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1. Department of Molecular and Cellular Biology of Cancer

Regulation of E-cadherin expression, E-cadherin/catenin adhesion complexes and β−catenin signaling during tumor progresion

Group Leader: Amparo Cano

Our studies are intended to understand the molecular mechanisms responsible for the downregulation of E-cadherin expression and function which occurs during tumor progression, and more specifically at tumor invasion in experimental systems (i.e. mouse skin carcinogenesis) and in human tumors. In addition, we are also interested in understanding the mechanisms involved in the modulation of β-catenin signaling during tumor progression. In relation with the mechanisms involved in the regulation of E-cadherin gene expression, our previous studies on the E-caherin promoter in different cell lines from the mouse skin carcinogenesis system indicated a major repressor role for the E-pal element (-90 to -70) as well as the modulatory role of a proximal CE region (-108 to -86) containing an Ets-binding site. Using the one-hybrid approach we have been able to identify three transcription factors interacting with the wild type version of the E-pal element. One of the factors identified correspond to Snail, a Zn-finger transcription factor of the Snail family previously showed to be involved in epithelial-mesenchymal transitions (EMTs) in embryonic development. We have now showed that Snail is a strong repressor of E-cadherin promotor activity, and its ectopic expression in different epithelial cell lines trigers EMT concomitantly to the loss of E-cadherin expression and the acquisition of invasive and tumorigenic properties. In addition, the analysis of endogenous Snail and E-cadherin expression in murine and human cell lines and tumors indicate a strong inverse correlation between the expression of both molecules, specifically at the invasive areas. These data indicate that Snail controls EMTs which occur at tumor invasion by repressing E-cadherin expression and may be considered as a marker of tumor invasion. At present we are characterizing the molecular mechanism of repression of the E-cadherin promoter activity by Snail, as well as characterizing the other two transcipriton factors identified by the one-hybrid approach.

With regard to the modulation of E-cadherin function our recent studies are focused on the effects of H-ras activation on the E-cadherin/catenin adhesion complexes and β-catenin signaling in mouse epidermal keratinocytes. Oncogenic activation of H-ras (V12H-ras) in Pam212 keratinocytes induced the destabilization of E-cadherin/catenin complexes from the cell-cell contacts and the cytoplasmic relocalization of β-catenin. These effects are concomitant to the loss of β-catenin/APC interaction and a significant decrease in P-Ser β-catenin, and lead to the metabolic stabilization of cytoplasmic β-catenin. Interstingtly, those effects are independent of the GSK-3β activity, involved in the phosphorylation of β-catenin and its cytoplasmic stability through the Wnt signaling pathway. However, the effects of V12H-ras on β-catenin relocalization and metabolic stabilization in Pam212 keratinocytes depend on the activity of PI3K, a H-ras effector. A specific β-catenin/PI3K complex has been detected in Pam212 keratinocytes, being significantly increased in Pam212 cells overexpressing V12H-ras. On the other hand, stable expression of a constitutively active form of PI3K (p110α-CAAX) in Pam212 cells is sufficient to inhibit the β-catenin/APC interaction and lead to the metabolic stabilization of β-catenin and its nuclear translocation. These results indicate that β-catenin signaling can be induced by

oncogenic activation of H-ras by mechanisms independent of the Wnt/GSK-3β pathway, or β-catenin mutation, and involving the participation of PI3K. At present, we are characterizing in further detail the specific mechanism by which PI3K contributes to β-catenin signaling, the specific kinase(s) involved in β-catenin phosphorylation in this system, as well as investigating the mechanims of nuclear translocation of β-catenin.

In addtion to the above mentioned specific research lines, we continue investigating other aspects of tumor progression, such as the relation between E-CD and/or β-catenin signaling and metalloproteinases expression (in collaboration with Dr. A. Fabra), the organization and function of tight junctions in relation to E-CD and H-ras activation (in collaboration with Dr. L. González-Mariscal), the expresion of APC and catenins in human breast carcinomas (in collaboration with Dr. C. Gamallo and Dr. J. Palacios) and the anti-tumor effects of the E1A gene of adenovirus (in collaboration with Dr. S. Ramón y Cajal and Dr. M. Quintanilla).

Signal transduction mechanisms altered after transformation by oncogenes of the Ras superfamily.

Group Leader: Juan Carlos Lacal

Understanding the molecular basis of carcinogenesis is of utmost importance for the development of new anticancer strategies. To that end, it is essential to unravel the regulation of both normal cell proliferation and its alterations in cancer cells. We have previously demonstrated that in oncogene-transformed cells such as *ras, raf* and *src*, there is an increased level of phosphorylcholine (*P*Cho) resulting from a constitutive activation of phospholipase D (PLD) and choline kinase (ChoK). We have also demonstrated the importance of ChoK for the regulation of cell proliferation, since inhibition of this enzyme drastically reduces entry into the S phase after stimulation with growth factors. Furthermore, *P*Cho itself has mitogenic activity. We have recently reported the synthesis of new highly specific inhibitors for ChoK that drastically reduce entry into the S phase after stimulation with specific growth factors. A more profound inhibition of cell proliferation was observed in cells transformed by oncogenes such as *ras*, *src*, *raf* and *mos* in the presence of ChoK inhibitors, compared to their parental cells. This effect was not observed in cells overexpressing the *fos* oncogene. While *ras*, *src*, *raf* and *mos* transformation is associated with elevated levels of ChoK activity, *fos* expression does not affect ChoK.

The inhibitory effect on proliferation of ChoK inhibitors correlates well with their ability to inhibit the production of phosphorylcholine in whole cells, a proposed novel second messenger for cell proliferation. Here we describe the characterisation of ChoK inhibitors with antiproliferative properties against human tumour-derived cell lines. The new molecules were tolerated in mice at doses that showed *in vivo* antitumor activity against human tumour xenografts derived from HT-29 and A431 cell lines implanted subcutaneously in nude mice. These results strongly support a critical role of choline kinase in the regulation of cell growth and makes this enzyme a novel target for the design of anticancer drugs. This first generation of inhibitors provide *in vivo* evidence that blockade of *P*Cho production is a valid strategy for the development of new anticancer agents, opening a new avenue for the development of antitumor drugs with a novel mechanism of action.

It has long been accepted that some oncogenes and oncosuppressor genes are involved in cell death as well as in proliferation. The Rho GTPases form a subgoup of the Ras superfamily of GTP binding proteins that regulate a wide spectrum of cellular fuctions. The Rho GTPases function cycling between an active GTP-bound state and an inactive GDP-bound state. Activated Rho GTPases interact with intracellular target proteins or effectors to trigger a wide variety of cellular responses including the reorganization of the actin cytoskeleton, cell cycle progression, adhesion, metastasis, and gene transcription. Some members of the Rho GTPases family also play a role in apoptosis. On such behalf we have reported that the human genes *rho* A, *rho* C and *rac* 1 are capable of inducing apoptosis in different cell systems like NIH 3T3 fibroblasts and the human erythroleukemia K562 cell line after serum deprivation. We have investigated the mechanism involved in this process and have demostrated that apoptosis induced by Rho proteins is independent of p53 and is sensitive to expression of Bcl 2 protein *in vivo* and *in vitro*. Furthermore, overexpression of *rho* induces the activation of an endogenous sphingomyelinase, and induction of apoptosis by overexpression of the human Rho A and Rho C proteins correlated with an increase in ceramide levels, a putative second messenger for apoptosis. Furthermore, It was then verified that Rho-induced apoptosis is indeed mediated by generation of ceramides. Moreover, *vav* and *ost,* two guanine exchange factors for Rho proteins with oncogenic properties, were also able to induce apoptosis under similar conditions. We have seen also that Rho proteins play an important role in the physiological regulation of the apoptotic response to stress-inducing agents since a dominant negative mutant of Rac 1 interferes with the induction of apoptosis by TNFa.

Molecular genetics of neurogenic tumors: diagnostic markers and progression mechanisms

Group Leader: Angel Pestaña

Current research interest of the group is centered on the gene expression profile of Ewing´s tumors. The Ewing family of tumors of neuroectodermal origin has been defined by the presence of an EWS-ets gene rearrangement whose fusion transcript acts as a transforming transcriptional factor. Most of the Ewing´s tumor (85 %) carries a t(11;22) translocation generating a EWS/FLI-1 fusion transcript. The remaining 15 % of the cases other chromosome 22 (EWS) translocations lead to the formation of other EWS/ets fusion transcript (EWS/ERG being the second most frequent). Besides these differences in fusion transcripts, the rearrangements show other kind of molecular diversity in the different combinations of exons from EWS and FLI-1 In this case the most frequent fusion (type 1) links EWS exon 7 in frame with exon 6 of FLI-1. Interestly, these fusion structures have been shown to be of prognosis value. Although the aberrant genes resulting from EWS/ets fusion may be related to clinical heterogeneity and response to treatment of Ewing´s tumors, little is known about the variety of gene expression induced by the fusion transcripts In an attempt to gain insight into this question we proposes to carry out a profiling of gene expression using SAGE (Serial Analysis of Gene Expression) and micro arrays in tumor cell lines expressing the EWS/FLI fusion transcript and in normal human cells transfected with the DNA rearrangements.

Intercellular communication and cellular signaling in normal and tumor cells

Group Leader: Antonio Villalobo

Our group is interested in the study of different mechanisms of intercellular communication and cellular signaling in normal cells and their alterations in tumor cells. Among the projects in progress are: i) The study of the phosphorylation of calmodulin by the epidermal growth factor receptor (EGFR) tyrosine kinase with emphasis on the functional consequence of this phosphorylation on the biological activity of calmodulin when acting on different calmodulin-dependent systems, and the regulatory role of phospho(Tyr)calmodulin on this receptor; ii) The study of the regulation of the EGFR by the calcium signal and the cross-talk between the calmodulin/phospho(Tyr)calmodulin system and the protein kinase C system regulating the receptor in intact cells; iii) The study of the physiological consequence of the mutagenesis of the calmodulin-binding site of the EGFR; iv) The identification of different calmodulin-binding proteins implicated in cellular proliferation mediated by the EGFR and the study of their functional roles; v) The characterization and the study of the physiological role of ecto 5'-phosphodiesterases/nucleotide-pyrophosphatases in normal and tumor cells; vi) The study of the regulatory roles of nitric oxide on EGFR-mediated cellular proliferation, the activation of the p38MAPK (p38 mitogen-activated protein kinase) pathway, and the activation of NOIPP-58, a newly discovered Nitric Oxide-Induced 58 kDa Phospho Protein; and vii) The study of the action of b-galactoside-specific animal lectins on cellular proliferation.

2. Department of Biochemistry and Genetics of Yeasts

ABC transporters: structure-function relationships

Group Leader: Pilar Eraso

Our main interest is the analysis of the structure-function relationships of ABC transporters. For this purpose we are using as a model the yeast cadmium factor, Ycf1, a vacuolar ABC protein involved in resistance to Cd^{2+} and to exogenous glutathione S-conjugate precursors in yeast. The ABC transporter superfamily includes human proteins such as CFTR (Cystic Fibrosis Conductance Transmembrane Regulator) or MRP1(Multidrug Resistance-associated Protein). Ycf1 has strong sequence similarity with CFTR and MRP1, which suggests a similar overall structure. In a first approach, we have performed a mutational analysis of the protein generating 22 mutations by site-directed mutagenesis in NBD1, NBD2, TMD2 and R domains. Characterization of the mutants led to the identification of essential residues for Ycf1 transport activity, biogenesis and regulation. In a second approach we have performed an intragenic suppressor analysis of six inactive mutations located in NBD1, NBD2 or R domains of Ycf1. In a third approach we are studying if the intragenic suppressor mutations identified in Ycf1 have similar properties when introduced into human CFTR.

Relations between glycolytic flux and the effects produced by sugars in yeast.

Group Leader: Carlos Gancedo

In the study of mutants that suppress the toxic effects of sugars on different glycolytic mutants we have shown that mutations in the genes *GAL2* or *GAL 4* are able to eliminate the effects of galactose on *gpm1* or *pyc1 pyc2* mutants in *Saccharomyces cerevisiae*. We are trying to characterize the genes responsibles of other mutations that suppress the toxic effects of different sugars in the above mentioned mutants.

We have shown that the gene the encodes the hexokinase from *Yarrowia lipolytica,* posseses an intron. The protein, very sensitive to inhibition by trehalsoe-6-P exhibits a loop absent from other hexokinases.

We have studied the gene *TPS1* from the yeast *Candida albicans* .Disruption of both chromosomal copies impairs hyphae formation and decreases infectivity of the organism.We have inititated the study of the gene *MIG1* from this yeast.

Catabolite repression in yeast and control of morphogenesis

Group leader: Juana M. Gancedo

Research topics: Role of cAMP in *FBP1* transcription; Elements which regulate pseudohyphae formation in *S. cerevisiae*; Glucose signaling; Regulation of the protein kinase Snf1.

We have shown that cAMP has a strong repressing effect on one of the UASs from the *FBP1* gene that may be mediated through its repression of the genes *CAT8* and *SIP4* which encode transcription activators. We found also that cAMP is able to induce pseudohyphal and invasive growth in *S. cerevisiae* in a variety of stress conditions. We have established that glucose signaling involves the interaction of the glucose sensors Snf3 and Rgt2 with the regulatory protein Mth1. Nevertheless we could show that glucose signaling may also occur in the absence of both Snf3 and Rgt2. We are investigating the existence of genes which can make the protein kinase Snf1 active in the presence of glucose, when overexpressed.

Turnover of plasma membrane proteins in yeast. Mechanisms of endocytosis

Group Leader: Rosario Lagunas

Addition of glucose to yeast cells starved of a nitrogen source triggers endocytosis and degradation in the vacuole of a number of plasma membrane proteins. This inactivation is called "catabolite inactivation" and our results suggest that it is due to the stimulation of the general protein turnover that occurs during nitrogen starvation. Endocytosis is inhibited by the presence of low concentrations of ethanol. We have found that yeast cells accumulate trehalose when a remodelation of the plasma membrane and, therefore, of endocytosis is required for yeast survival. We have also found that this accumulation circumvents the inhibition of endocytosis by ethanol. Using mutants defective in the heavy chain of clathrin and in several subunits of the COPI and COPII complexes, we have found that clathrin, as well as two cytosolic subunits of the COPII, Sec23p and Sec24p, could be involved in endocytosis of the 12-transmembrane segment

protein (12-TMS) maltose transporter. The results also indicate that endocytosis of this transporter and of the 7-TMS α–factor receptor could have different requirements.

Yeast Genes Functional analysis

Group Leader: María J. Mazón

1. Phenotypic analysis of the null mutants in six genes previously sequenced and cloned was performed. Strains carrying a deletion in *YGL133w* showed reduced mating efficiency and aberrant cellular morphology. This phenotype was only detected in the *MAT* α mutant but not in *MAT a*. Ygl133 protein was shown to localize to the nucleus using a GFP fusion protein. The mutant strain constitutively expresses *FUS1*, a pheromone induced gene in wild type cells.

2. We have shown that the 45 residue amino extension of **aminopeptidase I** (API) is necessary and sufficient to target GFP to the vacuole. We undertook a search for proteins able to interact *in vitro* and/or *in vivo* with this amino extension and found that Ssa1, a member of the Hsp70 chaperone family, is specifically retained by the prepro-peptide immobilized on a resine. We are studying API processing in a *ssa1^{ts}* mutant strain.

3. We are interested in the possible role of the amino terminal domain of the Ycf1 protein in its intracellular sorting to the vacuole and in protein function. We have shown that deletion of the 4 transmembrane segments of the amino terminal domain abolishes protein function while the protein is correctly localized. We are also studying the role of Ycf1 phosphorylation on its function. We have produced three mutants affecting the consensus site for PKA phosphorylation, located in the regulatory domain of Ycf1 : Ser^{908}/Ala, Ser^{908}/Asp and Ser^{908}/Glu, and are presently studying the transport ability of the mutant proteins and the effect of PKA phosphorylation on the wild type protein.

Molecular analysis of the yeast plasma membrane H^+-ATPase.

Group Leader: Francisco Portillo

Our main interest is to study the regulation of the yeast plasma membrane ATPase by glucose. Glucose triggers transcriptional and post-transcriptional mechanisms wich increase the level and the activity of the plasma membrane H^+-ATPase in *Saccharomyces cerevisiae*.

At the transcriptional level, we have found that glucose-dependent ATPase gen expresión is regulated by a lipid-mediated signal transduction pathway. In addition, we have investigated the post-transcriptional activation of the enzyme and have found that the Bck1/Mkk1/Mpk1 MAP kinase signalling pathway is implicated in this activation.

3. Department of Cellular and Molecular Endocrinology

Regulatory mechanisms by thyroid hormone in the Central Nervous System

Group Leader: Juan Bernal

Our work is directed towards understanding the basic processes whereby thyroid hormone influences brain development and function. In the past, we identified a series of genes whose expression is regulated by thyroid hormone in the rat brain. One of these genes is RC3/neurogranin, which encodes a protein kinase C substrate. RC3 is regulated by T3 directly at the transcriptional level and thus probably by interaction of the T3 receptor with genomic regulatory sequences. In previous work we isolated the promoter region and flanking sequences and found no T3-responsive elements (TRE) up to 6 kbp upstream of the promoter. To identify the TRE we used the whole human gene (11 kbp). Labelled restriction fragments were incubated with T3 receptor and immunoprecipitated with a receptor antibody. In this way we could isolate a DNA fragment that specifically bound the T3 receptor. Further analysis by footprinting, EMSA and transactivation of reporter genes demonstrated the presence of the TRE in the first intron, 3 kbp downstream of the origin of transcription.

To identify striatal genes regulated by thyroid hormone we screened a collection of cDNA clones corresponding to mRNAs that were enriched in striatum. One of these clones, SE6C, was found to dependent intensely of thyroid hormones. Isolation of the full length cDNA and conceptual translation of the ORF revealed that the SE6C clone encoded a novel protein with all the features of the small GTP binding proteins of the Ras family, which we have named Rhes (Ras homolog enriched in striatum). Rhes shares 65% identity with dexras, another Ras family member inducible by dexamethasone. Studies are in progress to define the Rhes effector pathways.

The concentration of T3 within the brain is critically dependent upon the activity of the deiodinases. These selenium-containing enzymes are of three types, of which type 2 (D2) and 3 (D3) are of special interest in the brain. D2 removes the iodine atom in the 5' position of T4 to generate the active hormone, T3. D3 generates inactive metabolites, rT3 from T4 and T2 from T3, after removal of the iodine in the 3 position. About 80% of brain T3 is generated locally through D2 activity. Also, D2 activity increases in hypothyroidism, and thereby tends to maintain T3 concentration in the face of a decreased T4. We have studied D2 and D3 expression in brain by in situ hybridization. D2 is expressed in glial cells (protoplasmic astrocytes and tanycytes), and is selectively increased by hypothyroidism in all the relay stations and cortical targets of the somatosensory and auditory pathways. This suggests that these pathways are specifically protected by D2 against hypothyroidism and that therefore thyroid hormone might have an important, previously unrecognized role in the development of these structures. On the other hand, D3 is expressed only in neurons. During the early postnatal period, D3 is selectively expressed in nuclei related to sexual differentiation of the brain, such as the bed nucleus of stria terminalis, the preoptic area and the medial amygdala. The results suggest that there is a need for exquisitely regulated T3 concentrations in these areas, although its biological meaning is still unknown.

Activaton and differential signaling of Ras proteins

Group Leader: Piero Crespo

Our group research interests are mainly focused in the regulation of Ras and other related GTPases and their signaling pathways, with special attention on MAP kinases routes. We are at present investigating the role of the DH domain harbored in Ras exchange factors in the regulation of Ras activity. We have found that Ras-GRF DH domain is essential for activating Ras/MAPK by regulating Ras-GRF access to the membrane. A process that we have found to be regulated by the GTPase Cdc42.

We are also investigating the differential roles of Ras proteins H, K and N-Ras. For that purpose we have generated dominant inhibitory mutants of each Ras isoform and found them to be specific in their inhibiting activities. We will process to use these tool in investigating the role of the three Ras in the regulation of cell cycle progression.

With regards to MAP kinases, one of our main interest is the role played by these in leukemogenesis. In this respect we have found that, unlike most cellular systems, myeloid leukemia cell growth and differentiation are independent of ERK1/2 activation.

Finally we are also focusing our attention on Mxi2, an splicing isoform of p38. Interestingly we have found that this MAP kinase exhibits differential regulation and substrate specificity when compared to p38.

Thyroid hormone status at different stages of development

Group Leaders: Gabriela Morreale de Escobar and Francisco Escobar del Rey

Extrathyroidal adaptations to thyroid hormone deficiency and excess: Research topics: a) Thyroid status of adult thyroidectomized rats on T3 infusions; b) Thyroid status of different tissues in adult rats on varying iodine intakes.

Maternal-fetal communication: Research topics: a) Thyroid hormones in early human embryonic compartments; b) Iodine nutrition of premature infants; b) Effects of maternal treatment with TRH and glucocorticoids on fetal thyroid status in rats with and without nitrofen-induced pulmonary immaturity.

Iodine deficiency studies: Research topics: a) Situation of iodine nutrition in different areas of Spain; b) Iodine deficiency in pregnant and lactating women; c) Experimental iodine deficiency disorders: treshold for brain damage; d) Brain damage in progeny of severely iodine deficient (hypothyroxinemic) rats: altered migration of brain cells.

Biology of nuclear hormone receptors: studies on brain development and cancer

Group Leader: Alberto Muñoz

Our group is focussed on the study of the effects of the nuclear receptors for thyroid and glucocorticoid hormones, vitamin D, and retinoic acid on different aspects of central nervous system development and cancer. In the developing rat brain we use diverse molecular approaches such as differential PCR or substractive hybridization to search for novel genes under thyroid hormone control. Identified target genes are studied in vivo in the rat brain and in cultured nerve cells to know the mechanism of control and their spatial and temporal pattern of regulation by standard molecular techniques as well as by *in situ* hybridization and immunohistochemistry.

In both colon and mammary epithelial cells, we investigate the effects of dihydroxyvitamin D3 and thyroid hormone on gene expression, proliferation, phenotype, and tumorigenicity. Finally, we also interested in the molecular mechanism of the antagonistic effect of activated nuclear hormone receptors on AP-1 action, and on the effects of v-*erb*A oncogene encoding a mutated form of the thyroid receptor in glial 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 of brown adipocytes are examined.

UCP-1 mRNA 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 (D2) 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.

Signal transduction and transcriptional mechanisms involved in the control of cell growth and differentiation: Role of tissue-specific transcription factors.

Group leader: Pilar Santisteban

Research topics: a) Hormonal regulation of thyroid-specific genes: role of transcription factors (tissue-specific and constitutive) and chromatin remodeling. b) Control of thyroid cell proliferation: signalling and cell cycle proteins. c) Tissue-specific transcription factors in the control of lung-specific gene expression. d) Transcriptional control of cytosolic malic enzyme (ME) gene expression.

The main interest of our group is focused in the transcriptional regulation and growth control by extracellular signals. To deep inside both mechanisms we have used different cell systems and functional approaches. One of the most used cell systems in our laboratory is the FRTL-5 thyroid epithelial cells. These cells express a set of tissue specific genes (thyroglobulin, thyroperoxidase and Na/I^- symporter) responsible for the thyroid hormone synthesis. We have studied the transcriptional regulation of these genes by hormones and growth factors demonstrating that thyroid-specific transcription factors and constitutive factors participate in this control. The thyroid specific factors are members of the homeo-box (TTF-1), forkhead (TTF-2) and paired-box (Pax-8) families. TTF-2 plays an essential role in the hormonal control of thyroid gene transcription interacting physically and functionally with CTF/NF1, a constitutive

transcription factor. Since TTF-2 is a forkhead transcription factor and due to the structural similarity of these factors with linker histone proteins, we have started the study of how changes in higher order chromatin structure control thyroid-specific gene transcription.Interestingly, the homeo-box gene TTF-1 is not only expressed in thyroid but also in lung, playing a decisive role in its development and morphogenesis. In a model of fetal lung hypoplasia we have shown that TTF-1 is down-regulated and that glucocorticoids revert this effect. We are currently studying the mechanisms of action of glucocorticoids and the differential expression of TTF-1 in thyroid and lung cells.

The cytosolic malic enzyme (ME) gene is a housekeeping gene whose transcription is controlled by thyroid hormones and insulin. We have identified in the promoter of this gene an insulin response element bound constitutively the transcription factors Sp1 and Sp3. The early growth response gene *Egr-1* binds to this element in response to insulin via Ras/MAPK. Recently we have shown that ME gene is up-regulated by dioxin-like compounds through a xenobiotic response elements (XRE) that matches in its position whith a thyroid hormone response element (TRE). Dioxin-like compounds induce, an still unidentified, nuclear protein that binds this XRE/TRE.

We are also interested in studying the control of thyroid cell growth. In the thyroid gland there are two types of cells: epithelial (or follicular) and parafollicular. Each one expresses different specific genes and have a different response to external signals. We have demonstrated the existence of an autocrine loop in the control of thyroid epithelial cells that involves the positive effector thyrotropin (TSH) and the general inhibitor somatostatin (SS). Our more recent data show that TSH control of epithelial thyroid growth involves both PKA and PI3-K pathways. PKA is responsible for TSH-induction of HMG-CoA reductase, RhoA and Cdk-2 activation, and down-regulation of $p27^{kip}$, whereas PI3-K increases cyclin E. Both pathways finally converge increasing cyclin E-Cdk2 complexes. SS exerts its antiproliferative effect upstream of PKA and PI3-K interfering with the TSH-induction of cAMP and adenylyl cyclase activity. We are now studying in more detail the existence of a PKA-dependent and a new one-independent PI3-K pathways in the TSH/cAMP mediated proliferation of epithelial thyroid cells.

The parafollicular thyroid cells are transformed in medullary thyroid carcinoma (MTC). In a MTC cell line, we have shown that the p53 locus is rearranged and in parallel these cells do not express the oncoprotein MDM2. The introduction of MDM2 in this p53-deficient MTT cells promotes apoptosis. MDM2-mediated programmed cell death is at least mediated by a down-regulation of the antiapoptotic protein Bcl-2 and an increase in caspase-2. We are now studying in the same mechanism in epithelial cells as well as the functional role of this apoptotic process induced by MDM-2.

Molecular mechanisms of neural differentiation

Group Leader: Mario Vallejo

Research interests in our laboratory are focussed on the transcriptional mechanisms that regulate cell-specific gene expression in forebrain neurons. We have established neural cell lines derived from rat embryonic cortex that retain phenotypic features of neural precursors. Using these cells, we found that activation of the cAMP-dependent signaling pathway induces astrocytic differentiation. These studies have been extended using primary cultures of cortical

precursor cells to identify neurotrophic factors that induce the differentiative response and to elucidate in detail the intracellular signaling mechanisms involved. Our ultimate goal is to understand the transcriptional mechanisms acting on target genes to trigger this response.

We are also interested in the study of homeodomain transcription factors that may play a role in brain development. In particular, we are focussing our attention on two homeodomain proteins identified in our laboratory, named Opx-1 and Drx-1, expressed in the perineural mesechyme and central nervous system during embryonic development. In addition, we have identified the expression of IDX-1 in the developing central nervous system. IDX-1 is a homeodomain transcription factor whose expression was previously thought to be restricted to pancreas and gut. Our studies are directed at understanding its possible role on central nervous system development.

4. Department of Enzymology and Molecular Patology

Structural bases of control, expression and interaction with microtubule formation of eukaryotic phosphofructokinases. Evaluation of intestinal lactase in vivo with galactosylxyloses.

Group Leader: Juan J. Aragón

Our research includes:

Structural analysis of control and expression of phosphofructokinase (PFK) from eukaryotic cells. Construction of chimeric PFKs by exchanging the N- and C-terminal halves between the M and C isozymes from muscle and ascites tumor, respectively, allowed us to examine the functional contribution of each domain to catalysis and allosteric control. This study, in conjunction with the characterization of several mutants of the non-allosteric enzyme from *Dictyostelium discoideum*, pointed to several amino acid residues which role in allosteric binding sites we are testing by specific mutations and other structural approaches. We are also investigating the control of expression of tumor PFK-C to account for its high content in malignant cells and its pattern of expression during tumor development.

Interaction of *D. discoideum* PFK with microtubule formation. This PFK was found to be a potent and specific inhibitor of tubulin polymerization. Now we have evidenced the formation of tubulin-PFK complexes and are investigating the possible role in vivo of this inhibitory effect by several approaches.

Utilization of 4-galactosylxylose in the evaluation of intestinal lactase in vivo as a possible diagnostic procedure for lactose intolerance. Oral administration of 4-galactosylxylose to suckling rats leads to urinary excretion of xylose which can be determined by a simple colorimetric method. Other advantages of this procedure related to the correlation with changes in intestinal lactase levels and the synthesis and doses of disaccharide made us to propose this procedure as a suitable non-invasive diagnostic method for lactase deficiency in humans. Efficiency of several galactosylxylose isomers has been evaluated and the correlation between blood xylose with enzyme level after administration of the disaccharide is now being assessed as an alternative procedure in pediatrics in near-future clinical tests.

Structural and functional characterization of novel human genes

Group Leader: Antonio Coloma

The research activities in our lab are focused in the characterization of novel human genes, which have been identified through its sequence homology to the corresponding genes from other organisms. We have recently reported on the structure of two genes: *NRGN* , positioned at 11q24, encoding neurogranin, a protein of neuronal expression regulated by thyorid hormone; and *POMT1* which encodes a putative O-mannosyltransferase, according to its homology to yeast Pmts. POMT1 is homologous to *Drosophila rotated abdomen*. We are currently investigating the function of the protiens encoded by both genes, as well as its possible involvement as a cause of genetic disorders.

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

Group Leader: Claudio Fernández de Heredia

We have continued the work on these two topics: a) study of mutual interactions in the metabolism of mono- and disaccharides in *Saccharomyces cerevisiae* and b) enzymes acting on 2',3' cyclic nucleotides. In relation with the first subject, we have seen that secretion of glucose from maltose, in response to the presence of fermentable hexoses in the media, requires the intracellular hydrolysis of maltose and involves, in addition to the maltose transporter, a very labile component(s) that is inactivated by incubating the cells with mixtures of maltose and any of the fermentable hexoses. This component(s) re-appears again by incubating the cells with maltose and this process is blocked either by cicloheximide or any of the fermentable hexoses. In relation with the second point, we have finished the characterization of the 2',3'-cyclic nucleotide 2'-phosphodiesterase from *Fusarium culmorum*, and we have developped an enzymatic method for the quantitative determination of micromolar concentrations of cytidine 2'-phosphate even in the presence of at least five times greater concentrations of a variety of related nucleotides.The method is also suitable for detection of 2',3'-cyclic nucleotide 3'-phosphodiesterase (EC 3.1.4.37) and its discrimination from the 2',3'-cyclic nucleotide 2'-phosphodiesterase (EC 3.1.4.16).

Mechanisms of cellular resistance to antifolates: Evaluation of folate-dependent enzymes inhibitors as antitumoral drugs.

Group Leader: Pilar Llorente

The inherent or induced resistance of certain tumors to cytotoxic drug therapy is a major clinical problem.There are many mechanisms of drug resistance elucidated principally from " in vitro " studies . These include mutation of target genes , amplification of target and mutated genes, differences in repair capacity altered drug transport , differences in nucleoside and

nucleobase salvage pathways and , in the case of antimetabolites as antifolates , polyglutamylation .

The essential requirement for folilpoliglutamates for celular viability and the importance of antifolates polyglutamates in cytotoxicity suggest that alterations in polyglutamate synthesis could have profound therapeutic effects.Alterations in polyglutamate synthesis can be arrived at indirectly, by changes in the one-carbon distribution in the folate pool ,o directly through effects on FPGS. In the latter regard our studies have been useful in developing compounds which interact and / or inhibet the FPGS activity.These compounds, mainly pterin derivatives, are hidrolysis intracelular products of natural folates and classical antifolates as MTX.

Metabolism and function of dinucleoside polyphosphates

Group Leaders: Antonio Sillero and María Antonio Gunther Sillero

Metabolic aspects of purine nucleotide metabolism in rat brain cytosol. The cytosolic IMP-GMP specific 5'-nucleotidase (c-N-II) from rat brain has been purified from rat brain cytosol and found that it is activated by dinucleoside polyphosphates and by inorganic polyphosphates (K_a, micromolar. We have made a systematic study of the metabolic fate of AMP throughout incubation of this nucleotide with fresh cytosol obtained from rat brain and following the kinetic of disappearance of AMP and the appearance of its degradative products, by HPLC. In order to quantify the kinetic behavior of these reactions, two complementary approaches are being followed: i) the Vmax and K_m values of the enzymes acting in the intermediate steps of the reactions were experimentally determined in fresh extracts; ii) these data were introduced into differential equations that describe the concentration of AMP and its degradative products along the time of incubation. The differential equations were solved with the help of the Program Mathematica 3 running in a Power Macintosh G3.
Mechanisms of the reaction catalyzed by firefly luciferase (EC 1.12.13.7). This mechanism is being explored through the use of potential specific inhibitors of two of the reactions catalyzed by this enzyme: light production and synthesis of dinucleoside polyphosphates. The syntheses of these compounds by cells carrying the gen for this enzyme is also being investigated.
Synthesis of dinucleoside polyphosphates. The synthesis of these compounds catalyzed by DNA and RNA ligases has been explored

5. Departement of Structure and Function of Biomolecules

Regulation and function of COT/Tpl-2 kinase in T cell activation

Group Leader: Susana Alemany

We have recently shown that COT kinase is implicated in the T lymphocyte G0/G1 transition. We are currently studying:

1) Implication of COT kinase in the G1/S transition of T lymphocytes and the regulation of cyclooxygenase 1 and cyclooxygenase 2 activity (A.Velasco, R. de Gregorio)

2) Regulation of COT promoter and COT mRNA levels by activatory T signals (R. de Gregorio).

3) Changes in the phosphorylation state of COT kinase and correlation with changes in its activity (M. Gandara and R. Hernando)

The rate limiting step in the synthesis of prostaglandins is the conversion of arachidonate to prostaglandin H_2, which is catalyzed by two isozymes COX-1 (cyclooxygenase-1) and COX-2 (cyclooxygenase-2) COX-1, that has been involved in "housekeeping" functions, is a constitutive enzyme expressed in many tissues and also in platelets In contrast, COX-2 is an inducible enzyme. The regulation of COX-1 activity is thought to be responsible for the gastric and renal side effects of nonsteroidal antiinflammatory drugs, as well as for their antithrombotic activity. On the other hand, the antiinflammatory capacity of the different nonsteroidal antiinflammatory drugs, is now proposed to be associated with the capacity of inhibiting COX-2 activity. We are currently the capacity of metamizol to inhibit COX-1 and COX-2 activities using different cell systems.

Molecular mechanisms implicated in megakaryocytic polyploidization

Grup Leader: Carmela Calés

We are interested in elucidating the molecular mechanisms by which human megakaryocytes become polyploid as part as their physiological maturation program. The experimental system we are using is based in the comparison of two established cell lines with megakaryoblastic features which respond to phorbol esters by differentiating towards mature forms of megakaryocytic lineage. However, whereas one of them (HEL) enters an endoreplication cycle and becomes polyploid, the other (K562) does not. We have demostrated that both G1/S cyclins (E and A) and their inhibitors ($p27^{kip1}$ and $p21^{cip1}$) are differentially regulated, and furthermore, that ectopic over-expression of cyclin E determines the polyploidization of K562 cells. We have also studied the putative role of transcriptional regulators in the establishment of such truncated cell cycle, and shown that ectopic expression of a *Drosophila* repressor and endocycle regulator, *esg*, inhibits HEL endoreplication.

We are also exploiting the fact that fetal megakaryocytes appear to have a diminished platelet production capability, compared to adult cells. We have observed that megakaryocytes cultured from umbilical cord blood do not become polyploid, in contrast with megakaryocytes obtained from peripheral adult blood, which attain DNA content up to 64N. We are currently combining both approaches (established cell lines and primary megakaryocytes) to further investigate the role of G1/S cyclins and inhibitors, as well as transcriptional regulators, in the establishment of endoreplication cycles and its effect on platelet production.

Biomedical Applications of Magnetic Resonance

Group Leader: Sebastián Cerdán

Our lab is involved in a variety of projects developing applications of Magnetic Resonance methods to biochemical and biomedical problems. Topics currently covered include; quantitative

analysis of neuronbal-glial interactions in the adult brain, pH homeostasis in cancer and pH imaging by 1H MRSI, Intelligent tumor diagnosis by MR, neural networks and multivariate analysis, water structure and dynamics in cells and tissues and intracellular organization of metabolism.

Cellular Regulation by Proteolysis.

Group Leader: José G. Castaño

The ubiquitin-proteasome pathway is responsible for most part of nuclear and cytoplasmic degradation within the cell. We are studying several aspects of the regulation of proteasome activity and assembly. Recent results (see publications) are centered in a new activity for proteasomes, limited proteolysis of a substrate (as demonstrated for GRK2). This newly identified activity opens another way of regulation of enyme activity by the proteasome, instead of total degradation to oligopepetides. In the study of the assembly line of 20S proteasomes within the cell we have shown that pro-βC5 N-terminal processing is coupled to its incorporation into precursor or mature proteasome complexes, depends on proteasome activity and takes place in the cytosol. Finally, we are also studying mitochondrial proteolyis and we characterized the human homologue (HClpP) of the bacterial ClpP protease, whose subunit composition and oligomeric structure is similar to the bacterial protein.

Study of the function of a casein kinase I from Dictyostelium discoideum

Group Leader: Margarita Fernández

The main goal of this project is to study the function of a casein kinase I in *Dictyostelium discoideum*.. We have cloned and characterized a 49 kDa form of casein kinase I (CKI) from this organism. The predicted amino acid sequence shares 70% identity with the catalytic domain of the mammalian ε and δ isoforms, the*Drosophila* CKIe and the*S.pombe* Hhp1, and 63% identity was also found with Hrr25, a 57 kDa form of casein kinase I from yeast involved in DNA repair.*Dictyostelium discoideum* CKI is expressed in vegetative asynchronous cells, as well as in differenciated cells as detected by northern analysis. The level of CKI expression does not change during the cell cycle. Antibodies raised against a truncated version of the protein recognize a 49 kDa protein from *Dictyostelium* extracts. The protein expression parallels the pattern found for the RNA. The *Dictyostelium discoideum* CKI expression in *Escherichia coli* resulted in an active enzyme that autophosphorylates and phosphorylates casein. Immunofluorescence assays shows that DdCKI is localized in the cytoplasm and nuclei of *Dictyostelium* cells.

We have been unable to obtain disruptants of the casein kinase I gene, suggesting that this protein is essential for vegetative growth of *D. discoideum*. Overexpression of DdCKI renders cells that are more resistant to hydroxyurea suggesting a potencial role for this kinase in DNA repair.We are at the present using other approaches to obtain further information on the function of this protein a) we are obtaining transformants using a construct of a dominat negative version of DdCKI under the control of genes expressed only in differentiation b) we are making a

construct in which the carboxi terminal portion has been deleted (Putative dominant version of the kinase) with the aim to study the phenotype of the transformants obtained.

The role of the Src family on the cellular proliferation/differentiation induced by cytokines

Group Leader: Jorge Martín-Pérez

Cytokines regulate a large number of physiological functions through interaction with their receptors. The cytokine receptors have not intrinsic enzymatic activity. Cytokine binding results in receptor dimerization, activation of the preassociated members of the Jak and Src family of tyrosine kinases and on tyrosine phosphorylation of cellular proteins, including the receptor and additional molecules implicated in signalling cascades, that in turn regulate survival, proliferation and/or cellular differentiation.

Since the observation that c-Src interacts and is activated upon prolactin receptor stimulation, we are looking for the biological consequences of this event. Prolactin receptor stimulates Jak2 and Src tyrosine kinases. The Src activation is not required for Jak2 stimulation nor for tyrosine phosphorylation of the receptor. In turn, Jak2 does not play a role on prolactin stimulation of Src. Both events, emanating from the receptor activation are independent and at the same time essential for cell proliferation. Prolactin is implicated in the maturation of the Immune System, the receptor expression has been detected in very early B cells precursors, its expression increases up to mature B cells, where prolactin acts as a mitogen. In the mouse pro-B cell line BaF-3, upon transfection of the prolactin receptor, the cytokine induces the expression of B cell differentiation markers (l5 or IL-2Ra chain) as well as anti-apoptotic factors as bcl-2. Indeed, prolactin promoted significant expansions of defined B-lineage cell populations in short-term bone marrow cell cultures. These findings suggest that PRL, in collaboration with other cytokines and hormonal influences, modulates B cell development.

There are increasing evidences supporting a role for prolactin in breast cancer. In T47D human breast cancer cells, prolactin increases the phosphotyrosine content of proteins of the focal adhesion complexes, such as FAK and paxillin; these effects appear to be mediated by the Src family as they are abolished by specific inhibitors of these tyrosine kinases. The stimulation of the MAPK activity and the proliferation of the T47D cells induced by prolactin also seem to be controlled by the Src family. Together, the results suggest that these tyrosine kinases play a central role in the proliferative and cell morphology changes induced by prolactin in T47D breast cancer cells.

Having these data in mind, experiments seeking to further define and evaluate the role of the Src and Jak kinases and the cytokine receptor tyrosine phosphorylation on cell survival, proliferation and differentiation are ongoing in the laboratory.

Meshotelial cells obtained from peritoneal effluent. Its relationship with peritoneal antecedents and functional parameters

Group Leader: Francisco Vara

Anatomical and functional integrates of mesothelial cell (MC) are necessary to secure peritoneal stability. Omentum or peritoneal biopsies cannot be easily taken from the majority of peritoneal dialysis (PD) patients. The objective of this study is to show that MC taken from PD bags are able to growth in culture.

Fifty-two non selected PD patients using dialysate containing glucose (2.27%) were studied. MC were obtained from bags of peritoneal nocturnal effluent. MC of 80.7 % patients showed appropriate proliferation in the culture flasks to reach the subconfluence state. After trypsinization, MC representation has been markedly increased, reaching the 95.5% of the total cells in the culture. The second seeding process in the multi-well plate show that there is a exponential cell growth until day 16.

MC growth rate was inversely related to PD duration. Neither peritonitis incidence nor other demographic characteristic were related to MC growth degree. Creatinine and urea MTCs but not UF capacity were significantly related to MC growth rate. This growth is influenced by some of the intrinsic peritoneal characteristics derived from the peritoneal dialysis process. This tool is useful to evaluate individual peritoneal conditions and probably as a method for peritoneal viability follow-up, although further research is required.

6. Department of Regulation of Gene Expression

Regulation of gene expression by nuclear receptors in hipophisiary 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 other transcription factors, coactivators, and membrane receptors with tyrosine kinase activity to regulate the expression of different genes involved in proliferation and differentiation of pituitary and neuronal cells. We have identified response elements in the growth hormone, prolactin, c-*myc* or RARβ2 genes which mediate transcriptional activation or repression by nuclear receptors and have analyzed the receptor domains involved in this regulation. We have also discovered a novel mechanism of nuclear receptor activation by interaction with the transcription factor Ets-1, and have defined mechanisms by which nuclear receptors modulate responses mediated by other signalling pathways

Function and control regulation of the paramyosin/miniparamyosin and Troponin T genes in Drosophila

Group leader: Margarita Cervera

Our main interest in the last two years has been centered in a) the study of the regulation of the expression of muscle genes, concretely the *Drosophila* paramyosin/miniparamyosin gene (PM/mPM), as well as the troponin T gene (TnT) which represent a good model system in order to clarify such a regulation mechanism in *Drosophila* and probably in mammals, too. To characterize the mechanism(s) of transcriptional regulation of these genes during development, the *in vivo* analysis of the expression of the β-galactosidase gene under control of selected sequences in germ line transfectans of *D. melanogaster* has been studied. b) The identification and study of TnT mutations of the familial hyper trophic cardiomyopathies and its heart effects for the establishment of new diagnostic test for detect those mutations and also the establishment of correlations among mutations and phenotype. In paralell and as a second aim we propose the development of an animal model for study this disorder, concretely the use of the the indirect flight muscles of *Drosophila* because this muscles have similar characteristics than cardiac muscles.

Characterization of novel human genes and its possible involvement in inherited diseases.

Group Leader: Jesús Cruces

The activities of this 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 *POMT1,* encoding a Protein-O-mannosyl transferase which might be involved in early muscle system formation.

The tw (twisted) gene, the *Drosophila* ortholog of human *POMT2* gene, involved also in muscle system formation.

The *UHG62* gene, encoding a protein with unknown function containing a polyglutamine tract, and possibly implicated in neurodegenerative diseases.

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. We are also trying to develop a mouse model for the disease generating the same molecular defect as in humans.

Expression of mitochondrial genes and of the transcription factors involved in the biogenesis of mitochondria Tfam, NRF-1 and NRF-2, in rat liver, testis and brain

Group Leader: Carmen García Vallejo

Mitochondrial function requires genes encoded in both mitochondrial and nuclear genomes. Tfam, the activator of mammalian mitochondrial transcription, is encoded in the nucleus and its expression has been shown in *in vitro* studies to be controlled by nuclear respiratory factors NRF-1 and NRF-2. In order to understand the physiological dependence of mitochondrial gene expression, we have analysed in rat liver, testis and brain the expression level of mitochondrial genes in parallel with those of the three transcription factors. We found that a) Tfam expression is down-regulated in rat testis, both at the protein and transcript level. The 3-fold reduction in the

abundance of Tfam protein in rat testis does not result in low steady-state levels of mitochondrial gene transcripts, suggesting that Tfam is in excess and does not limit transcription *in vivo*. b) NRF-1 and NRF-2 (α, β and γ subunits) mRNAs were analysed by Northern blotting; for each mRNA, several transcripts were observed as well as tissue-specific patterns of expression. The mRNA steady-state levels of NRF-1 and NRF-2 were higher in testis than in liver or brain. These data suggest that the low expression level of Tfam found in testis is not due to decreased NRF-1 and/or NRF-2 expression. c) The analysis of the protein levels of NRF-2 subunits indicated that there is no correllation between messenger and protein levels, further suggesting the existence of tissue-specific post-transcriptional regulatory mechanisms for the expression of NRF-1/NRF-2 genes. Similar observations were made with another key, nuclear-encoded mitochondrial protein, β subunit of ATP synthase complex. We are studying at present the regulation of these nuclear transcription factors in different physiological conditions.

Physiopathology of mitochondrial biogenesis

Group Leader: Rafael Garesse

Mitochondrial diseases comprise a group of disorders, usually degenerative in character and mainly affecting muscle and central nervous system, which are associated with mutations in mitochondrial DNA (mtDNA) or nuclear DNA (nDNA). 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. We are studying mitochondrial biogenesis at two different levels: i) using as model system *Drosophila melanogaster* we are characterizing regulatory proteins involved in mitochondrial proliferation and differentiation, and modifying *in vivo* mitochondrial DNA replication. ii) Using the cybrid technology we are studying at molecular level the cellular effects of new mtDNA mutations responsible of human mitochondrial diseases and identifying the nuclear genes involved in syndromes of mtDNA depletion..

The Troponin component of *Drosophila* muscle. Genes and isoforms.
Towards the establishement of a permanent *Drosophila* colony in Space.

Group Leader: Roberto Marco

We have started to characterize the properties of *Drosophila* troponin using the potent new techniques of mass spectrometry with the goal to identify more precisely the posttranslational modifications that occur in many of these isoforms. The project has been initiated on both troponin T and troponin H, a special high molecular weight tropomyosin isoform, which is believed to be exclusively expressed in the Indirect Flight Muscle. Troponin H is phosphorylated in threonines and we have already identified the modified residues in the sequence extension specific of these isoforms. At the same time, we have started studying the properties of *Drosophila* troponin C, the Ca binding troponin subunit, which in Drosophila is encoded by at least three different genes, clones of which are already available in our laboratory. We are

cloning the genes from related Drosophila species (*subobscura* and *virilis*) and we are raising antibodies to identify these components at the protein level. In relation to the Space program, the initiation of the assembly of the International Space Station has brought a certain reduction in the Space Biological Research activities. We are taking advantage of this slowing down to prepare the experiments that will be carried out in the ISS facilities, once they become available to the International Scientific Community. We are developing the hardware to grow and process the samples of *Drosophila* indefinitely cultured in Space. With this proposal, we would try to identify the genetic and phenotypic changes that a population of Drosophila may undergo in Space and the possible changes in the developmental mechanisms and/or in the aging process of this biological model system produced by this long-term exposure. It seems already amply verified that simple cell culture systems sense and respond to the changes in different Space environmental parameters such as the gravitational field. We are also optimizing certain techniques that may be difficult or dangerous to be performed in Space, For example, we are studying the microwave enhancement effects on fixation and on the bactericidal/sporicidal effects of certain chemical reagents with potential applications to Space and Ground Activities.

Regulation of expression of the β-Amiloid 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 β-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 nuclear receptors.

Group Leader: Ana Pérez Castillo

We have recently demonstrated that thyroid hormone is an important regulator of mitochondrial gene expression during brain development. In addition we have found that this effect, at least in part, seems to be mediated by the regulation of mitochondrial transcription factor Tfam. The promoter of this gene responds to T3 through an IR2 localized 600 bp upstream of the transcription initiation site. To gain further insights into the consequences of this regulation, we have performed functional analysis of brain mitochondria from control and hypothyroid neonatal rats. We have found that, in the hypothyroid cerebral cortex, striatum, and hippocampus, ATP synthesis is reduced by a 52, 59 and 31%, respectively, compared to control animals. The developing brain is a known target of T3 action, and many genes have been characterized so far

to be under T3 regulation. Among these we have found that CCAAT/enhancer binding protein beta gene expression is regulated by T3 in specific areas of the brain. This effect in due to a direct effect of T3 upon C/EBPb transcription since the promoter activity of this gene is induced by this hormone. Another gene whose expression is induced in the hyperthyroid brain is the transcription factor egr-1/NGFI-A. This gene, as well as C/EBPb, is involved in differentiation and cell death processes in neuroblastoma cells, processes in which T3 is also involved.

Regulation of gene expression in differentiation and development. Studies on the Serum Response Factor (SRF)

Group Leader: Leandro Sastre

Our studies on the regulation of gene expression during the embryonic development of the crustacean *Artemia franciscana* and during the differentiation of the social amoeba *Dictyostelium discoideum* have been focused in the following topics: a) Function of the transcription factor SRF in the differentiation of the fruiting body of *D. discoideum* and in spore formation. b) Molecular cloning, developmental expression and functional studies of the *A. franciscana* SRF homologue. c) Identification of regulatory sequences on the Sarco/Endoplasmic Reticulum Ca-ATPase promoter 2 from *A. franciscana*.. d) Study of the nucleotide polymorphism of *A. franciscana* Na/K ATPase a1 subunit gene.

7. Department of Cellular Signaling

Role of Akt/PKB kinase in neuronal survival.

Group Leader: Antonio Cuadrado

Akt/PKB kinase is one element of the kinase cascade PI3K/PDK1/PDK2/Akt, specifically related with cell survival. In our group we are analyzing the activation of this kinase by neurotrophins and oxidative stress in neural cells.

We have analyzed the inhibition of Akt by ceramide, a lipid second messenger involved in apoptosis signaling. We have reported the down-regulation of Akt by ceramide activated phosphatase in PC12 cells. Based on these results we have proposed a new mechanism for induction of apoptosis by ceramide based on the down-regulation of the Akt survival pathway. Moreover, we are also analyzing the possibility that activation of the Akt survival pathway might interfere with some elements of sphingomyelin metabolism.

Since oxidative stress appears to be the most likely cause of several neurodegenerative disorders we are studying the regulation of the PI3K/Akt pathway by reactive oxygen species and the potential role on cell viability of ectopically expressed hyperactive Akt versions. We have observed that PC12 cells overexpressing membrane anchored Akt by a myristylation tag are more resistant to cell death induced by MPP+ (1-methyl-4-phenilpyridinium). This drug is a widely used as an inducer of experimental Parkinson disease in laboratory animals. At present we are

studying the mechanisms involved in this protection and its possible use for future in vivo studies.

Hepatic metabolism of carbohydrates: Diagnostic, physiopathological and regulatory aspects.

Group Leader: Juan E. Felíu

Our work is mainly focused on the study of carbohydrate metabolism in liver, under the following aspects:

1) Biochemical and molecular diagnosis of glycogenoses, hereditary fructose intolerance and galactosemia.

2) Study of the molecular mechanisms underlying leptin- and TNF-alfa-dependent insulin resistance at the hepatic level.

3) Development of an animal model of hereditary fructose intolerance.

Molecular and cellular basis of malignant progression of squamous cell carcinomas

Group Leader: Miguel Quintanilla

Our laboratory is studying molecular and cellular events involved in malignant progression of squamous cell carcinomas by using the mouse skin model of carcinogenesis. We found that TGF-β_1 is a modulator of the epithelial phenotype of squamous carcinoma cells and induces, in vitro as well as in vivo, a squamous-spindle carcinoma transition associated with increased invasive and metastatic abilities. TGF-β_1 stimulates cell migration and invasiveness of transformed keratinocytes by activating pericellular proteolysis. Thus, the growth factor induces the expression / secretion of components of the plasminogen system, such as urokinase (uPA) and its inhibitor PAI-1. uPA was demonstrated to be functionally involved in TGF-β_1-stimulated cell motility and invasiveness by using synthetic peptides antagonizing the binding of uPA to its cell-surface receptors. We have analyzed the transduction pathways that mediate the growth inhibitory and invasive responses of normal and transformed keratinocytes to TGF-β_1. We have found that the growth factor rapidly and transiently activates both the Ras-MAP kinases and Smad pathways. TGF-β_1 utilizes one or another pathway to modulate the expression of specific genes or depending of the cellular state. Thus, upregulation of uPA by TGF-β_1 is mediated by the Ras-MEK-Erk pathway while upregulation of PAI-1 appears to be Smad-dependent. Furthermore, induction of the cell cycle inhibitor $p21^{Cip1}$ is modulated by the Smad pathway in normal keratinocytes, while, in transformed keratinocytes containing a Ras oncogene, $p21^{Cip1}$ induction requires Ras-MAP kinase signaling. We also have found a cross-talk between the Ras and Smad pathways in Ras-transformed keratinocytes. The blockade of Smad4 (with a dominant-negative construct) in these cells constitutively hyperactivates the Ras-MAP kinase pathway leading to progression to poorly differentiated carcinomas. This effect was not observed in normal (immortalized) keratinocytes containing normal Ras genes This finding might be

clinically relevant in certain cancers where inactivation of the Smad4 tumor suppressor gene coincides with oncogenic Ras mutations, such as in pancreatic and colon carcinomas.

In addition to our studies with TGF-β_1, we have identified and characterized the PA2.26 antigen as a novel mucin-type transmembrane sialoglycoprotein involved in cell migration and carcinogenesis. PA2.26 is induced in epidermal keratinocytes and dermal fibroblasts during carcinogenesis and skin remodeling processes, although it is present in normal tissues (brain, lung, kidney) in epithelial, mesothelial and endothelial cells. It is located on plasma membrane projections (microvilli, ruffles), where interacts with the actin cytoskeleton probably through association with the ezrin and radixin members of the ERM protein family. Transfection experiments in normal (immortalized) keratinocytes indicate that PA2.26 is functionally involved in cell motility and carcinogenesis.

Signaling pathways in stress, differentiation and apoptosis

Group Leader: Jaime Renart

Our group is studying signaling pathways in different cellular situations. 1. We are interested in the specific degradation of the R1 subunit of the NMDA glutamate receptor when cells (both rat cortical neurons in culture or mammalian non-neuronal cells expressing the receptor with a vaccinia virus system) are treated with drugs that induce endoplasmic reticulum stress (tunicamycin, calcium ionophores, brefeldin or tapsigargin). We find a proteasome-dependent specific degradation of R1 subunit (vs. R2A subunit) with some but not all these compounds. 2. We are carrying structural studies of NMDA receptors (expressed with vaccinia virus in a non-neuronal cell line) with respect to a) differential stability of the different subunits; b) degree of glycosylation; c) presence in the plasma membrane; d) role as calcium channels, and e) activity of homomeric R2A receptors. We are also producing recombinant vaccinia viruses that overexpress the different subunits of the NMDA receptors to determine the structure of these proteins. 3. We are studying the roles of ERK and JNK protein kinases in apoptosis induced by PKC inhibition in N2A cells. We find that JNK is activated and ERK inhibited when apoptosis is induced. 4. We are also studying MAPK profiles in differentiating cells (either by serum deprivation, overexpression of MEKK1 or Geldanamycin treatment). ERK activity is continuously activated during the process; JNK is also activated, but its kinetics is dependent on the stimulus used to differentiate the cells. 5. We are studying the effect of Geldanamycin in different cell lines. Whereas this drug induces apoptosis in PC12 cells, N2A cells differentiate, and C2C12 cells undergo morphological changes not related to differentiation or death. 6. We are studying gene expression induced by TNFα. This cytokine has been described to be involved both in induction and protection from apoptosis. To gel a more clear picture of TNFα action, we want to identify the genes that are regulated by it. To this goal, we are using the Serial Analysis of Gene Expression (SAGE) technique.

Molecular bases of the thyroid hormone action on differentiation of nerve cells

Group Leader: M. Angeles Rodríguez-Peña

Myelination of axons in the Central Nervous System (CNS) is a process controlled by thyroid hormone (T3). We have reported previously that thyroid hormone regulates the steady-state levels of the mRNAs encoding the major myelin protein components and that this effect is due to the T3 action on the oligodendrocyte generation. Thus, T3 promotes the differentiation of the oligodendrocyte precursor cells (OLP) into mature oligodendrocytes "in vitro". Thyroid hormone binds specific nuclear receptors (encoded by the a and b genes) that must be considered as ligand-regulated transcription factors. To establish the bases for the T3 action on the different aspects involved in oligodendrocyte differentiation program, we have studied 1) the contribution of T3 to the exit from the cell cycle of the OLP as the obligatory step prior differentiation and have found that thyroid hormone expression impairs cell proliferation by increasing the steady-state levels of the inhibitor p27. 2) The genetic program (activation/repression of a group of genes) in the precursor cell triggered by T3 has been analyzed by DDRT-PCR with several putative regulated genes that will be confirmed and characterized.

Diffusible factors and signalling pathways implicated in the control of the inner ear development: regulation of apoptotic cell death.

Group Leader: Isabel Varela

The main objective of the group is to characterise the difusible factors and molecular mechanisms of action that regulate the early development of the inner ear. This study is focused in the study of programmed cell death. This project is divided in three particular objectives:1) Study of the role of IGF-I in the developing inner ear, 2) Study of NGF apoptotic actions: the inner ear as a model of neurotrophic factors-induced apoptosis, 3) Crosstalk between IGF-I and NGF signalling pathways. All these studies will offer new insights into the molecular mechanisms that control cell proliferation and apoptosis during inner ear organogenesis which may contribute to the development of future therapeutical strategies for inner ear therapy.