Group Leader: Amparo Cano

Regulation of E-cadherin expression and β-catenin signaling during tumor progression

Our studies are intended to understand the molecular mechanisms responsible for the downregulation of E-cadherin (ECD) expression 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 ECD gene expression, our previous studies on the ECD promoter indicated a major repressor role for a palindromic element, E-pal, containing two adjacent E-boxes (-76 to -86) as well as the modulatory role of a proximal CE region (-106 to -92) 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: the Zn-finger factor Snail (represented in 49% of the clones) and two members of the bHLH family, E47 and E2-2A (represented in 32 and 12% of the clones, respectively). The functional characterization of Snail and E47 has shown that both factors are strong repressors of the ECD promoter activity by direct binding to the E-pal element. Furthermore, ectopic expression of either factor in epithelial cell lines triggers a full epithelial-mesenchymal transition (EMTs) concomitant to the loss of ECD expression and acquisition of invasive and tumorigenic properties. In addition, the endogenous expression of Snail and E12/E47 factors in murine and human carcinoma cell lines correlates inversely with ECD expression. A similar expression pattern was detected in early mouse embryonic development. These data indicate that Snail and E12/E47 control EMTs which occur during development and at tumor invasion by repressing ECD expression and may be considered as new markers of tumor invasion. Recenlty, we have also characterized another factor of the Snail family, Slug, showing that it also acts as a repressor of ECD expression and as an inducer of EMTs. However, detailed binding studies of the different factors to the E-pal element indicate that the affinity ranges from Snail>E47>Slug. These data, together with the expression pattern of the three factors in early embryonic development support a differential function of the three repressors, maybe modulated by the specific cellular or tumoral context. At present we are characterizing the molecular mechanism(s) of repression of the ECD promoter activity by the different factors, as well as the

potential cooperation between them and their additional target genes. The expression of the different repressors in human tumor biopsies is also being analysed. With regard to the modulation of β-catenin signaling during tumor progression our previous studies showed that activation of H-ras (V12H-ras) in mouse keratinocytes induces the destabilization of ECD/catenin complexes from the cell-cell contacts and the cytoplasmic stabilization of β-catenin. These effects are concomitant to the loss of β-catenin/APC interaction and a significant increase in the metabolic stabilization of cytoplasmic β-catenin. The effects of V12H-ras on β-catenin relocalization and metabolic stabilization depend on the activity of PI3K, and are associated to the induction of a specific β-catenin/PI3K complex. 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. Our present studies are focused on the functional role of the β-catenin/PI3K complex. β-catenin/PI3K complexes are able to significantly stimulate the transcriptional actitvity of β-catenin/Lef-1 complexes in different cell lines. Our present results support that translocation of β-catenin/p85α complexes are needed for this event. On the other hand, we are also investigating the modulation of β-catenin levels during the cell cycle. The results obtained so far support a strict control of cytoplasmic β-catenin levels during G2/M phase of the cell cycle both in normal and transformed cells carrying a wild type version of APC. In addtion to those specific research lines, we continue investigating other aspects of tumor progression, such as the relation between ECD and/or β-catenin signaling and the organization and function of tight junctions (in collaboration with Dr. L. González-Mariscal) and the anti-metastatic effects of the E1A gene of adenovirus 5 (in collaboration with Dr. S. Ramón y Cajal and Dr. A. Fabra).

Group Leader: Juan Carlos Lacal

Rho GTPases in transformation and apoptosis and design of new antitumor drugs

Our group has demonstrated the relevance of the alteration in mitogenic signal transduction pathways related to phospholipids metabolism during carcinogenesis in human tumors. Among those enzymes involved in the regulation of phospholipids metabolism, we have identified phospholipase D (PLD) and choline kinase (ChoK) as critical enzymes in oncogenes-induced transformation. Previous

studies of our group demonstrate that newly designed inhibitors of the enzyme ChoK are potent antiproliferative drugs *in vitro* and have antitumoral *in vivo* activity. Our knowledge on the mechanisms of ChoK regulation under physiological conditions and its alteration after oncogenic transformation is quite limited. We are currently investigating the mechanism of regulation of the enzyme ChoK under physiological conditions as well as its participation in human carcinogenesis. The following questions are addressed: * Regulation of ChoK after stimulation of normal cells by growth factors in both human primary cells and immortalized, non-tumorigenic cells. * Alteration of ChoK regulation after transformation. This is performed in cell lines derived from human tumors * Analysis of the levels of ChoK and its activity in human tumors and stablished cell lines. Correlation with genetic alterations * Correlation of ChoK activity in tumor-derived cell lines and its response to antitumor treatment.

A second line of research in our group involves the family of Rho GTPases. Rho proteins constitute a subfamily of the Ras superfamily of GTPases. There are at least fifteen members, including the prototypes RhoA, Rac1 and Cdc42. The function of Rho proteins has been related to cytoskeleton organisation. However their ability to activate signalling pathways that impinge into the regulation of cell proliferation and apoptosis, demonstrate that Rho proteins participate also in these events, leading to cell transformation and cancer. Our group has been pioneer in the identification of signalling pathways modulated by Rho proteins demonstrating their role in cell transformation, the acquisition of the metastatic phenotype and the regulation of apoptosis. Thus, recently we have demonstrated that Rho proteins activate the transcription factor Stat3 and that this activation is required for their transforming activity. Also, we have demonstrated that under specific growth conditions, Rho proteins induce the synthesis of Fas-ligand (FasL) resulting in the induction of apoptosis. We are investigating further the mechanisms of activation of intracellular signalling pathways by Rho proteins and their relationship with transformation, metastasis and apoptosis.

Group Leader: Rosario Perona

Mechanisms of cell death and drug resistance induced by chemotherapeutic drugs

Cisplatin is a drug widely used in the treatment of cancer. One of the main problems in cisplatin therapy is the development of resistance to the drug that is the main cause of treatment failure. Cisplatin resistance can involve the activity of

different gene products such as the ones involved in drug transport, DNA repair or genes involved in the process of apoptosis. We have aproached this problem in two different ways: by investigating the signal transduction pathways triggered by cisplatin that are related to cell death and by using expression cDNA libraries of human genetic suppresor elements in order to identify genes that are involved in cisplatin sensitivity

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Group Leader: Angel Pestaña

1) Screening for potential EWS/FLI-1 targets in a human model of Ewing tumour

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. Since these chimeric transcription factors are thought to play a pivotal role in the pathogenesis of EFT, we have established a human embryonic cell line expressing the chimeric EWS/FLI-1 and FLI-1 proteins, in order to study the pattern of gene expression regulated by these proteins. using cDNA arrays (with 2304 different annotated genes) and Serial Analysis of Gene Expression (SAGE) methodologies. This study will also be extended to characterize the gene expression profile in samples of Ewing tumors and several tumor-derived cell lines, expressing the quimeric transcript and protein.

2) Molecular diagnosis of retinoblastoma and Ewing's tumor family.

In retinoblastoma we have studied more than 60 patients of hereditary retinoblastoma, having detected new polymorphisms and mutations, including some splicing mutations that we are currently characterizing. We are starting co-operations with research groups in Cuba, Colombia and Ecuador, in order to identify carriers in their country's population. In Ewing's tumors we are trying to establish correlations between tumoral genotype and metastasis phenotype. To this end we have established a multicentric study comprising patients treated according to the EWING-SEOP protocol, in wich the clinical data will be confronted with the genetic characteristics of the tumors (using comparative genomic hybridization and gene expression profiles in DNA arrays) and their

metastatic behaviour (measured as circulating tumour cells using quantitative RT-PCR technology).

Group Leader: Antonio Villalobo

Intercellular communication and signaling in normal and tumor cells

Our group is interested in the study of different mechanisms of intercellular communication and cellular signaling and their alterations in tumor cells. Among the projects in progress are: i) Phosphorylation of calmodulin by both the epidermal growth factor receptor (EGFR) and Src with emphasis on the functional consequences of these phosphorylations in the biological activity of calmodulin, and the action of different phospho(Tyr)calmodulin species on the activity and fate of the EGFR in intact cells; ii) Regulation of the EGFR by the Ca^{2+} signal in intact cells and the cross-talk between the calmodulin/phospho(Tyr)calmodulin system and protein kinase C; iii) Functional role of the calmodulin-binding and calmodulin-like domains of the EGFR, and their implication in tumorigenesis; iv) Regulation of the activity and fate of other receptors of the ErbB family by calmodulin; v) Functional role of calmodulin on the adaptor protein Grb7; vi) Regulation of EGFR-mediated cell proliferation by nitric oxide and the implication of the p38 mitogen-activated protein kinase (p38MAPK) pathway and a newly discovered Nitric Oxide-Induced 58 kDa Phospho-Protein (NOIPP-58) in this process; and vii) Activation of protein-tyrosine kinases in cells transfected with PA2.26, a cell surface mucin-like glycoprotein involved in cell motility and malignant tumor progression.

Group Leader: Pilar Eraso

Structure and function of ABC transporters

Our research activities are centered on the structure and function of eukaryotic ABC transporters and our main interests are first, to study the mechanism by which these proteins couple the energy of ATP hydrolysis to drive vectorial transport across a lipid bilayer and second, the structural basis of their substrate specificity. Our current projects include: a) Analysis of intramolecular interactions crucial for function of the yeast cadmium factor, Ycf1, a vacuolar ABC protein involved in resistance to Cd^{2+} and to exogenous glutathione S-conjugate precursors in *S.cerevisiae*. To this end we chose an intragenic suppression analysis of inactive mutations located in the nucleotide binding or regulatory domains. Our

results indicate a direct involvement of NBD1 Walker B region in coupling ATPase activity and substrate binding and/or transport. b) Study of the molecular determinants of substrate recognition in the yeast ABC protein Ste6, the a-factor pheromone exporter. With this purpose we have overexpressed Ste6 in the membrane of secretory vesicles that accumulate in a *sec6-4* yeast mutant strain and developed an *in vitro* assay for a-factor transport into the vesicles. The a-factor is a 12-amino acid lipopeptide with a C-terminal farnesylation and methylation. The ability of truncated and carboxyl-terminal modified a-factor analogs to compete with unmodified a-factor in transport assays will be examined.

Group Leader: Carlos Gancedo

Regulation of the glycolytic flux in yeasts and its relationship with catabolite repression

We have shown that the expression of genes that encode hexose transporters is altered in *Saccharomyces cerevisiae* mutants affected in hexokinase 2 activity. The expression of heterologous genes encoding hexokinases is able to suppress the deffect of the S. cerevisiae mutants. We have isolated mutants resistant to the catabolite repression produced by galactose. These mutants have altered either the galactose transport or the function of an activator that controls the transcription of the GAL genes. Also we have isolated mutants that become simultaneously resistant to the repressvie action of glucose and of galactose. A study of the MIG1 gene from *Candida albicans* has shown that in contrast with what happens in *S. cerevisiae* the product of this gene does not participate in the process of catabolite repression in this species. We are studying a gene from *Yarrowia lipolytica* that when expressed in multicopy corrects the phenotype of tps1 mutants from *S. cerevisiae* that show a deregulation of glycolysis. The sequence of this gene has homology with that of one gene from *S. cerevisiae* of unknown function.

Group Leader: Rosario Lagunas

Role of ubiquitin in signaling endocytosis of the maltose transporter in yeast.

We found previously that internalization in yeast of the maltose transporter, a protein with 12 transmembrane segments, requires binding of ubiquitin (Ub). Based on this fact, we investigated whether this internalization involves binding of Ub chains, or if binding of one Ub molecule is enough to trigger the process. To this end mutant Ubs carrying Lys/Arg mutations, which prevent the formation of various kinds of Ub chains, were over-expressed in mutant yeast cells lacking free Ub. The obtained results showed that monoubiquitination is sufficient to promote a maximal internalization rate of the transporter and demonstrated that, against what has been reported, binding of UB-Lys63 chains is not a general requirement for the maximal internalization rate of the 12 transmembrane proteins

Inactivation of the sugar transporters and its role in the preferential use of glucose by yeast.

The yeast *Saccharomyces cerevisiae* uses glucose preferentially to any other carbon source. The consensus is that inactivation of sugar transporters other than glucose transporters is one of the specific mechanisms that favour this preferential use of glucose. However, our previous results suggested that inactivation of these transporters is not a specific control mechanism but just consequence of an unspecific increase of protein turnover. It should be noted that these studies are performed using media without a nitrogen source and that starvation of this nutrient triggers a rapid protein turnover. We have investigated this possibility and our results strongly indicate that, in fact, inactivation of the sugar transporters is consequence of a rapid turnover and not of a specific control mechanism. In addition, they indicate that the contribution of this inactivation to the preferential use of glucose is much lower than generally believed.

Group Leader: María Jesús Mazón

Mechanisms of transport of Aminopeptidase I to the yeast vacuole

The yeast vacuolar enzyme Aminopeptidase I (API) is synthesized in the cytoplasm as a precursor (pAPI). Under vegetative growth conditions pAPI is packed into vesicles and transported to the vacuole via the cytosol to vacuole targeting pathway (CVT pathway). Under nitrogen starvation conditions autophagy is induced and pAPI is transported to the vacuole by autophagosomes (APG pathway). Both the cvt and apg pathways largely overlap in terms of the machinery used and general mechanisms. Yol082/Cvt19, identified by its ability

to interact with pAPI in a two-hybrid screening, is required for transport of API to the vacuole. Absence of Cvt19 precludes the enclosure of pAPI into transport vesicles. Cvt19 has been proposed as the receptor responsible for pAPI targeting to the vacuole. To get more information on the involvement of Cvt19 on the transport of pAPI to the vacuole, we are trying to find possible interacting partners of Cvt19 by co-immunoprecipitation experiments and two-hybrid screening.

Functional analysis of ITC1, a subunit of the chromatin remodelling complex Itc1-Isw2

Previous work showed that disruption of the ORF YGL133w produces both a decrease in the relative mating efficiency and an aberrant cell morphology, resembling the characteristic shmoo formed by cells exposed to mating factor. These phenotypes were found to be specific of *MATalpha* cells. We analyzed the activation state of the pheromone signaling pathway by determining the transcriptional activation state of one of the targets of the pathway, FUS1, a pheromone responsive gene required for cell fusion during the mating process. The *MATalpha* mutant cells showed constitutive activation of FUS1. Activation of the pheromone pathway was confirmed by showing that, in the *MATalpha* mutant strain, the MAP kinases Kss1p and Fus3p are in their phosphorylated and therefore activated form. We also showed that an intact pheromone response pathway is required for the observed alpha mating type-specific constitutive signaling. Subsequently, the protein was shown by others to be a subunit of an ATP-dependent chromatin-remodeling complex, and was renamed *ITC1*. The complex, formed by Itc1p and the product of *ISW2*, represses transcription of early meiotic genes during mitotic growth. We have shown that, in contrast to wild-type cells, in *MATalpha itc1* mutant cells and in the diploid homozygous *itc1/itc1* mutant, the a-specific genes are derepressed, thus pointing to an extensive alteration of the transcriptional repression as the underlying cause of the mutant phenotype.

Postranslational modification of the yeast cadmium resistance factor Ycf1

The yeast cadmium factor Ycf1 is a *Saccharomyces cerevisiae* protein member of the ABC transporter family. The Ycf1 protein localizes to the vacuolar membrane. To study the function of a putative phosphorylation site in the regulatory domain, the mutations Ser908/Ala, Ser/Asp and Ser/Glu were introduced by site-directed mutagenesis. The Ser908/Ala mutant is strongly affected in its ability to detoxify

cadmium ions. When the *in vitro* transport capacity of the three mutants was studied in vacuolar membrane vesicles, we found a low vmax of transport, for the Ser908/Ala Ycf1 variant, while the Ser/Asp and Ser/Glu protein variants showed a near wild-type vmax. Accordingly, the corresponding mutants Ser/Asp and Ser/Glu had recovered the ability to grow in a cadmium-containing medium. In addition, a Ser/Thr mutant, isolated as an intragenic suppressor of the Ser908/Ala mutant was found to recover near wild-type transport capacity and ability to grow in cadmium-containing medium. All together these data strongly suggest that the Ser908 residue is phosphorylated *in vivo* and that its phosphoryation is required for the Ycf1 protein function. Ycf1 was found to localize correctly to the vacuole in all four mutants but western blot analysis showed the presence of a doublet that was not detected in the wild-type. Our results, using vacuolar membranes prepared in the presence of phosphatases inhibitors are compatible with the presence of a second phosphorylation site, whose phosphorylation is modified by the changes introduced in the Ser908 residue.

Group Leader: Francisco Portillo

Structure, regulation and biogenesis of an eukaryotic ion-pump

Our main interests in the last two years have been focused in 1) domains of the yeast plasma membrane H^+-ATPase (Pma1) involved in degradation of misfolded Pma1. Misfolded Pma1 is degraded by different quality control machinery, all of them required a Rsp5-dependent ubiquitination of the mutant protein. By intragenic suppression analysis of misfolded *pma1* mutants we have found a conserved motif required for ubiquitination and degradation of the misfolded proteins. And, 2) isolation of genes involved in regulation and biogenesis of Pma1. The systematic analysis of the mutant phenotype of yeast protein kinases have allowed the identification of two protein kinases (Ptk2 and Kas1) affecting those proccesses. Ptk2 mediates the glucose-dependent H^+-ATPase activation and Kas1 is involved in the transport of the protein to the plasma membrane.

Group Leader: Juana María Sempere

Catabolite repression in yeast. Metabolism of xylose by *S. cerevisiae*

We have shown that the decrease in cAMP levels which occurs in the absence of glucose allows a rapid expression of the *FBP1* gene, but is dispensable for its long-term derepression. We have established that different elements in the promoter of the *FBP1* gene respond differently to glucose-dependent signals. We have found that a deletion of the *GPR1* gene, encoding a putative glucose sensor, has no effect on the activity of different enzymes subject to catabolite repression in glucose-grown yeast. We are studying the capacity of an engineered *S.cerevisiae* strain to produce ethanol from xylose.

Group Leader: Juan Bernal

Role of thyroid hormone receptors in brain development and function

Thyroid hormone (T3) is needed for proper brain development in mammals, including the human being. In the past, our laboratory has contributed in the identification of most of the known genes regulated by thyroid hormone during brain development, using the neonatal hypothyroid rat as a model. Among the most recently identified genes, Rhes is a member of the Ras family expressed predominantly in the striatum. One of our present lines of research is the identification of the signaling pathways regulated by the Rhes protein in rat brain and in cultured neuronal cells. T3 controls gene expression in brain, as in other tissues, by binding to protein members of the nuclear receptor superfamily. T3 receptors are encoded by two genes located in different chromosomes, and are known as TRα and TRβ. Several protein products which differ in the carboxy terminus are produced from the TRα gene. From these, only TRα1 is a bona fide receptor. From the TRβ gene three other receptor proteins, TRβ1, TRβ2 and TRβ3, as well as a T3 binding, truncated protein, are produced by modifications of the amino terminus. These receptor isoforms have a defined spatial and developmental pattern of expression, but the role of the individual receptor isoforms in brain development and function is largely unknown. In our laboratory we are using T3 receptor knock out mice to define the role of individual receptor isoforms. TRα1 accounts for about 80 % of all T3 receptor proteins in developing rat brain. However, TRα1 knock out mice do not present obvious signs of neural involvement, despite the profound alterations typical of hypothyroid animals. We have recently investigated the reason for this paradox by studying cerebellar development. Thyroid hormone is needed in cerebellar development for the timely migration of granule cels from the external germinal layer to the internal granular layer and for proper differentiation of Purkinje cells. We observed that granule cell

migration and Purkinje cell differentiation are normal in TRα1-deficient mice, indicating that TRα1 is not needed for these processes. Furthermore, inducing hypothyroidism in these mice resulted in no alterations, in contrast to the effects observed in wild type mice, ruling out a compensatory effect of TRβ. Our results suggest that the hypothyroid phenotype is due to inhibitory influences of the unliganded TRα1, perhaps due to gene repression, and not to the lack of thyroid hormone per se. New physiological functions for TRα1 have also been disclosed in adult animals. We have found that TRα1-deficient mice have an abnormal response in the contextual fear conditioning test. The behavioral alterations correlated with morphological abnormalities in the hippocampus, consisting in a significantly reduced number of perisomatic GABAergic terminals from the parvalbumnin-positive interneurons to the pyramidal cells of the CA1 field. These cells express predominantly TRα1 and therefore, absence of this receptor isoform could be responsible for the morphological and behavioral abnormalities. As a control, parvalbumin-positive cells of the neocortex, which express predominantly TRβ, are not affected in TRα1-deficient mice. Our results suggest that alterations of the TRα gene could be implicated in psychiatric syndromes in humans.

Group Leader: Piero Crespo

Functions and regulation of Ras and MAP-kinases

Our laboratory is working on ellucidating the mechanisms by which Ras isoforms are differentially regulated. Another line of research is the regulation of MAP kinase pathways by direct interactions between MAP kinases, at present focusing on the interactions between p38 isoforms and ERKs.

Group Leader: Teresa Iglesias

Functional study of the novel neurospecific protein Kidins220 and PKD in the nervous system.

Protein Kinase D (PKD) is a kinase activated by the protein kinase C family, whose members play a pivotal role in synaptic plasticity and brain function. We have cloned the first PKD substrate, Kidins220 (Kinase D interacting substrate of

220 kDa), a novel neurospecific protein whose gene is highly evolutionarily conserved, from *D. melanogaster* through *C.elegans* to humans. Kidins220 is an integral membrane protein with a polarized distribution, being enriched at the tip of the neurites and at the axonal growth cone in neurons. Kidins220 is phosphorylated following PKD activation and neurotrophin and ephrin tyrosine kinase receptor stimulation. All these facts suggest an important role for Kidins220 in neuronal function and brain development. Our main interest is to identify Kidins220 and PKD function in neurons, undertaking confocal and time-lapse microscopy studies on neuronal differentiation, neuritogenesis, growth cone formation and guidance, and synaptogenesis in hippocampal neurons cultured from E18 rats. Kidins220 mutants are being generated and expressed in these primary cultures and in PC12 cells to analyze if they provoke changes in these parameters. We are also interested in identifying Kidins220 interacting partners by immunoprecipitation followed by mass spectrometry protein sequencing ("Cellular Map Proteomics"), and by the yeast two hybrid system.

Group Leader: Gabriela Morreale

Metabolism of thyroid hormones at different developmental stages and prevention of neurodevelopmental damage.

Group leader: G. Morreale de Escobar

1) Research topics: a) Metabolism of iodothyronines in different structures of the human fetal brain. b) The hypothyroxinemia of the preterm infants and neurodevelopment. c) Iodine nutrition of premature neonates on parenteral feeding. d) Maternal hypothyroxinemia in the iodine-deficient rat and early neurodevelopment: alteration of migration of cerebral cells into the cortex.

Group leader: F. Escobar del Rey

2) Research topics: a) Iodine deficiency and maternal first trimester hypothyroxinemia in Spain. b) Iodine deficiency and maternal hypothyroxinemia in Europe. C) Adaptation of the fetal thyroid to iodine deficiency.

Group Leader: Alberto Muñoz

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

Our group is focussed on the study of the effects of the nuclear receptors for thyroid and glucocorticoid hormones and vitamin D on different aspects of cancer and central nervous system development. We study the mechanism of action of vitamin D and non-hypercalcemic derivatives in human colon cancer cells and tumor biopsies, trying to characterize their protective effect and to identify target genes. In breast cancer, we intend to characterize the effect of thyroid hormone by studying their biological activity in cultured cells and the integrity and expression levels of the different thyroid receptors in human tumors. In addition, we have investigated the molecular basis for the activity of thrombospondin–1, one of the most potent natural antiangiogenic agents. Finally, we are also interested in the mechanism of the antagonistic effect of activated nuclear hormone receptors, particularly glucocorticoid receptor, on AP-1 action. In the developing rat brain we try to identify and study 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.

Group Leader: María Jesús Obregón

Regulation of UCPs, leptin and deiodinases in adipocytes

Our main interest is the study of several genes involved in the activation of thermogenesis and energy expenditure or in related functions in brown adipocytes.

1. Regulation of the UCP-1 gene. Its mRNA expression is estimulated in brown adipocytes by NE, T3, and these increases modulated by insulin and glucocorticoids. The regulation of the UCP-1 gene promoter is also studied using transient tranfections, specially its regulation by glucocorticoids, which effects are inhibitory near confluence and stimulatory during the period of differentiation.

2. The regulation of UCP-2 and UCP-3 mRNA had been studied using T3, Triac, NE, insulin and tiazolidiendiones (TZD), ligands or the PPARgamma receptor.

3. Triac, a natural metabolite of T3, is a potent thermogenic agent in brown adipocytes as compared with T3, increasing the adrenergic stimulation of UCP-1 mRNA and D2 activity, as well as D3 and LPL mRNA. Triac and T3 actions have also been compared in rats using constant infusion, using different tissues and comparing its actions on several genes. Triac concentrations were measured in plasma and tissues.

4. The regulation of leptin secretion and mRNA by T3, Triac, insulin and serum, has been studied using brown and white adipocytes.

5. Regulation of type II 5'Deiodinase (D2) activity and mRNA in cultured brown adipocytes. D2 is adrenergically stimulated by NE only if T3 is present. The actions of insulin and glucocorticoids are also reported.

6. Regulation of Deiodinase activities in fetal rat tissues and human developing brain. Those studies are carried out mainly in situations of iodine deficiency or diabetes in rats and in human fetal brain areas during the fetal development.

Group Leader: Pilar Santisteban

Mechanisms of transcription and signalling involved in the control of thyroid cell differentiation and proliferation

Research topics: a) Expression of tissue-specific genes in the mechanisms of thyroid cell differentiation: Role of transcription factors (activators and repressors) 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. 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 and PCC13 thyroid epithelial cells. These cells express a set of tissue specific genes (thyroglobulin, thyroperoxidase and Na+/I-symporter, NIS) responsible for the thyroid hormone synthesis. The expression of above genes is under hormonal control, being the thyrotropin (TSH) via cAMP and the insulin, thought the IGF-1 receptor, the main regulators. In addition, the above genes are expressed in a tissue-specific manner. Three thyroid specific transcripción factors mediate their expression. They are members of the homeo-box (TTF-1), forkhead (TTF-2) and paired-box (Pax-8) families of transcripcion factors and play an important role also in the hormonal control. Since TTF-2 is a forkhead transcription factor and due to the structural similarity of these factors with histone linker, we are studying how changes in higher order chromatin structure control thyroid-specific gene transcription. In the case of NIS, the regulation is more complex, as insulin/IGF-1 inhibits the TSH induction of NIS. This mechanism involved the PI3K. Other important modulator of NIS expression is TGFß, and this growth factor also sinhibis the TSH induction of NIS expression vía Smads proteins. 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. As the TTF-1 promoter does not have GRE elements our data suggest the glucocorticoids are regulating TTF-1 expresion in our model of lung hypoplasia by a mechanism involving the AP1 elements. More recently, we have shown that the transcriptional repressor DREAM play an important role in thyroid-specific gene expression, as a DRE element is part of the TTF-1 and Pax-8 binding sites. This repressor is under Ca2+ and cAMP control and our current hypothesis is that in thyroid cells the increase in cAMP levels, removes DREAM from its site in the promoters and then TTF-1 and Pax-8 bind, regulating the expression of their targets genes. 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 has a different response to external signals. Previously we 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). 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 p27kip, 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. As the TSH-R and SS-R are receptor of seven trans-membrane domains coupling to G proteins, we are studying the mechanisms of action of G proteins in this system. Ras is important in thyroid cells proliferation and transformation, this together with the observation that other member of the small GTPasa, RhoA, mediates the proliferation in response to TSH, we have studied the role of these proteins. Our results show that the cells over-expressing the constitutive active RhoA promotes transformation and loss of thyroid cell differentiation interfering with TTF-1 activity. Other important protein that we have identified in thyroid, as mediator in the signalling transduction, is DARPP-32, a PP1 inhibitor. We are currently studying the functional role of this molecule in the response to TSH and IGF-1 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 the same mechanism in epithelial cells as well as the functional role of this apoptotic process induced by MDM-2. In addition MDM2 sensitizes MTT cells to gamma radiation, a mechanisms that is mediated by E2F-1 .

Group Leader: Mario Vallejo

Cortical astrocyte differentiation during embryonic development

Previous studies in our laboratory indicate that stimulation of cAMP-dependent signaling in cortical precursor cells triggers their differentiation into astrocytes. An integral part of the astrocyte differentiation process is the activation of transcriptional mechanisms that regulate glial fibrillay acidic protein (GFAP) gene expression. For this reason, one of our lines of research deals with the analysis and characterization of regulatory elements in the GFAP gene promoter. We have identified one element, located in close proximity to the TATA box that regulates cAMP-dependent transcription. The sequence of this regulatory element is not related with previously known cAMP-response elements.

Regulation of gene expression by homeodomain transcription factors in the central nervous system

We are studying the mechanisms by which two homeodomain transcription factors, named Opx-1 and Drx-1, regulate embryonic neural development. One aspect in which we have focussed our attention is the regulation of GFAP gene expression by these proteins during cortical astrocyte differentiation. GFAP gene expression is essential to maintain normal astrocyte functions, the alterations of which are related with some neurodegenerative diseases of the central nervous system. In addition, we are studying the possible involvement in the mechanisms that control the closure of the neural tube. Defects in the closure of the neural tube give raise to congenital malformations that are relatively frequent in humans.

Transcriptional regulation of insulin gene expression in pancreatic islets

We found that Opx-1, besides being expressed in the central nervous system, is expressed in beta cells of the pancreatic islets of Langerhans. This prompted us to investigate whether Opx-1 contributes to the regulation of insulin gene transcription. Our studies revealed that Opx-1 recognizes a regulatory element of

this gene known as E2-A3/4. At this site, Opx-1 interacts functionally with other homeodomain and bHLH transcription factors.

Group Leader: Juan José Aragón

Molecular bases of the control of glucose consumption in eukaryotic cells. Non-invasive evaluation of lactose intolerance with galactosil-xylose.

Our research includes:

- Structure/function relationships of control of eukaryotic phosphofructokinase (PFK). Specific sequence manipulation of various eukaryotic isoforms that differ in their regulatory properties to identify amino acid residues involved in the response to specific ligands. We found that the unique C-terminal extension of the nonallosteric PFK from *Dictyostelium discoideum* (DdPFK) is responsible for its lack of specific control properties. Systematic deletions and point mutations at this region showed that the last C-terminal residue, Leu^{834}, is critical to produce an allosteric enzyme. This finding provided a new interpretation of the allosteric control of PFK. Structural studies of wild-type isoforms from different sources and allosteric mutants are in progress.

- Interaction of glycolytic enzymes with microtubule formation. DdPFK was found to be a potent inhibitor of tubulin polymerization. We are now investigating the structural motives responsible for this behavior by comparison with other proteins that negatively modulate microtubule dynamics and subsequent site-directed mutagenesis analyses. Other approaches to study the significance of this interaction as well as the function of DdPFK in vivo, such as its overexpression in *D. discoideum* and the generation of a null mutant, are being carried out.

- Evaluation of intestinal lactase with galactosyl-xylose as a non-invasive test for the diagnosis of lactose intolerance. Measurement of blood xylose after oral administration of 4-galactosyl-xylose to suckling rats allowed us to evaluate intestinal lactase activity in vivo. This technique was found to be as reliable as determination of the sugar in urine and required a minimal sample of blood, so it might be of potential interest in pediatrics. The contribution of the lactase and florizin-hydrolase catalytic sites of lactase to the hydrolysis of 4-galactosyl-xylose has been assessed in comparison with the regioisomers 3- and 2-galactosyl-xylose, which differ in catalytic efficiency. Preliminary assays in adult voluntaries have indicated that this methodology is valid for the non-invasive diagnosis of lactose intolerance in humans.

- Molecular genetics of intestinal Na^+-glucose cotransporter to evaluate the physiopathological role of mutations in the *SGLT1* gene in infants affected by glucose/galactose malabsorption.

- Structural studies with aspartate kinase from *Saccharomyces cerevisiae* to investigate the role of the oligomeric state on its threonine regulation and catalytic activity.

Group Leader: Claudio Fernández-Heredia

Interactions in the metabolism of mono- an disaccharides in *Saccharomyces cerevisiae*

We have continued the work on the secretion of glucose by yeast fermenting maltose, in response to the presence of hexoses in the medium, mainly on the kinetics and characteristics of the inactivation/reactivation of a protein factor involved in the process.

Group Leader: María Antonia Günther

Synthesis and modification of (di)nucleoside polyphosphates

Dinucleoside polyphosphates may have important signalling functions, both inside and outside the cell. Their intracellular concentration is determined by their rates of synthesis and degradation. In the last years our group has been mainly involved in the search of new ways of synthesis of those compounds. During this investigation it was found that T4 RNA ligase, an enzyme that catalyzes the formation of a phosphodiester linkage between neighboring 3'-hydroxyl and 5'-phosphoryl end groups in RNA or DNA, is able to catalyze the synthesis of dinucleoside polyphosphates; in addition these compounds behave as RNA analogues accepting cytidine 3',5'-bisphosphate in their 3'-OH groups. Studies with *E.coli* poly(A) polymerase revealed that this enzyme, besides adding poly(A) tails to RNA, is able to adenylate the 3'-OH residue of various nucleosides, nucleoside 5'-phosphates and dinucleotides polyphosphates. This last finding may have physiological significance as millimolar concentrations of nucleosides/ nucleotides may compete with the adenylation of RNA in vivo

Theoretical aspects of the purine nucleotide metabolism

This type of work comprised the following steps: setting up the velocity equation for each enzyme, taking into account the kinetic constants and the influence of potential effectors on the actual velocity; writing the differential equations describing the changes in the concentrations of substrates and intermediate products as a function of time, and solving the differential equations with the help of the Mathematica 3.0 program. This method allows to approach theoretically, situations that can not be experimentally tested in vitro; and to predict the metabolic pathways affected, observing the changes in the metabolite concentrations, as a result of a particular situation (stress for instance). This procedure has been applied so far, to the study of the purine metabolism in rat brain cytosol.

Group Leader: Pilar Llorente

Mechanisms of cellular resistance to antifolates: Evaluation of new FPGS inhibitors as possible anti-tumoral agents.

Biochemical events associated with resistance to MTX include changes in the ·target· enzyme DHFR, in the activity of other enzymes folate-dependent and in the drug uptake and its metabolism, specially its conversion to polyglutamyl derivatives (MTX-Gln), mediated by the FPGS enzyme.

We have continued the study of the FPGS enzyme, as a new drug ·target,· in MTX-resistant LLA-L5178Y murine cells, assaying: a) new pterine analogs and different MTX metabolites as inhibitors of FPGS. b) The action of these products on the biochemical events affected by the MTX-Gln: the purine biosynthesis and the folate-dependent enzymes GAR and AICAR formyltransferases.

C9-N10 FSE (Folate splitting enzyme) activity has been determined and the main products of the reaction, PABA-Glu and pterine derivatives, Pterine-6 aldehyde and Pterine-6 COOH, previously described as FPGS inhibitor, were identified by HPLC.

Group Leader: Antonio Sillero

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Enzyme kinetics: theoretical aspects

A combined analysis of enzyme inhibition and activation has been developed, based on a rapid equilibrium model assumption in which one molecule of enzyme binds one molecule of substrate and/or one molecule of a modifier. The modifier

acts as activator (essential or non-essential), as inhibitor (total or partial), or has no effect on the reaction rate, depending on the values of the equilibrium constants, the rate constants of the limiting velocity steps, and the concentration of substrate. Particular attention has been paid to the analysis of the crossing point of the straight lines of Lineweaver-Burk plots. As a corollary of this study, an easy method to transform the ratio of two polynomials of first degree into a Michaelis-Menten equation has been developed

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Group Leader: Susana Alemany

Cot kinase in T cell activation

The Cot/tpl-2 gene encodes a MAP kinase kinase kinase that is potentially capable of switching on several MAP kinase cascades. These signal transduction pathways connect Cot kinase activity with the up-regulation of several transcription factors, sush as AP-1 and NFkB, which leads to the actvation of the TNF-α and IL-2 promoters. In the past two years we have shown that Cot kinase activates COX-2 gene expression in Jurkat T lymphocytes, mainly by increasing the NFAT mediated transactivation activity (de Gregorio, et al 2001). We have also shown that T cell activating signals increase the transcriptional activity of Cot promoter and consequently the expression levels of Cot mRNA in T lymphocytes (Sanchez-Gongora, et al 2000). This observation indicated that Cot kinase could be also implicated not only in G0 to G1 phase cell cycle transition, but also in the G1 to S phase transition. Experiments performed in a IL-2 dependent T lymphocyte cell line pointed out that Cot kinase contributes to the G1 phase progression through the cell cycle by regulating the E2F activity and p27kip levels (Velasco-Sampayo, et al 2001).

de Gregorio, R., M. A. Iniguez, M. Fresno and S. Alemany (2001). "Cot kinase induces cyclooxygenase-2 expression in T cells through activation of the nuclear factor of activated T cells." J Biol Chem 276(29): 27003-9. Sanchez-Gongora, E., C. Lisbona, R. de Gregorio, A. Ballester, V. Calvo, L. Perez-Jurado and S. Alemany (2000). "COT kinase proto-oncogene expression in T cells: implication of the JNK/SAPK signal transduction pathway in COT promoter activation." J Biol Chem 275(40): 31379-86. Velasco-Sampayo, A. and S. Alemany (2001). "p27kip protein levels and E2F activity are targets of Cot kinase during G1 phase progression in T cells." J Immunol 166(10): 6084-90.

Group Leader: Carmela Calés

Molecular mechanisms of re-replication control: megakaryocytic endomitosis.

We are interested in the molecular mechanisms underlying the establishment of endoreplication cycles during megakaryocytic endomitosis. We have focussed on whether cell cycle regulators are differentially regulated to allow established megakaryoblastic cell lines to re-enter S phase without completing mitosis. We have determined that G1/S transition cyclin-cdk complexes remain active during endoreplication cycles, through stabilization of cyclin E and down-regulation of p27kip1 inhibitor. From these basic results, different lines of development have arisen. First, we have asked if such differential regulation is ultimately controlled through a transcriptional program. We have found that escargot, a transcriptional repressor belonging to the snail family of Zn-finger proteins interferes with megakaryocytic differentiation by impeding the establishment of endomitosis. Furthermore, we have identified endogenous nuclear proteins which DNA-binding activity is differentially regulated in cells that do and do not undergo endoreplication. We are at present investigating the nature of such factors, their possible targets and their putative implication in megakaryocytic differentiation program. A second question we have aimed to answer is whether DNA replication initiation machinery is regulated during endoreplication cycles. We have focussed on one of the factors implicated in origin assembly and in re-replication prevention, namely cdc6. We have found that cdc6 expression is differentially regulated during endomitosis through both maintenance of transcription and cyclin E-mediated protein stabilization. Work is in progress to determine if a similar regulation operates on the other essential licensing factor, cdt1, and its regulatory partner geminin. Finally, we have been investigating these events on primary megakaryocytes. By comparing the ability to establish endomitotic cycles of ex vivo cultured megakaryocytes from Umbilical Cord Blood (UCB) and Adult Peripheral Blood (APB), we have found that fetal megakaryocytes do not reach full maturity in terms of becoming polyploid, probably due to lack of regulated expression of G1/S cyclins. We are presently studying whether this relative immaturity is on the basis of the delay in platelet recovery observed in patients transplanted with UCB when compared with adult Bone Marrow or APB.

Group Leader: Víctor Calvo

Role of the EPH receptors and their ligands in the immune system

Tyrosine kinase receptors (RTKs) are a family of cell surface proteins whose cytoplasmic portion has tyrosine kinase enzymatic activity. The Eph subfamily includes A type receptors (Eph-A1 a A8), that bind preferentially to glycofosfatidylinositol (GPI)-linked ligands (ephrins-A1 to A5), and B type receptors (Eph-B1 to B6) that bind preferentially to transmembrane ligands (ephrins-B1 to B3). The functions of the Eph receptors and their ligands seem more related to cellular migration processes than to cellular proliferation. The detection of the mRNAs of some Eph receptors and their ligands in lymphoid organs suggests their possible involvement in the ontogeny and the functions of the Immune System. The confirmation of this hypothesis would allow to modulate the immune response for the benefit of the individual's health.

Group Leader: Sebastián Cerdán

Functional Magnetic Resonance Laboratory

The Functional Magnetic Resonance Laboratory develops novel Magnetic Resonance Approaches to understand the biochemistry, physiology and pathology of living systems. Current research interests involve: Magnetic Resonance approaches to Cancer diagnosis and prognosis, 13C NMR studies of cerebral metabolism, Studies of water trafficking, the basis of MRI contrast and the development of higher performance contrast agents for increased MRI resolution and specificity. The application of NMR methods to cancer research have provided algorithms for the diagnosis of tumors using artificial intelligence techniques and procedures to visualize extracellular pH in tumors in vivo, using a new series of pH sensitive contrast agents produced by our laboratories. 13C NMR studies of cerebral metabolism revealed the relative selectivity for glucose or lactate in primary cultures of neurons and astrocytes or in the intact, adult rodent brain. It was possible to show that glial cells depict an active oxidative metabolism and that the glutamine cycle demands oxidative activity in the astrocytes. Water metabolism was reviewed and studied with an enphasis on hydrogen turnover of 13C labeled isotopomers. Our results allowed to resolve for the first time the intracellular trafficking of glutamate and aspartate through the malate aspartate shuttle. Finally, a new series of highly efficient paramagnetic contrast agenst has been patented.

Group Leader: Jorge Martín

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

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 pre-associated 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 (Mol.Endocrinol. 11: 1461-1467; Biochem J. 345:17-24). The activation of both enzymes, emanating from the receptor dimerization, is independent from each other and at the same time is essential for cell proliferation (Biochem J. 345:17-24; Mol Biol Cell 12: 2171-83). 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 (lambda 5 or IL -2Ralfa 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 (Cell Growth Diff. 10:583-590). 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. Recently, our work was focused on studying the role of the SFK as mediators of PRL induced proliferation of T47D and MCF7 human breast cancer cell lines. The mitogenic activity of prolactin on these cells was confirmed and it was found that is associated with increased activity of kinases of the SFK, FAK/Erk1/2 as well as the PI3K dependent p70S6K and Akt kinases. All these prolactin-stimulated cellular events depend on the primary activation of c-Src, as they are abrogated by

the expression of a negative dominant of c-Src, and by the SFK inhibitor PP1. Inhibition of the Erk1/2 pathway revoked prolactin stimulation of T47D and MCF7 proliferation, without altering activation of the PI3K pathway signaling. Similarly, inhibition of PI3K blocked prolactin activation of p70S6K and Akt as well as cell proliferation, but did not alter prolactin activation of Erk1/2. Therefore, these findings define a prolactin signaling cascade in T47D and MCF7 where c-Src is the mediator for stimulation of Fak, which in turn controls Erk1/2 activation, subsequently required for induction of proliferation. Independently of Fak-Erk1/2 pathway, c-Src also moderates PRL-activation of the PI3K cascade, which is also needed for T47D and MCF7 proliferation (In preparation). Since in addition of prolactin other known mitogens are also involved in breast cancer development, we are analysing the interactive signalling mechanisms among them using anti-tumoral agents.

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 on hemopoietic and breast cancer cells are ongoing in the laboratory.

Group Leader: María Angeles Pajares

Structure-function relationships in proteins of pathological interest

We have been able to show the presence of an intrasubunit disulfide in methionine adenosyltransferase (MAT). This disulfide is established between residues C35 and C61, and its role seems to be related to the stability of tetrameric (MAT I) and dimeric (MAT III) forms of the enzyme. In parallel, a refolding procedure for MAT obtained from *E. coli* inclusion bodies has been established, and the protein forms obtained characterized and compared to liver-purified MAT isoenzymes. The data indicate that refolded forms are very similar to the liver enzyme, and thus we have used them for structural studies. MAT I structure has been solved, and the methionine binding-site identified. In addition, crystals of betaine homocysteine methyltransferase have been also obtained, but until the moment the structure has not been completely solved. Other projects of our laboratory are devoted to the study of PrP interactions with several ligands.

Group Leader: Ana Aranda

Mechanisms of transcriptional regulation by nuclear receptors

Nuclear receptors can activate or inhibit gene transcription in a ligand-dependent manner through binding to DNA hormone response elements (HREs). Using as a model both HREs ligated to heterologous promoters or natural HRE-containing promoters (such as the growth hormone or prolactin promoters, the HIV virus long terminal repeat etc), we are examining the mechanisms of stimulation of gene transcription by thyroid hormone, estrogen, retinoic acid and vitamin D receptors. Our results indicate the complexity of this regulation in which participate not only coactivators an corepressors whose interaction with the receptors depends on binding of the corresponding ligand, but also other transcription factors both ubiquitous and tissue-specific which can determine the receptor transcriptional activity. The situation is even more complex, since association of the receptors with components of the basal transcriptional machinery and with components of the RNA Polymerase II holoenzyme, as well as interactions of these factors with coactivators and corepressors modulate activation by nuclear receptors. In the case of negative HREs, the molecular mechanisms of transcriptional repression by the receptors are still poorly known. The unoccupied thyroid hormone receptors (TRs) cause an increase of transcription which is reversed by the ligand. In this paradoxical stimulation, post-translational modifications of chromatin components, such as histone acetylation or methylation appear to play an important role.

Mechanism of neuronal differentition by retinoic acid

Retinoic acid (RA) causes neurite extension in A126-1B2 cells, a PC12 cell subclone deficient in protein kinase A (PKA) activity. A126-1B2 cells express higher levels of RA receptors than parental PC12 cells, in which RA does not cause morphological differentiation A central process in differentiation of PC12 cells by neurotrophins such as nerve growth factor (NGF), is the rapid expression of the so called “immediate early genes” as a result of the activation of the mitogen activated protein kinase (ERK) cascade. Activation of this cascade leads to phosphorylation, among other transcription factors, of the transcription factor CREB (cyclic AMP response element binding protein), which appears to play an important role on NGF-mediated gene expression and neuronal differentiation. We have observed that RA causes a rapid and sustained CREB phosphorylation and an increase in the activity of this transcription factor in A126-1B2 cells. This activation requires previous stimulation of ERK activivity which occurs upon minutes of retinoid treatment. As a consequence, RA activate transcription of genes such as c-fos, c-jun or jun-B, which do not contain RA response elements

(RAREs), but contain CRE or TRE motifs. The data obtained suggest that RA stimulates signalling pathways and expression of early genes involved in neuronal differentiation by a non-genomic mechanism that does not appear to involve binding of its receptors to RA response elements.

Inhibition of ras-mediated responses by the thyroid hormone receptors

Thyroid hormone receptors (TRs) can modulate transcription by direct binding to response elements, or by modulating the activity of other signal transduction pathways. Both the thyroid hormone T3 and the ras oncogene, which is often mutated in tumors, play an important role in cell proliferation and differentiation. We have demonstrated that T3 strongly antagonizes Ras-induced proliferation in neuroblastoma N2a-b cells. One of the main targets for the proliferative effects of Ras is cyclin D1, and T3 blocks Ras-mediated transcriptional stimulation of the cyclin D1 gene. T3 also inhibits stimulation of the cyclin D1 promoter by Raf or MAPK (but not by RSK2). Antagonism of ras-mediated transcriptional responses by T3 is also observed in other cell types and with other promoters, including the p21 and p27 CKIs. In the case of cyclin D1 activation by Ras is mediated by proximal promoter sequences which contain a CRE. Mutation of this element abolishes the response to the oncoprotein. This sequence binds factors such as CREB or ATF-2, and their transcriptional activity is stimulated in N2a-b cells expressing oncogenic Ras. Furthermore, T3 directly represses transcriptional activity of both factors. These results indicate that thyroid hormones have profound modulatory effects on signalling pathways stimulated by the ras oncogene which could potentially play an important role not only in proliferation, but also in cellular transformation. This hypothesis is confirmed by the finding that oncogenic Ras is unable to cause transformation of fibroblasts transfected with TRs in foci formation assays upon incubation with T3. We are analysing the possibility that this receptor could also inhibit tumor cell growth “in vivo” in nude mice.

Transcriptional antagonism between the thyroid hormone receptor and the transcription factor CREB

Combinatorial regulation of transcription involves binding of transcription factors to DNA as well as protein-protein interactions among them. We have demonstrated the existence of a mutual transcriptional antagonism between the

thyroid hormone receptor (TR) and the cyclic AMP response element binding protein (CREB), which involves a direct association of both transcription factors. In pituitary cells, T3 reduces CREB phosphorylation in serine 133, inhibits CREB transcriptional activity, and represses activation of CREs (cAMP response elements)-containing promoters which lack thyroid hormone response elements, TREs. In turn, expression of CREB reduces TR-dependent transcriptional responses. Association of TR with CREB decreases “in vitro” binding of both transcription factors to their cognate DNA elements, inhibits the ability of protein kinase A to phosphorylate CREB at Serine 133, and leads to a reduction of the ligand-dependent recruitment of p160 coactivators by TR. These results indicate the existence of a transcriptional cross-talk between CREB and TR signalling pathways which can have important functional consequences in pituitary cells, since it modulates the expression of genes such as the transcription factor GHF-1/ Pit-1 which is required for the expression of growth hormone, prolactin or thyrotropin.

Group Leader: Margarita Cervera

Genomic approaches to muscle development: Identification of transcriptional regulatory elements involved in the regulation of Drosophila muscle genes by in vivo analysis.

Muscle genes required for the elaboration of the contractile apparatus are activated coordinately. Gene transcription is typically regulated by the interaction of multiple enhancers, each of which may contain the binding sites for multiple transcription factors. One of the goals of the lab was to determine whether these coordinately regulated genes share a common regulatory mechanism. To do this we have been studying the regulatory mechanisms controlling two distinct Drosophila muscle genes. First, the gene encoding TnT, which is a thin filament protein and the other one encoding paramyosin /miniparamyosin, which are protein components of the thick filament. The approach we have used has been the generation of transgenic lines using the P-element mediated transformation. We have linked LacZ gene to selected putative regulatory regions. In vivo transgenic expression was studied in the generated lines. The transgenic lines were identified by the presence of the white gene in the offspring. The efficiency of transcription among the different constructs was assessed measuring the timed appearance of β-galactosidase activity in larval and adult muscles. Comparison of the identified regulatory enhancers with other muscle enhancers allows to

determine whether these genes share a common regulatory mechanism. A second goal in the lab was to know the effect of overexpression or decrease of these muscle proteins in flight muscles. To investigate the function of Troponin T or miniparamyosin, we have produced transgenic organisms expressing distinct levels of TnT or mPM in the IFMs. We have tested the consequences of the distinct levels of protein and also the introduction of Familial Hipertrophic Cardiomyopathy-induced TnT mutations in the TnT sequence upon the structure and function in these muscles.

Group Leader: Jesús Cruces

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

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 muscle and nerve system formation.

- The Drosophila ortholog of human POMT2 gene, involved also in muscle and nerve 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.

- The novel gene encoding an adhesion protein possibly implied in non small cell lung cancer and other cancers.

Group Leader: Carmen García-Vallejo

Thyroid hormone regulation of mitochondrial function

Thyroid hormone (TH) regulates mitochondrial respiratory rate by activating coordinated transcription in the nucleus and mitochondria. Whereas TH activates transcription of mitochondrial genes directly, the activation of nuclear-encoded

mitochondrial genes is probably executed by indirect unknown mechanisms. Nuclear respiratory factors NRF-1 and GABP/NRF-2 may function as transacting genes, but regulation of these genes by TH is not demonstrated. We show that TH administration to hypothyroid rats promptly increases GABP/NRF-2 α-subunit mRNA levels in the liver, without significant changes in β, γ subunits. In run-on and time-course experiments, the transcription rate and protein levels increased three-fold in response to TH, indicating GABP/NRF-2 transcriptional regulation. The results also support the notion that ATP synthase β-subunit, a nuclear-encoded mitochondrial gene, is regulated by TH through the indirect activation of GABP/ NRF-2. Mitochondria localise in specific cytoplasmic regions with the aid of microtubules. To investigate whether TH may be involved in mitochondrial dynamics, we have analysed tubulin expression in two mammalian species, rat and mouse. In both, tubulin protein expression is highly tissue-specific but α and β subunits in a given tissue are observed at the same level. However, during the postnatal development of the rat liver, α subunit is found at high concentration soon after birth whereas β is very low. In the following days, the amounts of both subunits change with patterns that lead to similar concentrations in the adult liver. In hypothyroid animals, α subunit is however low after birth. In adult rats, tubulin expression also appears affected by TH. β subunit protein is lower in hypothyroid animals and increases after TH administration. To analyse the likely differential involvement of TH receptors in tubulin expression, we have measured the two subunits in wild-type mice and animals either lacking α or β receptors. β tubulin increases or decreases when either α or β receptors are lacking, respectively. In hypothyroid animals, the protein increases in animals lacking β receptor and decreases in the other two cases. TH administration reverses the effect. The results are compatible with the β receptor being required for TH action in tubulin expression and the TH-occupied α receptor having a repressor effect. The analysis by confocal microscopy shows that tubulin cytoskeleton is somewhat disorganized in knockout animals, especially in those lacking β receptor. However, mitochondria localization does not appear grossly affected.

Group Leader: Rafael Garesse

Pathophysiology of mitochondrial biogenesis

Mitochondria play a pivotal role in cell physiology, producing the cellular energy and other essential metabolites as well as controlling apoptosis by integrating

numerous death signals. Consequently, there is an extense group of multisystemic and devastating pathologies produced by mutations in mitochondrial genes. Mitochondrial biogenesis is an extremely complex and well orchestrated process that depends on the coordinated expression of two genomes, nuclear and mitochondrial. To provide cells with the correct number of structural and functional differentiated mitochondria, a variety of transcriptional and post-transcriptional mechanisms are involved, that are modulated by a variety of physiological signals including environmental stimuli and developmental cues. Our knowledge of the factors involved in the regulation of mitochondrial biogenesis is still in its infancy. In our group we are carrying on three different lines: i) Using *Drosophila melanogaster* as model system we are applying a combination of cell culture and in vivo approaches in order to characterize factors involved in the control of mitochondrial differentiation and mitochondrial DNA replication and maintenance. In particular we have focused our atention in the study of several genes of the replication/transcription machineries, including mt-DNA and mt-RNA polymerases, mt-helicase, mtTFA and mtTFB ii) Mitochondrial alterations underlying encephalomyopathies and neurodegenerative diseases involve mutations in genes encoded in mitochondrial DNA (mtDNA) or nuclear DNA. Although a high and increasing number of mutations have been described to date, in the majority of the cases the pathogenic mechanisms remain unknown. We are studying in molecular terms several mtDNA mutations responsible of mitochondrial encephalomyopathies in transmitochondrial hybrid cell lines. They include mutations in tRNA and protein coding genes. We are also interested in identifying the nuclear genes responsible of the mtDNA depletion syndrome. iii) We have started to develop an animal model of mitochondrial pathology in *Drosophila melanogaster*, a system succesfully used in the study of neurodegenerative diseases. We have overexpressed in transgenic lines a mutated version of the catalytic subunit of the mitochondrial DNA polymerase lacking proofreading activity. Mutant fly lines will be characterized under a cellular, biochemical and molecular point view in order to identify potential mitochondrial dysfunctions. We will extend this approach generating transgenic flies harbouring mutations in genes encoding factors of the mtDNA replication machinery already described in human pathology.

Group Leader: Roberto Marco

The Regulation of the muscle contraction in Drosophila and the adaptation to the Space Environment

During these years we have extended our studies initiated with Troponin T to the two additional Troponin components, Troponin I and Troponin C, as well as to Troponin H, an IFM specific isoform of Tropomyosin II, In the cases where they were unknown, we have identified the genes and the proteins. We have obtained antibodies against the proteins and we have studied their properties in other *Drosophilidae*.

In relation to our Space experiments, we have continued the preparation of the Hardware as well as the modification of the methodology that will make possible to perform meaningful experiments in the restricted conditions that will be available in the International Space Station. Among these, we are preparing a Fixation Unit that will be functional in Space to fix embryos and adults, as well as the preparation of samples for microscopic observation in Space. Finally, using the different Instruments that deliver altered gravity conditions on Earth, we are exploring the experimental conditions that will open the way to our final goal, the establishment of a permanent colony of *Drosophila* in Space to study the long-term effects on complex organisms of this strange environment and the changes that will accumulate in their long-term adaptation to this environment.

Group Leader: Angel Pascual

Molecular mechanisms involved in the regulation of the APP gene expression

The expression of the β-amyloid precursor protein (APP), which plays a key role in the development of Alzheimer's disease, is regulated by a variety of cellular mediators in a cell-dependent manner. In neuroblastoma cells, we have previously described that thyroid hormones negatively regulate the expression of the APP gene at transcriptional level by a mechanism that requires binding of the nuclear thyroid hormone receptor to specific sequences located in the first exon of the gene. The identification and characterization of those elements as well as the other proteins and factors involved in the response is a central aim of our research. Also in cells of neuronal origin, we have demonstrated that different growth factors, in particular BDNF, can induce signals that activate the APP transcription. The analysis of those pathways (Ras/MAPK or PI3K) also represents a priority in our laboratory.

Group Leader: Belén Peral

Genetic study of the polycystic ovary syndrome and some endocrine-metabolic pathologies: analysis of candidate genes and implementation of DNA microarray technology

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies among women of reproductive age, affecting to the 6.5% of women. Together with hirsutism, acne, chronic anovulation and infertility, PCOS is associated with type 2 diabetes mellitus and obesity, and is associated with an increased risk for cardiovascular and cancer morbi-mortality. PCOS is a complex disease: there are more than one gene implicated in the disorder.

In the lab we are analysing candidates genes associated to various metabolic or regulatory pathways of steroid hormone synthesis, regulatory pathways of gonadotropin action, the insulin-signaling pathway, and pathways regulating body weight. Already we have analysed the association of PCOS and SNPs in the Calpain-10 gene (CAPN10), which has been identified as a susceptibility gene for type 2 diabetes mellitus and also in the Tumor Necrosis Factor Receptor 2 Gene (TNFRSF1B) which mediates most of the metabolic effects of tumor necrosis factor-alfa. We have found SNPs in both genes associated with PCOS and hyperandrogenism. Now we are studying the association of various genes to these disorders as PTP-1B (protein tyrosine phosphatase 1B), PON-1 (paraoxonase), GBP28 (adiponectin), etc.

To better understand the genetic basis of PCOS and to study different expression patterns of genes between affected women and healthy ones, we want to perform DNA microarray analysis. The different gene expression pattern between the affected status and the normal one could show altered pathways (up-regulated or down-regulated) that will reveal novel candidate genes or will confirm the old candidate genes for the disease. Also we are starting to implement the bidimensional gel electrophoresis approach in order to identify modified proteins associated with the pathology.

Group Leader: Ana Pérez

Regulation of neuronal differentiation and cell death by members of the nuclear receptor superfamily and by the early genes C/EBPα, C/EBPβ, and egr-1.

The ligands of the nuclear hormone receptor superfamily (NHRs) are important effectors of development, cell growth and metabolism. To this family belong the receptors for thyroid hormone (T3, TRs), retinoic acid (RA, RARs), peroxisomal proliferators (prostaglandins, PPARs) and vitamin D3 (vitD3, VDR). These proteins are widely expressed in the Central Nervous System and, some of them, play an important role in brain development. Our group has demonstrated that the genes coding for the transcription factors egr-1, C/EBPα, and C/EBPβ, which have been related with differentiation and cell death processes, are under the regulation of some of these nuclear receptors. During these two years we have been involved in the study of the precise role of egr-1, C/EBPα, and C/EBPβ on the differentiation and cell death of neuronal cells in culture. We have shown that the proteins egr-1, C/EBPα and C/EBPβ induce neuronal differentiation and programmed cell death. As it has been suggested in adipocyte differentiation, we show that C/EBPβ induces the expression of the endogenous C/EBPα gene, and that this protein by itself is also able to induce a differentiated phenotype in Neuro2A cells. Neuronal differentiation induced by C/EBPβ requires activation of the phosphatidylinositol-3 kinase signaling pathway, whereas inhibition of the mitogen-activated protein kinase signaling does not have any effect. In addition we have demonstrated that C/EBPβ is expressed in the brain of neonatal rats, suggesting that this protein could play an important role in neuronal maturation. Finally, we found that C/EBPβ-induced cell death takes place through activation of the p53 protein and the cdk inhibitor p21. We are also interested in the study of the possible role of two members of the nuclear receptor superfamily: PPARgamma and vitamin D receptor on the differentiation, proliferation and cell death of neural progenitor cells. The ability of the adult central nervous system to produce new neurons is limited, rendering it vulnerable to injury and disease. In most mammals, the majority of neurons are born by the early postnatal period, but it has been demonstrated that neurons continue to arise in the adult in at least two identified regions of the mammalian brain, in the dentate gyrus of the hippocampus and in the lateral ventricles. These late-born neurons are believed to derive from populations of resident stem cells and their less multipotent progeny progenitor cells. In turn, these progenitor cells are believed to give rise to the more differentiated cell types. An understanding of the factors that promote stem cell division and that regulate the proliferation, migration and differentiation of its progeny, the more differentiated progenitor cells, is an important step towards the goal of being able to use the endogenous stem cell populations to induce the production of new neurons and glia. We have found that 15-deoxy-Δ-12,14-

prostaglandin J2 (15dPG-J2) and vitamin D3, ligands of PPARg and VDR, respectively, are able to inhibit the proliferation of these neural progenitor cells. In addition, both factors induce a neuronal phenotype when these cells are transferred to an adhesive substrate. VD3 also seems to regulate the expression of some adhesion molecules since treatment with this factor results in adhesion and migration of these cells without any transfer to an adhesive substrate.

Finally we have studied the effects of the activation of PPARgamma upon several breast cancer cell lines. We reported recently that PG-J2, a specific ligand of the nuclear receptor PPARg, inhibits proliferation and induces cellular differentiation and apoptosis in the breast cancer cell lines MCF-7, T47D, and SKBR3 at least in part through the inhibition of ErbB2 and ErbB3 tyrosine phosphorylation caused by neuregulin-1 and neuregulin-2. Although many genes are regulated by PPARg, how certain biological PG-J2 effects relate to individual gene regulation remains enigmatic. To address this question with respect to PG-J2-induced programmed cell death, we applied a differential screening technology to determine gene expression profiles in MCF-7 cells undergoing apoptosis. Several genes were found to be regulated by PG-J2 in the first 12 h of treatment. Analysis of the regulated genes revealed that several mitochondrial mRNAs were down regulated in MCF-7 cells treated with PG-J2. Moreover, analysis of the biochemical mechanisms involved showed that PG-J2 treatment resulted in a mitochondrial dysregulation leading to the release of cytochrome c, a drop in mitochondrial transmembrane potential, and an activation of caspase-7. Overexpression of the anti-apoptotic protein bcl-2 inhibited PG-J2-induced mitochondrial dysregulation and apoptosis. These changes were associated with inhibition of NFkB, that has been implicated in the suppression of apoptosis in other systems, and are independent of p53 activation. Together, these results suggest that the decrease in mitochondrial protein expression may play a role in PG-J2-induced apoptosis of MCF-7 cells.

Group Leader: Leandro Sastre

Functions of the transcription factor Serum Response Factor (SRF) in cell differentiation and morphogenesis

The transcription factor Serum Response Factor (SRF) was initially characterized for its role in the activation of transcription of immediately early genes in quiescent cells stimulated by serum. However, it is now well established that this factor also participates in cell differentiation processes, both in vertebrate and in

invertebrate animals. Actually, our laboratory has shown that the SRF homolog SRFA is necessary for spore differentiation and fruiting body morphogenesis in the social amoeba *Dictyostelium discoideum*. We have continued these studies in the last two years analyzing the mechanisms that regulate SRFA expression and identifying genes whose expression is regulated by this transcription factor. We have also studied the functional conservation of SRF domains between different animal species. Finally, we have continued the characterization of the two alternative promoters of the sarco/endoplasmic reticulum Ca-ATPase from the crustacean *Artemia franciscana*

Group Leader: Antonio Cuadrado

Regulation of AKT/PKB kinase activity by ceramide

Accumulation of ceramide has been reported in stress- and receptor-induced apoptosis in the nervous system. However, its role in apoptosis signaling remains elusive. We describe here the inhibition of the NGF-activated phosphoinositide 3-kinase (PI3K)-PKB/Akt1 survival pathway by the cell permeable analog C2-ceramide. C2-ceramide did not inhibit ERK, PI3K or PDK1 activities and did not alter the translocation of PDK1 and Akt1 to the plasma membrane, but blocked nuclear translocation of Akt1. Down-regulation of the Akt pathway was due to enhanced dephosphorylation of Akt1 at residues T308 and S473. Moreover, Akt1 was dephosphorylated in vitro by a cation-independent phosphatase involving ceramide-activated protein phosphatase (CAPP). Membrane anchored Akt1 was more resistant to dephosphorylation/inactivation by C2-ceramide than wild type Akt1. Consistently, N-myristylated-Akt1 conferred resistance to the apoptosis induced by C2-ceramide in PC12 cells. These results provide a novel mechanism for induction of apoptosis by ceramide in nerve-derived cells.

Neuroprotective effect of Akt/PKB against Parkinson-inducing toxins.

The phosphatidylinositol (PI)-3 kinase-Akt/PKB survival pathway protects neurons from apoptosis caused by diverse stress stimuli. However, its protective effect against neurotoxins that produce oxidative stress and neurodegeneration has not been investigated. We analyzed the effect of this pathway on the action of the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium (MPP+). Overexpression of a membrane-targeted, N-myristylated fusion protein of enhanced green fluorescence protein (EGFP) and mouse Akt1 attenuated the

apoptotic effect of the neurotoxin in PC12 cells. This effect was not due to protection of mitochondrial complex I activity or restoration of energy charge. Following MPP+-treatment, myr-EGFP-Akt1-transfected cells exhibited an unaltered mitochondrial membrane potential and lower ROS levels than control cells. These results provide a new site of action of Akt/PKB at the level of the oxidative detoxifying cell machinery and suggest that this effect may be responsible in part for the resistance of myr-EGFP-Akt1 expressing cells to oxidative stress and MPP+-induced apoptosis.

Effect of the Alzheimer amyloid fragment Abeta(25-35)

The phosphatidylinositol 3 kinase(PI3K)-Akt/PKB pathway protects neurons from apoptosis caused by diverse stress stimuli. However, its protective role against the amyloid beta peptide (Ab), a major constituent of Alzheimer's disease plaques, has not been studied. We investigated the effect of the Ab-derived Ab(25-35) peptide on apoptosis and on the Akt survival pathway in PC12 cells. Cells submitted to micromolar concentrations of Ab(25-35) exhibited increased production of reactive oxygen species (ROS) and morphological alterations consistent with apoptosis. Akt1 was activated shortly after incubation with Ab(25-35) and Ab(1 -40) with a kinetics different to that of nerve-derived growth factor. Akt1 activation was blocked by the PI3K inhibitor wortmannin. We tested the hypothesis that Akt1 might modify the vulnerability of neural cells to apoptosis induced by Ab(25-35). Overexpression of an active version of Akt1 attenuated the apoptotic effect of Ab(25-35) as determined by flow cytometry. Moreover, PC12 cells overexpressing a membrane-targeted N-myristylated fusion protein of enhanced green fluorescence protein (EGFP) and mouse Akt1 exhibited lower levels of ROS than control EGFP-transfected cells. The present findings demonstrate that Akt1 is activated in response to Ab(25-35) in a PI3K-dependent manner and that active Akt1 protects PC12 cells against the pro-apoptotic action of this peptide.

Group Leader: Juan Emilio Feliu

Biochemical and genetic analysis of inherited diseases of carbohydrate metabolism.

Our “Unidad de Investigación y Diagnóstico de Metabolopatías” acts as a Reference Laboratory for an increasing number of Spanish hospitals in the

diagnosis of inherited diseases of carbohydrate metabolism, such as hereditary fructose intolerance, galactosemia and all types of glycogenosis. In the last years, we are carrying out a Research Project entitled "Hereditary fructose intolerance and classical galactosemia: A genetic study in Spanish families". More recently, we are also interested on the molecular characterization of the mutations of both glucose 6-phosphatase and debranching enzyme genes –responsible of glycogenosis Ia and III- in the Spanish population

Molecular mechanisms of insulin resistance.

This research line was initiated in 1987. Since then, we have demonstrated that the glycosyl-phosphatidylinositol-dependent system is altered in different animal model of insulin resistance, such as streptozotocin diabetes, glucocorticoid excess, ageing and genetically determined obesity (Zucker fa/fa rats). In these obese rats, we have also studied the molecular mechanism implicated in the resistance of hepatic gluconeogenesis to the modulation by insulin, glucose and sulfonylureas. At the present time, we are interested on the study of leptin and TNF-alpha on hepatic glucose metabolism, as well as on the metabolic effects of overexpression of the uncoupling protein –2 in the liver of transgenic mice.

Metabolism in gastrointestinal cells.

In this research line, we have investigated the influence of ethanol and different non-steroidal anti-inflammatory drugs on various cellular parameters implicated in acid formation in isolated gastric glands and cultured parietal cells, obtained from rabbit stomachs.

Development and analysis of a transgenic animal model of hereditary fructose intolerance.

The objective of this research line has been to develop an animal model of hereditary fructose intolerance by overexpression of the fructokinase gene in the liver of transgenic mice. The rationale for this model is based on the increase of the physiological unbalance between fructokinase and the total rate of fructose utilization by the liver. The higher fructokinase activity causes a greater accumulation of fructose 1-phosphate, which is accompanied by a more marked reduction in ATP levels, in the liver of transgenic mice fed with fructose, in

comparison to control animals. The metabolic consequences of acute and chronic fructose administration has been studied.

Group Leader: Miguel Quintanilla

Mechanisms involved in malignant progression of carcinomas

Our group is investigating molecular and cellular events related to the evolvement of highly undifferentiated invasive carcinomas. We are analyzing signaling events triggered by TGF-β1: Smad and Ras/MAPK (and their interrelationships), since it has been demontrated that this growth factor acts as a stimulator of malignant progression in mouse as well as in human carcinogenesis models. Particularly, we are focused to study the cooperation between Ras oncogenic activation and inactivation of the Smad4 tumor suppressor gene in the progression of colon and pancreatic human tumor cells. We have also started a study about the role of endoglin, a co-receptor for TGF-β, in skin carcinogenesis using genetically modified mice. On the other hand, we continue our studies about the characterization of PA2.26 antigen, a novel transmembrane mucin-type glycoprotein associated with cell migration and metastasis, identified by us in murine skin carcinoma cells. Our recent findings with the human orthologous protein, whose cDNA was isolated and characterized by our laboratory, suggest that it is involved in progression of oral squamous cell carcinomas. PA2.26 is also a specific marker for lymphatic capillaries that discriminate the lymphatic from the blood endothelium in both normal ant tumoral tissues, and it has been proved to be very useful to study the lymphatic vasculature (and lymphangiogenesis) in human cancer.

Group Leader: Jaime Renart

Signaling pathways in stress, differentiation and apoptosis

Our group is studying different aspects of the signaling networks involved in stress, differentiation and apoptosis. 1. Regulation of the NMDA glutamate receptor by N-glycosylation and Endoplasmic Reticulum (ER) stress. We have observed differential maturation of the carbohydrates in the NR1 subunit with

respect to the NR2A, and a high sensitivity of NR1 to ER stress. We want to characterize the underlying mechanism of the degradative process affecting to NR1 and the role that the carbohydrate moiety has in folding and stability of these subunits. 2. Role of the ER stress responses in excitotoxicity and cerebral ischemia. Excitotoxicity due to overstimulation of NMDA receptors, and consequent changes in calcium homeostasis, is considered to play a fundamental role in processes such as hipoxia and ischemia, as well as several neurodegenerative disorders. Calcium toxicity is partially mediated by massive release of this ion from ER stores which triggers a specific ER stress response that ultimately can produce apoptotic cell death. Using both primary cultures of rat cortical neurons and animal models of focal ischemia, we want to characterize the contribution of ER stress responses to neuronal degeneration. 3. Role of protein kinase cascades for ERK, JNK, p38 and Akt in the survival and apoptotic responses. Inhibition of PKC results in apoptosis in the mouse neuroblastoma cell line N2A, although the overexpression of the BCL2 abrogates this effect. We have focussed our work in the study of the different protein kinases cascades, in relation to both survival (mainly ERK, JNK and Akt) and differentiation (ERK and p38), and we are also analyzing the involvement of these pathways in the cell cycle. The possible crosstalk between the ERK and p38 cascades is also being addressed, as well as how the mentioned pathways trigger the activation of the survival transcription factor NFkB. 4. Differential gene expression studies in N2A neuroblastoma cells treated with Tumour Necrosis Factor alfa, by means of Serial Analysis of Gene Expression. Using this high throughput technology, we have detected 28 differentially expressed genes, and some of them have been confirmed by northern analysis. We have focussed in the phospholipid hydroperoxyde-Glutation peroxidase (PH-GPx4) gene, involved in protection of membrane phospholipids from oxidative damage. We are studying the promoter regions of this gene with a reporter luciferase system, and also which of the different forms of the gene (cytoplasmic vs. mitochondrial) is induced by the cytokine.

Group Leader: María Angeles Rodríguez

Molecular basis of the thyroid hormone action on glial cell differentiation

Lack of thyroid hormone in humans leads to cretinism. Cretinism is also found in other mammals but the underlying mechanism of this condition is not well

understood. Hypothyroidism produces severe delays and reductions in the myelination of the central nervous system reflecting an abnormal development of oligodendrocytes. Likewise, lack of thyroid hormone impairs the normal differentiation of microglia and several types of neurons in different brain areas. Thyroid hormone regulates gene expression through the interaction with specific nuclear receptors (encoded by two genes, alfa and beta) that act as ligand dependent transcription factors. Our aim is to establish at the molecular level, the differentiation program governed by thyroid hormone and to study the specific contribution of each thyroid hormone receptor isoform to this process both in vivo and in vitro.

Group Leader: Isabel Varela

Auditive neurodegeneration : in pursuit of a model for neuroprotection with IGF-1

Deafness is a major health problem with a very high social impact. Up to 6% of the first world population suffers from this disease that will surely increase due to noise and other environmental noxious factors. In Spain, 200 profoundly deaf patients are born per year and more than one million and a half individuals suffer from auditory disorders. Our recent data show that Igf-1 knock out mice develop important postnatal alterations in the inner ear. On the other hand, evidence suggests the potential effects of IGF-1 in protecting neural tissue against mechanical or chemical aggression. Our project has been aimed at the study of the relationship between IGF-1 and auditory degeneration by a triple complementary approach: 1) To identify molecular targets of IGF-1 in the ear by following the expression of neural and other markers in the IGF-1 knock out mice. 2) To characterize the phenotype of IGF-1 (-/-) and (+/-) mice, which we propose as a model system for degenerative hypoacusia, and a test for assessing the impact of environmental and pharmacological trauma, and 3) To explore the signalling networks that regulate early inner ear neurogenesis in the chick embryo model, to understand further the molecular basis of neural regeneration, and to search for possible use of IGF-1 in improving neural regeneration. In summary, we are studying the cellular and molecular basis of neural degeneration in the ear, and the use of IGF-1 for neuroprotection.