed the transgene. These results show that HMW glutenins may be overexpressed in transgenic wheat with possible beneficial effects on bread-making quality.

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A POSSIBLE ALTERNATIVE MECHANISM FOR THE REDUCTION OF FORMALDEHYDE IN ANAEROBIC CONDITIONS. G.Vidal*, F. Omíl**, R. Méndez** and J.M. Lema**. *Universidad de La Frontera, Dpto. Ing. Qca., Casilla 54-D. Fono/Fax: 253177. E-mail: gvidal@werken.ufro.cl. Temuco, Chile. **Universidad de Santiago de Compostela, Dpto. de Ing. Qca., E-15706 Santiago de Compostela, Spain.

Formaldehyde is a raw material used in different industrial processes: pesticides, plastics, hardboard or medium density fiberboard manufacturing, industrial adhesives, etc.

The purpose of this study was to investigate the anaerobic biodegradation of formaldehyde, in particular, regarding the effect of the presence of particular co-substrates, such as Butyric acid (HBu).

The operational methodology was based in anaerobic biodegradability batch assays. CH_4, CO_2, formaldehyde and methanol concentration was measured during the assays. Besides, the adsorption and volatilization phenomena in sterilized assays was analyzed.

The formaldehyde anaerobic biodegradation kinetics can be modeled by Haldane kinetics, but the kinetic constants are different when the formaldehyde is degraded in presence of co-substrate. This phenomena can be explained through the metabolic route of formaldehyde degradation.

The latter follows by H_2 and CO_2 hydrolysis and a further methanisation by means of methanogenic hidrogenofilic microorganisms.

This work was supported by the DIDUFRO 9627 Project.

NOVEL MR

INDUCTOR DEL SUEÑO NATURAL

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Cada comprimido ranurado contiene
Melatonina sintética ..... 3 mg / 3000 mcg

Indicaciones
Para problemas de insomnio, alteraciones del sueño por cambios horarios, ya sea por jornadas laborales o por viajes que signifiquen cambios de hemisferios, con el propósito de conseguir un sueño reparador sin ocasionar efectos hang over, logrando un equilibrio del reloj biológico del paciente.

Contraindicaciones
Personas que ingieren medicamentos esteroidales, embarazadas, nodrizas, mujeres que deseen embarazarse, personas con enfermedades autoinmunes, con cánceres autoinmunes. Menores sin consultar previamente al médico.

Posología
Tomar 1/2 a 1 comprimido ranurado antes de acostarse.

Presentación
Frasco de 30 comprimidos ranurados.

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ADDENDUM

Abstract of the presentation of Leonor Cancela in Symposium 13, *Extracellular Matrix-Cell Adhesion and Recognition* (abstracts 64-67).

EXPRESSION OF TWO VITAMIN K DEPENDENT PROTEINS OF THE EXTRACELLULAR MATRIX DURING EARLY DEVELOPMENT OF THE TELEOST FISH SPARUS AURATA. L. Cancela, D. Power and J.E. Pinto. University of Algarve-UCTRA, 8000 Faro, Portugal. E-mail: lcancela@si.ualg.pt.

Matrix Gla (MGP) and Bone Gla (BGP) are small extracellular proteins of unknown function post translationally modified by a vitamin K dependent processus thus belonging to the large family of vitamin K dependent proteins. MGP is expressed in nearly all mammalian tissues analysed while BGP exclusively in bone and dentin. Since the structure of the MGP and BGP genes and studies on the control of their expression have been carried out exclusively in mammals, we have cloned the MGP and BGP cDNAs from a non-mammalian vertebrate and determined their expression in early development in an effort to bring some insight into the function of these proteins. Fish have a number of advantages over mammalian systems since the fertilized egg develops rapidly outside the parental organism and the free swimming larvae can be easily grown and collected. We have chosen as model system the gilthead seabream Sparus aurata because it is locally grown in various aquacultures. Sea bream larvae 12 to 90 days old were collected and development of cartilage and bone structures followed by histological techniques. Total RNA was extracted from fully calcified larvae, reverse transcribed and used as template for cDNA synthesis by the polymerase chain reaction. Specific MGP and BGP cDNAs were cloned and sequenced. Appearance of seabream MGP and BGP mRNA was followed throughout the early stages of development by in situ hybridization using antisense RNA probes generated from the cloned seabream cDNAs. BGP mRNA was first detected in the calcified structures of the mouth region, skin (due to the presence of calcified scales) and at the sites of fin insertion while MGP mRNA was localized mainly in cartilage and epithelia, in agreement with results previously obtained in mammals and suggesting that teleost fish are a valid model to study MGP/BGP control of gene expression.
(This work was supported in part by NATO grand GRG 940751)

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ROLE OF PROTEIN KINASE C IN THE REGULATION OF IODIDE UPTAKE BY CALF THYROID PRIMARY CULTURE.

Bocanera L., Nocetti G., Krawiec L., Silberschmidt D., Juvenal G., Ginzburg M. and Pisarev M.
Div. Bioquímica Nuclear,Comisión Nacional de Energía Atómica and CONICET, Buenos Aires, Argentina.

The present research was performed in order to investigate the effect of protein kinase C on a typical functional parameter and limiting step in thyroid hormone biosynthesis: the iodide uptake. Primary cultures of calf thyroid were used as a model. In the first studies, confluent cells were maintained with TSH (0.5 mU/ml) for 72 h and incubated with 100 nM PMA for 60 minutes. Then ^{125}I uptake was assayed. PMA caused an inhibition by around 70% in cells treated with TSH ($p<0.001$). This effect was mimicked by 10 µM forskolin (53% $p<0.001$) or 100 µM Bu_2 cAMP (51% $p<0.01$). PMA inhibitory action was blocked by a PKC inhibitor: bisindolylmaleimide (BS), suggesting that it is mediated by PKC pathway. In order to determine whether the inhibitory effect of PMA take place at level of iodide efflux or influx, we studied the halogen liberation and Na^+/K^+ ATPase activity. No change on iodide efflux was observed, however PMA caused a significant inhibition of TSH effect on the ATPase (65%, $p<0.01$). This effect was blocked by BS. In summary, PMA inhibits the TSH effects on iodide uptake, this action is mediated by PKC pathway and it takes place at level of Na^+/K^+ ATPase activity.

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1α,25-DIHYDROXY-VITAMIN D_3 STIMULATION OF THE ADENYLYL CYCLASE/cAMP PATHWAY IN MUSCLE CELLS : STUDIES ON THE UNDERLYING MECHANISM. G. Vazquez; AR de Boland, and RL Boland. Depto. Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. (8000) Bahía Blanca, Argentina.

We have recently established that the hormonally active derivative of vitamin D_3, 1α,25-dihydroxy-vitamin D_3 ($1,25(OH)_2D_3$), rapidly (1-5 min.) stimulates voltage-dependent Ca^{2+}-channel mediated Ca^{2+} influx into both chick and rat cultured embryonic muscle cells (myoblasts) by a non-genomic action which involves hormone-induced inhibition of a 40 kDa pertussis-sensitive G protein (Gi) with accumulation of cyclic AMP (cAMP) cellular levels, these events leading to Walsh inhibitor-sensitive phosphorylation of several membrane proteins. To further characterize the mechanism underlying $1,25(OH)_2D_3$ activation of the cAMP pathway, in the present study the effect of the hormone on both adenylyl cyclase (AC) and protein kinase A (PKA) activities as well as on the phosphorylation state of Gi was investigated. In membrane preparations from chick embryo myoblasts, $1,25(OH)_2D_3$ stimulated AC activity (% above basal) at 0.1 (31), 1 (54) and 10 nM (27) in a time (1-5 min.)-dependent fashion, provided GTP (0.1 mM) was included in the assay buffer. In the absence of GTP or in the presence of Mn^{2+} (20 mM) $1,25(OH)_2D_3$ stimulation of AC was completely abolished. Additionally, in hormone pretreated myoblasts (1 nM $1,25(OH)_2D_3$, 5 min.) in vitro PKA activity was increased (21,0 ± 2,0 vs. 36,0 ± 9,0 pmol ^{32}P/min/mg of protein, control vs. treated cells). Immunoprecipitation of Gαi from ^{32}P-labeled myoblast microsomal membranes shows that 2 min. exposure to $1,25(OH)_2D_3$ (1 nM) results in a marked increase (2-2.5 fold) in the phosphorylation of its α subunit. The present data suggest that in muscle cells, $1,25(OH)_2D_3$ activates AC by a non-direct, GTP-dependent action which in part implies amelioration of Gi function by hormone-induced αi phosphorylation. Augmented cAMP levels and in turn, PKA activation, are sequential events mediating $1,25(OH)_2D_3$-dependent membrane protein phosphorylation.

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ACTIVATION OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE SUPPLY BY CARBACHOL. Marchesini, N., Hernández, G., Bollo, M., Racagni, G., Garrido, M. and Machado-Domenech, E.E..Química Biológica, FCEFQN, Universidad Nacional de Río Cuarto, Córdoba, Argentina. e.mail: emachado@unrccc.edu.ar

PtdIns(4,5)P_2 serves as precursor of a diverse family of signalling molecules, including diacylglycerol, InsP_3 and PtdIns(3,4,5)P_3. The production of these messengers can be activated by agonists, and therefore the rate of utilization of PtdIns(4,5)P_2 can vary dramatically. In our previous study in *Trypanosoma cruzi* epimastigotes, carbachol-induced phosphoinositide turnover was demonstrated (Machado-Domenech et al. (1992) FEMS Microbiol. Lett. 95: 267-270). In further experiments, we found that carbachol influences rapid changes in generation of InsP_3 which would be through a receptor-mediated process linked to phospholipase C (PLC) by a G-protein (Garrido et al. (1996) Cell. Mol. Biol. 42: 221-225). In accordance with this, our aim was to study the PI cycle after parasite stimulation with carbachol in reference to its modulation during stimulation, for a period of at least 20 minutes. Endogenous phospholipids were phosphorylated with [γ-^{32}P] ATP at 30 °C for 5 minutes. The products were isolated by TLC and quantified by scintillography. The Ins*P*s were separated by anion-exchange chromatography on Dowex columns (formate form). $[Ca^{2+}]_i$ was measured in suspensions of Fura-2-loaded cells.
In the present study we demonstrate a rapid mobilization of polyphosphoinositide in *T. cruzi* epimastigotes stimulated with carbachol. It causes a rapid release of Ins*P*s, diacylglycerol and a transient rise in the cytoplasmic free Ca^{2+} concentration. Under this circumstance, the demand for PtdIns(4,5)P_2 can rise subtantially and an increase in its net synthesis might occur. The latter could be supplied by an increase in the rate of PtdIns 4*P* 5 kinase and/or a decrease in the rate of a PtdIns(4,5)P_2 degrading reaction.

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PHYTOHEMAGLUTININ STIMULATES THE T-LYMPHOCYTES ACTIVATING PROTEIN KINASE C.

Bustamante. M., Garca. A., [*] Olate. J. & [§] Gonzalez. M. Dpto de Biología Molecular, [*] Dpto de Fisiopatología, Facultad de Ciencias Biológicas, [§] Dpto. de Química Clínica e Inmunología, Facultad de Farmacia, Universidad de Concepción, Concepción, CHILE

The protein kinase C (PKC) family of isoenzymes is participating in a wide range of signal transduction pathways in many different cell types, particularly in haematopoyetic cells like T-lymphocytes. The effects of activators (Phytohemaglutinin (PHA) and the phorbol ester (PMA)) and inhibitor (RO 31-8220) of PKC on the T-cells proliferation were studied. Peripheral blood T-lymphocytes from normal donors were isolated, cultured and stimulated. The cell proliferation was measured using ^3H Thymidine uptake. 500 nM of RO 31-8220 was able to inhibit the PHA and PMA induced T-cell proliferation. This effect is reversed by fetal calf serum (FCS) suggesting that FCS do not stimulate T-cells using the PKC pathway. The PKC inhibitor and the solvent of PMA and RO 31-8220, Dimethylsulphoxide, at concentration higher than 3 µM and 0,5% respectively were toxic for the T-cells cultures. These results show that the PKC inhibitor can be very usefull for disection of PKC mediated signal transduction pathways.

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