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Instituto de Investigaciones Biomédicas del C.S.I.C.

y

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Department of Molecular and Cellular Biology of Cancer

Role of E cadherin and P cadherin in tumor progression. Regulation of the expression and function of both cell-cell adhesion molecules.

Group leader: Amparo Cano

Our studies on the implication of E cadherin (ECD) and P cadherin (PCD) in tumor progression are based in the experimental model of mouse skin carcinogenesis where we are specifically interested in the molecular and cellular mechanisms involved in the regulation of the expression and functional activity of both molecules, as well as in the characterization of the anti-invasive role of ECD. On the other hand, we are also analysing the potential prognostic value of both molecules in several models of human carcinomas. Our specific research lines are the following:
- Regulation of ECD and PCD gene expression during tumor progression in mouse skin carcinogenesis. Analysis of promoters.
- Modulation of the functional activity of ECD in tumor progression. Role of plakoglobin and α-catenin.
- Role of ECD in the invasion and metastatic processes. Relationship with oncogenic Ha-ras dosage.
- Influence of the p53 gene product in the expression of ECD, PCD and integrins during malignant progression (papilloma-carcinoma transition).
- Expression of ECD and PCD in breast carcinoma and basal cell carcinoma.

Role of intracelular pH in transformation and stress responses.

Group leader: Rosario Perona

Changes in intracelular pH are involved in cellular proliferation in response to mitogens. Our interest has been foccused in the determination of the signal transduction pathways activated by the gene of a yeast proton pump ATPase (*PMA-1*) whose expression in NIH3T3 cells is able to induce morphological transformation. Expression of the *PMA-1* gene induces activation of the c-fos and c-jun promoters. Activation of the c-fos promoter is mediated through the serum response element and of the c-jun promoted is mediated by the JUN1 and JUN2 sites of the promoter. On the other hand exposure of cells to UV light triggers a transcriptional response mediated partially by the jun/ATF-2 transcription factors. The activation of c-jun is carried out by a serine threonine kinase, named JNK Inhibitors of the sodium proton antiporter are able to block of activation of JNK induced by several agonists. We are actually investigating which element of the pathway activated by stress is pH dependent.

Malignant progression in mouse skin carcinogenesis.

Group leader: Miguel Quintanilla

Research topics:
- Identification of tumor antigens induced in malignant progression by the production of monoclonal antibodies.
- The growth factor TGFbeta as a modulator of the epithelial phenotype in carcinoma cells. Implications in invasion and metastasis.

- Role of E-cadherin in the metastatic ability of carcinoma cells. Correlation between the metastatic potential and H-ras oncogene expression.
- Analysis of cadherin expression as a marker of progression in carcinomas.
- Effects of the adenovirus E1A gene expression in the differentiation of carcinoma cells and sensitivity to chimio- and radio-therapy.

Intercellular communication in normal and tumoral cells.

Group leader: Antonio Villalobo

Our group is interested in the mechanisms involved in intercellular communication between normal cells and their alterations in tumor cells. Among the studies in progress are: i) The effect of nitric oxide on cell proliferation and its inhibitory action on the transphosphorylation and tyrosine kinase activity of the epidermal growth factor receptor, ii) The control of the epidermal growth factor receptor by calmodulin and phosphocalmodulin, iii) The expression of transforming growth factor β and their receptors in tumor cells, iv) The phosphorylation of connexin-32 by the epidermal growth factor receptor tyrosine kinase and its effect, v) The role of connexin-32 phosphorylation on its turnover, and vi) The function of plasma membrane adenylylated proteins from normal and tumor cells.

Department of Molecular and Cellular Biology of Signal Transduction

Role of Est kinase, AdoMet synthetase and AdoMet decarboxylase in T cell activation.

Group leader: Susana Alemany.

Studies in our laboratory have shown that the mRNA levels of the protooncogene est kinase are increased after T lymphocyte activation. We have also detected that T cell transfection with the oncogenic form of the kinase (cot kinase), increases the release of IL-2, these data implicated est kinase in IL-2 secretion.
On the other hand we have demonstrated that the expression of the g AdoMet synthetase and AdoMet decarboxylase genes are induced during T lymphocyte activation as a consequence of the interaction of IL-2 with its high affinity receptors, while AdoHcy hydrolase mRNA levels remain unchanged. Further studies with inhibitors indicate, that the induction of γ AdoMet synthetase mRNA levels is tightly related to lymphocyte proliferation.

Magnetic Resonance in Biology and Medicine

Group leaders: Sebastián Cerdán
Paloma Ballesteros

Research topics:

Water structure and function in biological systems.

We are studying the transport, metabolism, physical properties and structure of water in various biological systems using NMR methods. The residence time of water is approximatelly 30 s in the perfused liver, 10 ms in rat erythrocytes and < 0.1 ms in rat liver mitochondria. The effective viscosity of the cytosol is approximately twice that of distilled water and the effective viscosity of the mitochondria is fifteen times larger than that of the cytosol.

Quantitative approaches to cerebral metabolism.

We have studied quantitatively the metabolism of glucose in prymary cultures of neurons and astrocytes and compared it with metabolism of the neuronal and glial compartments of the intact brain.

Hormonal regulation of cerebral metabolism.

We have shown that adult onset hypothyroidism markedly affects the cerebral metabolism in the neuronal and glial compartments of the intact brain, altering mainly the relative exchange of glutamate, glutamine and GABA betwen the neuronal and glial compartments.

Magnetic resonance of neurodegenerative disorders.

We are currently analyzing the metabolic alterations induced during Focal Cerebral Ischemia (FCI) and reperfusion in the ischemic and contralateral hemispheres. In addition, a pattern recognition algotithm based on the analysis of neural networks is being developed for the automatic diagnosis of cerebral tumors by ^{1}H NMR.

Novel molecular probes for diagnosis by MRS and MRI methods.

We have synthesized a novel series of pH probes and Gd chelating agents for ^{1}H MRI diagnosis. We are also developing a strategy to apply molecular dynamic calculations to predict pharmacological activity.

Cloning and Expression of a Casein Kinase I in Dyctiostelium discoideum.

Group leader: Margarita Fernández.

Research topics:

Cloning and expression of a casein kinase I from Dictyostelium discoideum..

Our main goal is to study the function of this kinase in *Dictyostelium* cells, We have already cloned a truncated version of casein kinase I, the 5 prime has been localized by anchored PCR, we are searching for the 3 prime end of the gene. We have also performed studies of gene expression. This gene is expressed in large amounts late in differenciation. Althoug the level of expression is very low in vegetative asynchronously growing cells, in a synchronous population differences can be detected in its expression during the cell cycle. We have also generated antibodies against the amino terminal portion of the protein, and we are going to perform immunofluorescence studies. We are planning to perform overexpression and truncation experiments in *Dictyostelium* cells to study the phenotype of the transformed cells.

Activation of MAP and Raf kinase and Protein kinase C in response to different stimuli. .

We have been studying the activation of MAP and Raf kinase and Protein kinase C in response to different stimuli such as PDBu or interleukine 2. In the primary culture these enzymes are not activated in response to IL2, but they are activated in response to the phorbol esther. The Map kinase activation is the limiting step in the transition G1/S triggered by PDBu. Raf activation (measured as hiperphosphorylation) is required for proliferation but is not requred for MAP kinase activation.. We are currently studying the putative role of two protein phosphatases acting on phosphorylated MAP kinase to terminate the signal.

Metabolic regulation

Group leaders: José M. Mato
Isabel Varela
María A. Pajares

Research topics:

Alterations of methionine metabolism in hepatic diseases.

Regulation of S-adenosylmethionine synthesis.

Structure of S-adenosylmethionine synthetase.

Regulation of betaine: homocysteine methyltransferase.

Signal transduction mediated by lipids

Our work is aimed to determine the structure and biological actions of the mammalian inositol phosphoglycan (IPG) involved in signal transduction of the insulin family of factors, and to develop synthetic methods to obtain biologically active analogues of potential therapeutical interest. The basic mechanisms through which these molecules act will be studied. In this respect it will be investigated: 1) The role of IPG as a transducing signal for neuroendocrine hormones such as insulin and related insulin-like factors; 2) The role of IPG and IPG-analogues on the modulation of the expression of specific genes during cell growth and embryonic development; 3) Modulation by growth factors of the synthesis and turnover of glycosyl-phosphatidylinositol, the cellular IPG precursor. We are also investigating on the regulation of cell activation by sphingolipids. In particular, we are interested in the role of ceramides and ceramide

phosphates in cotrolling cell proliferation and apoptosis. Ceramides can induce cell differentiation and are potent inhibitors of cell growth. In contrast, we have observed recently that ceramide phosphate stimulates DNA synthesis and cell division. An inmediate objective of our work is now to determine the mechanism(s) whereby these effects are brought about *in vivo* and in cells in culture.

Protein phosphorylation in cellular signaling.

Group Leader: Jorge Martin

Protein phosphorylation, a mechanism of post-translational modification with relevant implications in many pathways of cellular signalling, is controlled by the counter-balance between the relative activities of protein kinases and phosphatases. We have studied the role of protein kinases and phosphatases in a variety of cell signalling mechanisms, including oncogenic transformation, apoptosis and proliferation. At the present time, our main emphasis is centered in gaining some understanding of the role play by the Src family of tyrosine kinases in the cellular mechanisms of signal transduction induced by prolactin.

Mitogenic Activities in the Peritoneal Effluent of Patients Treated with Continuous Ambulatory Peritoneal Dialisis (C..A.P.D.).

Group leader: Francisco Vara

Resesarch Topics:
Presence of Different Mitogenic Activities in the Peritoneal Effluent of Patients on CAPD.
Cell Populations Present in the Peritoneal Effluent of Patients on CAPD.

Peritoneal cells in CAPD patients are in continuous process of regeneration. We have described that NPE is mitogenic on human and mouse fibroblasts in culture, especially when a comitogen is present. The nature, origin and role of this mitogenic activity remains undetermined. The data following to dialysis process and different comitogen additions suggest that the peritoneal effluent contains different growth factors greater than 10000 daltons. Also, the presence of a growth inhibitor is plausible. In conclusion, different growth-promoting and inhibiting activities are present in peritoneal effluent, suggesting a complex cellular relationships as result of peritoneal dialysis with unknown consequences.

Department of Biochemistry and Genetics of Yeast

Molecular analysis of ionic transporters in yeast.

Group leader: P. Eraso

Research topics:

Molecular mechanism of regulation of yeast plasma membrane H^+-ATPase by glucose

New residues having an important role in the H^+-ATPase regulation by glucose have been identified using site-directed mutagenesis. A point mutation at the regulatory domain (H914Y) results in a fully activated enzyme which is able to eliminate both the lack of growth and the defect of ATPase activity of the double mutant S899A,T912A. Thr-912 is an essential residue for glucose activation of H^+-ATPase and forms part of a potential phosphorylation site for Ca^{2+}/Calmodulin-dependent protein kinase. We have investigated the role of some calmodulin antagonists on glucose activation of H^+-ATPase.

Structure-function relationship study on CFTR protein by directed mutagenesis and intragenic suppression analysis

FQ mutations are being created by site-directed mutagenesis in YCF, the yeast homologue of CFTR protein, to isolate and identify intragenic suppressor mutations.

Control of glycolytic flux in yeast

Group leader: C. gancedo

Research topics:

Control of glycolytic flux in yeast

We characterized the mutation *DGT1-1* (previously named *SMU1*). The corresponding gene regulates the expression of several genes related to sugar transport in yeast and the mutation obtained abolishes catabolite repression. A suppressor of the mutation *tps1* turned out to be *CAT3*.

We have developped an enzymatic assay method for the quantitative determination of trehalose-6-phosphate using purified hexokinase from the yeast *Yarrowia lipolytica*.

Secuencing of the yeast genome

Within the frame of the European programme of sequencing the whole yeast genome we have continued the sequence of a region of chromosme XV . We have identified in the left arm of this chromosome near the telomere several new ORFs not previously characterized.

Regulation of the expression of gluconeogenic genes in yeast.

Group leader: Juana M. Gancedo

Regulatory elements for the transcription of the *FBP1* gene from *S. cerevisiae* have been characterized. The purification of proteins involved in transcriptional activation is in progress. Regarding the post-transcriptional regulation of yeast fructose-1,6-bisphosphatase, domains of the protein related to catabolite inactivation have been investigated and a new allosteric regulatory mechanism, inhibition by trehalose-6P has been uncovered. Yeast strains

have been constructed to study the physiological significance of the different regulatory mechanisms of fructose-1,6-bisphosphatase. It can be concluded that the selective advantage conferred by these mechanisms is smaller than expected but sufficient to allow the rapid replacement of an unregulated strain by a regulated one. The study of mutants which affect the expression of a variety of enzymes subject to catabolite repression has been followed up.

Mechanism of glucose transport in yeast.

Group leader: R. Lagunas

Research Topics:

Mechanism of glucose transport in yeast.

It has been reported that the low-affinity component of the glucose transport in yeast is due to passive diffusion. We have found that the permeability coefficient of hexose in this organism is, at least, two to three orders of magnitude lower than required to account for this component and have concluded that it is not due to passive diffusion.

Mechanism of catabolite inactivation of plasma membrane proteins.

Catabolite inactivation of the transporters of the plasma membrane has been investigated using the maltose transport as an experimental model. We have found that this inactivation requires internalization by endocytosis, occurs in the vacuole and is independent of function of the proteasome and of the cAMP-dependent protein kinase.

Secuencing of the yeast genome

Group leader: Maria J. Mazón.

Resesarch topics:

Yeast Genome Sequencing Project

The nucleotide sequence of a fragment from the left arm of chromosome VII has been determined. Analysis of the sequence revealed nine complete ORFs and two incomplete ones. Five of these ORFs have been selected for phenotypic analysis and studies on gene expression and function.

Structure-function relationship study on Cystic Fibrosis Transmembrane Regulator (CFTR) protein by directed mutagenesis and intragenic supression analysis

Chimeric genes between the CFTR protein and its yeast homologue, YCF1, will be constructed to study the effect of cystic fibrosis (CF) mutations and to isolate and identify intragenic supressor mutations.

Molecular Analysis of the yeast plasma membrane H^+-ATPase

Group leader: Francisco Portillo

Resesarch Topics:

Genetic Analysis of the ATP Binding Site of the Yeast Plasma Membrane H^+-ATPase.

The highly conserved motif of H^+-ATPase ^{474}KGAP has been proposed to participate in the formation of the phosphorylated intermediate during the catalytic cycle. We have performed an intragenic suppressor analysis of the K474R mutation to identify the interacting regions involved in this function. One suppressor mutation (V396I), located 18 residues away from the Asp-378 residue, which phosphorylated during

catalysis, is allele-specific. This provides genetic evidence of a direct interaction between the KGAP motif and the phosphorylation domain during the catalytic cycle. Three mutations (V484I, V484I/E485K and E485K/E486K) may compense the structural alteration introduced by the K474R mutation. Three substitutions (A165V, V169I/D170N and P536L) may act as allele-nonspecific suppressors.

Cloning and characterization of genes involved in the glucose-dependent expression of the ATPase gene (PMA1).

The expression of PMA1 is regulated by glucose. We have isolated mutations on seven genes that affect this regulation. One of these genes (APA1) was cloned by complementation and shown to encode a protein with six putative transmembrane stretches. Deletion of APA1 affects the expression of several glucose-inducible genes. These results suggest a model in which Apa1 acts on a glucose-signaling pathway.

Department of Molecular Endocrinology

Expression of brain genes regulated by thyroid hormone

Group leader: Juan Bernal

Research topics:

Regional specificity of thyroid hormone on RC3 expression.
Distribution of T3 receptors in brain and colocalization with T3-sensitive genes
Hormonal regulation of RC3 in GT1-7 cell cultures
Identification of regulatory sequences in the RC3 gene
Expression patterns and hormonal regulation of a novel striatal-specific gene

The goals of our work are the identification of brain genes dependent of thyroid hormone, and the study of the mechanism of thyroid hormone action. The best studied so far is RC3/neurogranin. It encodes a 78-aminoacid protein located in the dendritic spines of cerebral neurons and it is not expressed in cerebellum. The protein is a calmodulin binding, PKC substrate presumably involved in synaptic plasticity. Expression of the gen is under tight control by T3 in layer VI of cerebral cortex, retrosplenial cortex, caudate nucleus and dentate gyrus of the hippocampus. Al these regions, which express RC3 after postnatal day 10, are dependent of thyroid hormone. Other regions, such as layers II-III of cerebral cortex, habenular nucleus, CA fields of hippocampus, amygdala, or medial geniculate nucleus are insensitive to T3. To study the determinants of T3 sensitivity we have studied the colocalization of RC3 with T3 receptor variants in sensitive and insensitive cells. The results are that sensitivity to T3 is not determined by differential distribution of receptors. On the other hand, we have found that the GT1-7, hypothalamic cell line express very low levels of RC3 mRNA but that the addition of T3 results in a fast (4 hours) and robust (20-fold at 24 hours) increase of RC3 mRNA. The effect is probably mediated directly by the T3-receptor complex since the T3 response is not inhibited by cycloheximide. Furthermore, GT1-7 cells express high levels of functional T3 receptors, as assessed by T3 binding assays and reporter gene induction by endogenous receptors. Despite this, the RC3 promoter is not responsive to T3 which raises the possibility of T3-responsive elements located in region of the gene different from the promoter.

Production and action of thyroid hormones at different stages of development

Group leader for maternal-fetal communication: G. Morreale de Escobar

Research topics:

Thyroid hormones in early embryonic compartments
Alterations of thyroid hormone transport, induced with a synthetic flavonoid, and consequences for the maternal and fetal thyroid hormone status.
Effects of the herbicide nitrofen on maternal-fetal communication and pulmonary immaturity.
Brain damage in progeny of severely iodine deficient rats: audiogenic seizures
Brain damage in Thyroid hormone receptor b null mutant mice: audiogenic seizures.

Group leader for extrathyroidal adaptations to thyroid hormone deficiency and excess: G. Morreale de Escobar

Research topics:

Thyroid hormone status in thyroidectomized rats on different T4 doses: euthyroidism of all rat tissues is not attained simultaneously with any dose.
Thyroid hormone status in thyroidectomized rats on different T3 doses: euthyroidism of all rat tissues is not attained simultaneously with any dose.
Thyroid hormone status in thyroidectomized rats on different combinations of T4 + T3: euthyroidism attained in all tissues.
Homeostasis of T3 in the adult cerebral cortex.

Group leader for iodine deficiency studies: Dr. F. Escobar del Rey

Research topics
Situation of iodine nutrition in different areas of Spain.
Iodine nutrition in schoolchildren of the Community of Madrid.
Iodine deficiency in premature infants
Iodine deficiency in pregnant and lactating women.
Experimental iodine deficiency disorders: treshold for brain damage.

Effects of the c-erbA and v-erbA genes and of thyroid hormone on cell growth and differentiation

Group leader: Alberto Muñoz

Research topics:
Effects of erbA genes and of thyroid hormone on glial cells
Regulation of the brain-specific prostaglandin D2 synthetase gene by thyroid hormone
Effects of erbA genes and of thyroid hormone on mammary epithelial cells
Search for brain genes regulated by thyroid hormone

Our group is focussed on the study of the biology of the erbA genes. We are analyzing the effects of c-erbA and v-erbA on the growth and differentiation, phenotype and gene expression of normal, non-tumorogenic cell lines of glial (B3.1) and mammary epithelial (EpH4) origin. On the other hand, we are searching for new genes regulated by c-erbA and its ligand thyroid hormone (T3). To this, genomic and differential display PCR approaches have been performed. A series of genes have been found that are transcriptionally regulated by erbA/T3 during rat brain development. In addition, we are studying the regulation by T3 of the prostaglandin (PG) D2 synthetase gene. The product of this gene is the enzyme responsible for the synthesis of PGD2, the major PG in the brain which is involved in the control of functions such as body temperature and the sleep-wake cycle. We have characterized a T3-regulatory element in the promoter region of the PGD2 synthetatse gene, and in situ hybridization and immunohistochemistry analyses have confirmed the control by T3 in vivo.

Regulation of brown adipocytes proliferation and differentiation. Regulation of deiodinase activity by thyroid hormones.

Group leader: María Jesús Obregón

Proliferation of brown adipocytes in primary culture. The mitogenic effects of growth factors, vasopresin and norepinephrine (NE) on brown preadipocytes are studied.
The regulation of the UCP gene in brown adipocytes by NE, thyroid hormones and glucocorticoids is examined at the promotor level, and the regulation of UCP mRNA

expression.

Regulation of 5'Deiodinase (5'D-II) and 5 Deiodinase (5D) activities in brown adipocytes.
The adrenergic stimulation of 5'D-II is potentiated by T3, while 5D activity is increased by several growth factors, as well by thyroid hormones and NE.

Regulation of nuclear T3 receptors (TR) mRNA in brown adipocytes.
T3 increases beta-TR mRNA species while insulin decreases alfa and beta-TR mRNAs.

Regulation of 5'Deiodinase activity in rat tissues by thyroid hormones. 5'Deiodinase activities has been examined in tissues from fetal and neonatal rats undergoing diabetes and undernutrition, flavonoids administration or iodine deficiency, or in adult rats replaced with T4 or T3.

Regulation of nerve cells differentiation and neuro-specific promoters expression by nuclear receptors.

Group leader: Angeles Rodriguez-Peña

The role of thyroid hormone and thyroid hormone receptor (TR) expression in the differentiation of nerve cells have been studied in primary cultures of oligodendrocytes progenitors and neuroblastoma cell lines.

We have shown that the generation of oligodendrocytes in vitro predominantly occurs in asymmetric division and differentiation of O-2A progenitor cells, process in which thyroid hormone increase the number of oligodendrocytes per clone, but does not change the timing of appearance.

Two lines of neuroblastoma cell lines differentiation after stable expression of TR have been studied, the N2a and the clones N1 and N7 from the neuroblastoma NB41A. Expression of TR decrease their growth capabilities and induces the expression of the neurotrophins receptor, TrkB.

The activation of two neuro-specific promoters by nuclear receptors have been studied: the myelin basic protein promoter which is activated upon stimulation with thyroid hormone and 9-cis retinoic acid through the same element, and the characterization of the trkB promoter and flanking sequences.

Department of Enzymology and Molecular Pathology

Control mechanisms of sugar metabolism

Group Leader : Juan J. Aragón

Research topics:

Molecular bases of the allosteric control of phosphofructokinase (PFK) of eukaryotic cells**.** To this aim, we use PFKs from *Dictyostelium discoideum,* a non allosteric isozyme, and ascites tumor cells, a regulatory one. The cDNA of *D. discoideum* PFK was expressed in a PFK^- strain of *Saccharomyces cerevisiae* and the recombinant enzyme purified in the amount of milligrams, thus enough for the production of mutant forms by specific manipulation of the sequence. The full-length cDNA of cDNA of PFK-C from ascites tumor has been isolated and residues proposed for the catalytic and regulatory sites were located in the amino acid sequence.

Metabolism of glutamine and glucose in hybridoma cells. The metabolism of glutamine and glucose is being investigated in hybridoma cells for the evaluation of their metabolic fates, their capacity as energy substrates and the possibility of interaction between both of them.

Utilization of galactosylxyloses in the evaluation of intestinal lactase *in vivo*. 2- 3- and 4-Galactosylxyloses have been synthesized enzimatically and proved to be hydrolyzed *in vivo* by rat intestinal lactase after oral administration of the disaccharide and measurement of xylose in the urine by a simple colorimetric procedure. Xylose elimination correlated with lactase activity when evaluated along growth of suckling animals. This method can be of application to the diagnosis of lactase deficiency in humans, with particular interest in pediatrics because of its simplicity and apparent innocuousness.

Human Molecular Genetics. The search and identification of human genes. Characterization of new mutations causing hemophilia B in the spanish population.

Group Leader: Antonio Coloma

Research topics:

The interest of our laboratory if focused on the characterization of new human genes, as a contribution to the Genome Project, as well as on the improvement of the molecular diganostics of recessive hereditary diseases by characterization of novel mutations causing diseasein families that are noninformative by RFLP analysis.

Our work on new human genes has consisted on the contribution to the identification of the Diastrophic Displasia gene, completed in 1994 in Eric Lander´'s lab, the search of the Spinal Muscular Atrophia gene, already identified by others in 1995, and the identification of the gene encoding human neurogranin (RC3), a neuronal protein which is a substrate of protein kinase C. Concerning the molecular diagnostics aspects, we have described 10 novel point mutations on the factor IX gene causing hemophilia B in spanish families.

Hormonal Regulation of Metabolism

Group Leader: Juan Emilio Felíu

Research topics:

Actions of sulnonilureas in extrapancreatic tissues.
Glycosyl-phosphatidylinositol signalling system and insulin resistance.
Diagnosis of inherited metabolic diseases related to carbohydrate metabolism.

Control of expression and modulation of enzyme activities in yeast and Artemia

Group Leader: Claudio Fernandez de Heredia

Research topics:

Transport of hexoses in yeast. The constitutive transport of hexoses in yeast has been re-examined with a new radioactive experimental approach devised to distinguish between association or independence of the transport step with phosphorylation of the sugar substrate. Our results with wild-type *Saccharomyces cerevisiae* support the view that the transport of hexoses in yeast does not involve phosphorylation of the substrate.

Sensitivity of glycolysis to inhibition by glucosamine**.** Inhibition by glucosamine (a metabolic glucose analogue) of the utilization of hexoses by *Saccharomyces cerevisiae* is induced by growing the cells in media with galactose. The changes that render the cells sensitive to glucosamine, are under the control of the *gal80* and *gal4* genes. The inhibition by glucosamine is pH dependent. Intracellular accumulated glucosamine derivatives impairs the transport of glucose and mannose in galactose, but not in glucose or ethanol, grown yeast, under conditions in which the utilization of these sugars is inhibited.

Synthesis of nucleosides 2'-phosphate**.** The purification and characterization of the *Artemia* 2',3' cyclic nucleotide phosphohydrolase has been completed

Mechanismos of resistance to Metotrexate (MTX) in LLA cells. Enzymology of salvage pathways in differentiation and pathological states.

Group Leader: Pilar Llorente

Research topics:

Inhibition of FPGS by metabolites of Folic acid and MTX hydrolisis**.** Pterine and aminopterin analogs, potential hydrolisis products of Folic acid and MTX respectively, have been synthesized and their action on the regulation of the plyglutamylation (FPGS) in leukaemic lymphoblasts cells (L 5178-Y) has been studied. These molecules are values tools for understanding the metabolism of antifolate drugs so as the mechanism (s) of the drugs resistance celular.

Purine metabolism in HGPRT-deficient patients Studies performed with effectors, Fe^{3+} ion , so as specific inhibitors of PNP and phosphatase(s) , support the sequential implication of both enzymatic activities in purine nucleosides PRPP- dependent synthesis , in erythrocytes from HGPRT- deficient patients (Lesch-Nyhan syndrome).This metabolic pathway may be an alternative route for hypoxanthine salvage and / or purine nucleotide formation in HGPRT-deficient erythrocytes.

Thermal stability of *Artemia* HGPRT: effect of substrates on inactivation kinetics.

In this work , we have carried out a study on HGPRT from Artemia cysts in order to further investigate the effect of temperature on the activity and stanhancement of its thermostability.

Nonenzymatic antioxidant levels in blood and arteries related to cardiovascular risk

Group leader: María Rosa de Sagarra

Research topic:

Ubiquinol is an extremely labile substance. In order to measure ubiquinol levels in surgical tissue samples, a preservation medium has been developed and assayed in guinea pig heart. With this medium, it has been possible to assay ubiquinol and Vitamin E in low density lipoproteins isolated from several blood samples. Due to Hospitalary problems, it has been particularly hard to obtain samples from the hospitals, so that the continuation of the work has been delayed.

Metabolism and function of dinucleoside polyphosphates

Group Leaders: Maria Antonia Günther and Antonio Sillero

Research topics:

Metabolism and Function of dinucleoside polyphosphates (Np_4N). a) Yeast Acetyl-CoA synthetase catalyzes the synthesis of adenosine 5'-tetraphosphate and adenosine 5'-pentaphosphate; b) Synthesis of labelled Np_4N has been obtained with firefly luciferase (EC 1.13.12.7); c) Synthesis of diadenosine tetraphosphate (Ap_4A) with luciferase takes place in the absence of oxygen; d) a non-specific adenylate deaminase from *Helix pomatia* transforms Ap_4A into diinosine tetraphosphate (Ip_4I); e) Encysted embryos of *Thamnocephalus platyurus* contain millimolar concentrations of diguanosine tetraphosphate; f) the Ap_4A, ATP and catecholamines content has been analysed in bovine adrenal medulla, chromaffin granules and chromaffin cells.

Purine nucleotide metabolism. The synthesis of uric acid from purine bases, nucleosides and nucleotides has been measured in rat liver supernatants

Enzyme kinetics. The reservoir model of enzyme kinetic has been applied to intuitively visualise the effect of the different types of reversible enzyme inhibition on the kinetic properties of an enzyme. The relationships between the concentration of an inhibitor producing 50 % inhibition of an enzyme reaction (I_{50}) and its inhibition constant has been discussed. A tridimensional representation of enzyme inhibition, useful for diagnostic purposes, has been developed.

Department of Regulation of Gene Expression

Regulation of gene expression during development and criptobiosis.

Group Leader: Leandro Sastre

Our group is working on the regulation of transcription during the activation of *Artemia* cryptobiotic embryos (cysts) and their consequent development. *Artemia* cryptobiotic embryos are characterized by the absence of any biological activity, including gene transcription. Their activation involves the resumption of these activities between minutes to a few hours. The study of the regulatory mechanisms that mediate transcriptional activation during this process has been approached through the isolation of a number of genes that are expressed soon after activation of the cysts and the characterization of their promoter regions. Once these promoters have been characterized we will study the mechanisms that regulate their expression. A second approach to the problem is the study of basic transcription factors that could mediate transcriptional activation and/or repression. We have initiated this project through the cloning of the TATA Binding Protein (TBP) and the study of its expression during cyst activation.
This general project has been divided in the following research topics:
- Regulation of the expression of *Artemia* sarco/endoplasmic reticulum Ca-ATPase gene.
- Regulation of the expression of *Artemia* Na/K ATPase α1 subunit gene.
- Regulation of the expression of three *Artemia* actin genes.
- Structure and expression of *Artemia* TATA Binding Protein (TBP) gene.

Paramyosin and other muscle proteins in *Drosophila*: Biochemical and Function analysis

Group Leader: Margarita Cervera

Analysis of the *in vivo* β−galactosidase expression has allowed us to identify "minimal" promoters as well as the "complete" promoter sequences that qualitatively reproduce the *in vivo* expression properties of PM and mPM. We are now initiating a more rigorous analysis of the quantitative expression in embryos and larvae by *in situ* hybridization to characterize in detail the different potentially regulatory sequence motives
The study of the TnT promoter has started isolating the genomic clones from *D. melanogaster* and *virilis* .The comparison of both promoters will allow the identification of "putative" functional motifs. We will identify the minimal promoter as well as conserved, known and unknown, specific motifs (E boxes, MEF2 sites, CArgG boxes) in the sequences of both genes.

Study of genes responsible for inherited neuromuscular diseases in humans. Analysis of human centromeric sequences

Group Leader: Jesús Cruces

Neuromuscular diseases are the main group of inherited diseases affecting humans. Our group' work is focused in two of them. First, the spinal muscular atrophy (SMA), a disorder in which affected individuals present with different degrees of degeneration of the anterior horn neuronal cells. SMA is, after cystic fibrosis, the second most frequent severe recessive genetic disease in humans: 1 in 8-10.000 live births. On the other hand, we are also

characterizing the human homolog of the *Drosophila* recessive muscular dystrophy mutation "rotated". To date, this *D. melanogaster* gene has not correspondence with any characterizated human gene. However, given its putative function and the "rotated" phenotype, the human homolog is a good candidate for involvement in human recessive dystrophies.

We are also studying human chromosome centromeric sequences in an attempt to understand the structure and minimal length of sequences required to make a functional centromere, with a final aim of generating mammalian artificial chromosomes (MACs) as possible vectors to introduce foreign genes into human cells, with possible applications in gene therapy. This general project has been divided in the following research topics:

-Spinal Muscular Atrophy: Physical and genetic map of the region of chromosome 5, containing the SMA locus
- Study of alpha satellite DNA at the centromere of human chromosome 7.
- Characterization of the human homolog of the *Drosophila* "rotated" gene

Regulation of mitochondrial genes expression during development.

Group Leader: Carmen G. Vallejo

During the early development of *Artemia* there is an increase in mitochondrial enzyme activities of about one order of magnitude, whereas the activities of two cytoplasmic enzymes tested as controls remain unaltered. The mitochondrial enzyme activation correlates with (i) large changes in mitochondrial morphology, (ii) a 5-fold increase in the amount of the H^+-ATP synthase ß-subunit and (iii) a dramatic increase in the steady-state level of mitochondrial mRNAs, whereas mitochondrial rRNA concentrations remain mostly unchanged. In contrast, the level of mitochondrial DNA does not change significantly during the first 20 hours after resumption of development. After hatching, the mitochondrial DNA content increases twice in parallel with one round of cellular division, thus indicating that mitochondrial and nuclear replication are coupled in mitochondrial postgastrular development. The data presented strongly suggest that mitochondrial maturation in the absence of significant mitochondrial proliferation is responsible for the dramatic increase in mitochondrial function that takes place after resumption of development in *Artemia*.

We have identified the transcription initiation sites and constructed the transcription map of the mitochondrial genome of *Artemia*. By in vitro capping of the nascent RNA 5' ends with guanylyltransferase, two transcription initiation sites have been identified in the heavy strand. The first is located in the 5' end of the 12S rRNA, at one end of the non-coding area, and it is heterogeneous. A second transcription initiation site, less frequently used, occurs at 250 nucleotides upstream from the former. The transcription initiation sites have been mapped by primer extension, S I protection and RNAse protection techniques. Regarding the light strand, although the in vitro capping technique has produced no results, it has been possible to identify at least one possible candidate for transcription initiation site. The transcription map has been constructed from results obtained with the Northern hybridization technique using double stranded DNA, RNA and single stranded oligonucleotides as probes. It can be concluded that (i) the *Artemia* mitochondrial genome is transcribed as polycistronic units beginning at one or few promotors, as in other animal mitochondrial systems (ii) the primary transcripts are then processed to render mature transcripts using the tRNAs as processing signals, processing being less efficient in those regions of the genome where a tRNA does not occur, (iii) the heavy strand is transcribed completely, including the main non-coding region from which stable transcripts have been mapped and (iv) the transcription is terminated bidirectionally at the leucine tRNA.

Artemia mitochondrial RNA polymerase has been isolated from the fist time from an

invertebrate. Mitochondria were purified and the RNA polymerase activity was solubilized and affinity-chromatographied on Heparin-Sepharose. The characterization of the purified preparation indicated that the enzymatic reaction was completely dependent on added DNA and rNTPs and that the reaction products were completely sensitive to RNAse. The reaction requires Mg^{2+}, being Mn^{+2} an inhibitor as well as the monovalent cations. The polymerase is insensitive to rifampicin and α-amanitin. Although the characteristics of the isolated polymerase are typical of the mitochondrial enzyme, the in vitro transcription of *Artemia* mtDNA fragments containing the initiation sites turned out to be unspecific. In connexion, the main initiation site was protected from DNAse I by the crude but not the purified mitochondrial RNA polymerase preparation. The results altogether suggest that a specificity factor is separated from the catalytic subunit during the chromatography and, since this does not happen in the systems described up to date (yeast and vertebrates), it may be inferred that the specificity factor in invertebrates is of a different nature.
No information is available on the likely role of phosphorylation/dephosphorylation mechanisms in mitochondrial biogenesis, despite the fact that the *Artemia* mitochondrial protein-coding genes contain phosphorylation consensus sites for different protein kinases. Likewise, protein kinases have not been described in mitochondria from invertebrates and there are only a few reports from mammalian mitochondria. As a first approach, we have isolated and characterized two protein kinases in *Artemia* mitochondria, casein kinase II and cAMP-dependent protein kinase. Whereas the first presents the same characteristics of the cytosolic enzyme, the second has a much higher affinity for the specific peptide Kemptide. Each kinase phosphorylate a different set of proteins in *Artemia* mitochondria.

Physiopathology of mitochondrial biogenesis

Group Leader: Rafael Garesse

Mitochondrial diseases are now recognised as a distinct class of disorders, usually degenerative in character, which are associated with mutations in mitochondrial DNA (mtDNA) mainly affecting muscle and central nervous system. MtDNA exists as a semiautonomous genome encoding only a small subset of the function of the organelle, which are nevertheless critical to respiration. The rest, are encoded in the nucleus, and therefore the biogenesis of functional mitochondria depends on the co-ordinated expression of both genomes. In order to know how nuclear and mitochondrial genes are expressed in physiological and pathological conditions we are using two different approaches: i) using as model system *Drosophila melanogaster* we are studying the promoter of several genes that play a key role in mitochondrial biogenesis. ii) we are studying at molecular level the cellular effects of new mtDNA mutations responsible of human mitochondrial diseases.

Genetic and Epigenetic Factors in Arthropod Development and Aging

Group leader: Roberto Marco

In the first topic, purification and properties of muscle proteins, we have checked the efficiency of new methods of separation of muscle proteins and applied them in arthropods to the identification and study of the calcium binding properties of Troponin T and C. In the second topic, epigenetic modulation of *Drosophila* and *Artemia* development and aging, we have performed several experiments in Space, in the Shuttle Columbia (NASA-ESA) and in Russian biosatellites (Biopan and Foton-10). We have extended the information on the effects of microgravity on *Drosophila* and *Artemia* development , as well as on *Drosophila* aging in close correlation with additional parameters modifying the aging response, such as the

temperature of rearing and selection for differential longevity..The results overall agree with the *rate-of-living* theory of aging but indicates that mitochondrial alteration is only one of the parameters involved in the aging response.In the fourth topic, mitochondrial DNA and phylogenetic studies in *Artemia*. we have completed the identification of the parthenogenetic strains in this genus.

Effect of perinatal hypothyroidism on brain development.

Group Leader: Ana Pérez Castillo

In vertebrates, the thyroid hormone, T_3, plays a critical role in the development of the central nervous system and its deficiency during the early neonatal period results in severe brain damage. However, the mechanisms involved and the genes specifically regulated by T_3 during brain development are largely unknown. We have identified an early gene: NGFI-A, to be a target of T3 action. In vivo the expression of this gene is up-regulated by T3 during brain development. The thyroid hormone receptors a1 and b1 are able to transactivate the NGFI-A promoter independently of ligand. On the other hand, by using a subtractive hybridization technique we have shown that T3 is an important regulator of mitocondrial gene expression during all the neonatal, and also prenatal, periods of brain development.
Expression of thyroid hormone receptor genes (TRs) and 5'deiodinase activities in the developing pituitary and regulation by T3. We have previously shown a regulation of TSH and GH genes by T3 very early in pituitary development. Now we have studied the developmental profile and regulation by T3 of the different TRs and the enzyme activities responsible for the plasma and cellular T3 concentrations. Both TRa and TRb genes are present very early in development and regulated by T3. Type I and type II deiodinase activities have different ontogenic patterns: type II is the predominant activity in fetuses whereas the level of type I increases with age. These results demonstrate that the mechanisms responsible for T3 action are mature and active very early in development and suggest an involvement of T3 in the establishment and/or maintenance of the somatotroph and thyrotroph phenotype.

Genetic Approaches to the study of signaling pathways in mammalian cells in culture

Group Leader: Jaime Renart

Research topics:

Obtention of cell lines resistant to the neurotransmitter glutamate. Our main goal is to develop an in vitro excitotoxicity assay to make mutant cell lines resistant to this neurotransmitter. To this end we are obtaining cells lines (derived from the human embryonic kidney cell line HEK293 and from the neuroblastoma N2A) that express different subunits of the NMDA receptor: NR1 and NR2A and NR2C. The expression of the NR2A and NR2C subunits is under the control of a regulated promoter, based on the tetracycline repressor.

Characterization of a cell line that expresses stably the NR1 subunit of the NMDA glutamate receptor. This line was constructed using the neuronal specific enolase promoter, and has two interesting properties: first, the extension of processes in the presence of serum, and second, the overexpression of the immediate early gen NGFI-A. We are currently studying the molecular bases of this phenotype. 3. induction of apoptosis by protein kinase C inhibition in a neuroblastoma cell line. When N2A cells are treated with the specific PKC inhibitors bisindolylmaleimide GF103209X and Gö 6976, apoptosis is triggered.Cells are protected from apoptosis by transfection with the *Bcl2* gene or

when the treatment is done in the presence of calpain I inhibitors. We are currently characterizing this system, as irreversibility of the process, and expression of different genes during the treatment.

Department of hormonal regulation

Tissue-specific transcription factors in the hormonal control of gene expression, proliferation and cellular diferentiation.

Group Leader: Pilar Santisteban

The main objective of our work is the identification of the *cis*-regulatory elements and the transcription factors mediating the hormonal regulation of gene expression. The fact that certain genes are transcribed only in a determined cell type make the thyroid cells an excellent model for both studies. The expression of Tg, TPO and TSH-R genes is restricted to thyroid cells since only in these cells are present three thyroid-specific transcription factors TTF-1, TTF-2 and Pax-8. This transcription factors are member of *homeo, fork head* and *paired box* families and are the responsible of thyroid phenotype determination. We have identified the TTF-2 binding site as a hormone response element. How TTF-1 works is much more complex, involving phosphorylation as the main mechanism of regulating its activity. We have also studying the role of PKA, PKC and MAPK in TTF-1 activation/inactivation process. Studying Harvey-ras transformed thyroid cells where TTF-1 is present but inactive we have confirmed the importance of protein phosphorylation in this transcription factor activation. TTF-1 and Pax-8 are involved in thyroid cell proliferation and differentiation, playing an important role in thyroid pathologies. We have described a familiar hipothyroidism due to low expression of the transcripction factor TTF-1.
Another important model for study hormonal regulation of gene expression is the regulation of malic enzyme by insulin and thyroid hormones. An element Sp1 like has been identified as mediator of insulin action in the promoter of this gene.

Regulation of gene expression in pituitary and neuronal cells by the thyroid hormone/retinoic acid/vitamin D3 subfamily of nuclear receptors.

Group Leader: Ana Aranda

The nuclear receptors for retinoic acid, vitamin D3 and thyroid hormone, as well as several "orphan" receptors such as PPAR are highly homologous. This homology makes possible that the different receptors can recognize similar DNA elements and regulate common gene networks. We are studying the molecular mechanisms by which those receptors interact among them and with other extracellular signals, growth factors and oncogenes to regulate somatic growth and neuronal cell proliferation and differentiation. Their effect on the expression of pituitary-specific genes (growth hormone, prolactin and the transcription factor GHF-1), "immediate early genes" (fos, jun, N10 and Egr-1) implicated in proliferation processes, as well as marker genes for neuronal differentiation (neurotrophin receptors, transin, tyrosine hydroxylase, and TGF-b) have been analyzed using pituitary and neuronal cell lines.

Signal transduction pathways regulated by growth factors and oncogenes. Biochemical and biological effects.

Group Leader: Juan Carlos Lacal

Our group is engaged in the characterisation of signal transduction pathways activated during regulation of cell proliferation induced by growth factors and cell transformation induced by oncogenes. Our major interest is dedicated to members of the Ras superfamily of

proteins, including both the Ras and the Rho branches. These two families of proteins belong to the Ras superfamily of monomeric GTPases, proteins with the ability to bind and hydrolyse GTP. Both Ras and Rho proteins are involved in the regulation of proliferation and differentiation processes. Special attention is also dedicated to the regulation of phospholipid metabolism.

Our studies have demonstrated the relevance of the activation of a phosphatidylcholine-specific phospholipase D (PC-PLD) in the induction of cell proliferation by both growth factors and oncogenes. We have also demonstrated the transforming potential of Rho proteins, although this activity is less potent to that of Ras proteins. In addition, oncogenic Rho proteins induce apoptosis under conditions of serum depletion, a process that is not observed in the *ras*-transformed cells. We have further identified the specific activated pathways for the transforming and apoptotic activity of Rho proteins. While PC-PLD is activated in both *ras*- and *rho*-transformed cells, only sphingomyelinase is found activated in the *rho*-transformed cells under conditions of induction of apoptosis. Thus, we suggest that ceramides are the messenger involved, as a progression factor, in the induction of apoptosis. Thus, generation of specific phospholipid-derived metabolites, such as PA, *P*Cho or ceramides, may be critical for the regulation of the biological responses induced by growth factors and oncogenes.

Finally, we are using the knowledge of the activation of specific signalling pathways after transformation by different oncogenes to design strategies to specifically interfere with these pathways in a search for the development of new anti-tumour drugs.

Regulation of the β-amyloid precursor gene expression.

Group Leader: Angel Pascual

β-amyloid, the major constituent of Alzheimer´s plaques is derived from an amyloid precursor protein (APP) which is expressed in several different isoforms spliced from a unique gene that has been mapped to human chromosome 21. Overexpression of APP, as well as a specific increase in mRNA encoding the Kunitz protease inhibitor containing isoforms (770 and 751-mRNA), might contribute to the pathogenesis of this pathology.

We have measured APP-mRNA in four different neural cell lines (PC12, N2A, SH-SY5Y and C6) after different treatments with several hormones and growth factors. Retinoic acid as well as vitamin D3 induce a positive effect on APP gene expression and increase APP-mRNA levels in C6 and SH-SY5Y cells. By contrast, thyroid hormone inhibits these levels in N2A cells when its receptor is overexpressed. In PC12 cells NGF and other growth factors such as b-FGF or EGF induce an increase of APP-mRNA through a ras-dependent mechanism. A similar effect is also observed in the SH-SY5Y cells.

Patterns of the mRNA isoforms have been studied by PCR. Cells of neuronal origin clearly express the three most prevalent isoforms (APP-770, APP-751 and APP-695), whereas the 695 form practically disappears in cells of glial origin. In addition, the existence of two new spliced mRNA isoforms is now under study in PC12 and N2A cells.