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Department of Molecular and Cellular Biology of Cancer

Role of E cadherin and P cadherin in tumor progression. Regulation of their expression and function

Group leader: Amparo Cano

Our studies on the implication of E cadherin (E-CD) and P cadherin (P-CD) in tumor progression are based in the experimental model of mouse skin carcinogenesis where we are specifically interested in the molecular and cellular mechanims 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 E-CD. The analysis of the E- and P-CD promoters have shown that the basal activity of both promoters are mediated trhough the 5' proximal GC-rich and CCAAT regions which are recognized by common factors in both promoters (Sp1 and CP1, respectively) and by additional factors in the E-CD promoter (AP2 and CP2, C/EBP). Furthermore, the E-CD promoter is negatively modulated by the palindromic E-pal element and an Ets-binding site, with a predominant role of the E-pal element in E-CD deficient cells. The basal activity of the P-CD promoter, on the other hand, is modulated by an AP1-binding site present in an enhancer located in the first intron of the gene. The studies on the E-CD/catenin complexes present in cell lines of different stages of tumor progression have shown the involvement of the complexes containing plakoglobin in the maintenance of stable cell-cell contacts and a partial tumor-suppressor role of the E-CD/plakoglobin complexes. The organization and functional activity of the E-CD/catenin complexes is negatively influenced by oncogenic H-ras in this system. On the other hand, our studies on the invasive and metastatic behavior of E-CD (+) and E-CD (-) keratinocyte cell lines strongly support that the anti-invasive role of E-CD in this system is mediated trhough the downregulation of the metalloproteinase MMP-9 .

Signal Transduction Pathways Regulated by Growth Factors and Oncogenes

Group Leader: Juan Carlos Lacal

Our group is engaged in the characterization of signal transduction pathways involved in the regulation of cell proliferation and apoptosis triggered by growth factors and oncogenes. While growth factors define the physiological conditions for the regulation of these events, the involvement of activated oncogenes define their pathologial state. Our major interest is dedicated to different members of the Ras superfamily of GTPases, including both the Ras and the Rho branches. Special attention is dedicated to two specific areas :

1) the role of the regulation of the metabolism of phospholipids

2) the involvement of Rho proteins in the regulation of transcriptional events

Our studies have demonstrated the relevance of the activation of a phosphatidylcholine-specific phospholipase D (PC-PLD) in the induction of cell proliferation by growth factors and oncogenes. We have also demonstrated the transforming potential of Rho proteins as well as their ability to trigger an apoptotic response under conditions of stress or cytokines treatment.

Biological responses induced by the DNA damaging agents: cisplatin and UV light.

Group Leader: Rosario Perona

The main objective of our group is the study of the mechanism of apoptosis induction by the DNA damaging agents UV light and cisplatin. We have found that jun kinase (JNK) is activated by both agents and the timing of activation of this enzyme is directly correlated with apoptosis induced by both mechanisms. Moreover, positive modulation of JNK activity by using tyrosine phosphatase inhibitors increases the apoptotic response to the platinum compounds. We have also found that inactive platinum compounds fail to induce persistent JNK activation because they very efficiently activate the synthesis of MKP-1 while the active compounds do not. On the other hand cisplatin activates JNK by the MEKK1-SEK1 cascade. Inhibition of these kinases by transfection of dominant negative mutantas against them, protects cells from apoptosis induced by cisplatin. We are studying the role of the activating and inactivating cascade of JNK in cell lines and tumours resistant to cisplatin. Finally we have generated a library of human genetic supressor elements and use it to isolate cell lines resistant to cisplatin. These sequences will be used to obtain bone marrow cells resistant to the drug and to study the behaviour of the cells in animals in response to intensive therapies with cisplatin.

Tumoral progression patterns in neurogenic neoplasms

Group Leader: Angel Pestaña

We are investigating the molecular mechanisms responsible for the malignant progression of neurogenic neoplasms, and evaluating the predictive value of the genomic anomalies characterizing each histological subtype of neoplasm.

Our previous data demonstrated that astrocytic and oligodendroglial tumors display different patterns of formation, and we are now analyzing the involvement of *MTS1, MTS2, PTEN/MMAC1* and *DMBT1* suppressor genes in neoplastic progression of both glioma subtypes.

In meningiomas we are mainly analyzing sporadic forms, which usually result from inactivation (deletion and mutation) of the *NF2* gene (located at 22q12). In atypical and anaplastic samples we have shown the accumulation of secondary anomalies : deletions at 1p and 14q.

We had previously found a high incidence of 1p deletions in oligodendrogliomas and neuroblastomas and, thus, we are now performing a high resolution deletion mapping analysis of this genomic region. Using microsatellite markers to analize 30 loci spanning the short arm of chromosome 1, we intend to delimit the loci involved in those neoplasms, mainly taking into consideration that previous cytogenetic and molecular data suggest that up to four tumor suppressor genes might be located on 1p. Our study will provide evidence on the involvement of distinct 1p subregions (that is distinct tumor supressor genes) in every neurogenic neoplasm: meningioma, oligodendroglioma, neuroblastoma.

Malignant progression in mouse skin carcinogenesis

Group leader: Miguel Quintanilla

Our laboratory is studying cellular and molecular events associated with malignant progression in mouse epidermal carcinogenesis. Our main achievement have been the demonstration of a role of TGF-β_1 as a modulator of the epithelial phenotype and the invasive and metastatic abilities of squamous carcinoma cells. On the other hand, we have completed the biochemical and molecular characterization of PA2.26 antigen, a sialylated glycoprotein of the cell surface induced in tumor and stromal cells during carcinogenesis, and, apparently, involved in cell migration. We have also continued our studies about the involvement of the cell-cell adhesion molecule E-cadherin in invasion and metastasis, and that of adenoviral E1A gene as a tumor suppressor and its implication for gene therapy, in collaboration with other groups.

Cellular communication in normal and tumor cells

Group leader: Antonio Villalobo

The scientific interests of our group are the study of different mechanisms involved in intercellular communication in normal cells and their alterations in tumor cells. Among the projects in progress are: i) Adenylylation of plasma membrane proteins in normal and tumor hepatic cells, ii) Calcium signalling and regulation of the epidermal growth factor receptor by the calmodulin/ phosphocalmodulin system, iii) Regulation of cell proliferation by nitric oxide, iv) Role of gap junction channels in cell proliferation, and v) Antimitogenic effects of β-galactoside-specific lectins.

Department of Biochemistry and Genetics of Yeasts

Molecular analysis of ionic transporters in yeast.

Group Leader: Pilar Eraso

The molecular mechanism of glucose-induced activation of H^+-ATPase is probably mediated by phosphorylation of the enzyme. The physiological role of a Ca^{2+}/Calmodulin-dependent protein kinase (CaM-kinase) was investigated by using specific calmodulin antagonists *in vivo* and *in vitro*. The results suggest a calmodulin-mediated regulation of the H^+-ATPase independent of glucose-induced activation. To conclusively unravel the role of a this kinase, deletion mutants of the genes encoding CaM-kinases (CMK1, CMK2, RCK1 and RCK2) are being constructed.

Human CFTR protein and the yeast YCF1 protein belong to the ABC transporters family. Several cystic fibrosis-associated alleles of the CFTR were introduced into YCF1. The ycf1 mutants have been analyzed at the phenotypic and biochemical level showing defects analogous to those seen in the CFTR. These studies indicate that YCF1 protein provides a good model system for the study of the structure-function relationship of CFTR.

Relationships between glycolytic flux and sygar effects in yeast

Group Leader: Carlos Gancedo

We have continued our study of mutations that suppress the toxic effects of sugars upon glycolytic mutants. We have identified the mutations in the gene *DGT1-1* and *BPC1-1* (alleles of *MTH1*).

We have characterized the hexokinases from *Schizosaccharomyces pombe* and *Yarrowia lipolytica* and cloned their coding genes. We have also cloned the *PYC1* gene from *Pichia pastoris* and have isolated a suppressor of the pyc1 phenotype.

Our laboratory has continued its participation in the Eurofan project for functional analysis of the yeast genome.

Regulation of the expression of the *FBP1* gene and catabolite repression in yeast

Group leader: Juana M. Gancedo

Research topics:
Characterization of elements whcich regulate *FBP1* transcription
Role of cAMP in catabolite repression
Galactose *versus* glucose signalling
Control of glycolytic flux

A functional analysis of the upstream acivating sequences in the *FBP1* promoter has been carried out. We are investigating the mode of action of an upstream repressing sequence which is able in certain circumstances to act as activator of transcription. cAMP has been shown to repress to different degrees the synthesis of a variety of enzymes subject to catabolite repression. We have found that glucose signalling in yeast is only partially mimicked by galactose. Glycolysis in yeast operates near its maximal capacity and appears resistant to attempts to increase it.

Turnover of plasma membrane proteins in yeast. Mechanisms of endocytosis

Group Leader: Rosario Lagunas

Sugar transporters in yeast cells are degraded in the vacuole after internalization by endocytosis. The factors that govern endocytosis and degradation of these proteins are being investigated. Using the maltose transporter as experimental modelúwe have shown that two enzymes, at least, of the ubiquitin pathway, an ubiquitin-ligase and an ubiquitin-hydrolase, are required for internalization of these proteins. In addition, we have shown that actin microfilaments are involved in this process whereas microtubules are not. Moderate concentrations of ethanol (2 to 6%, vol/vol) inhibit endocytosis of the transporters. The results show that this inhibition is due to alterations produced by ethanol in the organization of the plasma membrane. Apparently, endocytosis is particularly sensitive to these alterations as compared with other processes occurring at the plasma membrane.

Yeast Genes Functional Analysis

Group Leader: María Jesús Mazón

Six of the ORFs identified during the Yeast Genome Sequencing Project were disrupted and a basic phenotypic analysis was performed with the mutants. The results obtained allowed the identification of a gene, YGL142c, whose disruption is lethal and codes for a protein involved in the synthesis of the glycosylphosphatidylinositol (GPI) anchor. This gene is the structural and functional homolog of the human gene *PIG-B*.

We are studying the structure-function relationship of the CFTR protein using the yeast structural homolog protein, Ycf1p, as a model. To address the effect of PKA phosphorylation a glycine residue was substituted for the phosphorylatable Ser908. This mutation abolished the activity of the resultant protein although it was correctly localized to the vacuole. However the substitution of either aspartic or glutamic for Ser908 rendered a partially active Ycf1 mutant that was also correctly localized to the vacuole. We are also interested in the identification of sorting signals in Ycf1p that determine its transport to the vacuole as well as in the study of the secretory route used by this protein to reach the vacuole.

Molecular analysis of the plasma membrane H^+-ATPase from *Saccharomyces cerevisiae*.

Group Leader: Francisco Portillo

Glucose triggers transcriptional and post-transcriptional mechanisms wich increase the level and the activity of the plasma membrane H^+-ATPase in *Saccharomyces cerevisiae*. We have investigated the post-transcriptional activation by glucose of the enzyme and have found that Rsp5, an ubiquitin-protein ligase enzyme, Ubc4, an ubiquitin conjugating enzyme, and the 26S proteasome complex are implicated in this activation. These results suggest that activation of ATPase by glucose requires the ubiquitin-proteasome proteolytic pathway. This conclusion is supported by the fact that overexpression of the ubiquitin-specific protease Ubp2, wich cleaves ubiquitin from its branched conjugates, inhibits this activation. We propose that glucose triggers degradation of an inhibitory protein resulting in the activation of the enzyme.

Department of Molecular Endocrinology

Expression patterns of genes regulated by thyroid hormone in the developing brain

Group Leader: Juan Bernal

The goal of our work is to identify and characterize brain genes under control of thyroid hormone. Among the most important and fundamental actions of thyroid hormones are those exerted on brain development and fuction. To gain insight into the molecular basis of thyroid hormone action in brain development, we started about ten years ago a systematic work aimed at the identification of thyroid hormone regulated brain genes. Among the 20 or so identified genes, we report here our work on RC3, SE6C and tubulin. RC3 encodes a neuron-specific, kinase C substrate that binds calmodulin in the absence of calcium and is involved in

synaptic plasticity, and dendritic spine formation and remodeling, memory, etc. The protein is extremely conserved across species. Thyroid hormone controls transcription of the gene in selected populations of cells in vivo and in neuronal cells in culture. Control of hyroid hormone is exerted both during development and in adult animals. We have identified a thyroid hormone responsive element in the first intron of the human gene. SE6C is a 4 kb mRNA expressed almost exclusively in the caudate nucleus. Thyroid hormone is essential for normal expression of this gene. We have recently isolated the full-length cDNA, which encodes a 345 aminoacids, novel member of the Ras family of GTP binding proteins. We are presently expressing the protein in order to produce specific antibodies and to perform functional analysis. The alfa isoform fo tubulin is also under thyroid hormone control. Thyroid hormone downregulates the gene through an action at the transcriptional level. We have recently identified a T3 responsive element consisting of a direct repeat separated by three base pairs. This arrangement is typical of the vitamin D3 responsive elements. However, the tubulin T3RE is able to bind the RXR-T3R heterodimer and transduces thyroid hormone responses. Contrary to the usual position in the promoter region, this T3RE is located in the third exon of the gene. Results are under way to determine precisely the regional characteristics of T3 regulation of the gene by in situ hybridization and the role of the promoter sequences.

In addition to our studies on thyroid hormone-regulated genes, we have also examined the expression of the 5' type 2 deiodinase, the enzyme that converts T4 to T3 in the brain. We found that the mRNA is present in high amounts in the specialized glial cells named tanycytes, located in the tird ventricle, which suggests that these cells are involved in the regulation of T3 concentration in the CSF and in the median eminence. On the other hand, in other parts of the brain D2 is located in the astrocytes, suggesting that T4 reaches these cells after crossing the blood-brain barrier, is converted to T3 in the astrocytes, and is then released for neuronal use.

Thyroid hormones at different stages of development

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

Research topics: a) Thyroid hormones in early human embryonic compartments; b) Thyroid hormone status in premature infants; c) Alterations in fetal thyroid status, including cerebral T concentrations, in fetuses from mothers with the "low T3 syndrome" due to a maternal non-thyoidal illness (diabetes mellitus); d) Alterations of thyroid hormone transport, induced with a synthetic flavonoid, and consequences for the maternal and fetal thyroid hormone status; e) Effects of maternal treatment with TRH and glucocorticoids on fetal thyroid status in rats with and without nitrofen-induced pulmonary immaturity.

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

Research topics: a) Regulation of iodothyronine deiodinases by T4 and T3 in different tissues of the aduilt hypo, eu- and hyper-thyroid rat; b) Thyroid hormone status in thyroid hormone receptor β null mutant mice.

Group leader for iodine deficiency studies: Dr. F. Escobar del Rey Research topics: a) Situation of iodine nutrition in different areas of Spain; b) Iodine nutrition in schoolchildren of the Community of Madrid; c) Iodine deficiency in pregnant and lactating women; d) Experimental iodine deficiency disorders: treshold for brain damage; e) Brain damage in progeny of severely iodine deficient rats: delay in fetal brain maturation and seizure susceptibility.

Biology of erbA and related nuclear hormone receptors

Group leader: Alberto Muñoz

Research topics: a) Effects of erbA genes and thyroid hormone on glial and neuronal cells; b) Study of the nuclear receptors-AP-1 antagonism; c) Search for and study of brain genes regulated by thyroid hormone; d) Study of the antiangiogenic activity of nuclear receptors and thrombospondin-1

Our group is focussed on the study of the biology of the erbA genes encoding thyroid hormone (T3) receptors, and of related nuclear receptors such as those of glucocorticoids (GR), retinoic acid (RAR), and vitamin D3 (VitD3R).

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 neuronal (P) origin. On the other hand, we are searching for new genes regulated by c-erbA and its ligand T3 by differential display PCR.

In addition, we have studied the regulation by T3 of the prostaglandin (PG) D2 synthetase and tenascin-C genes. 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. In the case of tenascin-C, a complex pattern of regulation has been found in the developing rat brain.

Recently, we have defined a novel mechanism for the anti-AP-1 activity of liganded nuclear hormone receptors. Hormone-bound receptors inhibit the JNK signalling pathway activated by either UV radiation or tumor necrosis factor-alpha. By blocking c-Jun N-terminal phosphorylation glucocorticoids, retinoids, thyroid hormone, and vitamin D3 may exert their antiproliferative, antiinflammatory and immunosupressive activities.

Finally, we are initiating the analysis of the antiangiogenic action of thrombospondin-1 and of glucocorticoids and retinoids on cultured human and bovine microvascular endothelial cells.

Regulation of brown adipocytes proliferation and differentiation. Regulation of deiodinases by thyroid hormones.

Group leader : María Jesús Obregón

Research topics:

Our main interest is the study of the activation of brown adipose tissue, a highly thermogenic tissue. For this, we use primary cultures of brown adipocytes, and the pathways that lead to the activation of proliferation and differentiation are examined.

Proliferation of brown adipocytes in primary culture. Norepinephrine (NE) is able to potentiate the mitogenic effect of serum, growth factors, vasopresin and other mitogens as araquidonic acid and endotelin-1. The effects of NE seem to be mediated via PKC. The cells obtained are true adipocytes as express the differentiation marker: UCP.

UCP gene expression is regulated in brown adipocytes by NE, thyroid hormones, retinoic acid, insulin and glucocorticoids. Besides UCP mRNA expression, the regulation of UCP promoter is also studied.

Regulation of type II 5'Deiodinase (5'D-II) and 5 Desiodinase (5D) activity and mRNA in cultured brown adipocytes. 5'D-II is adrenergically stimulated by NE and T3 is required for such stimulation; on the other hand growth factors, thyroid hormones and NE induce 5D

activity and mRNA.

Regulation of Deiodinase activities in fetal rat tissues. Those studies are carried out mainly in situations of iodine deficiency or thyroid hormone substitutive therapy.

Regulation of transcription: Analisis of molecular events involved in the hormonal control and in the tissue-specific gene expression.

Group Leader: Pilar Santisteban

The goals of our work is to study the regulation of tissue-specific gene transcription. For this purpose, we have chosen the follicular thyroid cells as a model for study. Differentiation of these cells is controlled by a group of specific transcription factors (TTF-1, TTF-2 and Pax-8), which belong to the *homeo-box*, *fork-head* and *paired-box* families, control the expression of two thyroid-specific genes thyroglobulin and thyroperoxidase. We have identified the TTF-2 binding site as a hormone response element being the factor TTF-2 transcriptionally regulated by TSH/cAMP and insulin/IGF-I. However, the function of TTF-2 is dependent of the close position of an ubiquitous factor that we have identified as a member of the family of constitutive factors CTF/NF-1. TTF-1 and CTF/NF-1 interact and cooperate in the regulation of thyroproxidase gene. The regulation of TTF-1 is much more complex, involving phosphorylation as the main mechanism controlling its activity. We have also studying the role of PKA, PKC and MAPK in TTF-1 activation/inactivation process. Since the knock-out mice for the above transcription factors, obtained in different laboratories, give a phenotype with thyroid alterations, we have started a new project studying the role of this transcription factors in human pathologies such as hypothyroidism, agenesias and ectopias. TTF-1 is also expressed in lung and is the main regulator together with HNF-3 of the surfactant protein gene expression. The function of these transcription factors in lung is another project of our interest.

The above study has been made in thyroid epithelia cells. Another thyroid cells type, are the parafollicular cells that are the responsible of the medullary thyroid carcinoma. In this cells we have show that the p53 locus is severely rearranged and the introduction of p53 induces cell-cycle arrest.

Another important model for study hormonal regulation of gene expression is the regulation of malic enzyme promoter by insulin and thyroid hormones. We have identified a response element for insulin. To this element different transcription factors are able to bind : Sp-1, Sp-3 and erg-1/NFG-IA. Sp-1 is a functional factors able to cooperate with members of the nuclear receptor family and erg-1 is induced by insulin. Finally, we have identified Xenobiotics Response Elements in the malic enzyme gene promoter that response positively to the induction of dioxin receptor in liver.

Department of Enzymology and Molecular Pathology

Mechanisms of control of carbohydrate metabolism

Group leader: Juan José Aragón

Our research is focused on the molecular and physiological bases of the function of enzymes involved in the regulation of carbohydrate metabolism. Structure-function relationship studies are carried out in eukaryotic phosphofructokinases, namely the C-isozyme from ascites tumor cells, the M-isozyme from human muscle and the non-allosteric isozyme from *Dictyostelium discoideum* by expression of their cDNA in yeast and specific manipulations of their sequences. Other studies are related to *i)* the interaction of tubulin with phosphofructokinase from rabbit muscle and *D. discoideum*, the latter of which has been found to play a role in microtubule dynamics; *ii)* the non-invasive evaluation of intestinal lactase activity *in vivo* by using 2-, 3- and 4-galactosyl-xyloses, with potential application to the diagnosis of the deficiency of this enzyme in humans, and iii) the glutamine and glucose metabolism in hybridoma cells

Characterization of Novel Human Genes And its Possible Involvement in Inherited Diseases.

Group Leaders: Antonio Coloma and Jesús Cruces

The activities of our group are focused in the field of Human Molecular Genetics, and our main interest is the characterization of novel human genes. Current studies deal with the analysis of the structure and function of:

- The gene *NRGN,* encoding neurogranin a neuronal protein with specific expression in the brain.
- The gene *POMT1,* encoding an O-mannosyl transferase which might be involved in early muscle system formation
- The genes contained in the deleted region of the Williams- Beuren Syndrome, a complex alteration of development affecting the nervous system and cardiovascular apparatus, as well as connective tissues.

Also, we are studying the organization of centromeric sequences from human chromosome 7 and their putative contribution to the function or/and structure of the centromere.

Control of expression and modulation of enzymatic activities in yeast and developing systems

Group Leader: Claudio Fernández de Heredia

Our main interest during the last two years has been centered in: a) study of the mutual interactions in the metabolism of mono- and disaccharides in *Saccharomyces cerevisiae* and b) characterization of enzymes acting on 2',3' cyclic nucleotides. In relation with the first subject, we have found that hexoses (galactose, mannose, fructose and their non-metabolizable structural analogs) interfere with the metabolism of maltose in *Saccharomyces cerevisiae* at two levels: transport of the

disaccharide inside the cell and phosphorylation of the glucose generate by intracellular hydrolysis of maltose. In relation with the second subject, we have partially characterized in *Fusarium culmorum* two phosphodiesterases which hydrolyze respectively the 2' or 3' ester bond of 2',3' cyclic nucleotides to give the corresponding nucleosides monophosphates.

Mechanism of Methotrexate resistance in murine L.L.A cell lines

Group leader: Pilar Llorente

Adquired resistance to MTX newer folate analogs under development will remain a major limitation to their effective clinical utility. One of the determinants of cytotoxicity shared by these drugs is the process of intracellular polyglutamylation synthesis mediated by the enzyme folylpolyglutamate synthetase (FPGS).

We have undertaken a study of the possible mechanism(s) of cellular resistance to MTX focusing in the properties of the FPGS activity in murine acute lymphoblastic leukemic cells , L5178Y, with different sensitivity to MTX and checking the FPGS as a new drug target in the parental and MTX resistant cells, evaluating previous isolation, characterization and synthesis of folate and MTX hydrolisis metabolites as FPGS inhibitors.

Metabolism and fuction of dinucleoside polyphosphates

Group Leaders: Antonio Sillero and Mª Antonia Günther

Nucleotide metabolism in brain. The IMP/GMP specific 5'-nucleotidase (EC 3.1.3.5) has been purified to homogeneity from the cytosol of rat brain. The enzyme is activated by dinucleoside polyphosphates and by polyphosphates. These compounds are also positive efectors of the phosphotransferase activity of the enzyme.

Metabolism of 2´.3´-dideoxynucleotides. 2',3´-Dideoxynucleoside triphosphates (ddNTP) and di-2`,3'-dideoxynucleoside tetraphosphates (ddNp4ddN) behave differently to the corresponding NTP and Np4N counterparts as substrates of firefly luciferase, dinucleoside tetraphosphatase and phosphodiesterase. The possibility of using ddNp4ddN or Np4ddN as a source of the active retroviral agent ddNTP, for example in HIV infection, is outlined.

Mechanism of reaction of luciferase. The formation of dehydroluciferin (L) from luciferin (LH_2) in the reaction catalyzed by firefly luciferase (EC 1.13.12.7) has been studied. The E•LH_2-AMP complex may follow two different pathways: towards production of light and towards the synthesis of the E•L-AMP complex. This last step has an inhibitory effect on light emission as molecules of enzyme are trapped in a light unproductive complex. The effect of CoA and nucleoside 5´-triphosphates (NTPs) on light emission are quantitatively different. CoA combines with the L moiety of the E•L-AMP complex, yielding L-CoA, promoting liberation of free luciferase and increasing light yield. NTP reacts with the AMP moiety of the same complex generating adenosine(5´)tetraphospho(5´)nucleoside (Ap_4N) and, probably, the E•L complex, and scarcely increasing light production.

Synthesis of dinucleoside polyphosphates. Acyl CoA synthetase from *Pseudomonas* catalyses the synthesis of adenosine 5'-polyphosphates and dinucleoside polyphosphates.

Department of Structure and Function of Biomolecules

COT kinase regulates T lymphocyte activation

Group Leader: Susana Alemany

COT kinase has been related as a MAP kinase kinase kinase that play a role in T lymphomas development. We have recently shown that COT kinase regulates the transcription of IL-2 and TNF-α genes, by activating the AP-1 and NFAT response elements. This increase in the expression of these genes renders to an increase in the extracellular levls of these cytokines and can explain the role of COT kinase in T lymphocyte activation.

Proteasome modulation and autoimmune diseases

Group Leader: José G. Castaño

We are interested in the study of modulation of proteasome activity. We have found that several proteasome subunits are phosphorylated by casein kinase I and c-src, having identified the phosphorylated subunits by anti-subunit specific antibodies and demonstrated that the phosphorylation occurs both, in vivo and in vitro. We are also interested in the proteasome function in CNS proteolysis and have continued to characterise its involvement on the degradation of myelin basic protein. Another line of research with a protease from E. coli (EClpP), related in specificity to the proteasome, has allowed us the characterisation of specific antibodies against this E. coli protease in patients with primary biliary cirrhosis and we continued working with the study of the autoimmune response against the eukaryotic proteasome.

Magnetic Resonance in Medicine and Biology

Group Leader: Sebastián Cerdán

Our laboratory is developing novel Magnetic Resonance (MR) strategies to (i) understand the biological basis of contrast in MR images and its relationships with water metabolism in different cells and tissues (ii) asses quantitatively the metabolic interactions between neurons and glial cells under physiological or pothological situations, (iii) investigate the regulation of intra- and extracellular pH in tumors, (iv) develop automatic diagnostic MR procedures based in the use of artificial intelligence and (v) implement a novel series of contrast agents for the non invasive MR imaging of intra- and extracellular pH in non transparent samples.

Study of the function of casein kinase 1 from *Dictyostelium discoideum*

Group leader: Margarita Fernández

Cloning and expression of a casein kinase 1 from *Dictyostelium discoideum*..
Our main goal is to study the function of this kinase in Dictyostelium cells. We have

cloned and sequenced a casein kinase 1 from a *dictyostelium* cDNA library, this gene shows a high homology with other members of the casein kinase 1 family, in particular with those isoforms that present nuclear localization.We have generated antibodies against a truncated version of the protein produced in bacteria. Northern and western analysis indicate that this gene is expressed in vegetative as well as differenciated *Dictyostelium* cells, without significative changes in its expression through the *Dictyostelium* vital stages, suggesting that it could be an important protein for the life of this organism.

Immunofluoresce studies performed with the affinity purified antibody, suggest that the localization of the protein changes during the cell cycle and that migth be involved in mitosis.

In order to investigate the function of the protein, we have make a construct in which the coding sequence has been interrupted with a gene that codify for a blasticidine resistance. We are currently analyzing, the transformants which growth in the presence of the drug.

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

We had study the activation of MAP, Raf kinase and protein kinase C in response to different stimuli such as PDBu or IL2 in rat lymphoblasts. In the primary cultures these enzymes are not activated in response to IL2 but they are activated in response to the phorbol ester, which is a mitogen for these cells. The activation of the MAP kinase is the rate limiting step in the activation process, this activation is transient due to the presence of different protein phosphatases involved in its dephosphorylation and inactivation. We have been studying the dephosphorylation process and we have concluded that there are inducible as well as constitutive protein phosphatases involved in the process.

Regulation of the hepatic metabolism of methionine: structure/function relationships

Group Leader: María de los Angeles Pajares

The main interest of the laboratory is the study of the regulation of the liver methionine metabolism, as well as the structure/function relationships of several of the enzymes involved in it. For these purposes we prepare mutants of residues that seem to be important in the structure, or for the function of the enzymes of our interest. These mutants are used for structural studies after its characterization. Moreover, experimental models are prepared in order to study the influence of several metabolites in the function of the methionine cycle and its regulation.

Mitogenic Activities in the Peritoneal Effluent of Patients Treated with C.A.P.D.

Group Leader: Francisco Vara

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 Regulation of Gene Expression

Regulation of gene expression by nuclear receptors in pituitary and neuronal cells: interaction with other transcription factors and with mitogenic and neurotrophic factors.

Group Leader: Ana Aranda

The aim of our work is to analyze the molecular mechanisms by which the nuclear receptors cooperate with membrane receptors with tyrosine kinase activity to regulate the expression of genes involved in proliferation and differentiation in pituitary and neuronal cells. The interaction among different ligands of nuclear receptors with other transcription factors and growth factors to regulate transcription of the growth hormone, prolactin and the retinoid receptor RARb2 genes has been analyzed analyzed in pituitary cells. In PC12 and neuroblastoma cells, in which nuclear receptor ligands act in conjunction with neurotrophins to elicit differentiation and antimitogenic effects, the expression of the RARb2 gene, of components of the AP-1 complex, or of genes directly involved in cell cycle control (the c-*myc* oncogene, the CKI p27, cyclins and CDKs), has been analyzed. Finally, and since the human immunodeficiency HIV-1 virus causes relevant neurological effects, the role of the functional interaction between nuclear receptors and neurotrophic factors to activate HIV-1 gene expression in neuronal cells has also been analyzed.

Control Regulation Of The Paramyosin/Miniparamyosin And Troponin T Genes In *Drosophila*

Group Leader: Margarita Cervera

Mucle differentiation is performed through the combination of multiple molecular mechanisms, which includes differential expression of muscle protein genes and specific isoform generation. Transcription regulation of tissue-specific genes is regulated by enhancers and cis-acting regulatory regions o modules which interact with unique combinations of transcription factors in each cell type. Muscle gene regulation is different from houskeeping gene regulation, and the *Drosophila* paramyosin/ miniparamyosin gene, as well as the troponin T gene, are good model systems in order to clarify such regulatory mechanisms in *Drosophila* and in mammals, too. In addition, the analysis of the mPM promoter, which controls the expression of one of the few adult-specific muscle proteins, probably will allow us the identification and characterization of novel muscle transcription factors acting specifically in the adult stages. TnT and paramyosin are two contractile proteins localized in the same structure, the sarcomere, although in different filaments, and show the same temporospatial expression pattern. A comparison between the features of the regulatory elements acting in these two genes will permit us the searching of common elements wich could be involved in the coordination of the expression of the components of a defined structure.

Regulation of the expression of mitochondrial genes

Group Leader: Carmen García-Vallejo

Our interest continues to be focused on the regulation of mitochondrial gene expression. At the moment, we are attempting the purification of the mitochondrial poly A polymerase which has not been identified in any system so far and, therefore, remains as a piece of the transcriptional machinery still to be characterized. The comparative initial characterization of an enzymatic preparation from the cytoplasm and another one obtained from purified mitochondria suggests that the poly A polymerases from the two compartments are different entities. Our aim is to clone and characterize the mitochondrial poly A polymerase.

To gain insight into the mechanisms that regulate mitochondrial gene expression, we are studying the expression patterns and steady-state levels of mtTFA, the only transcription factor described up to date in mammals, and two nuclear transcription factors, NRF-1 and NRF-2, on which the mtTFA expression is dependent, in the context of the mitochondrial gene expression of different tissues. We expect that the integrated analysis of all these data will allow us to better understand the role of the different transcription factors in the expression of the mitochondrial genes.

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 characterizing transcription factors involved in mitochondrial differentiation, and modifying *in vivo* mitochondrial DNA replication. 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

During the period covered by this report, our group has continued working on the different sublines started in the past, namely, a) on the purification and properties of Troponin proteins in *Drosophila* and other arthropods, b) on the epigenetic modulation of *Drosophila* and *Artemia* development and aging by alterations in environmental conditions including exposure to abnormal graviational forces like those existing in Space and c) applications of Microwaves to Biological Technology. While these sublines will be actively pursued during the next research biennium, our previous line linking mitochondrial DNA to phylogenetic studies in *Artemia*. has being finished with the overall characterization in te World of the

different species in this *genus*.

Regulation of expression of the β-amyloid protein gene

Group Leader: Angel Pascual

β-amyloid protein is the major component of the senile plaques observed in the brains of humans with Alzheimer's disease. This 39-43 aminoacids peptide is a cleavage product of the different isoforms of the amyloid precursor protein APP, and an over-expression of this protein leads to a higher formation of the b-amyloid protein and is associated with neurotoxicity, thus contributing to the development of the pathology. Ligands of receptors with tyrosine kinase activity, as well as ligands of the nuclear receptor superfamily appear to regulate APP gene expression through yet unknown mechanisms. Using cultured cells of neural origin, we will analyze the molecular mechanisms by which these ligands regulate APP gene expression. Because most of these effects migth be directly mediated throughout promoter elements, we wiil analyze the sequences involved in the regulation, as well as the contribution of the two AP-1 binding sites contained in the regulatory región of the gene.

Regulation of gene expression by thyroid hormone during development.

Group Leader: Ana Pérez-Castillo

Effect of congenital hypothyroidism on the morphology and function of brain mitochondria. We have recently demonstrated that thyroid hormone is an important regulator of mitochondrial gene expression during brain development. To gain further insights into the consequences of this regulation, we have performed functional and structural analysis of brain mitochondria from control and hypothyroid neonatal rats. Flow cytometry analysis showed a significant decrease in the mitochondrial transmembrane potential in hypothyroid animals as compared to controls, which was reversed after 48 hours, but not after 2 hours, of thyroid hormone administration, suggesting that the functional alterations observed are the consequence of changes in mitochondrial gene expression. Electron microscope analysis of cerebral cortex, striatum and hippocampus revealed marked differences in the morphology of neuronal mitochondria from control and hypothyroid neonates. Hypothyroid mitochondria presented a decrease in the area of the inner membrane plus cristae in all the areas studied, except for the hippocampal CA1 neurons and non-neuronal cell types. In addition, transfection experiments showed a direct regulation of mtTFA promoter activity by thyroid hormone, suggesting a possible nuclear mechanism for T3 action upon the mitochondrial genome. These observations provide a basis for the known biochemical action of thyroid hormone on brain development.Regulation by thyroid hormone of the C/EBP alpha and beta genes during liver development. The effect of thyroid hormone and retinoic acid on the expression of CCAAT/enhancer binding proteins (C/EBPs) α and ß was investigated in rat liver during development. Congenital hypothyroidism caused a significant decrease in both C/EBPα and C/EBPß gene expression at early stages of postnatal development. C/EBPα and ß protein levels were also markedly diminished in hypothyroid neonates and the kinetics of induction of these proteins by thyroid hormone was faster than the one observed for the corresponding transcripts, suggesting a translational regulation of these genes. However, this regulation must be more complex since preliminary experiments using 1.2 kb of the promoter region of C/EBPα show a direct regulation by T3. Tyrosine phosphorylation of transmembrane ligands for ErbB receptors. Many cellular processes such as differentiation,

proliferation and cell death are mediated in part by signaling events involving members of the ErbB receptor tyrosine kinase family and their membrane-bound ligands the neuregulins. Since the structure of these neuregulins resembles those of membrane receptors we have studied whether these proteins could also act as receptors. We have found that the cytoplasmic domain of the transmembrane ligands NRG1 and NRG2 became phosphorylated on tyrosine residues after serum stimulation, which suggests that both neuregulins have receptor-like intrinsic signaling potential.

Transcriptional Regulation in Developmental Systems.

Group leader: Leandro Sastre.

Transcriptional regulation during the activation and early development of *Artemia franciscana* encysted embryos. *Artemia franciscana* cysts are obtained metabolically inactive in a cryptobiotic state. Under experimental conditions they can be activated and resume their metabolic activity and developmental program. Our group is interested in the elucidation of the mechanisms that produce the transcriptional arrest in the cysts and those implicated in its later activation. This problem is being approached through the study of the promoter region of some genes whose expression has been shown to be induced during this process. In particular, we are functionally characterizing the promoters of three actin genes and the two alternative promoters of the sarco/endoplasmic reticulum Ca-ATPase gene. We are also studying the transcription factors that binds to these promoters in cryptobiotic and developing embryos.

Structure and function of the Serum Response Factor (SRF) in *Artemia franciscana* and *Dictyostelium discoideum*. The SRF has been shown to be very conserved during evolution. In fact, its binding site, the Serum Response Element, is present in one of the *Artemia* actin promoters. However, while the SRF seems to participate in the regulation of cell proliferation in mammals, its has been shown to be required for the specification or differentiation of some cell types in *Drosophila*. This apparent paradox has motivated our interest in the study of this transcription factor in the crustacean *Artemia franciscana* and in a simpler model, the slime mold *Dyctiostelium discoideum*. cDNA clones coding for the SRF homologues have been isolated from both organisms and being studied. Gene interruption experiments have shown that spore maturation is dependent on SRF in D. discoideum.

Department of Cellular Signaling

Role of Akt kinase in neuron survival induced by neurotrophins and G protein coupled receptors

Group Leader: Antonio Cuadrado

The nervous system is subjected to apoptotic and survival signals which drive its embryonic development and estress response. Akt, a Ser/thr protein kinase, appears to be a key element in the transduction of these signals. Based on our experience on lipid second messengers and signal transduction we are opening a lane of research on the role of Akt in signalling by NGF receptors (TrkA), with Tyr kinase activity, and by muscarinic receptors, coupled to trimeric G proteins. We are also analysing the efector mechanisms of Akt related

to esphingomielin metabolism, and looking for relevant substrates for its antiapoptotic function. These studies are of interest in aspects of neuronal regeneration and neurodegenerative processes.

Hormonal Regulation of Metabolism

Group Leader: Juan Emilio Felíu

We are interested in the study of the modulation of hepatic glucose production by insulin, glucose and sulfonylureas in the Zucker obese (*fa/fa*) rat, a genetic model of obesity. Our results indicate that the adaptation of key glycolytic enzymes, as well as the cellular concentration of F,2,6-P2 , to hyperinsulinemia present in this animal contribute to the resistance of gluconeogenesis to short-term modulation by insulin, glucose and sulfonylureas. We are also involved in a clinical program of biochemical and molecular diagnosis of inherited metabolic diseases of carbohydrate metabolism .

Cytokine signal transduction mechanisms: Prolactin

Group Leader: Jorge Martín-Pérez

Prolactin (PRL) is a cytokine exercising multiple biological functions throughout a dimmer of a monomeric type I cytokine receptor. Therefore the prolactin receptor (PRLR) does not have any known enzymatic activity, however upon interaction with the hormone, the PRLR interacts and activates the Jak and Src family of tyrosine kinases that in turn induce tyrosine phosphorylation of cellular proteins including the receptor itself. By mutation of the rat PRLR long form we have been able to show that the association and activation of c-Src is independent of the Jak kinases and of the tyrosine phosphorylation of the receptor. We have evidences that phosphorylation of the PRLR is catalyzed by the Jak kinases.

To gain some understanding on the role of tyrosine phosphorylation of the receptor on the signal transduction mechanisms induce by PRL, we have produced a number of deletion and point-substitution (F/Y) mutants of the PRLR. Since we have observed that PRL induces both proliferation and differentiation of some hematopoietic cell precursors, we will use this model system to analyse the functionality of those mutants as well as the role of the Src family in the PRL signalling mechanisms.

Genetic approaches to the study of signalling pathways in mammalian cells in culture

Group Leader: Jaime Renart

Our group has two main research projects; one is the study of NMDA-type of glutamate receptors; the second is the characterization of the induction of apoptosis by PKC inhibition.

In the first project, we have expressed the NR1 and NR2A receptor subunits with the aid of recombinan vaccinia virus. We get with this system functional receptors that allow calcium influx dependent on receptor agonists and that induces cell death after 24 hr of treatmente witn NMDA. Antagonists of the NMDA receptor (DL-AP5, MK801) inhibit calcium influx and protect cells from death. Both subunits are N-glycosylated, but NR1 subunit is extremely sensitive to treatment with tunicamycin, being degraded in a short period of time. We are currently studying this degradation.

In the second project, we have found that inhibition of PKC in turn inhibits the stimulation of MAPK. This latter inhibition, however is not sufficient for apoptosis, as MAPK can be inhibited by other means (inhibiting MEK) without induction of apoptosis. In a cell line that overexpresses Bcl-2 and is resistant to apoptosis, we have found distinct effects of inhibitors of classic PKC (Gö6976) and all PKC (GF109203X): the former inhibits basal MAPK activity, but has no effect on the stimulation by phorbol esthers; GF109203X, however, inhibits both basal and stimulable activity. Given the isoforms present in N2A cells (alfa, epsilo and zeta), these results demonstrate that PKC epsilon has a specific effect in Bcl-2 overexpressing cells. We are currently characterizing the involvement of other signaling pathways (PI3K, JNK, p38) in this apoptotic system.

In relation with the second project, we are studying the effect of Geldanamycin in different cell lines. This antibiotic has antitumor activity and is an inhibitor of hsp90. It has been shown that GA treatment desestabilizes Raf-1. In N2A cells, GA induces differentiation; Raf-1 is degraded in 24 hr, and in this period MAPK activity raises (maximum at 5 hr) and then disappears after 24 hr; the same pattern is observed for Raf-1 protein. PC12 cells treated with GA are induced to enter apoptosis, but C2C12 cells differnetiate, like N2A.

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

Group Leader: Angeles Rodríguez-Peña

The role of thyroid hormone and thyroid hormone receptor (T3R) 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. The specific role of each T3R isoform (a and b) has been studied in different cell lines. Expression of T3R impairs proliferation. Interestingly, the b isoform is stronger than a, and such effects correlated with the increase in the cell cycle inhibitor p27/kip1.

The activation of two neuro-specific promoters by nuclear receptors have been studied: the myelin basic protein promoter is activated upon stimulation with thyroid hormone and 9-cis retinoic acid independently and through different elements. The neurotrophin receptor trkB promoters have characterised and the effect of thyroid hormone on trkB expression during brain development studied. Lack of thyroid hormone increases the trkB transcripts levels, by increasing the transcription rate. The T3-dependent repression is mediated through interaction an array of T3RE half sites located downstream of the transcription start.

Intracellular signals and proto-oncogenes involved in the development of the inner ear

Group Leader: Isabel Varela

One of the most appealing systems to study embryonic development both at the cellular and at the molecular level is the developing vertebrate inner ear. In the last few years the signalling networks responsible for the induction, growth and differentiation of vertebrate inner ear have started to be unravelled. We are interested in the molecular mechanisms by which these signals initiate and pattern the vertebrate inner ear. In this context, our laboratory

is interested in studying the biological activity and mechanism of action of lipid second messengers that are derived from glycosyl-phosphatidylinositol (GPI) and sphingolipids.

Recently, we found that IGF-I is a strong promoter of cell growth and morphogenesis for the developing inner ear. IGF-I modulates the hydrolysis of GPI leading to inositol-phosphoglycan (IPG) generation. The latter, can modulate the expression of c-*jun* and c-*fos* genes. Blockage of IPG generation abolishes IGF-I-induced cell proliferation and c-*jun* and c-*fos* expression. IGF-I and IPG are also involved in the modulation of cochleovestibular ganglia differentiation. We have developed the methodology to study the effects of overexpressing or blocking the action of proto-oncogenes and growth factors during chicken development by using retroviral vectors RCAS.

We are also investigating on the regulation of cell activation by sphingolipids. In particular, we are interested in the role of ceramides and ceramide phosphate in controlling cell proliferation and programmed cell death or apoptosis. Regulation of normal development involves a dynamic balance of the mechanisms regulating cell division, differentiation and death. We have investigated the signalling mechanisms involved in regulation of the balance between cell proliferation and apoptotic cell death in the otic vesicle. The sphingomyelin pathway signals apoptosis for nerve growth factor upon binding to p75 receptors. It is initiated by sphingomyelin hydrolysis to generate the second messenger ceramide. Nerve growth factor stimulates sphingomyelin hydrolysis and the concomitant ceramide release in organotypic cultures of otic vesicles. Both nerve growth factor and ceramide induce apoptotic responses to a different extent. Ceramide-induced apoptosis was suppressed by IGF-I and ceramide-1-phosphate protected the explants from apoptosis induced by serum withdrawal. Our results suggest that sphingomyelin-derived second messengers might be key modulators of programmed cell death during development.