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Centre
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Genòmica

Annual Report 2006

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CONT

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ENTS

A T I O N A N N U A L R E P O R T 2 0 0 6

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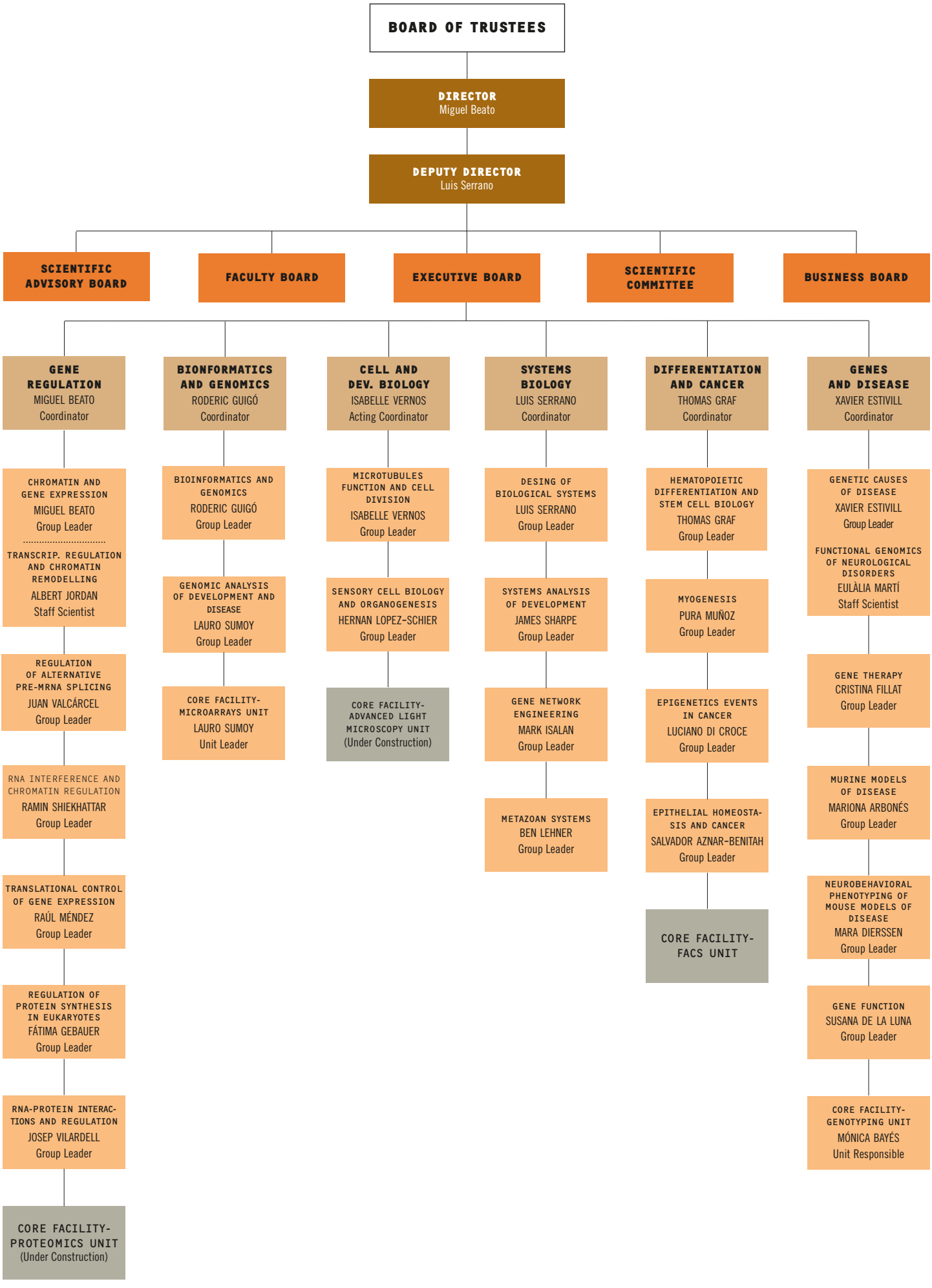
SCIENTIFIC STRUCTURE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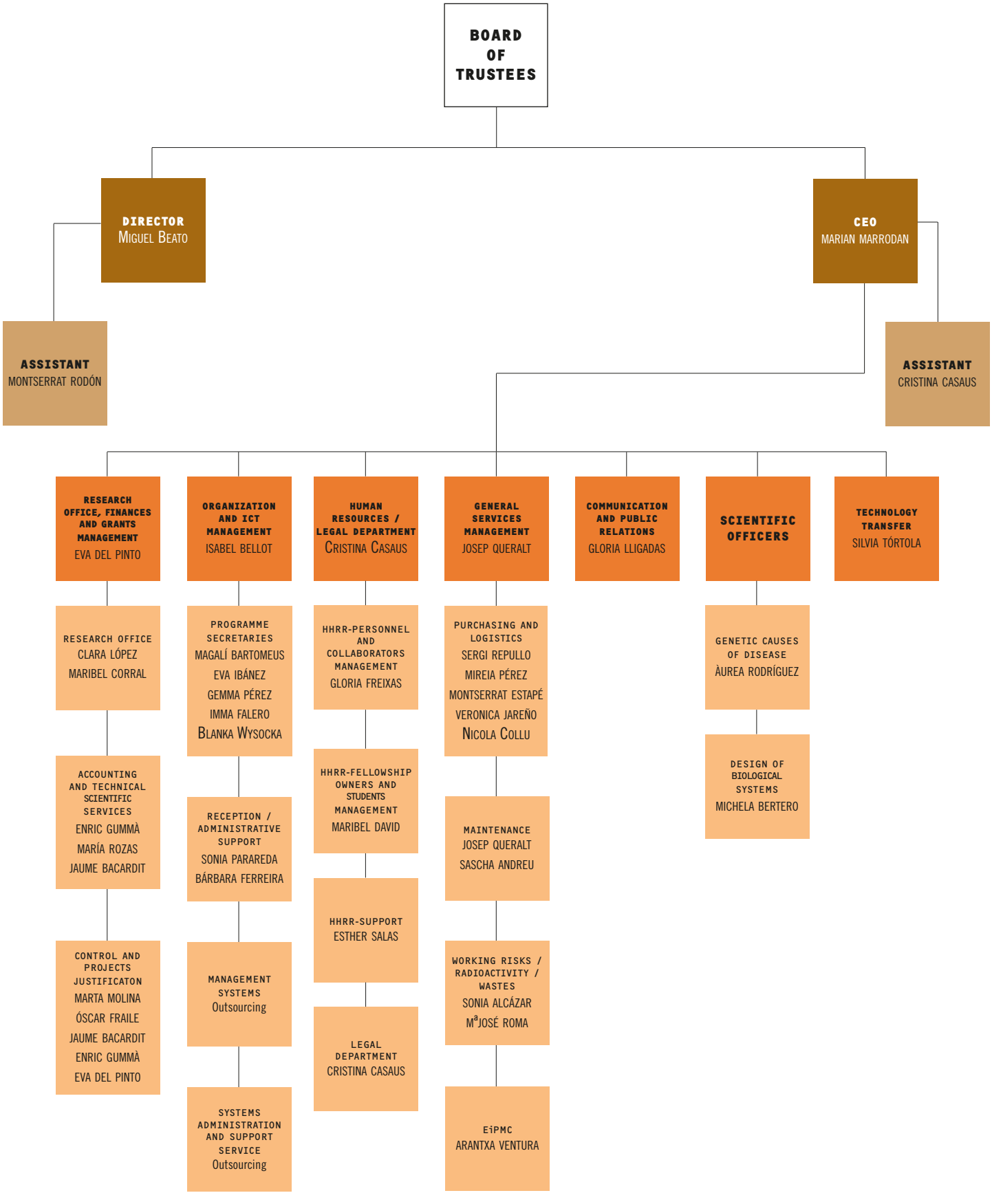
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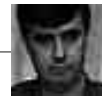

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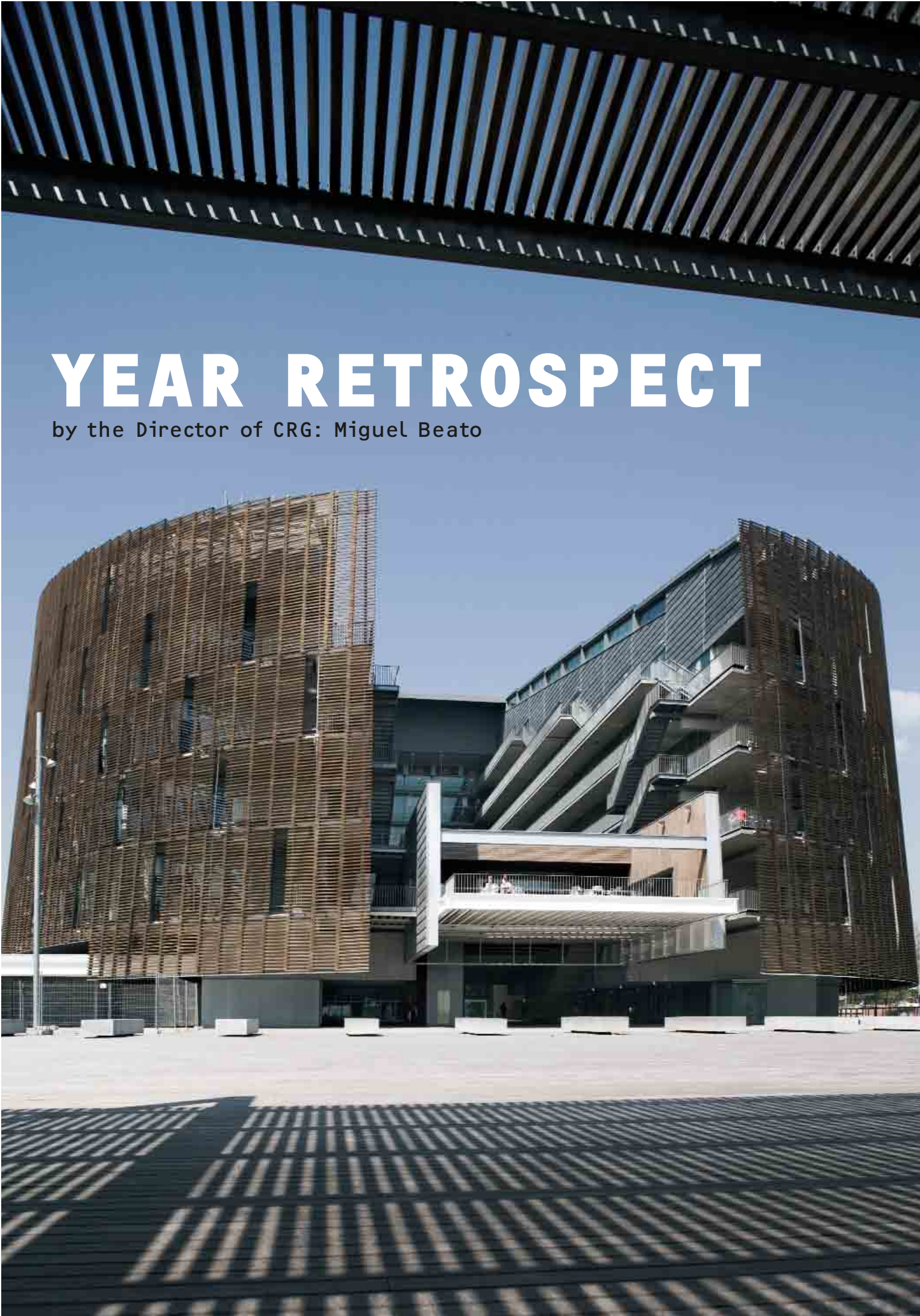
YEAR RETROSPECT

by the Director of CRG: Miguel Beato



The past year has been dominated by the moving to the new building of the PRBB. Although new groups started to work in the new building during the summer, the majority of the new and established groups moved between October and December. By the end of the year only the Genotyping Facility and the Microarray Unit were still in the CMIMA building, waiting for the installation of appropriate spaces in the PRBB building.

Another important event was the official signature of the partnership agreement with the European Molecular Biology Laboratory (EMBL), for the creation of an EMBL-CRG Research Unit in Systems Biology. The presentation to the media took place on September 7. The agreement foresees financing of four independent research groups for a period of 9 years subjected to periodic evaluations. The Unit will be directed by Luis Serrano, the coordinator of the CRG Systems Biology programme.

These important events took place in the context of a crisis in the coalition of political parties in power that lead to the outing from the Catalan Government of the Minister in charge of Research. Until the foreseen elections in November, a provisional government was named with changes in the research competences. Under these conditions it was not possible to secure a finance planning for the next years despite a personal engagement of the President of the Generalitat. Nevertheless we have managed to go on with our roadmap and, after long rounds of interviews, we have recruited six new group leaders and a unit leader, five of them foreigners.

The increasing number of groups and units has imposed a new organization of the leading structure. The former Direction Committee has split into a **Scientific Committee**, formed by all the senior scientists, unit heads and the administrative director, and an **Executive Committee**, formed by the programme coordinators, and the administrative director. Both committees are headed by the Director. The Scientific Committee is a debate and discussion organ that elaborates projects and proposals to be presented to the Executive Committee, which takes the final decisions. Moreover, Luis Serrano was nominated **deputy director** with the function to help the director and eventually replace him in institutional activities.

To document the increasing weight of the PhD Students in the CRG, we have created a **Graduate Committee** that organizes all issues related to our PhD students, in particular establishing the conditions for entering the Institute, selection of candidates, coordination with the CEXS/UPF and follow up of the progress of the students.
In the past year eight new independent scientists have joined the CRG: 4 senior scientists and 4 junior group leaders. Six of those new scientists

are foreigners, thus elevating to 30 % the proportion of foreigners among group leaders. Four of the new group leaders have obtained an ICREA senior contract. Among them Thomas Graf, a German scientist from the Albert Einstein Institute in New York, who has been acting as an advisor and is now the coordinator of the Differentiation & Cancer programme. He brings to the CRG his expertise in differentiation and reprogramming of mouse hematopoietic cells. Another senior scientist to join the CRG was Ramin Shiekhattar, a leading American biochemist from the Wistar Institute in Philadelphia with an impressive record in RNA interference and chromatin regulation. The third one is Luis Serrano, former director of the Structural and Computational Biology programme at the EMBL in Heidelberg, who is coordinating our Systems Biology programme. Finally, James Sharpe, a British colleague from the MRC in Edinburgh has joined the CRG as a senior scientist in the Systems Biology programme with a focus on limb development and new microscopic methods.

Among the new junior group leaders, one of them, Hernan Lopez-Schier, an Argentinian scientist from The Rockefeller University in New York, obtained a Ramon y Cajal position. He brings to the CRG his knowledge on organogenesis of sensory systems in zebra fish. Salvador Aznar-Benitah from the Cancer Research UK London, who works on skin homeostasis and cancer, obtained an ICREA junior position. Two others are members of the **EMBL-CRG Systems Biology Research Unit**: Mark Isalan, a British scientist from the EMBL, works on the engineering of gene networks; and Ben Lehner, another British colleague from the Sanger Institute in Hinxton, works on regulatory networks in *C. elegans*.

During 2006 scientists from the CRG organized nine scientific events, of which I want to mention just two. The first Student Symposium organized by the graduate students of CRG and CMIMA the July 25/26 with excellent external and local speakers, and the ***Fifth CRG Symposium*** held on December 15/16, 2006 and organized by Luis Serrano on "*Systems Biology: A Cell in the Computer*".

To make the activities of the CRG comprehensible for the general public, we started a project with a science journalist and a design team for publishing a popular version of the main scientific projects of the centre in form of a an intriguing story. The booklet entitled "In search of the genome score" was finished in September and distributed during the inauguration of the new CRG facilities at the PRBB building, on the 10th October.

Numbers. On December 31 2006 there were 204 scientists working at the CRG, among them 24 group leaders (9 with an ICREA contract and 5 with a Ramón y Cajal contract), 59 postdocs (11 with a Ramón y Cajal contract, 2 with a FIS contract; and from the overall 36 are foreigners), 48 technicians, 73 graduate students (30 of them are foreigners). In addition there were 32 people in administration and support, which makes a total number of persons working at the CRG of 236. Relative to 2005, this represents a 40% increment.

The very active recruitment policy has added a heavy load of search committees meetings and interviews on top of the effort devoted to planning the new labs. Nevertheless, the scientific activities in terms of faculty meetings, data seminars and external seminars have continued to expand. The same applies to the volume of the external support for CRG scientists, with a most significant increase in European projects, and the two new Spanish founding models: two of the four Biomedical oriented Consolider Projects are coordinated by CRG scientists, who also participate in three CIBER projects. Finally the number of peer reviewed publications were 78 and the average Impact Factor 8.186.

Retrospectively 2006 has been the most demanding year of the young CRG history. But finally in the new building we have been able to continue our planed expansion. As it became evident during our Christmas dinner December 18, the CRG is becoming a really international research center with a bright future due to the excellent group of people, both scientists and administrators that have joint the project. I want to thank all them for the enormous effort they have made to continue working with very high standards despite the numerous difficulties and uncertainties. The CRG faces now the urgent need to establish state of the art core facilities that will contribute to the scientific productivity of the whole PRBB community.

GENE REGULATION

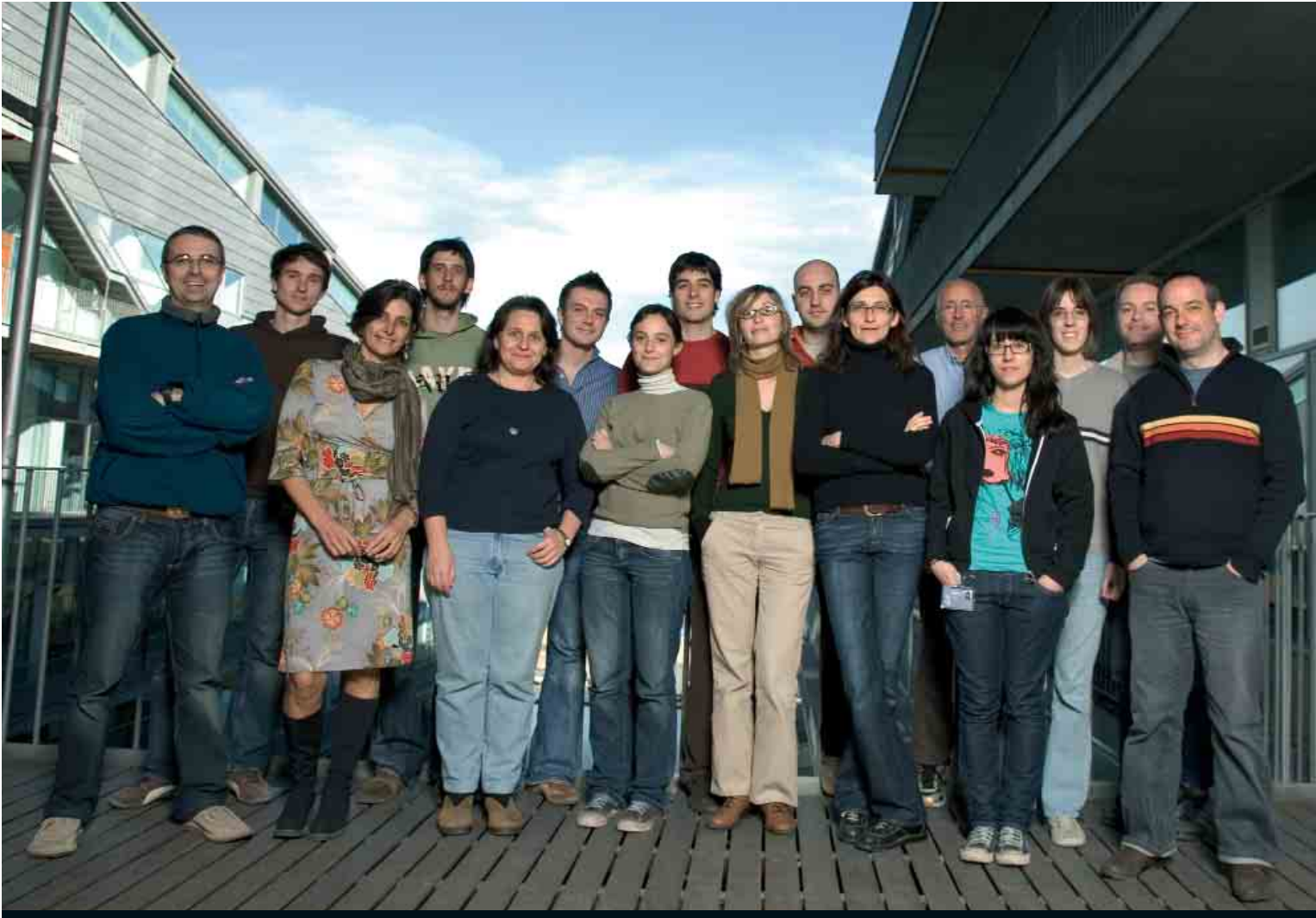
Coordinator: Miguel Beato

In 2006 the programme has expanded to six groups due to the incorporation in October of a new Senior Group Leader, Ramin Shiekhattar from the Wistar Institute in Philadelphia, USA. Ramin Shiekhattar brings to the programme his expertise in the biochemical isolation and characterization of multisubunit complexes with a special interest in chromatin modifying enzymes and RNA interference, two emergent areas of research that were not sufficiently implanted in the CRG.

Juan Valcárcel has elaborated further the strategy to establish a *Proteomics* facility coordinated with the actual proteomic facility of the UPF/CEXS. The CRG will fill the position of an expert technician and will finance the acquisition of new equipment including a LTQ Orbitrap machine. Moreover, we plan to establish a facility for the expression and purification of recombinant proteins using baculovirus-based vectors.

Thus, the present structure of the programme is:

- 6 Research groups:
  - Chromatin and Gene Expression (Miguel Beato/Albert Jordán)
  - RNA Interference and Chromatin Regulation (Ramin Shiekhattar)
  - Regulation of Alternative Pre-mRNA Splicing (Juan Valcárcel)
  - RNA-Protein Interactions and Regulation (Josep Vilardell)
  - Translational Control of Gene Expression (Raúl Méndez)
  - Regulation of Protein Synthesis in Eukaryotes (Fátima Gebauer)
- Associated Core Facility: Proteomics Unit (under construction)



GENE REGULATION

Chromatin and Gene Expression

The group is interested in understanding how eukaryotic cells respond to external signals, in particular how different signals are integrated and transduced to the nucleus to modulate gene expression. The main experimental model is gene regulation by steroid hormones in breast and endometrial cancer cells. More specifically, the crosstalk of estrogen and progesterone receptors with other signalling pathways originating in the cell membrane and how this network of signalling is interpreted at the level of chromatin. The role of steroid hormones in breast and endometrial cancer cell proliferation and apoptosis is another research line of the group.

GROUP STRUCTURE

Group Leader:	**Miguel Beato del Rosal**
Postdoctoral Fellows:	Cecilia Ballaré
	Mike Edel (till October)
	María Jesús Meliá (till March)
	Guillermo Vicent (Ramón y Cajal)
PhD Students:	Thomas Bechtold (till August)
	Verónica Calvo
	Jaume Clausell
	Michael Firgens
	Vladimir Maximov (till June)
	Roser Zaurin
	Michael Schaub (since December)
Technician/s:	Jofre Font
	Silvina Nacht
Visitors:	Patricia Saragüeta
Subgroup:	**Transcriptional Regulation and Chromatin Remodelling**

SUBGROUP STRUCTURE

Staff Scientist:	Albert Jordan
	(subgroup leader, Ramon y Cajal)
PhD Students:	Edurne Gallastegui
	Ignacio Quiles
	Mónica Sancho
	Alicia Subtil

RESEARCH PROJECTS

1. CROSSTALK BETWEEN HORMONE RECEPTORS AND OTHER SIGNALLING PATHWAYS

C. Ballaré, T. Bechtold, A. Jordan, I. Quiles, A. Subtil.

We have reported that progesterone can activate transiently the Src/Ras/ Erk pathways (Migliaccio *et al* EMBO J 17, 2008-18,1998) via an interaction of two domains of the progesterone receptor (PR) with the estrogen receptor alpha (ERα), and that the kinase activation is essential for the proliferative response of breast cancer cell lines (Ballaré *et a*l Mol Cell Biol 23, 1994-2008, 2003). We have now identified the amino acids implicated in the interaction between PR and ERα and have generated stable cell lines expressing PR mutants with defects in ERID-I, the AF2 or the DNA binding domain useful for defining the role of different PR domains in progestin activation of the Src/Ras/Erk cascade and the regulation of chromatin dynamics.

We have analyzed the initial response to progestins and have shown that already 5 min after progestin treatment PR_B is phosphorylated at serine 294 and forms a ternary complex with activated Erk1/2 and activated Msk1 (Figure 1). Inhibition of Erk1/2 or Msk1 compromises progestin induction of MMTV and other target genes (Vicent et al Mol Cell 27, 367-81,2006). Thus, induction of the Src/Ras/Erk pathway is important not only for the proliferative response of cancer cells to hormones but also for the transcriptional regulation of hormone target genes.

2. REGULATION OF MMTV TRANSCRIPTION IN THE CHROMATIN CONTEXT

G. Vicent, J. Clausell-Menero, A. Jordan, V. Maximov, R. Zaurin, S. Nacht, J. Font

After clarifying the complex role of nucleosome positioning and histone H1 in the induction process, the group has studied the structural changes accompanying activation of MMTV promoter chromatin and how they are catalyzed. Within 5 minutes of progestin addition to breast cancer cells carrying an integrated copy of the MMTV promoter, a ternary complex of activated PR and two activated kinases, pErk1/2 and pMsk1, is recruited to the promoter and leads to phosphorylation of histone H3 at serine 10, accompanied by acetylation at lysine 14. This changes lead to dissociation of a repressive complex containing HP1g as a prerequisite for the recruitment of ATP-dependent chromatin remodelling complexes (Snf2h and Brg-1), co-regulators (CBP, PCAF, Src1) and RNA polymerase II (Vicent et al Mol Cell 27, 367-81,2006). Shortly thereafter we detect the displacement of histones H2A and H2B from the promoter nucleosome containing the HREs but not from the adjacent nucleosomes (Vicent et al Mol Cell 16, 439-52, 2004) (Figure 1).

On nucleosomes assembled with recombinant histones, purified yeast SWI/SNF catalyzes displacement of H2A/H2B dimers from MMTV promoter but not from positioned nucleosomes containing ribosomal promoter DNA (Vicent et al Mol Cell 16, 439-52, 2004), suggesting the existence of topological information on the DNA that determines the outcome of the remodelling process. We are extending these studies to other nucleosomes in order to understand the properties of the DNA sequence res-

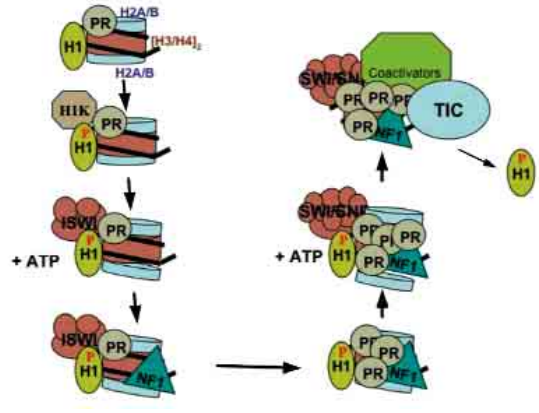


Figure 1. Steps in the activation of the MMTV promoter. PR binds preferentially to the exposed HRE1 on the MMTV nucleosome. It first recruits a histone H1 kinase (HIK) that phosphorylates H1, as a prerequisite for the ATP-dependent remodelling by the recruited ISWI-containing complex. This remodelling enables binding of NF1 that stabilizes the open conformation and facilitates binding of further PR molecules to the internal HREs. Complete binding of PR may require a SWI/SNF-like remodelling complex, which catalyzes the ATP-dependent displacement of H2A/H2B dimmers. Subsequently, PR recruits transcriptional co-activators and the transcription initiation complex (TIC). During initiation of transcription phospho-H1 is displaced from the promoter.

ponsible for the differential responses to SWI/SNF action.

We found that histone H1 enhances the activation of the MMTV promoter by PR and NF1 (Koop *et al* EMBO J 22, 588-99, 2003) and are now studying the role of various isoforms of linker histones on the remodelling and transcription of MMTV minichromosomes assembled in *Drosohila* embryo extracts.

3. GLOBAL ANALYSIS OF HORMONE-RESPONSIVE PROMOTERS

C. Ballaré, M.J. Meliá, B. Miñana (MAU), R. Zaurin

In collaboration with the Microarray Unit (MAU) of the CRG we are performing gene profiling and tiling studies in breast cancer cell lines to study the chromatin structure and the different response patterns to estrogens and progesterone of hormone-sensitive promoters. The focus is on the identification of the signalling pathways mediating the regulation of different clusters of genes with the aim of modulating specific aspects of hormone action such as cell proliferation, apoptosis or cell differentiation. In the context of the Integrated European project HEROIC, a selection of hormone responsive promoters is also analyzed by high resolution ChIP-on-chip to define their nucleosomal structure and the changes in chromatin structure and factor binding during the induction process.

4. ROLE OF STEROID HORMONES IN BREAST CANCER AND ENDOMETRIAL PHYSIOLOGY

C. Ballaré, V. Calvo, M. Edel, L. Rocha-Viegas (UBA), G. Vallejo (UBA), P. Saragüeta (UBA)

In collaboration with the Department of Pathology of the Hospital del Mar, Barcelona, we are studying the gene networks regulated by estrogens and progestins in breast cancer cells and in primary tumour material and how this parameter is influenced by the products of the BRCA genes. In collaborating with Belen Miñana y Lauro Sumoy of the MAU of the CRG we have develop a microarray containing over 800 cDNA from genes relevant for breast cancer and hormone action. We are combining the array information with conventional molecular biology techniques for analyzing how the interactions between hormone receptors and BRCA proteins modulate gene expression and chromatin remodeling.

In a collaboration with the group of Adalí Pecci, University of Buenos Aires, we are studying the role of glucocorticoids and progestins in the control of apoptosis in various cell types via a regulation of *bcl-X* transcription and splicing. We have identified the HREs responsible for progesterone induction of the bcl-X gene in mammary epithelial cells (Rocha-Viegas *et al* J biol Chem 279, 9831-9, 2004) and are now analyzing the molecular mechanism involved in the tissue-specificity of the apoptotic response, focusing on the differential effect of glucocorticoids in thymocytes,

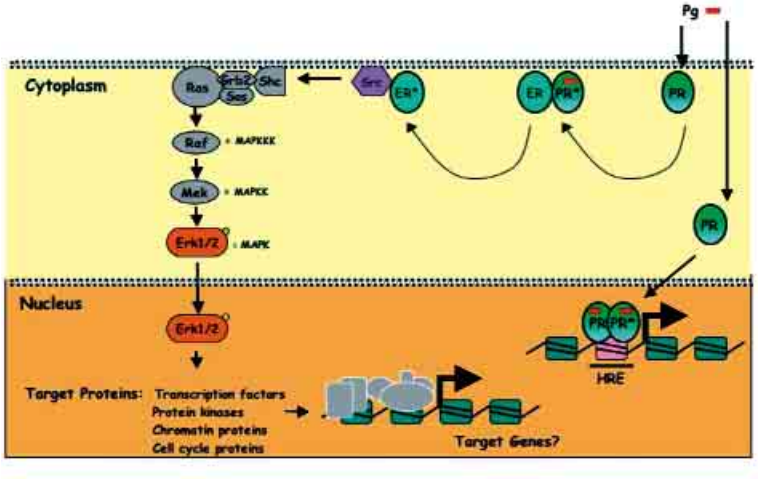


Figure 2. Crosstalk between PR and ER in the inner side of the cell membrane. In addtion to the direct activation of PR followed by homodimerization and binding to HREs in chromatin (right side), progesterone can induce the activation of a preformed ER-PR complex, leading to interaction of ER with c-Src and activation of the mitogenic Ras > Raf > Mek > Erk1/2 cascade (left side). In the nucleus Erk1/2 can phosphorylate transcription factors, cell cycle proteins and chromatin proteins, directly or via activation of downstream kinases.

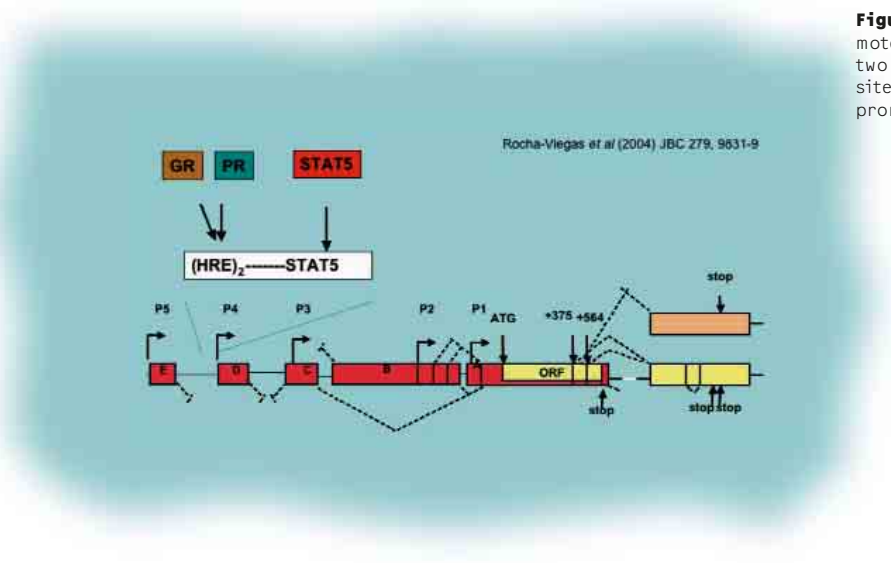


Figure 3. The mouse *bcl-X* promoter with the location of the two HREs and the STAT5 binding site indicated upstream of the promoter.

where they induced apoptosis, and in mammary epithelial cells, where they prevent apoptosis. We find that this difference is mediated by selective functional interactions with members of the STAT5 family of transcription factors (Rocha-Viegas *et al* J biol Chem 281, 33959-70, 2006) (Figure 2).

In collaboration with the group of Patricia Saragüeta, University of Buenos Aires, we are studying the response of endometrial stromal cells to estrogens and progesterone using cell biologial techniques and cDNA microarrays, with a focus on understanding the decidual reaction and the mechanism of blastocyst implantation (Figure 3). We found that very low concentrations of progestins induce cell proliferation via activation of the crosstalk of PR with ERß and the mitogenic kinase cascades (Vallejo *et al* Mol Endocrin 18, 3023-37), and are now studying how the combination of estrogens and progestins induces decidual differentiation.

Research Subgroup:

TRANSCRIPTIONAL REGULATION AND CHROMATIN REMODELLING

A. Jordan, E. Gallastegui, I. Quiles, M. Sancho, A. Subtil

1. TRANSCRIPTIONAL REGULATION AND CHROMATIN REMODELLING OF PROMOTERS RESPONDING TO STEROID HORMONES IN BREAST CANCER CELLS. DISTINGUISHING BETWEEN DIRECT EFFECTS AND THOSE MEDIATED BY SIGNAL TRANSDUCTION PATHWAYS.

We are interested in distinguishing between direct effects of nuclear hormone receptors on transcription of target genes and those mediated by crosstalk with other signal transduction pathways. For this, we have constructed breast cancer-derived cell lines that express tagged forms of PR mutated at residues involved either in the nuclear action of the receptor (DBD and AF-2) or in its ability to interact with components of signal transduction pathways (ΔERID-I). The MMTV promoter is used as a reporter to study the transcriptional effect of receptor variants. Microarray experiments are being performed to define the subsets of genes affected on its response to hormone by the different PR deffects. In addition, expression of tagged receptor is being used to perform proteomic studies of nuclear, cytoplasmic and membrane-associated purified complexes containing PR in the absence or presence of hormone.

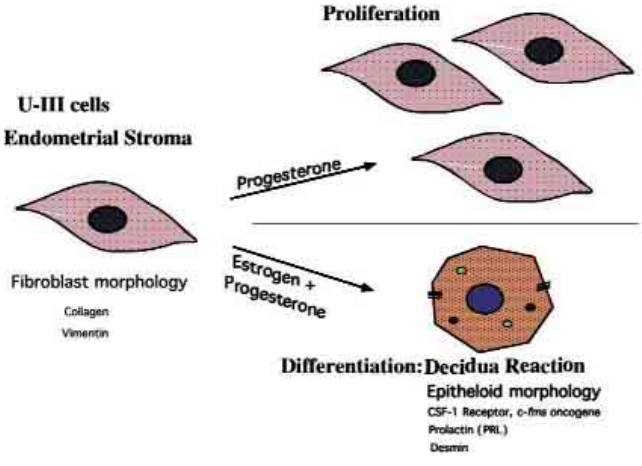


Figure 4. UIII endometrial stromal cells respond to progesterone with cell proliferation and to a combination of Estrogens and Progesterone with decidual differentiation.

We are also performing the characterization of the progesterone-responsive 11ß-HSD type 2 promoter: its kinetics of hormonal activation, involvement of signalling pathways, identification of HRE sequences, nucleosome positioning and chromatin remodelling in response to hormones. We are going to use chromatin immunoprecipitation (ChIP) to study histone modifications, as well as the composition of associated chromatin remodelling complexes and transcriptional complexes.

2. ROLE OF LINKER HISTONE H1 VARIANTS IN CHROMATIN AND TRANSCRIPTION.

During the process of MMTV promoter activation by PR in *Drosophila* extracts, histone H1 is phosphorylated and leaves the promoter (Koop *et al*, EMBO J 22, 588, 2003). At least six H1 variants exist in mammalian somatic cells that bind to the nucleosome core particles and linker DNA. We have been developing inducible RNA interference to create stable breast cancer cell lines lacking expression of each of the H1 variants specifically. The resulting phenotype is being characterized, as the inhibition of some of the isoforms produces a proliferation defect. With these cells we are investigating the role of each variant on MMTV promoter repression, activation and chromatin remodelling, as well as on global gene expression by using microarrays. In parallel, we have developed specific antibodies for H1 isoforms 1 to 5, and generated cell lines expressing HA-tagged H1 isoforms that will be used on ChIP-on-chip experiments devoted to determine the presence of each form in several promoters of interest.

3. INFLUENCE OF CHROMATIN AT THE INTEGRATION SITE ON THE TRANSCRIPTIONAL ACTIVITY OF THE HIV PROMOTER.

Finally, we are also studying the influence of chromatin at the integration site on the transcriptional activity of the HIV promoter. HIV integrates at a multitude of sites without any clear preference in the human genome. The chromatin environment at the integration site influences the nucleosome structure of the viral promoter and consequently its basal and Tat-induced transcriptional activity (Jordan *et al*, EMBO J 20, 1726, 2001), in a way that is independent of the degree of methylation of the proviral DNA (Pion *et al*, J Virol 77, 4025, 2003). In this respect, we have shown that at low frequency integration occurs at regions of heterochromatin (i.e. pericentromeric) leading to promoter repression and to a state of viral latency that can be reactivated upon T cell activation (Jordan *et al*, EMBO J 22, 1868, 2003). We plan to compare the chromatin structure, histone code and protein recruitment to the proviral promoter when integrated in transcriptional-competent euchromatin or in repressed heterochromatin. In addition, by using RNA interference we are investigating the hypothetical participation of several chromatin components or histone modifying enzymes on the establishment and

maintenance of the repressive promoter state, which could be candidates for the therapeutic intervention against the latent state.

PUBLICATIONS

Rocha-Viegas L, Vicent GP, Barañao JL, Beato M, Pecci A "Glucocorticoids repress bcl-X expression in thymocytes by recruiting STAT5B to the P4 promoter." J Biol Chem 281, 33959-33970 (2006)

Vicent GP, Ballaré C, Nacht AS, Clausell J, Subtil-Rodríguez A, Jordan A, Beato M "Induction of progesterone target genes requires activation of Erk and Msk kinases and phosphorylation of histone H3." Mol Cell 24, 367-381 (2006)

Ballaré C, Vallejo G, Vicent, GP, Saragüeta P, Beato M "Progesterone signaling in breast cancer and endometrium." J Steroid Biochem Molec Biol 102, 2-10 (2006)

Vicent G P, Ballaré C, Nacht AS, Clausell J, Subtil-Rodríguez A, Quiles I, Jordán A, Beato M "Convergence on chromatin of non-genomic and genomic pathways of hormone signaling." J Steroid Biochem Mol Biol (in press)

BOOK CHAPTERS

Vicent GP, Ballare C, Zaurin R, Saragüeta P, Beato M "Chromatin remodeling and control of cell proliferation by progestins via cross talk of progesterone receptor with the estrogen receptor and kinase signaling pathways." In *Estrogens and Human Diseases,* H. L. Bradlow, and G. Carruba, eds. (New York: Blackwell), pp. 59-72 (2006)

NE REGULATIO

GENE REGULATION

Regulation of Alternative pre-mRNA Splicing during Cell Differentiation, Development and Disease

Alternative pathways of RNA processing lead to the synthesis of different mRNAs from a single gene, thus expanding the coding potential of the genome. Our group investigates how different cell types, or the same cell under different conditions, decide which mRNA to produce from the same mRNA precursor. This involves selective removal of some sequences —known as introns- and splicing together of the remaining sequences —exons— to generate mature mRNAs. During 2006 we have made progress to understand how accuracy in 3' splice site recognition can be achieved. We have also found a variety of molecular mechanisms by which splicing regulators modulate splice site selection, and a mechanism by which signalling by the apoptotic receptor Fas regulates alternative splicing of its own pre-mRNA. Finally, we have gained insights into how cells program changes in splicing that occur during fly sex determination, mammalian cell differentiation and Hodgkin tumor progression.

J. VALCÁRCEL HAS A SENIOR ICREA GROUP LEADER POSITION.

GROUP STRUCTURE

Group Leader:	**Juan Valcárcel**
Postdoctoral Fellows:	Claudia Ben-Dov Sophie Bonnal Britta Hartmann Josefin Lundgren Veronica Raker Joao Tavanez (since August 2006)
Students:	Mafalda Araujo Nuria Majos Luis Miguel Mendes Soares Anna Corrionero
Technicians:	Alicia Ezquerra Elisabet Muñoz

RESEARCH PROJECTS

1. ACCURACY IN 3' SPLICE SITE RECOGNITION

One outstanding question in the field of RNA splicing is how splice site sequences can specify with high accuracy the boundaries between exons and introns, while very similar sequences present throughout the transcriptome are not involved in the splicing process. We have found that the chromatin-associated protein DEK provides a proofreading activity that enforces accuracy in 3' splice site recognition. 3' splice sites are initially recognized by the 65 and 35 Kda subunits of the splicing factor U2AF, which bind –respectively- to the pyrimidine-rich tract and AG dinucleotide present at the 3' end of the intron. DEK forms a complex with U2AF35 and this association is necessary for recognition of the 3' splice site AG by U2AF35. In the absence of this recognition, U2AF65 becomes displaced from the RNA, thus preventing stable binding of U2AF to pyrimidine-rich sequences not associated with 3' splice sites. Remarkably, in the absence of DEK splicing is inhibited but formation os spliceosomal complexes is not, suggesting that the proofreading function of DEK is required for catalytric activation of the spliceosome (Figure 1).

2. MECHANISMS OF ALTERNATIVE SPLICING REGULATION

We have continued our analysis of alternative splicing of the apoptotic receptor Fas. Inclusion or skipping of exon 6 leads to mRNAs encoding the pro-apoptotic or anti-apoptotic forms of the receptor, respectively. One factor that promotes exon 6 inclusion is the protein TIA-1. TIA-1 binds to uridine-rich sequences immediately downstream of exon 6 5' splice site and facilitates recruitment of U1 small nuclear Ribonucleoprotein Particle (U1 snRNP) to the 5' splice site. We have investigated the possibility that TIA-1 activity is modulated by the signalling pathway induced upon binding of the Fas ligand. This was of particular interest because the factors and mechanisms that mediate the effects of intracellular signalling cascades on alternative pre-mRNA splicing are poorly understood. We found that Fas-Activated Serine/Threonine Kinase (FAST K), which is known to interact with and phosphorylate TIA-1, increases TIA-1 activity in U1 snRNP recruitment and Fas exon 6 inclusion, thus creating an autoregulatory loop that can serve to amplify Fas responses (Figure 2).

The protein SPF45 promotes skipping of Fas exon 6. In collaboration with the group of Michael Sattler (EMBL, Heidelberg) we have carried out a structure-function analysis of SPF45 activity. SPF45 contains a U2AF Homology Motif (UHM), which is a variant form of the common RNA Recognition Motif (RRMs) which mediates protein-protein interactions. We have found that the UHM domain is essential for SPF45 activity in Fas splicing regulation, identified protein partners of this motif and generated mutants with distinct binding specificities. Our results suggest that various independent interactions

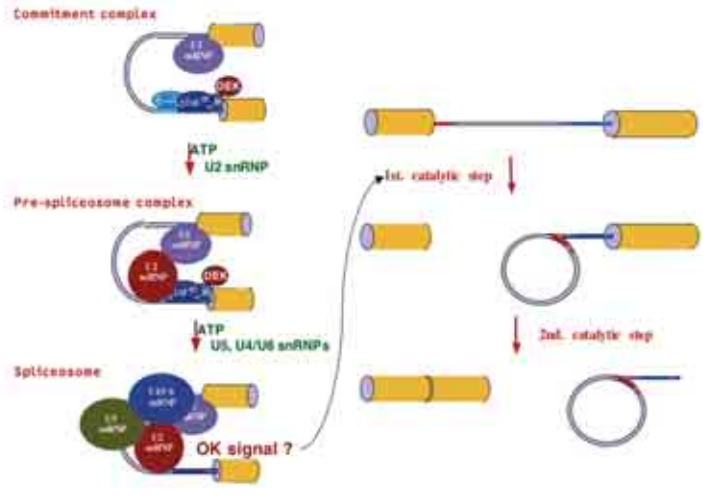


Figure 1. Proofreading of 3' splice site recognition by DEK is important for catalytic activation of the spliceosome. Association between DEK and the splicing factor U2AF is required for proper recognition of the 3' splice site by U2AF35. The proofreading activity of DEK is then required for catalytic activation of the spliceosome, even though U2AF and associated factors may be absent from fully assembled splicing complexes. In the absence of DEK, spliceosomes can assemble on the pre-mRNA but catalytic activation of the spliceosome is stalled.

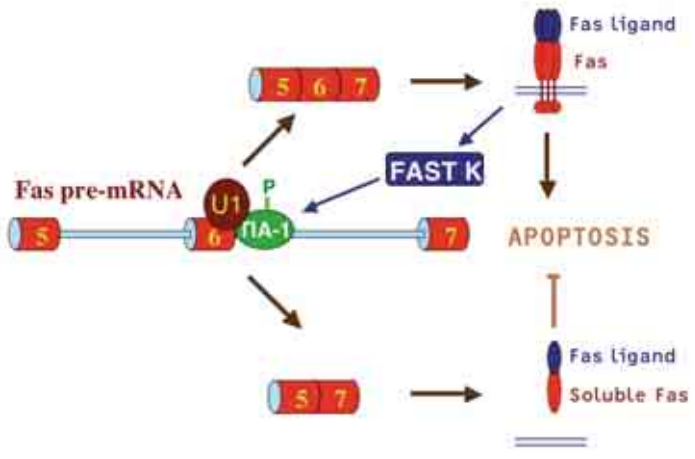


Figure 2. Regulation of the splicing factor TIA-1 by activation of Fas signalling. Binding of the Fas ligand to the Fas receptor induces activation of the FAST kinase. FAST phosphorylates TIA-1, which leads to increased activity of the protein in U1 snRNP recruitment to Fas exon 6 5' splice site. This results in higher levels of exon 6 inclusion and therefore in the accumulation of mRNAs encoding the pro-apoptotic form of the receptor, thus creating a positive regulatory loop that can amplify apoptotic signals.

mediated by the UHM domain are required for alternative splicing regulation.

3. CELLULAR PROGRAMS OF SPLICING REGULATION

We have designed microarrays able to distinguish between alternatively spliced mRNA isoforms and used them to analyze mechanisms of alternative splicing regulation. Several designs were completed using Agilent ink-jet platform technology, including genes encoding splicing factors, proteins of relevance for muscle differentiation and proteins related to cell transformation and cancer progression. Splicing events were identified computationally by alignment of genomic sequences to cDNA and EST libraries. Up to 22 probes were selected per event to detect constitutive and alternative exons as well as their corresponding splice junctions. Methods were developed to assess the statistical significance of variations in probe sets underlying changes in alternative splicing. These methods were further refined on the basis of experimental validation by quantitative RT-PCR.

We have applied these designs to the analysis of changes in alternative splicing in parallel with changes in the expression of all known components of the spliceosome, splicing regulatory factors and their isoforms. We have compared a variety of biological samples, including different tissues, cell lines undergoing muscle differentiation and cell lines derived from Hodgkin lymphoma tumors at different stages of tumor progression.

One first insight obtained from these studies was that relatively low number of changes in the expression of splicing factors is associated with the diverse array of biological processes studied. Interestingly, some of these changes are associated with variations in the expression / alternative splicing of factors which act at late steps of the assembly of splicing complexes. This observation suggests that in addition to promoting or inhibiting early splice site recognition, physiological mechanisms of splicing regulation can also target subsequent steps in spliceosome assembly and splice site pairing.

Sex-determination in *Drosophila* offers a textbook example of an alternative splicing regulatory cascade affecting a handful of genes. We have investigated to which extent the fly transcriptome shows sex bias at the level of AS using splicing microarrays. We have found that over 400 genes show differences in the relative abundance of mRNA isoforms between male and females flies. Interestingly, this category is enriched in factors involved in mRNA translation and pre-mRNA splicing. Searches for binding sites for the sex-determination factors SXL and TRA/TRA-2 revealed that only a small number of events harbor such sites, suggesting the existence of additional regulatory mechanisms for sex-specific splicing in Drosophila.

PUBLICATIONS

Mendes Soares LM and Valcárcel J "The expanding transcriptome: the genome as the "Book of Sand"." EMBO Journal, 25, 923-931 (2006)

Mendes Soares LM, Zanier K, Mackereth C, Sattler M and Valcárcel J "Intron removal requires proofreading of U2AF / 3' splice site recognition by DEK." Science, 312, 1961-1965 (2006)

Izquierdo JM and Valcárcel J "A simple principle to explain the evolution of pre-mRNA splicing." Genes & Development, 20, 1679-1684 (2006)

Guigó R and Valcárcel J "Unweaving the meanings of messenger RNA sequences" Molecular Cell, 23, 150-151 (2006)

Izquierdo JM and Valcárcel J "Fas-activated serine/threonine kinase (FAST) synergizes with TIA-1 / TIAR proteins to regulate Fas alternative splicing" Journal of Biological Chemistry, 282, 1539-1543 (Nov 29 2006 [Epub ahead of print])

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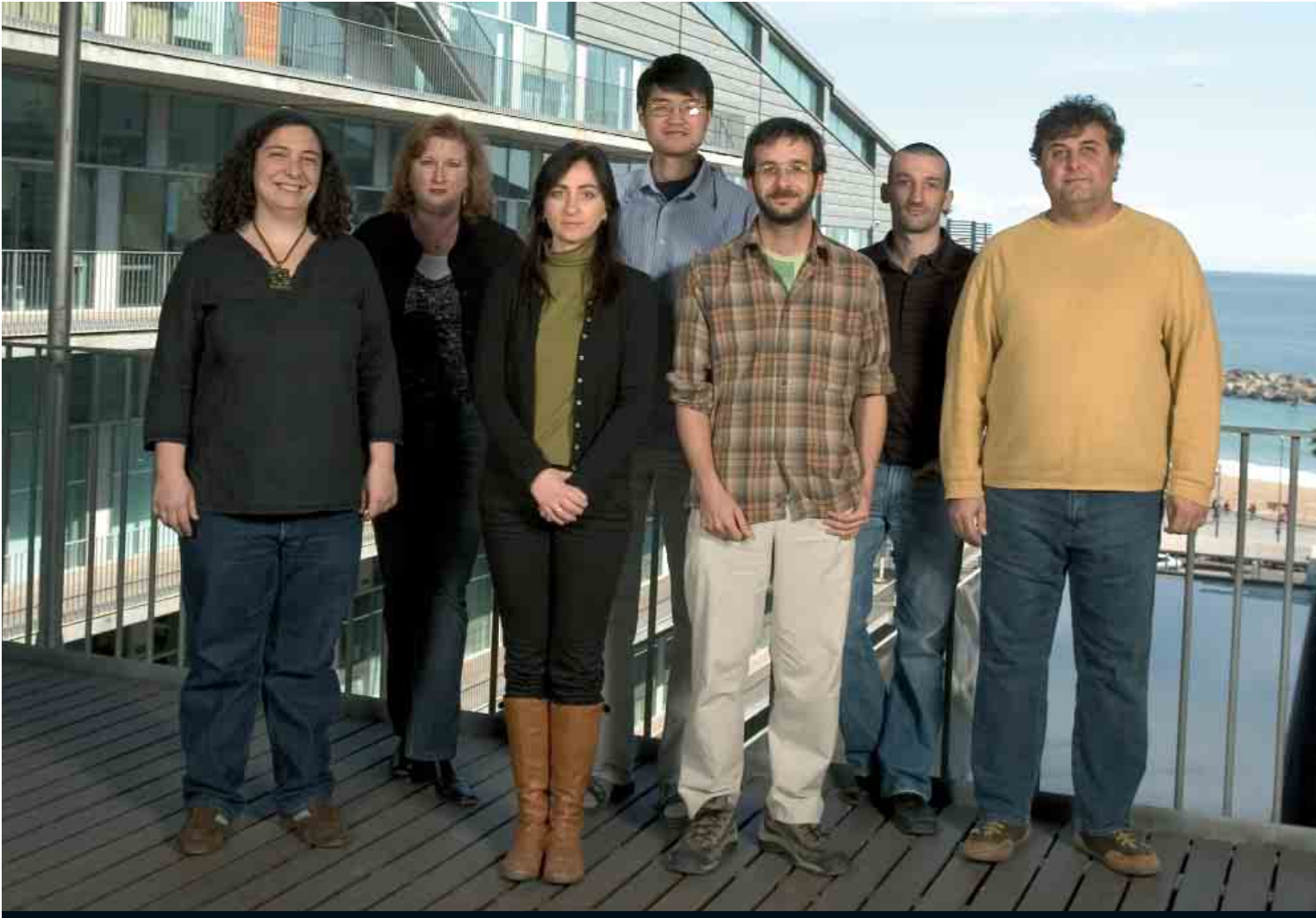

GENE REGULATION

RNA Interference and Chromatin Regulation

We are pursuing research in two major areas. The first is the molecular mechanism of cancer. We are working to address the mechanism by which tumor suppressors such as BRCA1 and BRCA2 exerted their biological effects. Our second avenue of research entails the delineation of the mechanisms by which the genome is silenced through chromatin modification and small regulatory RNA. The laboratory's goal is to understand the epigenetic regulation of gene expression in mammalian development and genetic disease. Histone modifying enzymes are a crucial component of the epigenetic control of gene activity through the regulation of chromatin state. We are also elucidating the pathways that direct transcriptional and posttranscriptional repression by small regulatory RNAs in human cells. These studies will also intersect with our ongoing studies on the BRCA1 and BRCA2 proteins as complexes containing BRCA1 and BRCA2 may play a role in directing the formation of a non-permissive chromatin through association with Xist RNA. Moreover, such studies will undoubtedly shed light on the mechanism of X-inactivation and imprinting as similar effector complexes may be responsible for other RNA-mediated transcriptional repression.

R. SHIEKHATTAR HAS A SENIOR ICREA GROUP LEADER POSITION.

GROUP STRUCTURE

Group Leader:	**Ramin Shiekhattar**
Staff Scientist:	Glenda Harris
Postdoctoral Researchers:	David Baillat Frederic Tort Laure Weill Fan Lai

RESEARCH PROJECTS

1. TITLE MODULATION OF CHROMATIN STRUCTURE BY TUMOR SUPPRESSORS AND REGULATORY RNAS IN HUMAN CELLS

Our initial experiments started by isolating BRCA1- and BRCA2- containing complexes using a combination of conventional chromatographic and affinity-purification techniques from HeLa nuclear extract. These studies resulted in the characterization of the BRCA1 interaction with the chromatin remodeling factor SWI/SNF (Bochar et al. 2000a) and the association of the BRCA2 protein with a novel HMG-containing protein BRAF35 (Marmorstein et al. 2001). We developed stable cell lines expressing an epitope-tagged BARD1, a binding partner of BRCA1. Isolation of the BARD1-containing complex resulted in the characterization of BRCC, a multiprotein E3 ubiquitin ligase complex that contains both BRCA1 and BRCA2 proteins (Dong et al. 2003). We have also identified a number of novel BRCC components (including BRCC36 and BRCC45) having similarities to the subunits of the proteasome and the signalosome. These studies have not only revealed a regulatory role for BRCC36 and BRCC45 in modulating the enzymatic activity of BRCA1/BARD1 but also point to the aberrant expression of these genes in sporadic breast cancers. Collectively, our studies have strengthen the hypothesis suggesting a dual role for BRCA1 and BRCA2 in transcription and DNA repair through modulating chromatin structure.

We have previously described a multiprotein complex termed the BHC or BRAF-HDAC complex, which is required for the repression of neuronal-specific genes. We have shown that the BHC complex is recruited by a neuronal silencer, REST (RE1-silencing transcription factor), and mediates the repression of REST-responsive genes. BHC is a multiprotein complex consisting of two enzymatic activities: a histone deacetylase (HDAC1 or 2) and a recently described histone demethylase (BHC110, also known as LSD1 or AOF2). We have reported (Lee et al., 2005) that BHC110-containing complexes show a nearly fivefold increase in demethylation of histone H3 lysine 4 (H3K4) compared to recombinant BHC110. Furthermore, recombinant BHC110 is unable to demethylate H3K4 on nucleosomes, but BHC110-containing complexes readily demethylate nucleosomes. In vitro reconstitution of the BHC complex using recombinant subunits reveals an essential role for the REST corepressor CoREST, not only in stimulating demethylation on core histones but also promoting demethylation of nucleosomal substrates. We find that nucleosomal demethylation is the result of CoREST enhancing the association between BHC110 and nucleosomes. Depletion of CoREST in in vivo cell culture results in de-repression of REST-responsive gene expression and increased methylation of H3K4. Together, these results highlight an essential role for CoREST in demethylation of H3K4 both in vitro and in vivo.

Differentiation of progenitor cells into post-mitotic neurons requires the engagement of mechanisms by which the repressive effects of the neuronal silencer, RE-1 silencing transcription factor (REST), can be overcome. Previously, we described a high-mobility group (HMG)-containing protein, BRAF35, which is a component of a co-repressor complex that is required for the repression of REST-responsive genes. We have reported (Wynder et al., 2005) that the BRAF35 family member inhibitor of BRAF35 (iBRAF) activates REST-responsive genes through the modulation of histone methylation. In contrast to BRAF35, iBRAFexpression leads to the abrogation of REST-mediated transcriptional repression and the resultant activation of neuronal-specific genes. Analysis of P19 cells during neuronal differentiation revealed an increased concentration of iBRAF at the promoter of neuronal-specific genes coincident with augmented expression of synapsin, recruitment of the methyltransferase MLL and enhanced trimethylation of histone H3 lysine 4 (H3K4). Importantly, ectopic expression of iBRAF is sufficient to induce neuronal differentiation through recruitment of MLL, resulting in increased histone H3K4 trimethylation and activation of neuronal-specific genes. Moreover, depletion of iBRAF abrogates recruitment of MLL and enhancement of histone H3K4 trimethylation. Together, these results indicate that the HMG-domain protein iBRAF has a key role in the initiation of neuronal differentiation.

We have shown (Lee et al., 2006) that HDAC inhibitors diminish histone H3 lysine 4 (H3K4) demethylation by BHC110 in vitro. In vivo analysis revealed an increased H3K4 methylation concomitant with inhibition of

nucleosomal deacetylation by HDAC inhibitors. Reconstitution of recombinant complexes revealed a functional connection between HDAC1 and BHC110 only when nucleosomal substrates were used. Importantly, while the enzymatic activity of BHC110 is required to achieve optimal deacetylation in vitro, in vivo analysis following ectopic expression of an enzymatically dead mutant of BHC110 (K661A) confirmed the functional cross talk between the demethylase and deacetylase enzymes. Our studies not only reveal an intimate link between the histone demethylase and deacetylase enzymes but also identify histone demethylation as a secondary target of HDAC inhibitors.

2. TITLE MECHANISM OF POSTTRANSCRIPTIONAL SILENCING BY SMALL NON-CODING RNA

To address the role of microRNAs in RNA interference, we have begun a systematic isolation of RNAi-mediated effector complexes from human cells. We have developed stable cell lines expressing an epitope tagged forms of a number of known RNAi components, including Dicer, Ago1 through Ago4, Drosha and many of the RNA helicases, known in the genetic model systems to play a role in the RNAi pathway. Concomitantly, we have generated polyclonal antibodies to these components which will assist us in isolating native complexes. To again insight into the nuclear complexes as effectors of RNAi in gene expression, we are isolating such complexes not only from the cytoplasmic fraction but also the nuclear and chromatin-bound fractions.

MicroRNAs (miRNAs) are generated by a two-step processing pathway to yield RNA molecules of approximately 22 nucleotides that negatively regulate target gene expression at the post-transcriptional level. Primary miRNAs are processed to precursor miRNAs (pre-miRNAs) by the Microprocessor complex. These pre-miRNAs are cleaved by the RNase III Dicer to generate mature miRNAs that direct the RNA-induced silencing complex (RISC) to messenger RNAs with complementary sequence. We have reported (Chendrimada *et al.*, 2005) that TRBP (the human immunodeficiency virus transactivating response RNA-binding protein), which contains three double-stranded, RNA-binding domains, is an integral component of a Dicer-containing complex. Biochemical analysis of TRBP-containing complexes revealed the association of Dicer-TRBP with Argonaute 2 (Ago2), the catalytic engine of RISC. The physical association of Dicer-TRBP and Ago2 was confirmed after the isolation of the ternary complex using Flag-tagged Ago2 cell lines. In vitro reconstitution assays demonstrated that TRBP is required for the recruitment of Ago2 to the small interfering RNA (siRNA) bound by Dicer. Knockdown of TRBP results in destabilization of Dicer and a consequent loss of miRNA biogenesis. Finally, depletion of the Dicer-TRBP complex via exogenously introduced siRNAs diminished RISC-mediated reporter gene silencing. These results support a role of the Dicer-TRBP complex not only in miRNA processing but also as a platform for RISC assembly.

The C-terminal domain (CTD) of RNA polymerase II (RNAPII) is an essential component of transcriptional regulation and RNA processing of protein-coding genes. A large body of data also implicates the CTD in the transcription and processing of RNAPII-mediated small nuclear RNAs (snRNAs). However, the identity of the complex (or complexes) that associates with the CTD and mediates the processing of snRNAs has remained elusive. Here, we describe an RNA polymerase II complex that contains at least 12 novel subunits, termed the Integrator, in addition to core RNAPII subunits. Two of the Integrator subunits display similarities to the subunits of the cleavage and polyadenylation specificity factor (CPSF) complex. We have reported (Baillat *et al.*, 05) that Integrator is recruited to the U1 and U2 snRNA genes and mediates the snRNAs' 3' end processing. The Integrator complex is evolutionarily conserved in metazoans and directly interacts with the C-terminal domain of the RNA polymerase II largest subunit.

RNA interference is implemented through the action of the RNA-induced silencing complex (RISC). Although Argonaute2 has been identified as the catalytic center of RISC, the RISC polypeptide composition and assembly using short interfering RNA (siRNA) duplexes has remained elusive. We have found (Gregory *et al.*, 2005) that RISC is composed of Dicer, the double-stranded RNA binding protein TRBP, and Argonaute2. We demonstrate that this complex can cleave target RNA using precursor microRNA (pre-miRNA) hairpin as the source of siRNA.

Although RISC can also utilize duplex siRNA, it displays a nearly 10-fold greater activity using the pre-miRNA Dicer substrate. RISC distinguishes the guide strand of the siRNA from the passenger strand and specifically incorporates the guide strand. Importantly, ATP is not required for miRNA processing, RISC assembly, or multiple rounds of target-RNA cleavage. These results define the composition of RISC and demonstrate that miRNA processing and target-RNA cleavage are coupled. (Gregory *et al.*, 2005)

PUBLICATIONS (*)

Yang W, Chendrimada TP, Wang Q, Higuchi M, Seeburg PH, Shiekhattar R, and Nishikura K "Modulation of microRNA processing and expression theorugh RNA editing by ADAR deaminases." Nature Sruct Mol Biol, 13, 13 (2006)

Ross DA, Hannenhalli S, Tobias JW, Cooch N, Shiekhattar R, and Kadesch T "Functional analysis of Hes-1 in preadipocytes." Mol Endocrinol, 20, 698 (2006)

Norman JA, and Shiekhattar R "A proteomic approach to ubiquitin-like modifications." Biochemistry, 45, 3015 (2006)

Da G, Lenkart J, Zhao K, Shiekhattar R, Cairns BR, and Marmorstein R "Structure and function of the SWIRM domain, a conserved protein module found in chromatin regulatory complexes." Proc Natl Acad Sci, 103, 2057 (2006)

Lee GM, Wynder C, Schmidt DM, MaCafferty DG, and Shiekhattar R "Histone H3 lysine 4 demethylation is a target of nonselective antidepressive medications." Chemistry and Biology, 13, 563 (2006)

Lee GM, Wynder C, Bochar DA, Hakimi MA, Cooch N, and Shiekhattar R "Functional interplay between histone demethylase and deacetylase enzymes." Mol Cell Biol, 26, 6395 (2006)

Nicolas E, Lee MG, Hakimi MA, Cam HP, Grewal SI, Shiekhattar R "Fission yeast homology of human histone H3 lysine 4 demethylase regulate a common set of genes with diverse functions" J Biol Chem, 281(47), 35983-8 (2006)

Gregory RI, Chendrimada TP, Shiekhattar R "MicroRNA biogenesis: isolation and characterization of the microprocessor complex" Methods Mol Biol, 342, 33-47 (2006)

Lee MG, Wynder C, Norman J, Shiekhattar R "Isolation and characterization of histone H3 lysine 4 demethylase-containing complexes" Methods, 40(4), 327-30 (2006)

(*) All these publications are the result of the work of Dr. Ramin Shiekhattar at the Wistar Insitute, Philadelphia, USA

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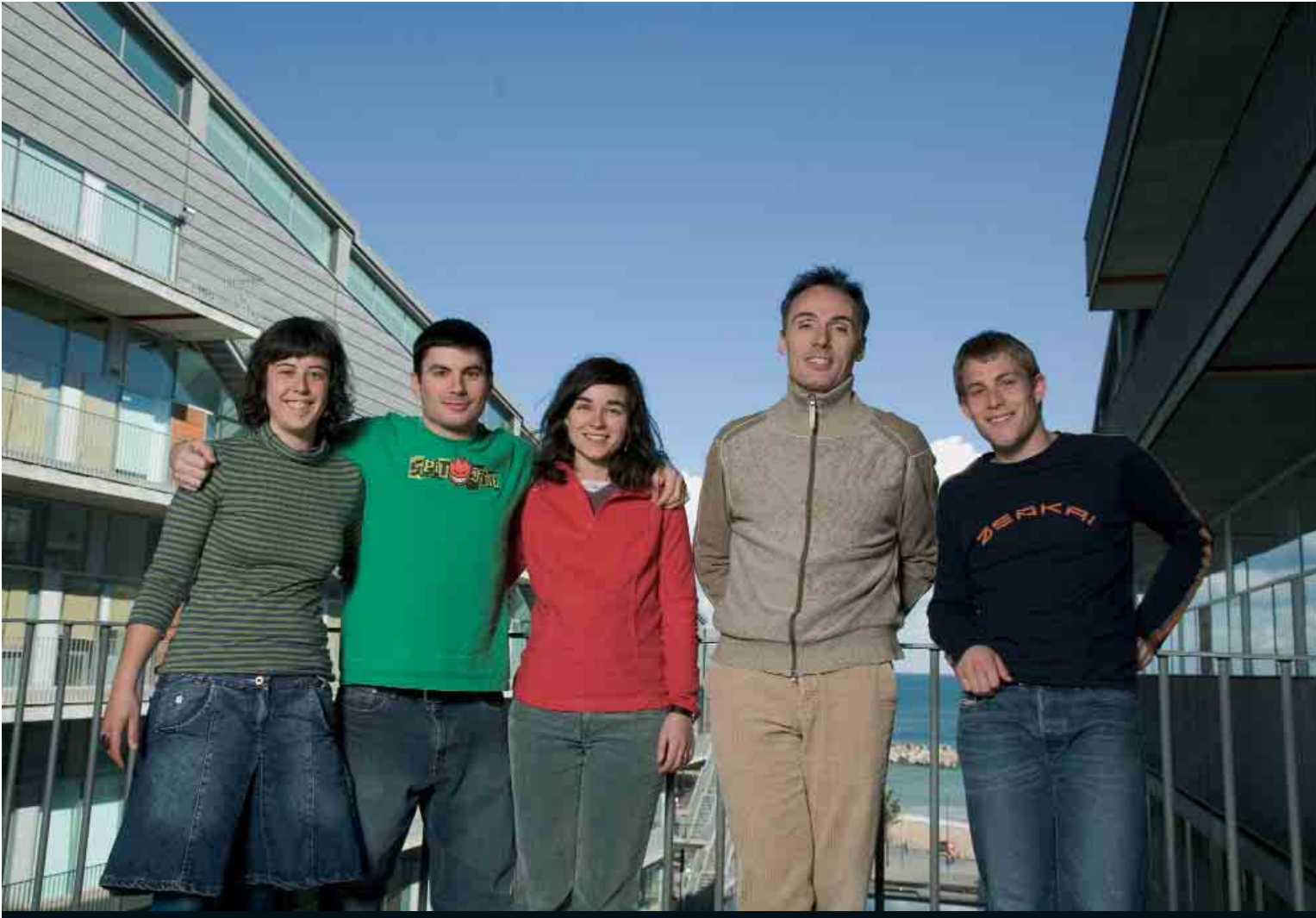


GENE REGULATION

RNA-Protein Interactions and Regulation

We are focused on the study of the molecular mechanisms of regulation of gene expression by RNA-protein interactions, using the model organism *Saccharomyces cerevisiae*. We have two main research goals in our laboratory. First, to dissect the molecular interactions involved on RPL30 regulation of splicing. Second, to further characterize the extent in which splicing is regulated in *Saccharomyces*.

RPL30, one of the best understood models of splicing regulation in *Saccharomyces,* encodes the ribosomal protein L30. Thru binding to a structure present in its own transcript L30 can regulate RNA processing at several steps. Our main interest is on control of splicing (see Fig 1), and the L30 system of regulation should provide insights on how RNA sequences and structures near the 5' splice site (ss) can affect spliceosome assembly and splicing.

In addition, in collaboration with other groups, we are undertaking bioinformatic and genomic approaches to uncover new instances of regulation, and to investigate how spread is this control of gene expression and its degree of coordination.

GROUP STRUCTURE

Group Leader:	**Josep Vilardell**
Postdoctoral Researchers:	Estefanía Muñoz John LaCava
PhD Students:	Mireia Bragulat Sara Macias Markus Meyer
Diploma Thesis Student:	Silvia Ramundo
Technician:	Asunción Romero

RESEARCH PROJECTS

1. GENETIC SCREEN TO SELECT MUTANTS IN REGULATION OF SPLICING

M. Bragulat

Employing refined screening methods we have isolated mutants that alter L30 regulation of splicing in either way: some mutants display a phenotype in which L30 can not regulate splicing of a target transcript; while others behave in the opposite way, in which L30 can regulate splicing of a transcript bearing a mutation that blocks L30 inhibition in wild type cells. One of the latter mutations is located in the gen *ST01*, encoding the large subunit of the cap binding complex (CBC), Cbp80. CBC has been shown to be required for the proper stability of U1 snRNP bound to the pre-mRNA, and we are investigating how this can affect L30 regulation. Our data indicates that the observed effect of Cbp80 could be unrelated to its role on U1 snRNP binding, providing data on a new function of this important factor.

2. BIOCHEMICAL ANALYSIS OF THE MECHANISM OF RPL30 CONTROL OF SPLICING.

S. Macias

As shown in Fig. 1, during L30 regulation of splicing a new complex is formed. We are interested on the nature of this complex, its components and their interactions. Crosslinking and western approaches together with biochemical purification procedures are being followed. We have generated interesting data showing that U1 RNA can be crosslinked to the 5' SS and by co-IPs with extracts with tagged splicing factors a picture of the regulation mechanism is becoming clear, suggesting that L30 is likely to disrupt the crosstalk between splicing signals. We are trying to refine the particular step that is being altered by L30, with data pointing to the stable association of U2 snRNP to the transcript, after U1 snRNP has committed the substrate to splicing. We have been able to link the role of L30 as a splicing factor with transcription, providing novel evidence about the connection of regulated splicing and pre-mRNA synthesis.

3. PROTEOMIC AND STRUCTURAL ANALYSES OF THE INHIBITED COMPLEX

John LaCava

We have the possibility to explore, analytically and structurally, a regulated splicing complex. We can purify the L30-stalled pre-spliceosome, or *inhibited complex*, in analytical amounts, either from *in vitro* approaches or from specially designed strains, *in vivo*. Our aim is to identify all the components present in this complex. Their interactions will be further analyzed by cross-linking experiments; and finally, if technically feasible, by cryo-EM, in collaboration with other leader groups in the field (Reinhard Luhrmann).

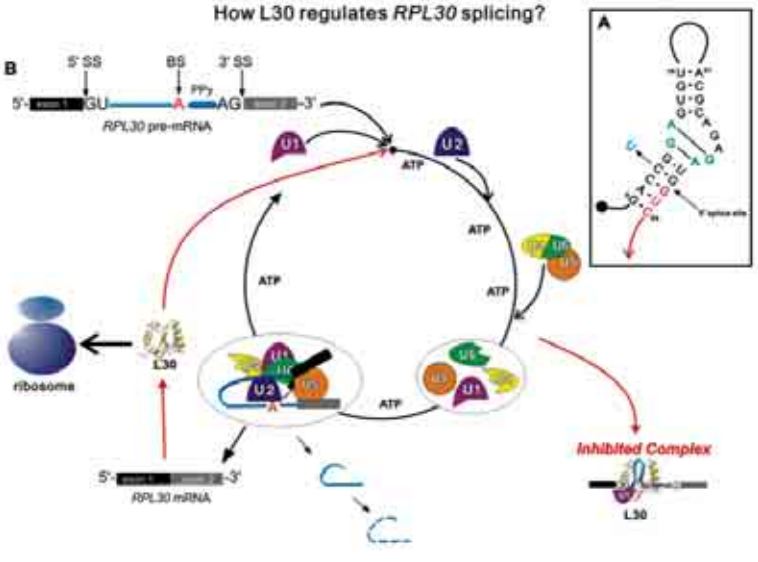


Figure 1. Regulation of RPL30 splicing by L30. (A) Secondary structure of the RNA element required for L30 biding. Nucleotides 17-50 are not involved. Mutation C9 to U (blue) abolishes regulation, probably by weakening L30 binding. The 5' splice site and nucleotides complementary to U1 snRNA are indicated. (B) L30 blocks spliceosome assembly at an early step, generating a new stable complex, the "inhibited" complex.

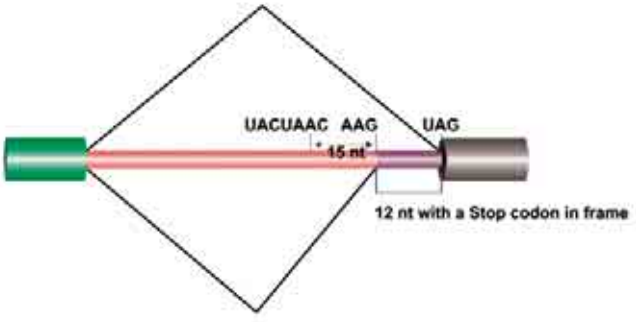


Figure 2. *DMC1* shows dual use of its 3'SS. The shorter version of the intron produces an mRNA with a premature stop codon and it's degraded by the non-sense mediated decay pathway.

In addition, we are collaborating with the group of Dr. Jean Beggs (University of Edinburgh) to apply genomic approaches to this question. We have supplemented their *Saccharomyces* splicing microarray with additional probes that can detect new, predicted, 3'SS splicing events.

4. GENETIC INTERACTIONS IN POSITIONS 3 AND 4 OF THE INTRON

J. Vilardell (in collaboration with Charles Query (Albert Einstein College, NY; and Magda Konarska, Rockefeller University)

The consensus sequence of the yeast 5' ss is GUAUGU, while that of L30 is GUCAGU, with A3C+U4A changes, evolutionarily conserved. However, it is known that A3C can be deleterious for splicing, while U4A has practically no effect. Studying the genetic interactions between positions 3 and 4 of a yeast intron we have been able to propose a new model for spliceosomal function, in which the first nucleotides of the intron would interact with two distinct regions of U6 snRNA. These interactions have to be disrupted to allow the spliceosome a conformational shift between the first and second step of splicing, implying also a repositioning of the substrates between both steps. This has been submitted, and published in *Molecular Cell* on 2006.

5. OTHER *S. CEREVISIAE* GENES WITH REGULATED SPLICING

Markus Meyer, E. Muñoz, S. Ramundo, Asun Romero

To what extent splicing regulation plays a role in the control of gene expression in *Saccharomyces*? Using bioinformatics, several studies are being performed. We are looking at (1) the possible folding around 5' splice site regions; and (2), putative alternate 3'SS. In both cases we include phylogenetic comparisons in our studies. We are collaborating in these efforts with the group of Dr. Eduardo Eyras (UPF).

As a fruit of these efforts a novel case of dual use of 3'SS has been uncovered (Fig. 2), and other cases show discrepancies with the annotated sequences in our experimental conditions.

PUBLICATIONS

Konarska MM, Vilardell J, Query CC "Repositioning of the reaction intermediate within the catalytic center of the spliceosome". Molecular Cell, 21, 543-553 (2006)

GENE REGULATION

Regulation of Protein Synthesis in Eukaryotes

We are interested in the regulation of mRNA translation by RNA-binding proteins and by elongation of the mRNA poly(A) tail (i.e. cytoplasmic polyadenylation). We study these mechanisms under three different biological contexts: X-chromosome dosage compensation, early embryonic patterning and cell cycle progression.

GROUP STRUCTURE

Group Leader:	**Fátima Gebauer**
Postdoctoral Researcher:	Rafael Cuesta
Students:	Irina Abaza Solenn Patalano Aida Martínez
Technicians:	Olga Coll Elisabeth Muñoz

RESEARCH PROJECTS

1. TRANSLATIONAL CONTROL OF DOSAGE COMPENSATION

Dosage compensation in *Drosophila* is achieved by hypertranscription of the male X chromosome via the action of a ribonucleoprotein complex known as the MSL (for male specific lethal). This process is inhibited in female flies primarily because of the lack of expression of a critical MSL subunit, the protein MSL-2. Expression of *msl-2* is prevented by the female-specific RNA-binding protein Sex-lethal (SXL). SXL binds to uridine stretches present in the 5' and 3' UTRs of *msl-2* mRNA and inhibits its translation. Translational repression requires an additional factor that is recruited by SXL to the 3´UTR of *msl-2*. We have identified this factor as the *Drosophila* homolog of mammalian Upstream of N-ras (UNR). UNR is an ubiquitous cytoplasmic protein present in both male and female flies (Figure 1). Translational repression of *msl-2* occurs by a "double-block" mechanism: the SXL/UNR complex bound to the 3' UTR inhibits the stable recruitment of the small ribosomal subunit, while SXL bound to the 5' UTR inhibits the scanning of those subunits that presumably have escaped the first control (Figure 2). Our results indicate that SXL provides a sex-specific function to UNR, and suggest that UNR is a novel regulator of dosage compensation in *Drosophila*.

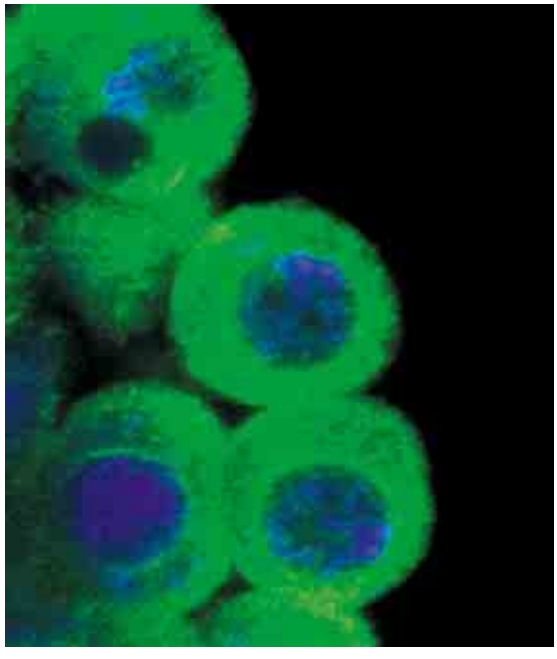


Figure 1. Intracellular localization of UNR. UNR (green) is a cytoplasmic protein.

2. TRANSLATIONAL REGULATION OF EARLY EMBRYONIC PATTERNING

A number of transcripts encoding factors important for antero-posterior and dorso-ventral axis formation in the *Drosophila* embryo are translationally activated by cytoplasmic polyadenylation. The cis-acting sequences and the factors regulating cytoplasmic poly(A) tail elongation in *Drosophila* are largely unknown. We are using a cell-free cytoplasmic polyadenylation/ translation system to study the translational regulation of toll mRNA and have identified novel cytoplasmic polyadenylation elements.

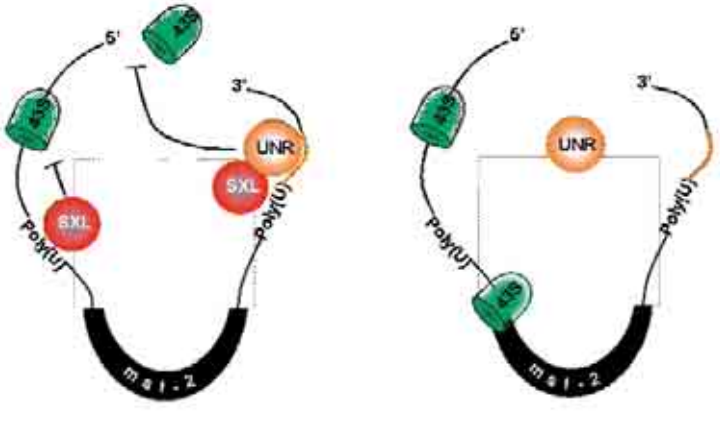


Figure 2. Translational control of msl-2 mRNA. SXL binds to poly(U) stretches in the 5' and 3' UTRs of *msl-2*. 3'-bound SXL recruits UNR, and the complex inhibits the association of the 43S pre-initiation complex with the mRNA. 5'-bound SXL inhibits the scanning of those 43S complexes that presumably have escaped the 3'-mediated control.

3. REGULATION OF P27^{KIP} MRNA TRANSLATION

p27^{kip} is a cyclin-dependent kinase (cdk) inhibitor that blocks the mammalian cell cycle in G1. Proper modulation of p27^{kip} levels is essential for cell proliferation. One of the mechanisms that dictate the level of p27^{kip} is the translational regulation of its mRNA. Our goal is to identify factors that specifically regulate p27^{kip} mRNA translation. We are currently trying to identify regulatory sequences for translation in p27^{kip} mRNA by using cell transfection approaches as well as in vitro translation in extracts generated from synchronized cells.

PUBLICATIONS (*)

Gebauer F and Hentze MW "Studying translational control in Drosophila cell-free systems". In: *Methods in Enzymology. Mechanistic studies of eukaryotic translation initiation*. Jon Lorsch (Ed). Academic Press (in press)

Hentze MW, Gebauer F and Preiss T "Cis regulatory sequences and trans- acting factors in translational control." In: *Translational control in Biology and Medicine,* Mathews, M. B., Sonenberg, N. and Hershey, J. W. B. (Eds). Cold Spring Harbor Laboratory Press (in press)

Abaza I, Coll O, Patalano S and Gebauer F "Drosophila UNR is required for translational repression of male-specific-lethal 2 mRNA during regulation of X chromosome dosage compensation." Genes Dev., 20, 380-389 (2006) (*)

Duncan K, Grskovic M, Strein C, Bechmann K, Niggeweg R, Abaza I, Gebauer F, Wilm M and Hentze MW "Sex-lethal imparts a sex-specific function to UNR by recruiting it to the msl-2 mRNA 3' UTR: translational repression for dosage compensation." Genes Dev., 20, 368-379 (2006) (*)

(*) *News and views* by A-B. Shyu. "UNRaveling the regulation of dosage compensation." Nat. Struct. Mol. Biol., 13, 189- 190 (2006).

NE REGULATIO

GENE REGULATION

Translational Control of Gene Expression

The primary interest of our group is to understand the molecular mechanisms that control the temporal and spatial translation of mRNAs during the cell cycle progression and early embryonic development. These events are programmed, at least in part, by maternally inherited mRNAs whose translation is specifically regulated by sequences located at the 3´-untranslated region (3´-UTR) of the mRNA and their binding proteins. Over the past few years, our work has focused on cytoplasmic polyadenylation as perhaps the most important mechanism for regulating translation in these systems. The knowledge of the molecular mechanisms that govern translational control during meiotic progression will then be applied to other mRNAs during cell cycle progression and stress response in somatic cells.

GROUP STRUCTURE

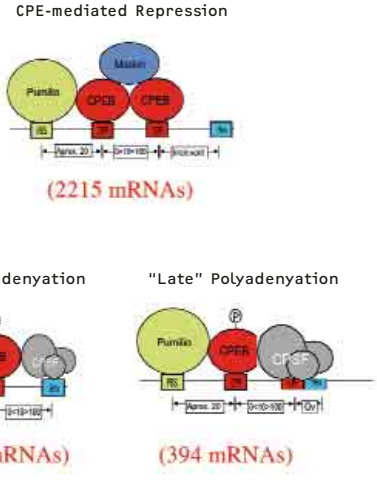
Group Leader:	**Raul Méndez**
Postdoctoral Researchers:	Isabel Novoa (Ramón y Cajal-awarded) Maria Pique
Students:	Carolina Eliscovich (Graduate Student) Eulalia Belloc (Graduate Student) Ana Igea (Graduate Student)
Technician:	Javier Gallego

RESEARCH PROJECTS

1. DETERMINATION OF THE 3'-UTR FEATURES THAT DEFINE THE TIMING OF CYTOPLASMIC POLYADENYLATION AND THE SILENCING OF AN MRNA.

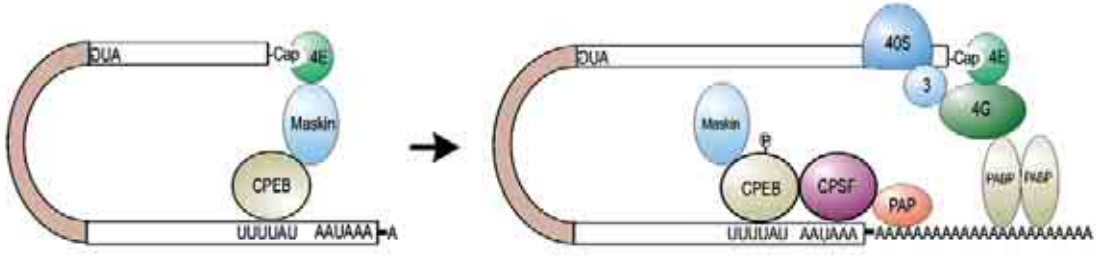
Mos, cyclin B1, and several other dormant mRNAs in oocytes contain short poly(A) tails (~20-40 nts), and it is only when these tails are elongated (to ~150 nts) does translation takes place. Cytoplasmic polyadenylation requires two elements in the 3'-UTR, the hexanucleotide AAUAAA, which is also necessary for nuclear pre-mRNA cleavage and polyadenylation, and the nearby cytoplasmic polyadenylation element (CPE). The CPE is bound by CPEB, a highly conserved zinc finger and RRM type RNA-binding protein. The CPE is not only necessary for cytoplasmic polyadenylation-induced translation in maturing oocytes, it also mediates translational repression (masking) in unstimulated oocytes. This event is mediated by Maskin, a protein that interacts with CPEB as well as the cap binding protein eIF-4E.

The Cyclin B family is composed of five functionally redundant members that are differentially expressed during oogenesis and meiosis. The detailed analysis of the cis-acting elements present in those mRNAs has allowed us to propose a global model of CPE-mediated translational regulation that, not only explains the different behaviour of the cyclins mRNAs mentioned above, but that can also be extrapolated to explain the differential translational control of all known cytoplasmically polyadenylated mRNAs and even to predict the translational regulation of mRNAs with putative CPEs. This model is based in a combinatorial model of three cis-acting elements (i.e., NRE, CPE and Hexanucleotide), which recruit three trans-acting factors (i.e., Pumilio, CPEB and CPSF). The number, relative position and exact sequence of these elements determine the specific time and amount of polyadenylation, as well as the active repression of the mRNA, allowing for a very accurate control of gene expression. Thanks to this approach, and in collaboration with the group of Roderic Guigó (CRG), we have generated algorithms to identify new mRNAs regulated by cytoplasmic polyadenylation and to predict their time and extent of activation.



2. CYTOPLASMIC POLYADENYLATION ROLE IN THE MITOTIC SPINDLE FORMATION AND CHROMOSOME SEGREGATION DURING CELL DIVISION

Once polyadenylation takes place during oocyte maturation, most of the CPEB (~90%) is destroyed; virtually all that remains



Translationally dormant

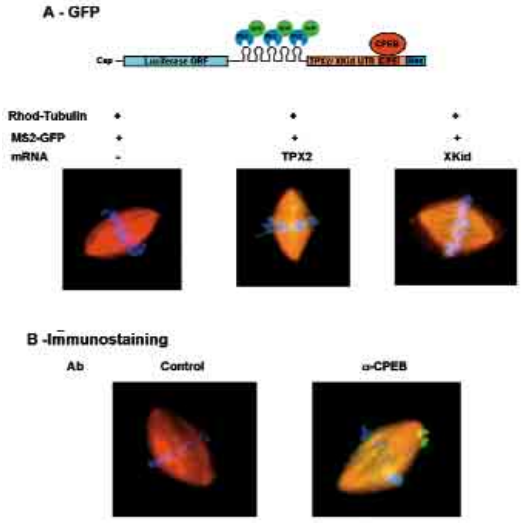
Translationally active

stable is confined to animal pole blastomeres where it is strongly associated with spindles and centrosomes. When injected into embryos, reagents that are known to disrupt polyadenylation-induced translation (e.g., CPEB antibody or a CPEB dominant negative mutant) inhibit cell division and produce abnormal mitotic structures. These results suggest that cell division requires polyadenylation-induced translation, but they do not indicate which mRNA(s) might be involved.

Based on the combination of cis-acting elements defined above. We have identified maternal mRNAs, encoding for proteins that regulate spindle formation, which are polyadenylated in response to progesterone and targets for CPEB-regulated translation. In collaboration with Isabelle Vernos group (CRG) we have shown that the 3'-UTRs of these mRNAs mediate spindle-localized translation and that localized CPE-mediated translation is required for meiotic progression.

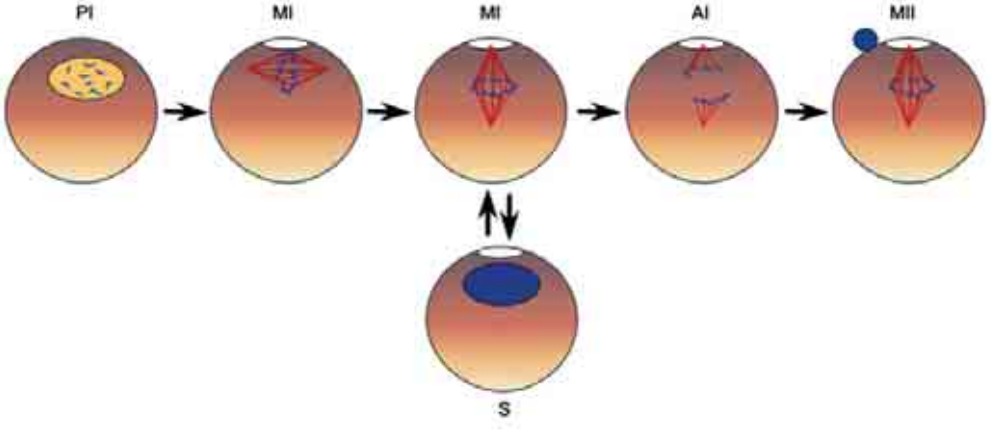
3. FUNCTIONAL SCREENING TO IDENTIFY NEW CYTOPLASMICALLY POLYADENYLATED MRNAS THAT REGULATE CELL CYCLE PROGRESSION

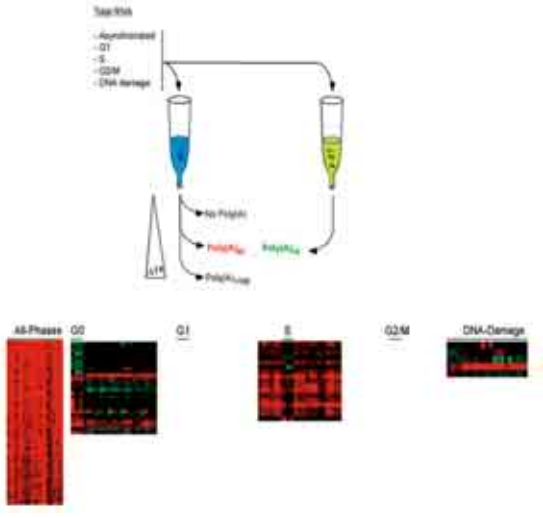
Up to the date, only a small number of mRNAs with functional CPEs have been identified, all of then involved in the regulation of cell cycle. However, these few examples are far from accounting for all the targets of the CPE-mediated translational control during meiotic progression. Therefore, we have designed a functional screening to identify new cytoplasmically polyadenylated mRNAs,



both during the PI→MI transition and the MI→MII transition.

The results from the screening have allowed us to validate the model proposed above and to identify many new mRNAs that are potentially regulated by CPEB. The next step we are undertaking is to determine which of the identified mRNAs have to be translated during meiotic progression. For that purpose we are cleaving the 3'-UTRs of the target mRNAs by microinjecting antisense-oligonucleotides and analyzing the effect of this treatment on the PI-MI and MI-MII transitions. With this approach we have already identified new CPE-regulated maternal mRNAs encoding for proteins that control meiotic progression. Among them, we have characterized in detail a CPE-regulated mRNA encoding for a Zinc finger protein that, in turn, induces deadenylation of ARE/CPE containing mRNAs and regulates metaphase arrest.





4. TRANSLATIONAL CONTROL OF MITOTIC CELL CYCLE

Using the knowledge acquired in Xenopus oocytes we are trying to determine whether cytoplasmic polyadenylation also regulates cell cycle progression in somatic cells. We have identified mRNAs that encode for proteins which participate in cell cycle checkpoints and that contain potential CPEs in their 3'-UTRs. Then, we have determined whether the CPEs are functional in oocytes and whether the mRNAs undergo changes in polyadenylation during the cell cycle in somatic cells.

In addition, we have adapted the above-mentioned functional screening to identify mRNAs that display changes in poly(A) tail length during cell cycle. We have identified 166 mRNAs, encoding for cell cycle related proteins, which are deadenylaed at the S-Phase and polyadenylated again at G2/M-Phases. We have also identified over 300 mRNAs putative targets for cytoplasmic polyadenylation dependent translational control in response to stress and DNA damage.

PUBLICATIONS

Book chapter

Pique M, López JM, Mendez R "Cytoplasmic mRNA polyadenylation and translation assays." In: Methods in Molecular Biology, 322, 183-198 (2006)

NE REGULATIO


16
17
18
19
20
21
22
23

DIFFERENTIATION AND CANCER

Coordinator: Thomas Graf

During most of 2006, the programme consisted of three groups. With the arrival of Salvador Aznar Benitah in December an additional group has now joined the programme. A call is out to hire two more group leaders to complete the program. In October the coordinator moved from New York to Barcelona to work full time at the CRG.

Research within the Programme emphasises adult stem cell biology, mechanisms of cell fate instruction and cancer. More specifically, it covers the areas of:

- Transcription factors and signaling pathways in the development, regeneration and function of muscle cells
  (Pura Muñoz)
- Epigenetic events in PML-RAR induced leukemia
  (Luciano Di Croce)
- Hematopoietic stem cells, cell differentiation and reprogramming
  (Thomas Graf)
- Epithelial stem cells in the skin and cancer
  (Salvador Aznar Benitah)
- Associated Core Facility: FACS Unit

All groups work with mammalian cell lines and with mice, sharing their expertise in various technologies, such as FACS analyses, fluorescence microscopy, histopathology and mouse genetics.

DIFFERENTIATION AND CANCER

Hematopoietic Differentiation and Stem Cell Biology

The lab is interested in mechanisms of blood cell differentiation, in particular, the role of transcription factors, and in the biology of hematopietic stem cells.

THOMAS GRAF HAS A SENIOR ICREA GROUP LEADER POSITION.

GROUP STRUCTURE:

Group Leader:	**Thomas Graf (ICREA, CRG)**
Postdoctoral Fellows:	Florencio Varas (FIS, CRG) Alexis Schubert (Marie Curie) Ester Sanchez Tillo (CRG) Francisca Rubio Moscardo (HEROIC)
PhD Students:	Lars Bussmann (CRG)
Technicians:	Luisa Irene de Andrés (CRG)

RESEARCH PROJECTS

1. CONVERSION OF B LINEAGE CELLS INTO MACROPHAGES BY ENFORCED TRANSCRIPTION FACTOR EXPRESSION: A MODEL TO UNDERSTAND DIFFERENTIATION AND CELL REPROGRAMMING

During blood cell formation, the earliest multipotent progenitors branch into a common lymphoid and a common myeloid precursor. However, it is largely unknown which transcription factors determine the branching between the two compartments. In attempts to answer this question, we have chosen the approach used in our earlier work with chicken cell lines, consisting in the perturbation of transcription factor networks by enforced transcription factor expression in differentiated ('committed') blood cells.

We found that the bZip type transcription factor C/EBPalpha, which is expressed in macrophages but not in B cell precursors, effectively induces a switch of B cell precursors towards functional macrophages. The activation of myeloid genes requires the collaboration between C/EBPalpha and the transcription factor PU.1, which is expressed in both B cell precursors and immunoglobulin positive B cells. In contrast, the extinction by C/EBPalpha of the late B cell marker CD19 is PU.1 independent, and is caused by an inhibition of the CD19 regulator, Pax5. That the induced changes are due to a true trans-differentiation and not to the selection of inadvertent myeloid contaminants in the cells examined could be shown by an in vivo lineage tracing system. In addition, the macrophages generated exhibited immunoglobulin rearrangements.

We have now developed an inducible system that consists of a fusion between C/EBPalpha and the estrogen hormone binding domain. Primary B cell precursors expressing this protein can be induced to synchronously reprogram into macrophage-like cells within a few days. This involves the up-regulation of nearly 1,000 genes within 3 hours after induction and the down-regulation of a similar number of genes. After 5 days, these numbers are about 5,000 genes that go up and about 5,000 that go down. We have also been able to generate an inducible differentiation system in a B cell line. Induced differentiation becomes irreversible after the cells are exposed for 6 hours to the hormone and subsequent hormone withdrawal. This system is being used to unravel the molecular mechanisms of cell reprogramming, using gain and loss of function approaches and by the study of epigenetic changes in the cells' chromatin.

2. REPROGRAMMING OF COMMITTED T CELL PRECURSORS INTO MACROPHAGES

Hematopoietic stem cells are capable of differentiating into a variety of lineages but how this occurs is still largely unknown. T cell development is a unique developmental pathway to identify transcription factor networks that are operational during the commitment of hematopoietic progenitors, and how these are modulated by extracellular signals.

Commitment towards the T cell lineage occurs after bone marrow progenitors enter the thymus. Differentiation occurs in discrete stages, with pro-T cells being able to differentia-

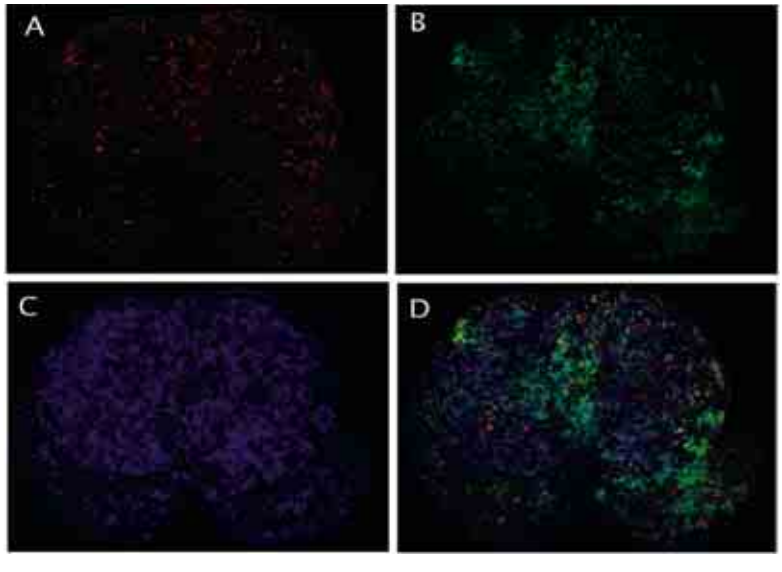


Figure 1. In vivo imaging of fetal liver megakaryocytes, myeloid cells and erythroid cells. The liver of a E13.5 embryo from a cross between CD41-YFP, lysozyme GFP and globin CFP mice was placed in a dish and imaged under the fluorescence microscope, using appropriate filters. A, YFP positive megakaryocytes false colored in red. B, GFP positive myelomonocytic cells in green; and C, CFP positive erythroid cells in blue. D, overlay of images A to C. Stadtfeld, M. Ye, M. and Graf, T. Identification of interventricular septum precursors in the mouse embryo (2007) Developmental Biology, Sep 16; [Epub ahead of print]

te into T cells, dendritic cells and macrophages, and pre-T cells being fully committed to the T cell lineage. Consistent with their multilineage potential, pro-T cells express the T cell transcription factors GATA-3 and Notch1 as well as the myeloid factors C/EBP alpha and PU.1. We now found that during commitment to the T cell lineage, at the pre-T cell stage, C/EBP alpha and PU.1 become down-regulated. In addition, T cell commitment can be reversed by the enforced expression of these myeloid transcription factors and in a highly selective way: PU.1 reprograms them into dendritic cells while C/EBP alpha induces them to become macrophages. Both processes that are antagonized by Notch signaling. This suggests that in early T cell progenitors, subtle changes in the balance between T cell and myeloid restricted transcription factors determines lymphoid versus myeloid cell fate and that activated Notch receptor directly feeds into these regulatory networks.

3. TESTING WHETHER TRANSCRIPTION FACTORS WITH BLOOD CELL INSTRUCTIVE CAPACITY CAN REPROGRAM MESENCHYMAL CELLS

It is widely assumed that the only truly totipotent cells of the body are embryonic stem (ES) cells whose chromatin becomes irreversibly modified as they progress through differentiation. However, the fact that mammals can be cloned by transfer of nuclei of differentiated cells into oocytes shows that even specialized somatic cells can be epigenetically reprogrammed to acquire totipotency. Therapeutic cloning, in which ES cells are first developed from the nuclei of somatic cells which can then be used to generate desired cell types, is based on these insights. Recent experiments suggest that it is even possible to reprogram somatic cells in culture: Ectopic transcription factor expression in fibroblasts induced the acquisition of an ES cell phenotype, including the capacity of the cells to generate all three germ layers. However, as in the nuclear transfer experiments (Hochedlinger and Jaenisch, 2006), the induced reprogramming was very inefficient (Takahashi and Yamanaka, Cell, 2006). At present the possibility that only a small percentage of somatic cells retain a chromatin configuration susceptible to remodeling cannot be distinguished from the possibility that technical inadequacies have prevented better outcomes.

An explanation for the apparent ease with which committed hematopoietic cells can be converted into one another is that they are closely developmentally related, thus expressing overlapping sets of transcription factors and sharing similar chromatin configurations. Implied in this assumption is that hematopoietic transcription factors should not be capable to reprogram more distantly related cells. We are therefore currently testing whether expression of specific combinations of hematopoietic instructive transcription factors can alter the phenotype of fibroblasts, a more distantly related cell type derived from mesenchymal stem cells, as are adipocytes, myocytes, osteoblasts and chondrocytes.

4. CD41-YFP KNOCK-IN MICE ALLOW IN VIVO LABELING OF MEGAKARYOCYTIC CELLS AND IDENTIFY NEWLY MADE PLATELETS

CD41/GpIIb is one of the earliest markers for definitive hematopoietic stem cells and is also a lineage specific marker of megakaryocytes/platelets. To study the development of this lineage from stem cells we have introduced the YFP gene into the CD41 gene by homologous recombination. In this mouse, megakaryocytes and platelets are EYFP labeled, while no cells from other lineages express the reporter gene. CD41-YFP mice contained a subset of YFP labeled megakaryocytes and platelets, whose proportions varied depending on the genotype and individual animal, while lymphoid, myelomonocytic and erythroid lineages were negative. In addition, a fraction of hematopoietic stem cells and intermediate progenitors expressed YFP at low levels. Crossing CD41-YFP mice with lysozyme GFP and globin CFP mice, followed by in vivo imaging of fetal liver, permitted the in vivo distiction of megakaryocytic cells from myeloid and erythroid cells (Fig.1). This experiment is also the first to show the distribution of three hematopoietic lineages in a minimally perturbed organ in vivo, requiring no manipulations such as tissue fixation.

In another line of the work we found that surprisingly, the fraction of CD41-YFP$^+$ platelets was more responsive to thrombin stimulation than the YFP$^-$ subset. Experiments aimed at determining the half-life of the YFP$^+$ platelets showed that after lethal irradiation of CD41-YFP mice the proportion of labeled platelets in the blood declined more rapidly than the bulk of the platelets. Since YFP has a shorter half-

life than platelets these results suggest that the YFP$^+$ subset is enriched for newly made platelets. They also imply that platelet activity decreases rapidly during physiological aging.

5. SEARCH FOR GENES THAT ENHANCE STEM CELL EXPANSION

Retroviral insertions in host cell DNA are biased towards transcriptionally active cellular genes. When retrovirus infected hematopoietic stem cells (HSCs) are transplanted into myeloablated recipients, a few repopulating HSC clones become dominant. This may be due to the activation of cellular genes flanking the retroviral insertion site that favor the clonal expansion of HSCs. A similar process has been shown earlier to contribute to the development of leukemias. Based on this rationale we searched for retroviral insertions associated with HSC clones showing an enhanced repopulating ability. We now have mapped over 40 retroviral integration sites in dominantly repopulating hematopoietic clones and have identified the corresponding closest cellular genes. Among these is the Meis1 gene that is known to play a role in HSC expansion and leukemogenesis, validating our approach. Another gene identified by this retroviral insertion approach is Abcc1, a member of the ATP-binding cassette (ABC) transporter superfamily, recently reported to increase repopulating activity by overexpression in HSCs. Currently we are testing the ability of a candidate gene, when expressed in a retrovirus vector, to rapidly expand HSCs in vitro and in vivo.

6. IDENTIFICATION OF INTERVENTRICULAR SEPTUM PRECURSOR CELLS IN THE MOUSE EMBRYO

Using an indicator mouse line for lysozyme M, a model that we have previosulsy exploited for hematopoietic lineage analyses, we unexpectedly discovered that the gene is also expressed in the embryonic heart. This was confirmed and extended with the lineage tracing line Lysozyme M-Cre crossed with ROSA 26 reporter mice. We found that lysozyme expressing cells give rise to the interventricular septum (IVS) and to a part of the left ventricular free wall, demonstrating that these heart regions are developmentally related. Lysozyme positive precursors were found not to be of hematopoietic origin and also developed in the absence of PU.1, a transcription factor that regulates lysozyme expression in macrophages. Lysozyme M deficient mice lack an overt cardiac phenotype, perhaps due to compensation by the related lysozyme P, which we found likewise to be expressed in the developing heart. Direct visualization of lysozyme expression, using Lysozyme M-EGFP knock-in mice, revealed that ventricular septation is initiated at embryonic day 9 by the movement of myocardial trabeculae from the primitive ventricle towards the bulbo-ventricular groove. After ventricular septation is completed, lysozyme expression continues to mark newly formed muscular components of the IVS until the second postnatal week. The identification of a novel population of myocardial progenitor cells of the interventricular septum is significant since IVS development is a central event during cardiogenesis and ventricular septal defects are common congenital disorders. In addition, our work predicts that Lysozyme M-Cre mice will become a valuable tool to inactivate genes in developing interventricular septum precursors.

PUBLICATIONS

Lee JS, Yu Q, Shin JT, Sebzda E, Bertozzi C, Chen M, Mericko P, Stadtfeld M, Zhou D, Cheng L, Graf T, MacRae CA, Lepore JJ, Lo CW, Kahn ML "Klf2 is an essential regulator of vascular hemodynamic forces in vivo." Dev Cell, 11(6), 845-57 (2006) (*)

Schulze H, Korpal M, Hurov J, Kim SW, Zhang J, Cantley LC, Graf T, Shivdasani RA "Characterization of the megakaryocyte demarcation membrane system and its role in thrombopoiesis." Blood, 107(10), 3868-75 (2006) (*)

Laiosa CV, Stadtfeld M, Graf T "Determinants of lymphoid-myeloid lineage diver-sification." Annu Rev Immunol, 24, 705-38 (2006) (*)

Laiosa C, Xie H, Stadtfeld M, de Andres L and Graf T "C/EBP alpha and PU.1 reprograms pre-T cells into macrophages and dendritic cells" Immunity, 25(5), 731-44 (2006). See also Perspective by Iwasaki and Akashi.

Stadfeld M, Ye M, Graf T "Identification of interventricular septum precursor cells in the mouse embryo." Dev Biol (Sept 17 2006 [Epub ahead of print: doi:10.1016/j.ydbio.2006.09.25]) (*)

(*) All these publications are the result of the work of Dr. Thomas Graf at the Albert Einstein College of Medicine, New York, USA

DIFFERENTIATION AND CANCER

Myogenesis

The main interest of our group is to elucidate the mechanisms controlling myogenesis in vitro and in vivo, with an emphasis in skeletal muscle regeneration and inherited myopathies. Myogenesis is largely controlled by the basic helix-loop-helix (bHLH) family of muscle regulatory transcription factors (MRFs), including MyoD, Myf5, myogenin and MRF4, and by the myocyte-enhancer factor-2 (MEF2) family of proteins, which regulate the expression of muscle-specific genes, such as muscle creatine kinase (MCK) and myosin heavy chain (MHC). Several intracellular signaling pathways (phosphatidylinositol 3-kinase (PI3K), NF-kB, and members of the mitogen-activated protein kinase (MAPK), such as ERK, JNK and p38), have been implicated in the control of muscle differentiation. However, their individual role in this process remains controversial. There have been several reports showing that p38 MAPK activity is induced during myogenic differentiation, being this activation required for myoblast fusion and differentiation in vitro. We are interested in analyzing the molecular mechanisms responsible for the promyogenic effect of p38, i.e. identification of transcription factors as downstream effectors of this MAPK during myogenesis. Moreover, we aim to investigate the role of the distinct isoforms of the p38 MAPK in vivo, using mice deficient in each isoform. Based on our earlier work, a strong emphasis is also devoted in our laboratory to the analysis of the role of the plasminogen activation (PA) system components in skeletal muscle regeneration (after injury or in inherited myophinopathies). Finally, Antonio Serrano (a Ramón y Cajal investigator in our laboratory) is analyzing the mechanisms involved in the regulation of muscle fiber type and size in vivo. In summary, our laboratory is pursuing three main lines of research:

- Control of muscle-specific gene transcription by p38 MAP kinases.
- Role of the plasminogen system in skeletal muscle regeneration in vivo.
- Molecular mechanisms regulating the muscle phenotype in vivo.

GROUP STRUCTURE:

Group Leader:	**Pura Muñoz Cánoves**
Ramón y Cajal Investigator:	Antonio Serrano
Postdoctoral Fellows:	Mònica Suelves Eusebio Perdiguero Esther Ardite
PhD Students:	Bernat Baeza-Raja Berta Vidal Vanessa Ruíz Pedro Souza-Victor
Technicians:	Mercè Jardí Isabel-Cuartas Gemma Cónsol

RESEARCH PROJECTS

1. CONTROL OF MUSCLE-SPECIFIC GENE TRANSCRIPTION BY P38 MAP KINASES

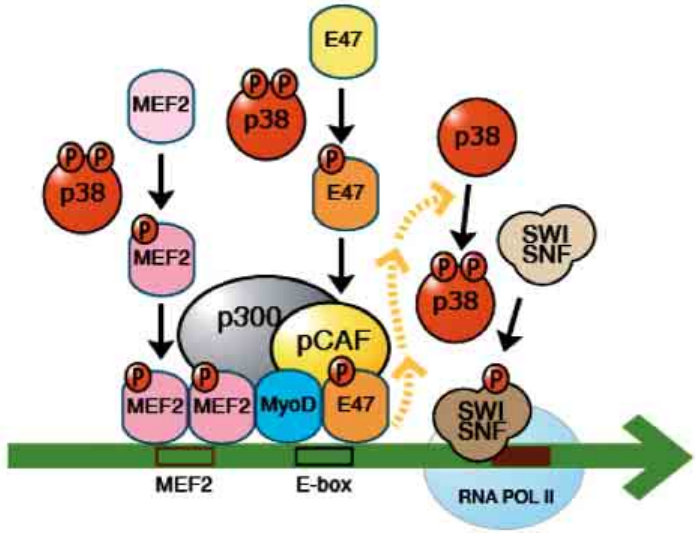
Myogenesis is largely controlled by the basic helix-loop-helix (bHLH) family of muscle regulatory factors (MRFs), including MyoD, Myf5, myogenin and MRF4. The MRFs exert their function by promoting muscle-specific gene transcription through a specific DNA sequence, the E-box. Selective and productive recognition of chromatin targets requires heterodimerization of MRFs with the ubiquitous E proteins, E12 and E47. Thus, formation of the functional MRF/E47 heterodimer is pivotal in controling muscle gene expression. We have investigated the potential mechanisms underlying the role of p38 MAP kinases in this process. Our results so far have been the following:

- p38 MAPK activity regulates MyoD/E47 association *in vitro* and *in vivo* by phosphorylation of the E protein at serine 140, thereby promoting muscle-specific gene transcription
- p38 MAPK represses the transcriptional activity of MRF4 (involved in late stages of myogenesis), but not of MyoD, resulting in downregulation of specific muscle genes.
- NF-kB activation is dependent on p38 activity during differentiation, being NF-kB an effector of p38, thus providing a novel mechanism for the promyogenic effect of p38
- p38alpha (but not p38beta, p38gamma or p38delta) is necessary for myogenesis. p38alpha controls myoblast proliferation both in vitro as in neonatal muscle. Indeed, myoblasts derived from p38alpha-deficient mice show persistent proliferation, and a subsequent block in myoblast differentiation. This result uncovers a novel mechanism explaining the fundamental role of p38 in myogenesis, and constitutes the first dissection of the relative contribution of the four p38 MAP kinases to this process.

2. ROLE OF THE PLASMINOGEN SYSTEM IN SKELETAL MUSCLE REGENERATION *IN VIVO.*

We had previously observed a muscle regeneration defect in uPA (urokinase plasminogen activator)-deficient mice after injury, which correlated with fibrin deposition and a decreased recruitment of blood-derived monocytes and lymphocytes to the damaged muscle. Our results are the following:

- uPA deficiency exacerbates dystrophy in *mdx* mice, an animal model of Duchenne Muscular Dystrophy (DMD), and injury-induced muscle regeneration, via two mechanisms: fibrin degradation and mobilization of bone marrow cells during muscle repair. *(submitted).*
- Fibrin accumulates in the muscle of mdx mice, exacerbating dystrophy progression, and have analyzed the underlying mechanisms mediating the deletereous role of fibrin.



p38 MAPK in the control of muscle-specific gene expression. Possible phosphorylation targets of p38 that may account for the key role of this MAPK in myogenic differentiation

From: Lluis F, Perdiguero E, Nebreda AR, Muñoz-Cánoves P. Trends in Cell Biology 16: 36-44, 2006

- Preliminary results show that the muscle size of PAI-1-deficient mice is larger than that of wild type mice, suggesting that PAI-1 may be negatively regulating muscle growth. The mechanisms underlying PAI-1 function in muscle regeneration are being analyzed at present.

3. MOLECULAR AND CELLULAR MECHANISMS INVOLVED IN THE REGULATION OF MUSCLE PHENOTYPE: THERAPEUTICAL IMPLICATIONS

Mammalian skeletal muscle fibers comprise four major fiber types, including slow or type 1 and three subtypes of fast or type 2 fibers, type 2A, 2X and 2B. Each fiber type is defined by the presence of a specific isoform of myosin heavy chain and by a distinct program of gene expression. Skeletal muscle has also a remarkable capacity of self-regeneration due to the presence of a specialized cell type, the satellite cells. Neural activity controls muscle gene expression and regulates fiber size. Despite of the fact that changes in fiber types, reduction of fiber size (muscle atrophy) and a decrease of the muscle regenerative capacity are detected in many neuromuscular pathologies, the basic mechanisms underlying these processes have not been yet well characterised.

Our main objective is to gain an insight into the knowledge of the molecular and cellular bases that control the muscle phenotype and their implications in physiopathology. In particular, by using gene transfer, pharmacological and biochemical approaches in various animal models combined with analyses of gene expression, we propose:

- To investigate the signaling pathways, the transcription factors and their putative interactions, as well as the target genes involved in fiber type-specific gene expression and in the control of fiber size

- To explore the relevance of the experimental manipulation of these networks for neuromuscular pathology in different animal models

PUBLICATIONS

Lluis F, Perdiguero E, Nebreda AR, Muñoz-Cánoves P. "Regulation of skeletal muscle gene expression by p38 MAP kinases". Trends Cell Biol, 16, 36-44 (2006)

Perdiguero E, Muñoz-Cánoves P "Trancriptional regulation by the p38 MAPK signaling pathway in mammalian cells". Volume on "SAPKs" Topics in Current Genetics (in press)

Perdiguero E, Ruiz-Bonilla V, Lionel Gresh, Lijian Hui, Ballestar E, Souza-Victor P, Baeza-Raja B, Jardí M, EstellerM, Caelles C, Serrano AL, Wagner EF, Muñoz-Cánoves P "Genetic analysis of p38 MAP kinases in myogenesis: fundamental role of p38alpha in abrogating myoblast proliferation" EMBO J (in press)

DIFFERENTIATION AND CANCER

Epigenetics Events in Cancer

The recent explosion in our knowledge of how chromatin organization modulates gene transcription has highlighted the importance of epigenetic mechanisms in the initiation and progression of human cancer. These epigenetic changes - in particular, aberrant promoter hypermethylation and histone modifications associated with inappropriate gene silencing - affect virtually every step in tumour progression. Our research investigation is focused on epigentic alterations that occurs during leukemia, as model cancer system.

LUCIANO DI CROCE HAS AN ICREA GROUP LEADER POSITION.

GROUP STRUCTURE:

Group Leader:	**Luciano Di Croce**
Postdoctoral:	Marcus Buschbeck Holger Richly Ana Sofia Quina Luciana Rocha Viegas
PhD Students:	Lluís Morey Raffaella Villa Iris Uribesalgo Micás Joana Ribeiro
Predoctoral Student:	Ohiana Iriondo
Technician:	Arantxa Gutierrez

RESEARCH PROJECTS

1.BIOCHEMICAL LINK(S) BETWEEN DNA METHYLATION AND TRANSCRIPTIONAL SILENCING

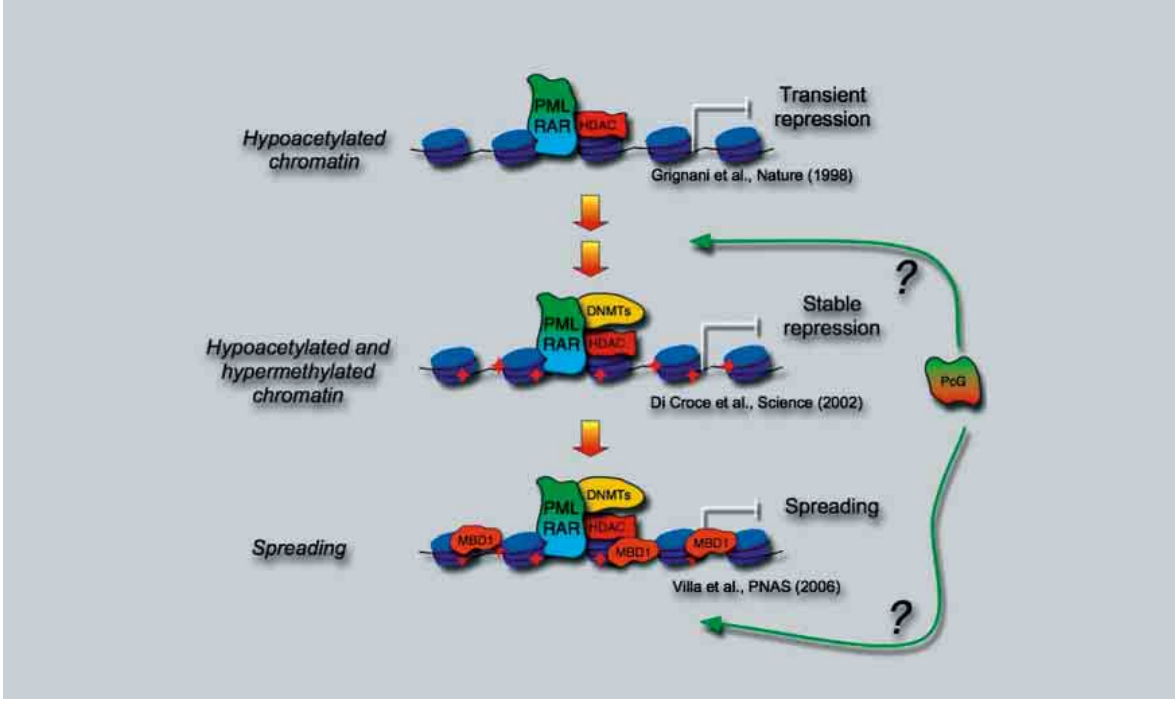
Our scientific interest is focused on the role of proteins involved in the recognition and binding of methylated CpGs (MBDs) in several leukemia models. Our experiments suggest that MBD1, a PML-RAR associated protein, is required for gene repression in APL cells. Indeed, MBD1 and PML-RARa are both required for fully silencing PML-RAR target genes. PML-RAR recruits MBD1 on its target promoter through an HDAC3-mediated mechanism. Furthermore, retroviral infected-hematopoietic precursors with MBD1 mutants (in either the MBD or the TRD domain) compromise the ability of PML-RAR to induce differentiation block, thus identifying MBD1 as a important player in PML-RARa promoter silencing subsequent to promoter hypermethylation, and as a potential candidate for cancer therapy. This research line thus has a strong potential impact on clinical aspects, as a point-mutated version of the MBD1 protein both prevents and reverts the PML-RARa hematopoietic differentiation block.

2. ROLE OF EPIGENETIC MODIFICATION IN CANCER

We are also investigating the regulation of several PML-RAR target genes (such as p21, RARa, c/EBP etc.). Our preliminary experiments suggest a strong correlation between the presence of a CpG island, promoter hypermethylation, chromatin structure/alterations and gene silencing, while promoter silencing of those genes that do not contain a CpG island is not only transitory but also is exclusively dependent on histone deacetylation. This results will help us in understanding the molecular mechanism by which PML-RARa (de-)regulates gene transcription, and will allow us to discriminate which drugs (among several available) are more appropriate for a given set of repressed genes.

3. HISTONE TAIL MODIFICATION AND HETEROCHROMATIN

Heterochromatin DNA is characterized by the presence of both a "closed" chromatin conformation and the presence of the Polycomb group (PcG) of proteins. The recruitment of the PcG protein complexes, as well as their contribution to cancer progression, is also investigated, in collaboration with PG. Pelicci/S.Minucci (IEO, Milan), F.Fuks (Univ. of Brussels), and K.Helin (BRIC, Copenha-



gen). Preliminary results suggest that members of the PcG interact with PML-RAR and are recruited to its target genes. Indeed changes in the "histone code" are also observed in proximity of PML-RAR binding sites. The heterochromatin formation could be thus responsible for the observed stable gene silencing.

PUBLICATIONS

Buschbeck M, Uribesalgo I, Ledl A, Gutierrez A, Minucci S, Muller S, and Di Croce L "PML4 induces differentiation by Myc destabilization." Oncogene (Dec 4 2006 [Epub ahead of print])

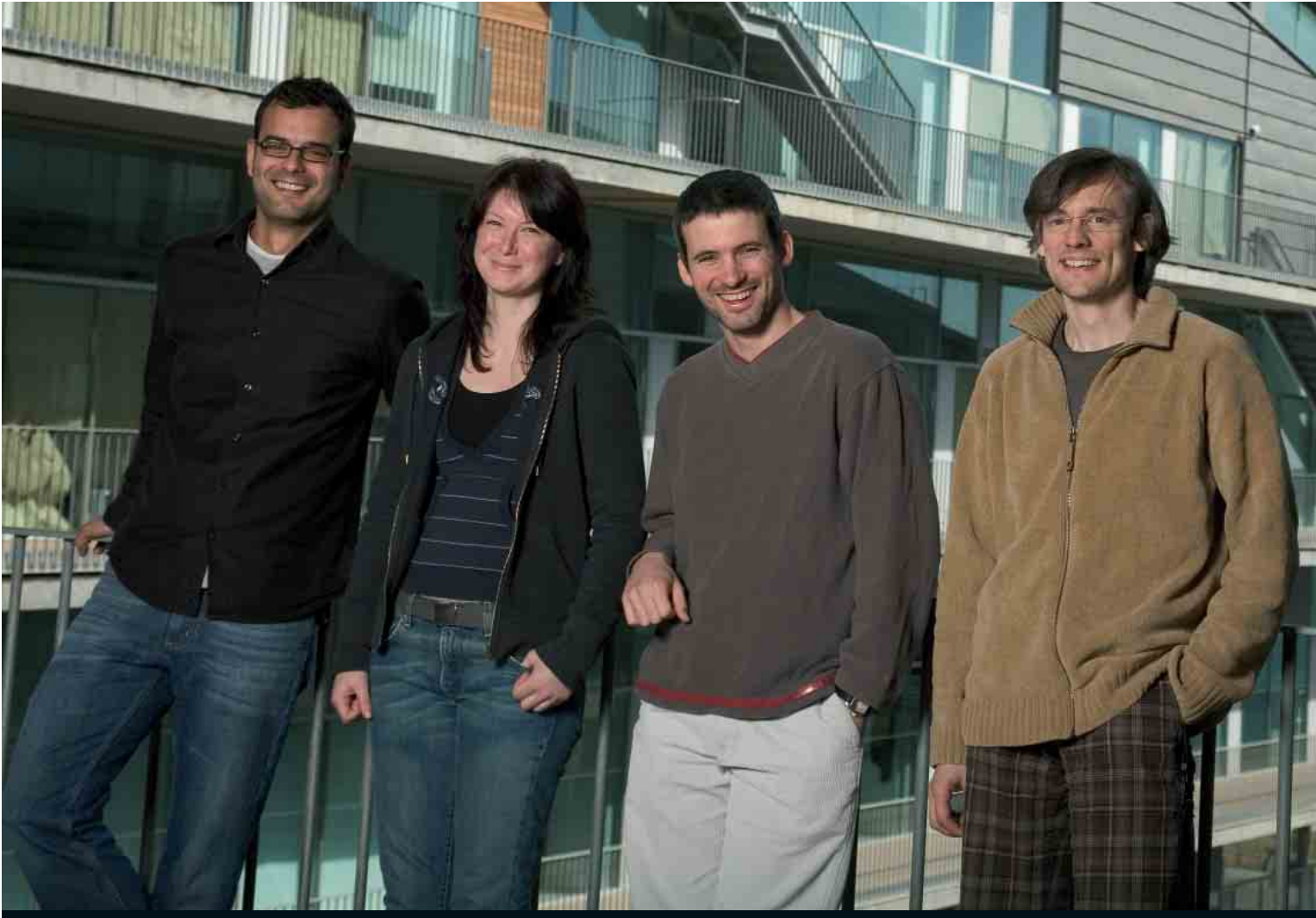
Quina AS, Buschbeck M, and Di Croce L "Chromatin structure and epigenetics." Biochem Pharmacol, 72, 1563-1569 (2006)

Villa R, De Santis F, Raker V, Corsaro M, Gutierrez A, Buschbeck M, Morey L, Varas F, Bossi D, Minucci S, Pelicci PG and Di Croce L "The methyl-CpG binding protein MBD1 is required for PML-RARá function." PNAS 103, 1400-1405 (2006)

Viré E, Brenner C, Deplus R, Didelot C, Morey L, Bernard D, Vanderwinden J, Bollen M, Di Croce L, de Launoit Y and Fuks F "The Polycomb group protein EZH2 directly controls DNA methylation." Nature, 439, 871-874 (2006)

Carbone R, Botrugno O, Ronzoni S, Insinga A, Di Croce L, Pelicci PG and Minucci S "Recruitment of the histone methyl-transferase SUV39H1 and its role in the oncogenic properties of the leukemia-associated PML-RAR fusion protein." Mol Cell Biol, 26, 1288-1296 (2006)

FERENTIATION AND CANCE



DIFFERENTIATION AND CANCER

Epithelial Homeostasis and Cancer (New Group December 2006)

We are interested in studying pathways involved in self-renewal and homeostasis of adult epithelia and how these contribute to the progression and aggressiveness of human tumours.

SALVADOR AZNAR BENITAH HAS A JUNIOR ICREA GROUP LEADER POSITION.

GROUP STRUCTURE:

Group Leader:	**Salvador Aznar Benitah**
Postdoctoral Fellows:	Lluís Riera
PhD Students:	Peggy Janich
Technicians:	Bernd Kuebler

RESEARCH PROJECTS

1. IMPACT OF RAC1/PAK2/MYC PATHWAY IN EPIDERMAL HOMEOSTASIS AND CANCER *IN VIVO*.

Adult epithelia are in constant need of renewal. A population of adult stem/progenitor cells (SCs) ensure maintenance of the undamaged tissue but also integrity in response to external stimuli. In our lab, we primarily use the skin as a model of epithelium with a high rate of turn-over and with well defined somatic epSCs populations. Homing and exit of these from their niche are tightly regulated processes that integrate extracellular cues and cell autonomous genetic programmes. We aim to identify some of the molecular mechanisms that modulate the behaviour of adult stem cells and how the tight control of these signals is lost in tumours.

Our recent work has shown that the interplay between Rac1 GTPase and the proto-oncogene Myc is essential for balancing epidermal renewal and differentiation (Benitah *et al.*, 2005; Benitah and Watt, 2007). The family of Rho GTPases is involved in changes in cell morphology, adhesion, invasion, polarization and proliferation, among others (Benitah and del Pulgar *et al,* 2005). Moreover, Rho GTPases are of clinical interest since deregulation of several members of its family is a common feature in human cancers that correlates with aggressive tumour behaviour (Benitah and del Pulgar *et al,* 2005).

We have deleted Rac1 in the undifferentiated compartment of adult epidermis, hair follicle and sebaceous glands in an inducible manner (Keratiin-14-CreER/Rac1$^{flox/flox}$). In these mice activity of Cre recombinase is dependent on administration of tamoxifen. Deletion of Rac1 in K14CreER/Rac1KO mice causes a rapid proliferation and irreversible mobilization of the epidermal and hair follicle SCs (epSCs) from their niche (Figure1). Depletion of epSCs upon Rac1 deletion ultimately results in alopecia, due to loss of hair follicle cycling, and failure of epidermal renewal. A similar effect was observed upon embryonic deletion of Rac1 in the epidermis (Keratin-5-Cre/Rac1$^{flox/flox}$ mice; Benitah and Watt, 2007). Accordingly, Rac1, together with the hematopoietic specific Rac2, is essential for haematopoietic stem cell maintenance and proper haematopoiesis (Cancelas et al, 2005).

At the molecular level, Rac1 maintains epSC self-renewal by modulating the activity of the transcription factor Myc (Benitah *et al,* 2005). Myc is one of the first and best characterized human oncogenes found to date, and much effort is being invested to elucidate its precise functions in normal tissues and in

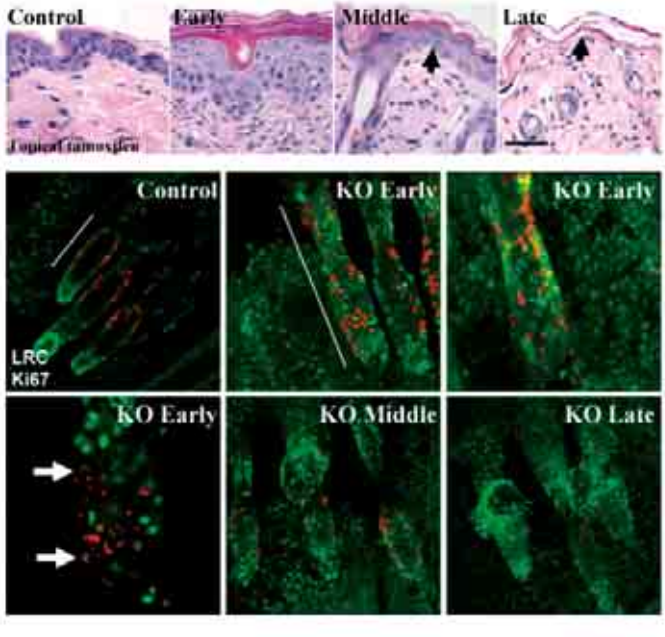


Figure 1. Inducible deletion of Rac1 in adult epidermis of K14CreER/Rac1^{flox} mice. Upper Panel: Loss of Rac1 causes an early hyperproliferative phase (Early) followed by irreversible terminal differentiation (Middle). Ultimately, the epidermis looses its self-renewal capacity and only remnants of the cornified stratum remain (Late). Lower Panel: Hair follicle stem cells, depicted as Label Retaining Cells (LRCs), mobilize out of the niche and proliferate upon epidermal KO of Rac1. Wholemounts of mouse tail epidermis show that upon KO of Rac1, LRCs egress out of the hair follicle stem cell niche (KO Early) and proliferate (Ki67 positive; KO Early and Middle). Ultimately, mobilization out of the niche coupled to proliferation results in loss of LRCs (KO Late).

tumours. In steady-state conditions, Myc induces exit of epSCs from their niche to permit their subsequent differentiation (Waikel *et al,* 2001; Arnold *et al,* 2001; Frye *et al,* 2003). However, sustained overexpression of Myc in the epidermis causes a decline of epSCs number and a hyperproliferative state that predisposes skin to the development of squamous tumours (Waikel *et al,* 2001). The effect of Myc on epSCs takes place through a bifunctional mechanism: induction of proliferation and de-adhesion from the stem cell niche (Gebhardt and Frye *et al,* 2006).

We have described that the serine/threonine kinase PAK2 colocalizes to, and is activated by, Rac1 in the epidermis. Once active, PAK2 phosphorylates Myc at three C-terminal aminoacids, Thr358, Ser373 and Thr400 (Benitah et al, 2005; Huang *et al,* 2004). Phosphorylation of Myc at these residues modifies its transcriptional activity, preventing its function over epSCs (i.e exit from the stem cell niche). We are further characterizing the impact of the Rac1/PAK2/Myc pathway on the homeostasis of the skin *in vivo* using novel epidermal mouse models.

2. MOLECULAR AND GENETIC MECHANISMS INVOLVED IN EPIDERMAL SELF-RENEWAL AND DIFFERENTIATION

We have performed Affymmetrix microarray analysis of primary human keratinocytes in which the pathway of Rac1/PAK2/Myc has been modulated. In addition, we are using a proteomic approach to identify proteins that interact with members of this signalling cascade. Since the Rac pathway is important for balancing epidermal homeostasis, we are combining these two approaches, microarray data and proteomics, to identify new players relevant for this process. Selected targets are being validated using cellular and molecular biology tools with primary cultures, as well as with our *in vivo* mouse models.

3. EFFECT OF RAC1/PAK2/MYC PATHWAY IN EPIDERMAL TUMOUR ONSET AND PROGRESSION

Adult stem cells are potentially the few long term tissue residents that in time may accumulate enough somatic oncogenic mutations which result in the development of neoplasias. Moreover, the behaviour and molecular signature of a small percentage of cancer cells, known as cancer stem cells, recapitulate those of adult stem cells in the normal tissue. Cancer stem cell self-renewal, high potential of invasion and homing into a specific niche, with direct consequences over tumour maintenance and metastasis, are most probably characteristics inherited from normal adult stem cells. However, very little is known about the signalling events and the molecular signature that contribute to the behaviour of cancer stem cells in tumours of epithelial origin.

Since Rac1 and Myc are essential regulators of epSCs behaviour, we are analyzing the impact of this pathway in the onset and progression of squamous neoplasias (Squamous Cell Carcinomas, SCCs). Using a panel of 50 human SCCs at different stages of tumour progression, we have shown that Rac1 is highly expressed in the undifferentiated and invasive front of these tumours (Benitah *et al,* 2005). Interestingly, using *in vivo* models of squamous carcinogenesis we have shown that Rac1 contributes to tumour progression by modulating the activity of the TGFb pathway (Benitah *et al,* 2007; sub). These results suggest that the Rac1 pathway may contribute to the aggressiveness of human neoplasias. We are currently generating novel mouse models of epidermal tumorigenesis to address these issues.

PUBLICATIONS (*)

Benitah SA*, Homma M*, Watt FM "Role of LIM kinases in normal and psoriatic human epidermis." Mol Biol Cell, 17(4),1888-96 (2006) (*co-authors)

Gebhardt A, Frye E, Herold S, Benitah SA, Braun K, Watt FM, Elsässer HP, Eilers M "Myc regulates keratinocyte adhesion and differentiation via complex formation with Miz1." J Cell Biol, 172, 139-49 (2006)

(*) All these publications are the result of the work of Dr. Salvador Aznar-Benitah at the London Research Institute, Cancer Research UK, London, UK

FERENTIATION AND CANCE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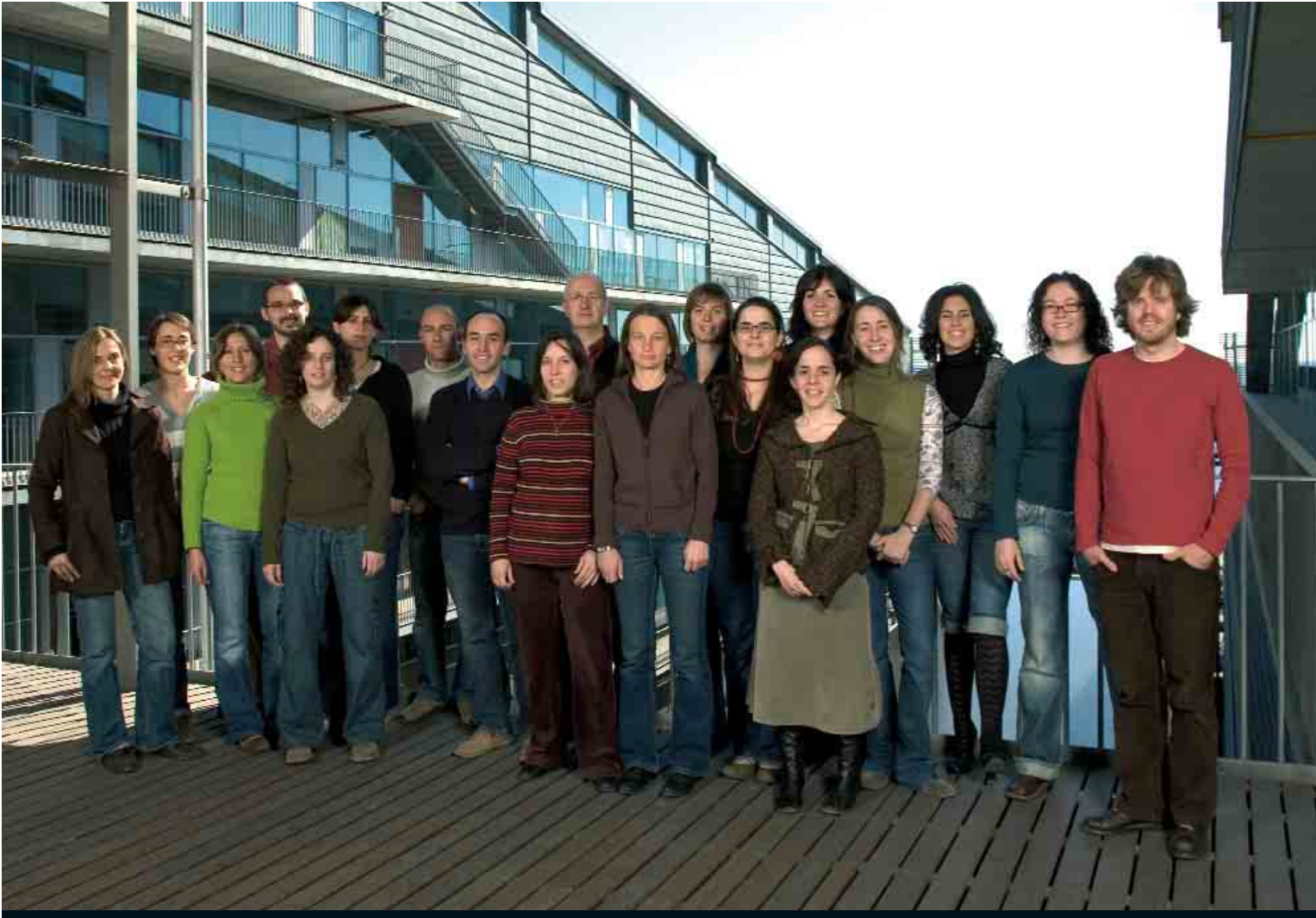
GENES AND DISEASE

Coordinator: Xavier Estivill

With the human genome sequenced, the genomes of model organisms deciphered, and a massive amount of information on the genome variability in hand, we now have the challenge to dissect the molecular components of common human disorders. This basic understanding should help us to define pathogenic pathways and to develop strategies to correct biological defects that lead to disease. The Genes and Disease Programme of the CRG aims to use genomic variability to identify the molecular basis of disease, to investigate the function of genes with a potential role in common human disorders, and to develop therapeutic approaches to correct some diseases. The programme combines large-scale experimental approaches with forward genetic strategies to elucidate some of the biological determinants of human disease. The programme is currently organised in five research groups and a core facility.

Current structure of the programme:

- 6 Research Groups:
  - Genetic Causes of Disease (Xavier Estivill)
  - Gene Function (Susana de la Luna)
  - Murine Models of Disease (Mariona Arbonés)
  - Neurobehavioral Analysis (Mara Dierssen)
  - Gene Therapy (Cristina Fillat)
- Associated Core Facility: Genotyping Unit



GENES AND DISEASE

Genetic Causes of Disease

The group focuses on the study of the variability of the human genome at the nucleotide and genomic levels and the use of these genetic tools to evaluate the predisposition/resistance to disease. Several psychiatric disorders are being investigated at different levels and the group have made some progress in the identification of SNPs in BDNF and NTRK2 that protect or predispose to eating disorders, and gene variants involved in hearing loss. The group has contributed to achieve the first-generation map of copy number variants (CNVs) of the human genome. The group is conducting projects on the identification of gene modifiers for non-syndromic hearing loss, the analysis of clinical variability in the response to methadone and to nicotine treatments, and the study of several psychiatric disorders (eating, depression, anxiety and obsessive compulsive disorders, among others). The group is also exploring the contribution of non-coding RNAs (and their target genes), particularly snoRNAs and miRNAs, in the susceptibility to complex diseases.

GROUP STRUCTURE

Group Leader:	**Xavier Estivill**
Staff Scientist:	Eulàlia Martí
Scientific Officer:	Àurea Rodríguez
Postdoctoral Fellows:	Lluís Armengol Mario Cáceres (Ramón y Cajal) Yolanda Espinosa (Ramón y Cajal) Miroslava Ogorelkova Kelly Rabionet (Ramón y Cajal)
PhD Students:	Ester Ballana Nina Bosch Celia Cerrato Monica Guidi Josep Maria Mercader Margarita Muiños Elena Miñones Lorena Pantano Ester Saus Marina Ventayol (until June) Francesca Vivarelli
Technicians:	Anna Carreras Manel García Marta Morell Imma Ponsa Sergi Villatoro

RESEARCH PROJECTS

1. COPY NUMBER VARIANTS, SEGMENTAL DUPLICATIONS AND HUMAN DISEASE

Large-scale segmental duplications have played an important role in hominoid evolution and can be hotspots for non-allelic homologous recombination leading to deletion, duplication, inversion or translocation. Many of these segmental duplications coincide with structural variations or copy number variants (CNVs) of the human genome. We have used bioinformatics and experimental approaches to evaluate the variability of the human genome. Our group has contributed to the development of the first whole genome map of structural variants of the human genome in collaboration with members of the International Consortium of Copy Number Variants. Over 1,400 CNVs have been detected using SNP and BAC arrays in an analysis of the 270 samples of the HapMap collection. On average between two individuals around 100 CNVs, which represent around 20 Mb, were detected. These figures correspond to a genomic variability due to CNVs of around 12% of the DNA sequence. The group has applied this information to evaluate the content of the ENCODE (Encyclopedia of DNA Elements) Project regions in segmental duplications and CNVs. ENCODE involves 35 groups that have provided more than 200 experimental and computational datasets that examined in detail a targeted 30 Mb of the human genome. This analysis has provided the grounds for further investigations at a large scale to characterize smaller CNVs, which could be important for disease and evolution studies.

The group has continued the characterisation of specific regions, such as 15q11-q13 and 8p23.1. The 15q11-q13 region contain three clusters of small nucleolar RNAs (snoRNAs), expressed in the central nervous system. One of these snoRNA clusters show a high number of nucleotide changes indicative of gene conversion events. Another interesting region is a five-Mb segment of human chromosome 8p23.1, which is inverted in a significant proportion of individuals in the general population. One of the genes located at segmental duplications that flank this inversion is primate-specific and shows a wide variability in copy number in humans and non-human primates.

We have used and implemented multiple new technologies to further analyse segmental duplication CNVs. We have developed and validated clone-based chips for comparative genomic hybridization (arrayCGH). We have also worked with Agilent and Illumina platforms to co-implement the latest oligonucleotide-based scanning approaches, as well as used multiplex ligation probe amplification (MLPA) and multiple amplification probe hybridization (MAPH) assays for rapid validation of primary genome scanning data. The group is using these approaches to analyse several complex disorders.

2. NEUROTROPHIC FACTORS AND NEUROTRANSMITTERS IN PSYCHIATRIC DISORDERS

The neurotrophin family of regulatory factors promote neuronal proliferation, regeneration and connectivity during development and participates in the plasticity and maintenance of neurons throughout adulthood. Based on clinical findings, animal and pharmacological studies, which mainly focused on depression, neurotrophins have been proposed as susceptibility factors for several psychiatric disorders and phenotypes. Nevertheless, genetic studies exploring the potential relationship between variations of these genes and psychiatric diseases such as bipolar disorder, eating disorders, major depressive disorder or schizophrenia have produced conflicting results. Taking into account these controversial results, and recent findings suggesting the involvement of other neurotrophins and/or their high affinity receptors in the regulation of mental disorders, we hypothesize that alterations in the function or expression in members of the neurotrophin family could affect the central neurotransmission and manifest as genetic liability to a psychiatric disorder. In concrete, variants (179 SNPs) located in the genomic regions of NGF, NTRK1, BDNF, NTRK2, NTF3, NTRK3, CNTF, CNTFR, NT4-5 and p75 are being studied through a case-control or family-based association approach in patients with anxiety disorders (panic disorder and obsessive-compulsive disorder), schizophrenia, eating disorders (anorexia nervosa and bulimia nervosa), affective disorder (bipolar disorder and major depression) and substance abuse disorder (opiate dependence).

We have previously reported an association between the Met66 allele of the Val66Met BDNF variant and restricting AN (ANR) and low minimum body mass index in Spanish patients. This association for BDNF has been confirmed in a large collection of trios from different European countries (France, Germany, Italy, Spain and United Kingdom). To further test the role of this variant in humans we have screened 36 SNPs in the BDNF gene and have tested for their association with ED and plasma BDNF levels as a quantitative trait. We have performed a family-based association study in 106 ED nuclear families and analyzed BDNF blood levels in 110 ED patients and in 50 sib-pairs discordant for ED. Our data strongly suggest that altered BDNF levels modulated by BDNF gene variability are associated to the susceptibility to ED, providing physiological evidence that BDNF plays a role in the development of AN and BN, and strongly arguing for its involvement in eating behaviour and body weight regulation.

In eating disorder patients, we have examined the involvement of the 5HT2C and SLC6A4 genes in the psychopathological symptomatology of ED. We have genotyped four SNPs within the 5HT2C gene and two sequence variants within the SLC6A4 gene to evaluate their involvement in the psychopathological symptomatology. Significant evidence of association between the a specific haplotype of the 5HT2C gene and different anxious and depressive subscales, that included Somatization, Obsessive-Compulsiveness, Depression, Anxiety, Hostility, Phobic Anxiety and Paranoid Ideation, was observed in BN patients. We also detected a strong association between the SLC6A4 genotype and Anxiety in the same group of BN patients. The analysis of epistatic effects between 5HT2C and SLC6A4, however, showed that the effect of the haplotype on the different anxious and depressive subscales was independent of the 5HTTLPR genotype. Moreover, ghrelin, a recently identified orexigenic peptide involved in the regulation of energy balance and food intake, was studied in an European collaboration project. Family trios and case–control studies of three ghrelin gene single nucleotide polymorphisms (SNPs), Gln90Leu, Leu72Met and Arg51Gln, were performed in 692 Caucasian cases (366 AN and 326 BN) and in 529 AN and BN trios recruited from seven European countries (Italy, Spain, Germany, Slovenia, France, Austria and UK). We did not detect significant associations for the three examined genetic variants in the two different study designs performed, making it unlikely that these polymorphisms in the ghrelin gene are predisposing factors to eating disorders in the European population.

Finally, murine models of AN provide useful tools to evaluate the effects of reduced caloric intake and body weight. The anx/anx mouse is the only spontaneous murine model of anorexia. The locus involved in the anx phenotype has been mapped to mouse chromosome 2 by linkage. We are currently refining the location of the anx locus on mouse chromosome 2 by linkage studies. We have also performed expression array experiments in the anx/anx model and identify biochemical pathways that are altered in the murine model of AN. Several neurotransmitter and hormone pathways are altered in these mice, which should provide useful hints for the study of the molecular basis of ED in patient samples.

3. GENETIC FACTORS THAT PREDISPOSE TO HEARING IMPAIRMENT

Genes encoding for beta-connexin proteins are involved in hearing impairment. The group has shown that most cases of congenital deafness are due to mutations in GJB2. The group has found that a mutation in the mitochondrial genome (A1555G) is the commonest genetic cause of late-onset familial progressive hearing loss, but presents with a wide range of clinical phenotypes. The group is currently searching for genetic and environmental factors that modify hearing impairment phenotype in subjects carrying mutation A1555G. The group has made progress in the characterization of a region of human chromosome 8p23 associated with the A1555G deafness. Several genetic and genomic features have been analysed in chromosome 8p23.1, such as the polymorphic nature of a cluster of alpha-defensin genes (*DEFA1A3*), the expression of a mitochondrial ribosomal protein pseudogene (*MRPS18CP2*) and the involvement of a component of tight junctions (*CLDN23*). The group has developed DHPLC and Pyrosequencing assays for reliable and accurate quantification of heteroplasmy. The study of the inheritance of heteroplasmic mitochondrial DNA muta-

tions has provided insights into the mechanisms of mitochondrial DNA transmission through generations.

4. FUNCTIONAL GENOMICS OF NEUROLOGICAL DISORDERS

Oxidative stress is a common hallmark of many neurodegenerative disorders involved in neuronal dysfunction and/or neuronal death. Our group identified some years ago the gene *DSCR1* (Down Syndrome Candidate Region 1, now renamed *RCAN1*, Regulator of Calcineurin), overexpressed in Down syndrome and Alzheimer disease patients and possibly contributing to the neuropathology associated to these diseases. The group has performed functional studies using primary neuronal cultures obtained from mice null for *RCAN1*, in collaboration with other members of the program, the CRG and the PRBB. Through these studies we found that *RCAN1* is a deleterious factor contributing to increased neuronal susceptibility to oxidative stress. The study provides insights to the signalling pathways by which *RCAN1* regulates the neuronal response to oxidative stress and suggests that *RCAN1* is a dosage sensitive gene in the context of neurodegeneration.

Special interest is also focused on neurodegenerative related regulatory networks involving mRNA and small RNA altered expression in human brain samples and in model systems for neurodegenerative processes, including mouse models and primary neuronal cell cultures. Neurodegeneration-linked common processes, such as impaired metabolic pathways, oxidative stress, necrosis and apoptosis are being analyzed. The final purpose is to gain information about gene regulatory networks modulating neurodegenerative processes, which might be suitable for diagnosis and identification of putative therapeutic targets.

PUBLICATIONS

Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, …, González, JR, Gratacòs, M, …, J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, CarterNP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME "Global variation in copy number in the human genome." Nature, 444(7118), 444-54 (2006)

Khaja R, Zhang J, Macdonald JR, He Y, Joseph-George AM, Wei J, Rafiq MA, Qian C, Shago M, Pantano L, Aburatani H, Jones K, Redon R, Hurles M, ArmengolL, Estivill X, Mural RJ, Lee C, Scherer SW, Feuk L "Genome assembly comparison identifies structural variants in the human genome." Nat Genet, 38(12),1413-8 (2006)

Pujana MA, Ruiz A, Badenas C, …, Cerrato C, Madrigal I, de Cid R, …, Estivill X, Puig S "Molecular characterization of a t(9;12)(p21;q13) balanced chromosome translocation in combination with integrative genomics analysis identifiesC9orf14 as a candidate tumor-suppressor." Genes Chromosomes Cancer, 46(2), 155-62 (2006)

González JR, Wang W, Ballana E, Estivill X "A Recessive Mendelian Model to Predict Carrier Probabilities of DFNB1 for non-Syndromic Deafness." Hum Mutat, 27 (11), 1135-1142 (2006)

Ribases M, Fernandez-Aranda F, Gratacos M, Mercader JM, Casanovas C, Nunez A, Vallejo J, Estivill X "Contribution of the serotoninergic system to anxious and depressive traits that may be partially responsible for the phenotypical variability of bulimia nervosa." J Psychiatr Res (Oct 19 2006 [Epub ahead of print])

Estivill X "De la epidemiología genómica a la biomedicina de sistemas: disección de los componentes biológicos y ambientales de la enfermedad." JANO, 7134 (2006)

Goidts V, Cooper DN, Armengol L, Schempp W, Conroy J, Estivill X, Nowak N, Hameister H, Kehrer-Sawatzki H "Complex patterns of copy number variation at sites of segmental duplications: an important category of structural variation in the human genome." Hum Genet, 120(2), 270-84 (2006)

Ballana E, Morales E, Estivill X "Reply to correspondence by Abreu-Silva et al. regarding Ballana et al.: Mutation T1291C in the mitochondrial 12S rRNA gene involved in deafness in aCuban family belongs to the macrohaplogroup L1 of African origin." Biochem Biophys Res Commun, 346(3), 619-20 (2006)

Guan MX, Yan Q, Li X, Bykhovskaya Y, Gallo-Teran J, Hajek P, Umeda N, Zhao H, Garrido G, Mengesha E, Suzuki Ts, del Castillo I, Peters JL, Li R, QianY, Wang X, Ballana E, Shohat M, Lu J, Estivill X, Watanabe K, Fischel-Ghodsian N "Mutation in TRMU related to tRNA modification modulates the

phenotypic expression of the deafness-associated mitochondrial 12S rRNA mutation." Am J Med Genet, 79(2), 291-302 (2006)

Yan Q, Bykhovskaya Y, Li R, Mengesha E, Shohat M, Estivill X, Fischel-Ghodsian N, Guan MX "Human TRMU encoding the mitochondrial 5-methylaminomethyl-2-thiouridylate-methyltransferase is a putative nuclear modifier gene for the phenotypic expression of the deafness-associated 12S rRNA mutations." Biochem Biophys Res Commun, 342(4), 1130-1136 (2006)

Cellini E, Nacmias B, ..., Estivill X, ..., Gratacos M, *et al.* "Case-control and combined family trios analysis of three polymorphisms in the ghrelin gene in European patients with anorexia and bulimia nervosa." Psychiatr Genet, 16(2), 51-52 (2006)

Del Campo M, Antonell A, Magano LF, Munoz FJ, Flores R, Bayes M, Perez Jurado LA "Hemizygosity at the NCF1 Gene in Patients with Williams-Beuren Syndrome Decreases Their Risk of Hypertension." Am J Hum Genet, 78(4), 533-542 (2006)

Bravo O, Ballana E, Estivill X "Cochlear alterations in deaf and unaffected subjects carrying the deafness-associated A1555G mutation in the mitochondrial 12S rRNA gene." Biochem Biophys Res Commun, 344(2), 511-516 (2006)

Celerier E, Gonzalez JR, Maldonado R, Cabanero D, Puig MM "Opioid-induced hyperalgesia in a murine model of postoperative pain: role of nitric oxide generated from the inducible nitric oxide synthase." Anesthesiology, 104(3), 546-555 (2006)

Donaudy F, Zheng L, Ficarella R, Ballana E, Carella M, Melchionda S, Estivill X, Bartles J, Gasparini P "Espin gene (ESPN) mutations associated with autosomal dominant hearing loss cause defects inmicrovillar elongation or organization." J Med Genet, 43(2), 157-61 (2006)

Peña E.A., Slate EH, González JR "Semiparametric Inference for a General Class of Models for Recurrent Events" JSPI (2006)

Pontual L, Pelet A, Trochet D, Jaubert F, Espinosa-Parrilla Y, Munnich A, Brunet JF, Goridis C, Feingold J, Lyonnet S, Amiel J "Mutations of the ret gene in isolated and syndromic Hirschsprung disease in human disclose major and modifier alleles at a single locus." J Med Genet, 43(5), 419-23 (2006)

Goidts V, Armengol L, Schempp W, Conroy J, Nowak N, Muller S, Cooper DN, Estivill X, Enard W, Szamalek JM, Hameister H, Kehrer-Sawatzki H "Identification of large-scale human-specific copy number differences by inter-species array comparative genomic hybridization." Hum Genet, 5, 1-14 (2006)

Ballana E, Morales E, Rabionet R, Montserrat B, Ventayol M, Bravo O, Gasparini P, Estivill X "Mitochondrial 12S rRNA gene mutations affect RNA secondary structure and lead to variable penetrance in hearing impairment." Biochemical and Biophysical Research Communications, 341, 950-957 (2006)

de la Luna S, Estivill X "Cooperation to amplify gene dosage imbalance effects." Trends in Molecular Medicine, 12, 451-454 (2006)

Rabionet R, Morales-Peralta E, López-Bigas N, Arbonés ML, Estivill X "A novel G21R mutation of the GJB2 gene causes autosomal dominant non-syndromic congenital deafness in a Cuban family." Genetics and Molecular Biology, 29,3,443-445 (2006)

Sahun I, Gallego X, Gratacos M, Murtra P, Trullas R, Maldonado R, Estivill X, Dierssen M. "Differential responses to anxiogenic drugs in a mouse model of panic disorder as revealed by Fos immunocytochemistry in specific areas of the fear circuitry." Amino Acids (Nov 20 2006 [Epub ahead of print])

Dierssen M, Gratacòs M, Sahún I, Martín M, Gallego X, Amador-Arjona A, Martínez de Lagrán M, Murtra P, Martí E, Pujana MA, Ferrer I, Dalfó E, Martinez-Cué C, Flórez J, Torres-Peraza JF, Alberch J, Maldonado R, Fillat C, Estivill X "Transgenic mice overexpressing the full-length neurotrophin receptor TrkC exhibit increased catecholaminergic neuron density in specific brain areasand increased anxiety-like behavior and panic reaction." Neurobiology of Disease, 24(2), 403-18 (2006)

Huch M, Abate-Daga D, Roig JM, Gonzalez JR, Fabregat J, Sosnowski B, Mazo A, Fillat C "Targeting the CYP2B1/Cyclophosphamide Suicide System to Fibroblast Growth Factor Receptors Results in a Potent Antitumoral Response in Pancreatic Cancer Models." Hum Gene Ther, 17(12), 1187-1200 (2006)

GENES AND DISEASE

Gene Therapy

Gene therapy is an emerging field that holds the promise of treating a wide variety of diseases. However, before this can be achieved, successful vector systems must be developed to deliver therapeutic genes and successful preclinical studies in animals models need to be carried out. Moreover a broad understanding of the disease pathology is required to be able to design candidate gene transfer approaches. The group is interested in understanding the pathophysiology and molecular aspects of Pancreatic cancer and Down syndrome and in the development of optimal gene therapy approaches. Gene delivery vectors that can selectively and efficiently target the cells of interest are being developed and their gene transfer efficiency is being evaluated in living animals by the use of non-invasive molecular imaging techniques. Pharmacokinetic and pharmacodynamic studies are being conducted to evaluate the therapeutic response in preclinical mouse models. Disease amelioration or partial phenotypic correction as consequence of particular genetic interventions become key determinants towards the understanding of complex phenotypes.

In these last years the group has also been involved in the development of new gene delivery systems for the local or systemic production of therapeutic proteins. These approaches have been successful to study the role of hHGF as a renoprotective factor and to model the misuse of gene therapy for doping control.

GROUP STRUCTURE

Group Leader:	**Cristina Fillat**
Postdoctoral Fellows:	Xavier Altafaj
PhD Students:	Meritxell Huch Jon Ortiz Laura Garcia Daniel Abate Daga Anabel Jose
Masters:	Alex Bio de Rojas
Technicians:	Núria Andreu

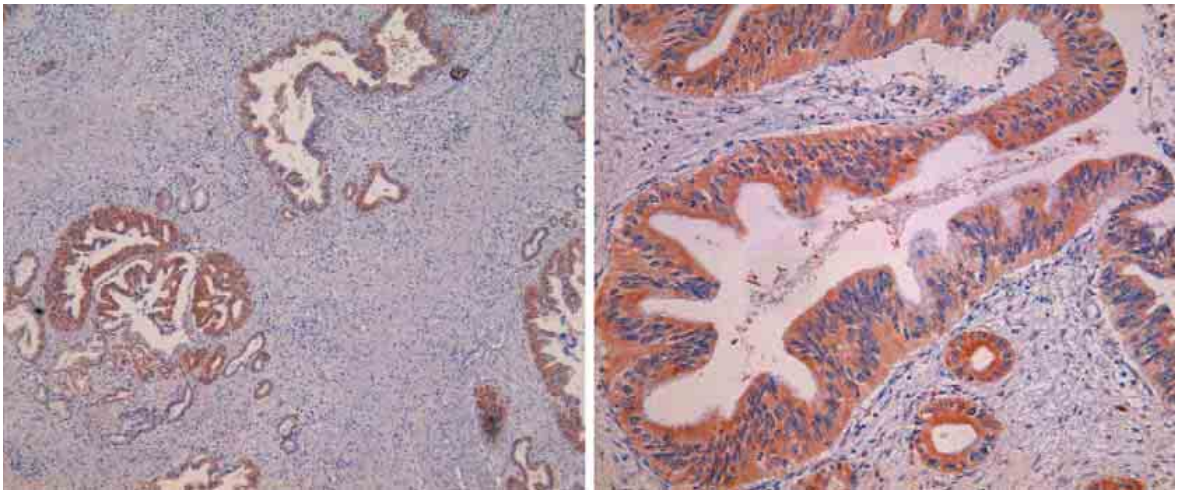


Figure 1. FGFR-1 expression in pancreatic tumors

RESEARCH PROJECTS

1. PANCREATIC CANCER

One of the most devastating diseases that our society is presently facing is cancer. Particularly pancreatic cancer is the fifth cause of cancer deaths in industrialized countries. This neoplasia has a very bad prognosis mainly due to the late diagnosis together with the fact that current therapies are very inefficient. Gene therapy emerges as a candidate approach for their treatment The group has been involved in the past few years in exploring the feasibility of suicide gene therapy in pancreatic cancer, showing some but limited effects. Based on those observations we are presently interested in the development of more potent and selective agents. To be able to increase their potency we are working with different systems that may facilitate the spreading of the cytotoxic compound into the tumor mass, such as by the use ot PTDs fused to suicide genes or the modulation of intercellular gap junctions. Targeting adenovirus vectors to tumoral cells is being explored as a way to increase the potency of antitumoral therapies. In that direction we have recently shown that by redirecting adenovirus to FGFR receptors the effectiveness of suicide gene therapy in pancreatic cancer is enhanced as was shown by the potent antitumoral effects and the increased survival rate achieved. In parallel to increase potency we are very much interested in selectivity and adenoviral vectors particularly active in tumoral cells are being studied. Moreover the group is also interested in the understanding of the basic mechanisms of tumor cell killing induced by particular suicide systems and in the definition of the genetic signatures that can sensitize pancreatic tumors to these particular therapies.

2. DOWN SYNDROME (DS)

Down syndrome is the most common autosomal trisomy; the trisomy 21. It is a multisystem disorder with a wide range of physical features, health and development problems. As we begin to understand the role of specific genes and we can identify the contribution of individuals genes to the overall phenotype thinking on partial gene therapy for specific defects might be a good approach for the treatment of certain diabilities. Alternatively the use of gene therapy approaches can be a very useful tool to validate potential targets for therapy and to contribute to understand the role of particular genes to a defined phenotype. From the results of our group and others we have strong evidences to believe that Dyrk1A can be one of the genes that

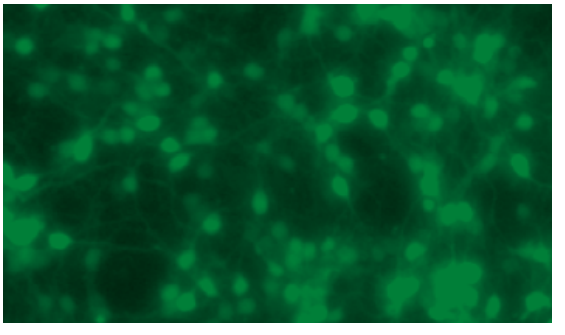


Figure 2. EGFP expression in viral transduced cerebellar granular cultures

will have a relevant role in Down syndrome. In fact, transgenic mice that overexpres Dyrk1A, present neurodevelopmental delay, motor alterations and some cognitive deficits, similar to those described in Down syndrome patients. We are currently testing the impact of reducing DYRK1A overexpression in brain by RNA interference technology to rescue defined phenotypes in TgDyrk1A and in the most complete DS model described, theTs65Dn mouse.

PUBLICATIONS

Andreu N, García-Rodríguez M, Volpini V, Frecha F, Molina IJ, Fontan G, Fillat C "A novel WASP complex mutation identified in a WAS patient results in an aberrant product at the C-terminus from two transcripts with unusual poly A signals." J Hum Genet, 51, 92-7 (2006)

Dierssen M, Ortiz Abalia J, Arqué G, Martínez de Lagran M, Fillat C "Pitfalls and hopes in Down syndrome Therapeutic approaches: In the search for evidence-based treatments" Behavioural Genetics, 36, 454-68 (2006)

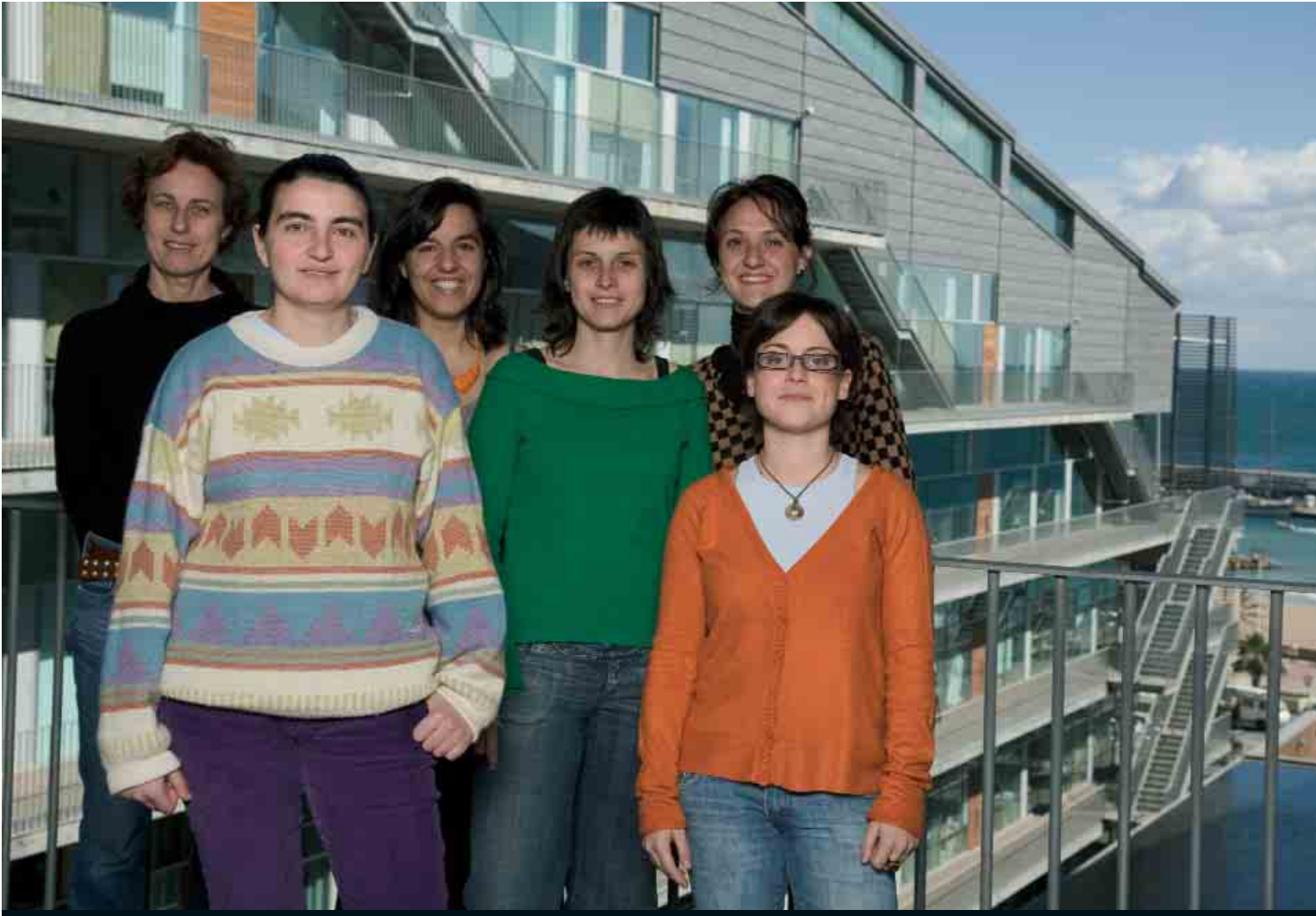
Burgos J, Guzman-Sánchez F, Sastre I, Fillat C, Valdivieso F "Non-invasive bioluminescence imaging for monitoring herpes simplex virus type 1 hematogenous infection" Microbes and Infection, 8, 1330-8 (2006)

Herrero-Fresneda I, Torras J, Franquesa M, Vidal A, Cruzado JM, Lloberas N, Fillat C, Grinyó M. "HGF gene therapy attenuates renal allograft scarring by preventing the pro-fibrotic inflammatory-induced mechanisms" Kidney Int, 70, 265-74 (2006)

Bayo-Puxan N, Cascalló M, Gros A, Huch M, Fillat C, Alemany R "Role of the putative HSG-binding site of the adenovirus type 5 fiber shaft on liver detargeting and knob-mediated retargeting" J Gen Virol, 87, 2497-95 (2006)

Dierssen M, Gratacos M, Sahun I, Martin M, Gallego X, Amador-Arjona A, Martinez de Lagran M, Murtra P, Marti E, Pujana MA, Ferrer I, Dalfo E, Martinez-Cue C, Florez J, Torres-Peraza JF, Alberch J, Maldonado R, Fillat C, Estivill X. "Transgenic mice overexpressing the full-length neurotrophin receptor TrkC exhibit increased catecholaminergic neuron density in specific brain areas and increased anxiety-like behavior and panic reaction." Neurobiol Dis, 24, 403-418 (2006)

Huch M, Abate-Daga D, Roig JM, González JR, Fabregat J, Sosnowski B, Mazo A, Fillat C "Targeting the CYP2B1/CPA suicide system to FGF receptors results in a potent antitumoral response in pancreatic cancer models." Hum Gene Ther, 17, 1187-1200 (2006)



GENES AND DISEASE

Murine Models of Disease

The overall goal of the laboratory is to investigate the in vivo function of genes involved in diseases affecting the central nervous system. Our current research focuses on the analysis of human chromosome 21 genes that are sensible to changes in gene dosage and therefore might be relevant for the cognitive deficits associated to trisomy 21 and to rare cases of monosomy 21. Since these are developmental disorders we are particular interested in genes that are proven to have or that are predicted to have an impact in neurogenesis. We, in the pass, have shown that the chromosome 21 gene *DYRK1A* is a good candidate to explain some of the developmental brain alterations in Down syndrome. We are continuing studying the role of this multifunctional protein kinase in central nervous system. In addition, we have contributed to the functional analysis of *DSCR1,* another human chromosome 21 gene that encodes a regulator of calcineurin activity.

GROUP STRUCTURE

Group Leader:	**Mariona Arbonés**
PhD Students:	Elisa Balducci (since September) Ariadna Laguna Laura Martín (until April) Silvia Porta
Technicians:	Erika Ramírez

RESEARCH PROJECTS

1. DYRK1A

DYRK1A is a serine/threonine kinase capable to phosphorylate a variety of substrates. We have shown in the pass that DYRK1A is involved in growth since, mice with only one functional copy of the gene present intrauterine growth retardation and microcephaly. More recent studies have revealed that changes in the amounts of DYRK1A protein lead to altered numbers of particular neural cell types in mouse brain and retina. We have carefully analysed the retina cell types that are sensible to changes on *Dyrk1A* dosage by using gain and loss of function transgenic mouse models. We have found that mice with one or three functional copies of *Dyrk1A* have retinas of an altered size. This phenotype is due to a significant alteration in the number of differentiating neurons that die by apoptosis during retinogenesis leading to retinas with an aberrant cellularity in the ganglion and inner nuclear layers of the retina.

To provide some insights into the possible role of DYRK1A in adult brain neurogenesis we have started experiments (collaboration with I. Fariñas, Universidad de Valencia) to evaluate the role of this protein kinase in the maintenance and proliferative capacities of the neurogenic niche of the mouse subventricular zone.

2. DSCR1/RCAN1

RCAN1, the gene product of *DSCR1*, is a functional inhibitor of calcineurin, a calcium-

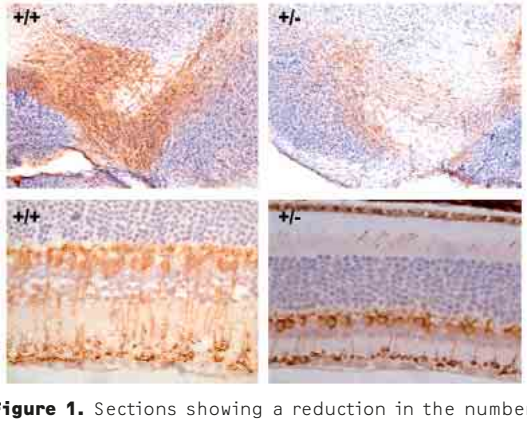
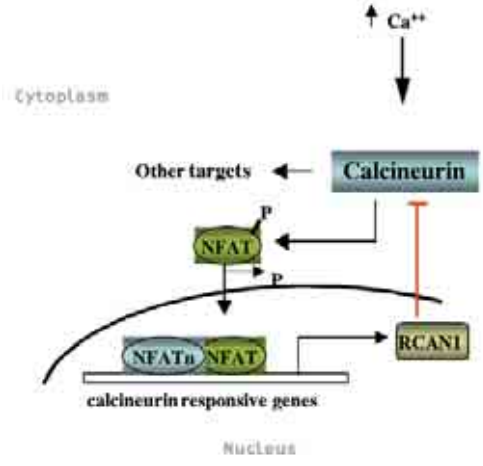


Figure 1. Sections showing a reduction in the number of brain dopaminergic neurons (upper panels) and retina rod-bipolar cells (lower panels) in mice heterozygous for a Dyrk1A null mutation.



activated protein phosphatase involved in many brain functions, including neuronal differentiation and learning and memory. We have shown that expression of RCAN1 in adult mouse brain follows a distribution pattern that overlaps to that of calcineurin. We have performed a preliminary phenotypic characterization of a *Dscr1* knockout mouse model that included morphological, molecular and behavioural tests without finding any significant alterations. In collaboration with the *Neurobehavioral Phenotyping* group at the CRG, we began to perform specific behavioral test to uncover possible neurological and cognitive alterations in these mice. Finally, we have collaborated with the *Genetic Causes of Disease* group at the CRG in a project aimed to elucidate the involvement of RCAN1 in neuronal death mediated by oxidative stress.

PUBLICATIONS

Rabionet R, Morales-Peralta E, López-Bigas N, Arbonés ML, Estivill X "A novel G21R mutation of the GJB2 gene causes autosomal dominant non-syndromic congenital deafness in a Cuban family" Genetics and Molecular Biology, 29,3,443-445 (2006)

Venail F, Wang J, Ruel J, Ballana E, Rebillard G, Eybalin M, Arbones M, Bosch A, Puel JL "Coxsackie adenovirus receptor and alpha nu beta3/alpha nu beta5 integrins in adenovirus gene transfer of rat cochlea" Gene Ther (Aug 3 2006 [Epub ahead of print]

Martínez de Lagrán M, Bortolozzi A, Millán O, Gispert JJ, Arbonés M, González JR, Artigas F, Dierssen M "Dopaminergic deficiency in mice with reduced levels of the dual-specificity tyrosine-phosphorylated and regulated kinase 1A, Dyrk1A(+/-)" Genes, Brain, Behav (2006 Nov 28 [Epub ahead of print]

GENES AND DISEASE

Neurobehavioral Phenotyping of Mouse Models of Disease

The overall goal of our research is to understand the role of putative candidate genes for human complex genetic diseases that affect the structural elements connecting the neurons with consequences on brain circuits that underlie cognitive systems. Genetically modified mouse models are a critical resource in the characterization of genes of biological importance, and in the dissection of the pathogenesis of neuropsychiatric and neurological disorders. A mouse model of human disease or gene function is, however, of limited value unless properly characterized and accurate phenotype assessment is a core issue of genetic manipulation. This will lead to a better knowledge of the genetic substrates regulating the expression of complex behavioral traits.

Our experimental approaches include high-throughput first behavioral analysis with multiple assessment tools that will detect basic alterations in nervous system function, and second level specialized analysis of motor and cognitive function. Our assessment targets are basic neurological function, motor function/control centers, exploratory activity, anxiety-related responses, depression, social interaction, and learning and memory. We also use neurohistological and morphometric approaches to determine a structural correlation for the detected phenotypic traits and cellular/molecular biology techniques to get insight in the underlying mechanisms of the specific phenotypic traits.

GROUP STRUCTURE

Group Leader:	**Mara Dierssen**
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Technician:	María Martínez de Lagrán Cabredo
Specialized Technician in phenotyping assay:	Ignasi Sahún Abizanda
Database manager:	David Fernandez
Visiting Predoctoral Fellow:	Garikoitz Azcona

RESEARCH PROJECTS

1. DOWN SYNDROME

M. Martínez de Lagrán, G. Arqué

The neuropathological processes underlying Down syndrome (DS) mental retardation and their genetic dependence remain an open question. The crucial problem is to define how does an excess of normal gene products in interaction with the environment direct and constrain neural maturation, and how does this abnormal development translate into cognition and behavior. Using a wide variety of relevant behavioral paradigms, our laboratory is investigating specific links between cognitive impairments and memory disorders in patients with DS and behavioral deficits in mouse models of this disease. We are also currently working on candidate genes involved in dendrites/spine dysmorphology and altered neural plasticity in learning and memory brain circuits. The aims of the project are: a/ to identify the physiological role of gene products and the dosage-dependent effects on neurodevelopment, learning and memory, and neurodegenerative processes in DS; b/ to identify cellular and molecular substrates that regulate the emergence of different forms of learning and memory.

The mouse is a model organism of choice to study human genetic diseases such as autosomal aneuploid syndromes, like DS. First, mice are mammals with similar physiology and embryonic development. Also, they share almost its whole genome with human. Indeed, the sequencing of the mouse and human genomes has shown that less than 1% of the genes of both genomes does not share any homology and that homologous genes are found as conserved syntenic regions in which the genes order and relative orientation are. Finally, mouse genetic offers a large panel of techniques allowing very precise genomic manipulation in associated with the development of battery of clinical tests to study the consequences of genetic modification. We are coordinating the work package for mouse models in a EU Integrated Project with the aim to make use of mouse models to understand the phenotypic consequences of gene dosage imbalance and to elucidate the role of particular chromosomal regions or specific genes in the pathogenesis of aneuploid syndromes. We use a global model of the disease the trisomy 16 mouse models Ts65Dn and Ts1Cje and partial trisomy and monosomy models such as Ts1Rhr, Ms1Rhr, Ts43H, Tbx1De5 then Ts2Yah (Y. Herault, CNRS). We also explore the role of specific candidate genes using conventional transgenic and gene targeted ("knock out") mice of single candidate genes generated in our Program. These include DYRK1A (Drosophila minibrain homolog), a serine/threonine kinase involved in neuronal development; DSCR1 (Calcipressin 1), an inhibitor of calcineurin-mediated signaling pathways, and BACE2 (ß-site APP cleaving enzyme 2), an aspartyl protease with APP ß-secretase activity.

Based on evidence from people with DS and studies in mouse models, we hypothesize that cognitive impairment in DS is due to abnormalities in the structure and function of dendrites and synapses with specific consequences in neuronal network formation or in properties of brain plasticity. DS shows several cerebral morphological alterations, altered cortical development and a reduction in connectivity. Therefore, the mental discapacity present in individuals suffering this syndrome is due to prenatal (embryonic cortical development) as well as postnatal alterations (cortical maturation and connectivity).

Thus, we study:

- different forms of learning and memory and other cognitive domains, and the microstructural characteristics of the brain regions involved, basically cerebral cortex and hippocampus, and the hippocampal–cortical networks since the hippocampus is crucial in integrating information from distributed cortical modules
- the geometry and microarchitecture of the dendritic trees and the morphology of the dendritic spines by stereological and michroarchitectural analysis of specific cell types. We use primary cultures of dissociated neurons of WT and DS model mice transfected with GFP-actin, and recorded and analyzed quantitatively to study growth cone morphology, dynamics of neurite outgrowth and branching and the actin cytoskeleton by confocal microscopy of fluorescent labeled neurons followed by quantitati-

ve image analysis and video-time-lapse microscopy and confocal bleaching to study the dynamics of actin.
- the experimental study of cellular mechanisms affecting cortical structure in animal models of DS.
- the analysis of genetic and epigenetic contributions in the functional and structural anomalies of cortical neurons in Down syndrome mouse models.

We have demonstrated decreased dendritic branching and reduced spine density in Ts65Dn and TgDyrk1A. To address the question if pyramidal cells in postnatal development do not achieve the structural complexity determined by intrinsic genetic programs, or if they undergo greater dendritic retraction of those in euploid brains during maturation due to extracellular factors, we will generate mice with chimeric cortices in which neuronal precursor cells of trisomic or transgenic (DS) genotypes are in contact and under the influence of non-transgenic/trisomic host neurons and vice versa.

The second phase of this project is oriented to determine how the interaction between these gene products and the environment contribute to the expression of learned behaviors in vivo neuroplasticity models. Moreover, we have continued the analysis of the synaptic features in the hippocampus of Ts65Dn mouse (collaboration with DC Davies, U.K.) and we will analyze the spinal cord development in TgDyrk1A mice (collaboration with Anna Casanovas, University of Lleida). Our work has shed new light on the possible mechanisms underlying the cognitive deficits and the defects in neural plasticity of DS. Besides we have observed significant changes in age-associated neurobehavioral and neuromorphological aspects in a murine model of overexpression of Dyrk1A (TgDyrk1A), a candidate gene for DS. Besides, we continue the characterization of transgenic and knockout models for Dyrk1A, DSCR1 and BACE2.

2. PATHOGENETIC AND MOLECULAR MECHANISMS INVOLVED IN PANIC DISORDER

A. Amador, X. Gallego, C. Obradors

Patients with neuropsychiatric disorders, such as panic/anxiety disorders, have an altered emotionality profile and abnormal social behaviors. A second research line in our group is aimed at identifying genetic causative and vulnerability factors underlying anxiety-related behavior and that could predict the onset of panic disorder. To this aim we use genetically modified animal models that help to elucidate mechanisms that may be acting in humans. Our current project is focused on the biochemical changes that occur in the CNS during development that are determinant of emotionality-related behaviors and to the development of therapeutic strategies that may overcome and/or prevent the brain alterations leading to panic attacks. Our interest is also aimed at elucidate the deficits of specific neurotransmitter systems that possibly underlie the inability of persons with anxiety disorders to correctly identify the fear-related information and the possible common neurobiological pathways responsible for co-morbid processes.

We are interested in candidate genes that participate in the dysfunction of brain circuits involved in fear-related memories and in mouse behavioral traits relevant to panic and to anxiety. The involvement of neurotrophic factors in neuronal survival and differentiation is well established. The more recent realization that these factors also play pivotal roles in the maintenance and activity-dependent remodeling of neuronal functioning in the adult brain has generated excitement in the neurosciences. Neurotrophic factors have been implicated in the modulation of synaptic transmission and in the mechanisms underlying learning and memory, mood disorders, and drug addiction. Here the evidence for the role of neurotrophins and other neurotrophic factors–and the signaling pathways they activate–in mediating long-term molecular, cellular, and behavioral adaptations associated with drug addiction is analyzed. We have demonstrated that NTRK3 (TrkC) can exert a major role in anxiety disorders based on the observation that its overdosage leads to an increased anxiety-like behavior and panic reaction, possibly due to the trophic effect attained on the catecholaminergic nuclei. Now we evaluate the mechanism of this effect studying if noradrenergic neuron neurogenesis, survival or plasticity can be affected. We have demonstrated

that overexpression of TrkC in mouse leads to an increase in number of catecholaminergic neurons in the LC and SN and show elevated anxiety and panic reaction. The open questions are now:

- if this effect is achieved by specifically promoting TH-positive neurons or by affecting survival and/or apoptosis
- if it affects other brain regions involved in fear circuits.
- if TrkC could be a transversal shared genetic facto in co-morbid disorders such as substance abuse or stress disorders.

To explore these aspects we are studying the development and function of the fear system in these mice, using behavioral, electrophysiological, and cellular experimental approaches. To this end we will evaluate the functional and structural modulations that occur upon application of specific neuromodulators or non-pharmacological stimuli that may regulate overall structure and plastic features of neurons and synapses. Finally we explore specific factors affecting the course of the disease in particular those that are associated with the regulatory capacity of the stress system. In collaboration with Dr. R. Maldonado (Pompeu Fabra University) we have initiate a project for studying the implication of NTRK3 in processes co-morbid to panic disorder, such as predisposition to substance abuse. Also we will analyze the predisposition to stress (collaboration with Dr. A. Armario, Autonomous University of Barcelona).

The role of other gene products like different nicotinic receptor subunits in behavior is also considered. Mice overexpressing alpha 7, alpha 5, beta 2, or beta 4 subunits, will be used to study the contribution of different nicotinic receptor subunits to (a) the expression of normal behaviors, (b) the sensitivity to the behavioral effects of panicogenic/panicolitic agents, and (c) the development of dependence and tolerance.

3. NEURODEGENERATIVE DISORDERS: ALZHEIMER'S TYPE NEURODEGENERATIVE PROCESS IN DOWN SYNDROME

G. Azcona, A. Türtzel

Alzheimer disease (AD) is a progressive neurodegenerative pathology that has elevated prevalence in Down syndrome (DS) patients, but very little is known about genetic and epigenetic factors underlying mental disability and AD in DS. Numerous studies have documented the presence of senile plaques and neurofibrillary tangles, hallmarks of Alzheimer's disease (AD). Subsequently, development of dementia symptoms is frequent in adults with DS. Very little is known about genetic and epigenetic factors underlying mental disability and AD in DS brain. Our goal is to elucidate the contribution to AD pathology found in DS, and in the events involved in the pathogenicity of amyloid formation of Dyrk1A, a serin treonin kinase involved in neurogenesis, cognitive processes, and DS and AD neuropathology and the influence of micro-environment in the function of altered neurons in the cerebral cortex using chimeric mice. The study will focus on AD symptoms, cortical neurogenesis, plasticity and behavioral analysis for cognitive impairment. We will use:

- Targeted-oriented array of AD mouse models to specifically address the role of Dyrk1A on neurodegenerative process by analyzing a) Dyrk1A expression pattern, proteome and phsophoproteome in AD mouse models and b) the impact of Dyrk1A overexpression on neuritogenesis/synaptogenesis/plasticity and on neurodegeneration using transgenic mice (TgDyrk1A).
- AD-like pathology induced by cytotoxic protofibrils from peptides derived of natural amyloid proteins a) in vivo by intracerebroventricular administration, and b) in vitro by addition of protofibrils onto primary cortical cell cultures in wild type and TgDyrk1A
- Generation and functional analysis of mice with chimeric cortices (Tg neurons in WT animals and viceversa) to establish the weight of genetic and microenvironmental factors in neuropathological aspects of AD present in DS.
- Differential proteomic analysis and identification of the phosphorylated subproteosome of specific cell types in TgDyrk1A mouse model, in basal conditions and after in vivo AD-induced pathology (protofibrils) and in mice with chimeric cortices.

This project will generate of new models of

in vivo AD-induced pathology (protofibrils), will provide information on the possible role of Dyrk1A in AD and its therapeutical use and will explore the involvement of epigenetic factors in the progression of the disease, an aspect that may be of enormous importance to develop protective and preventive strategies.

3. TECHNICAL DEVELOPMENT

I. Sahún, A. Amador, D. Fernandez

a. Neurobehavioral: aversive radial arm maze
b. Non-harmful immobilization device for immobilization stress studies
c. Specialized software analysis package
d. Development and validation of novel behavioral models for obsessive-compulsive disorders

Three patents on these projects are in progress.

4. PHENOTYPE ONTOLOGY PROJECT

I. Sahún, D. Fernandez

Phenotype analysis of mice has tended to be qualitative rather than quantitative in nature, but new more sophisticated tests of locomotor and cognitive function to mice are emerging. In the context of an Integrated European Project, our group is the Phenotypic Unit responsible for the second stage specialized phenotypic analysis of an in vivo library of partial trisomies/monosomies of MMU16. The results of the phenotypation will be standardized and categorized; to ensure that behavioral, functional and morphological characterization methods should be directly comparable between different groups to build a useful body of data. Traditional phenotypic descriptions are captures as free text but the information retrieval based on free text is extremely limited because of the inherent lack of accuracy and specificity. It is thus necessary to develop phenotypic descriptors that allow the creation of phenotypic annotation, so that one or more phenotypic descriptors will provide the phenotype of a particular model.

PUBLICATIONS

Sahún I, Gallego X, Gratacòs M, Murtra P, Trullás R, Maldonado R, Estivill X, Dierssen M "Differential Responses to Anxiogenic Drugs in a Mouse Model of Panic Disorder As Revealed by Fos Immunocytochemistry in Specific Areas of the Fear Circuitry" Amino Acids (2006 Nov 20 [Epub ahead of print])

Martínez de Lagrán M, Bortolozzi A, Millán O, Gispert JJ, Arbonés M, González JR, Artigas F, Dierssen M "Dopaminergic Deficiency In A Dual-Specificity Tyrosine-Phosphorylated And Regulated Kinase 1A Heterozygous Mice (Dyrk1A+/-)" Genes Brain Behav (2006 Nov 28 [Epub ahead of print])

Dierssen M "Genetic and environmental factors in the maldevelopment of the cerebral cortex in Down syndrome." Amino Acids (in press)

Dierssen M, Ramakers G "Dendritic pathology in mental retardation: from molecular genetics to neurobiology." Genes Brain Behav, 5 Suppl 2, 48-60 (2006)

Lumbreras M, Baamonde C, Martínez-Cué C, Lubec G, Cairns N, Sallés J, Dierssen M, Flórez J "Brain G protein-dependent signaling pathways in Down syndrome and Alzheimer's disease" Amino Acids, 31(4), 449-56 (2006)

Dierssen M, J. Ortiz Abalia, G. Arqué, M. Martínez de Lagran, C. Fillat "Pitfalls and hopes in Down syndrome Therapeutic approaches: In the search for evidence-based treatments" Behavioural Genetics, 36(3), 454-68 (2006)

Dierssen M, Martínez de Lagrán M "DYRK1A (dual-specificity tyronesine-phosphorylated and regulated kinase 1A): a gene with dosage effect during development and neurogenesis" Scientific World Journal June, 17, 6, 1911-1922 (2006)

Dierssen M, Gratacòs M, Sahún I, Martín M, Gallego X, Amador-Arjona A, Martínez de Lagrán M, Murtra P, Martí E, Pujana MA, Ferrer I, Dalfó E, Martinez-Cué C, Flórez J, Torres-Peraza JF, Alberch J, Maldonado R, Fillat C, Estivill X "Transgenic mice overexpressing the full-length neurotrophin receptor TrkC exhibit increased catecholaminergic neuron density in specific brain areasand increased anxiety-like behavior and panic reaction." Neurobiology of Disease, 24(2), 403-18 (2006)

GENES AND DISEASE

Gene Function

Research in the past few years has revealed that a number of human chromosome 21 (HSA21) genes are overexpressed in Down syndrome by, at least, 50% due to gene dosage. Because of the complexity of the Down syndrome phenotype, it is very likely that the increased expression leads to perturbations in a great variety of biological pathways. Furthermore, it is predictable that many HSA21 genes can interact functionally with each other within particular signalling pathways. Understanding the functional roles of the overexpressed genes will help not only to delineate the specific biological or biochemical processes affected but also to identify pathways that are particularly sensitive to dosage variations in any of their components. The group works in studying the functional roles of several HSA21 genes.

SUSANA DE LA LUNA HAS AN ICREA GROUP LEADER POSITION.

GROUP STRUCTURE

Group Leader:	**Susana de la Luna**
Postdoctoral Fellows:	Lali Genescà
PhD Students:	Sergi Aranda Eulàlia Salichs Krisztina Arató
Diploma Student:	Chrisovalentis Papadopoulus (from February to October)
Technician:	Alicia Raya

1. DYRK1A: A CROSSROADS FOR SIGNAL TRANSDUCTION PATHWAYS

Sergi Aranda, Eulàlia Salichs, Krisztina Arató

DYRK1A is one of the HSA21 genes for which changes in gene doses result in neuropathological alterations. It encodes for a protein kinase of the DYRK family of kinases. DYRK kinases (DYRK and HIPK subfamilies) constitute one of the families that belong to the CMGC group of protein kinases, formed by the CDKs, MAPKs, GSKs, CLKs and SRPKs. There are several reasons for choosing DYRK1A as one of our target molecules. First, we regard as pretty exciting the phenotypes shown by mouse transgenic mice in which the gene is either overexpressed or has been deleted, and second, we believe that DYRK1A might act as a crossroads for different signalling pathways since its substrates list consists of a variety of both cytosolic and nuclear proteins, transcription factors included.

Although DYRKs phosphorylate their substrates on serine and threonine, they autophosphorylate their activation loop on an essential tyrosine. This event is the result of an intramolecular phosphorylation reaction coupled to DYRKs translation that renders fully active enzymes. For that reason, and since an activating kinase appears not to be necessary, one might think that there is no room for activity regulation. However, DYRK1A seems to be extremely sensitive to gene dosage, and thus it is sensible to think that minimal changes in its activity would give rise to profound effects on the pathways it might control. With this in mind, we are interested in finding mechanisms that could possibly regulate the activity of DYRK1A.

We identified 14-3-3b during a screen for DYRK1A-interacting proteins and explored this interaction further. We have been able to show that DYRK1A autophosphorylates, via an intramolecular mechanism, on Ser-520, in the PEST domain of the protein. Phosphorylation of this residue, which seems to be subjected to dynamic changes *in vivo*, mediates the interaction of DYRK1A with 14-3-3b. We have also identified a second 14-3-3 binding site within the N-terminal of the protein. In the context of the DYRK1A molecule, neither site can act independently of the other. Finally, we have proved that 14-3-3b binding significantly increases the catalytic activity of DYRK1A.

Our results suggest a model in which the catalytic activity of DYRK1A is regulated by autophosphorylation and binding to 14-3-3b protein (Figure 1). In this model phosphorylation on Ser-520, outside the DYRK1A catalytic domain, would trigger the association with 14-3-3b. This interaction would induce a conformational change, resulting in increased DYRK1A catalytic activity. Being DYRK1A a highly gene-dosage sensitive gene, even small variations in activity could result in measurable changes in defined biological outputs. Finally, our work argues against the current view of DYRK1A as a

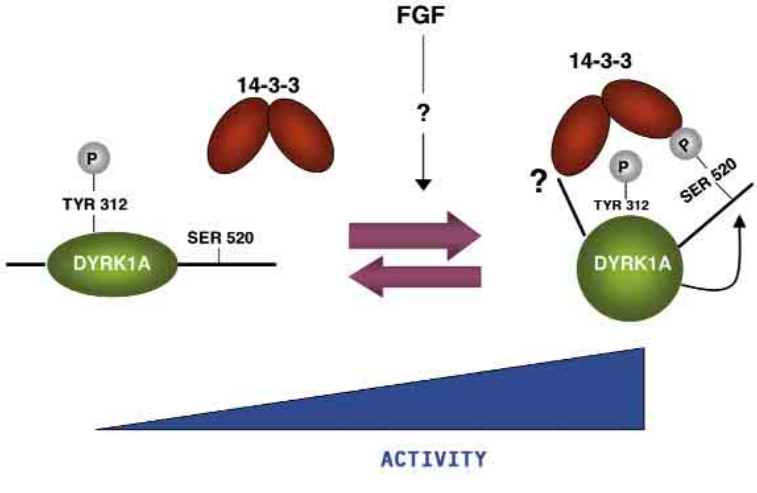


Figure 1. 14-3-3 binds to DYRK1A via a phospho-serine 520. Binding results in an increase in the catalytic of DYRK1A towards exogenous substrates. Phosphorylation levels of serine residue 520 are modulated by fibroblast growth factor (FGF) stimulation.

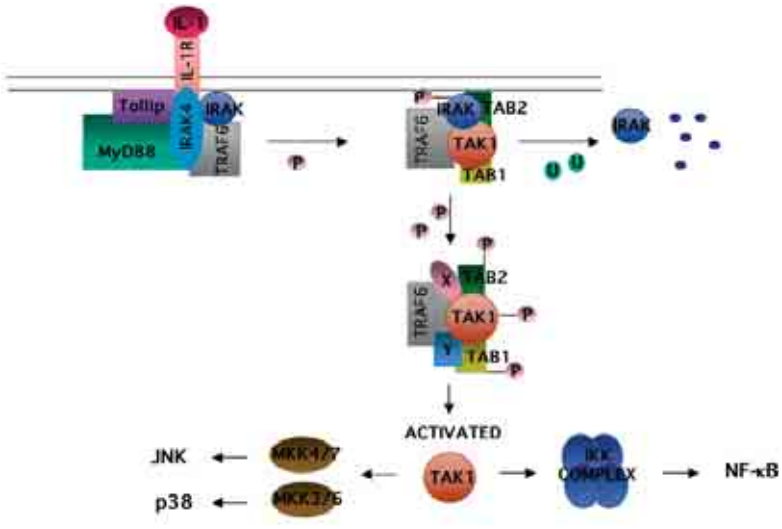


Figure 2. Schematic representation of the IL-1 signallling pathway. This pathway could be altered by overexpression of C21orf7

constitutive kinase that acquires full competency during its translation, and provides the first clear evidence of a mechanism of regulation for DYRK1A activity.

Other putative DYRK1A interacting-proteins are being explored. These include not only new substrates for DYRK1A (downstream targets), but also molecules than can act as effectors and thus, represent upstream modulators of DYRK1A in signalling cascades.

2. C21ORF7: A NOVEL HSA21 GENE INVOLVED IN THE REGULATION OF INFLAMMATORY CASCADES

Lali Genescà, Alicia Raya

We believe that an increased knowledge of the functional roles of HSA21 genes will greatly contribute to a better understanding of how overexpression affects the biological processes linked to HSA21-encoded protein products in Down syndrome. With this aim, we have undertaken the characterization of *C21orf7*, one of the HSA21 predicted genes with no function associated yet. Based on the similarity of its encoded products with the C-terminus of the protein kinase TAK1, we have focused our analysis in the signalling pathways controlled by this kinase.

TAK1, a member of the mitogen-activated protein kinase (MAPK) family, is activated by several stimuli such as the pro-inflammatory molecule interleukin-1 and Wnt, among others. TAK1 regulates the activity of various downstream signalling proteins including the MAPKs p38 and JNK and NF-kB, crucial players of many cellular activities. TAK1 participates in these pathways through the formation of signalling complexes in which several TAK1 binding proteins such as TAB1, TAB2 or TAB3 are present (Figure 2). Given that the homology region of TAK1 with *C21orf7* ORFs overlaps with the domain that the kinase uses to interact with TAB2 and TAB3, the hypothesis we are trying to test is that *C21orf7* ORFs bind TAB2 and/or TAB3 and compete them out for their binding to TAK1. In this way, *C21orf7* would act as an endogenous inhibitor in signalling cascades relying on the TAK1-TAB2/3 interaction.

Our results show that *C21orf7* ORFs are able to bind TAB2 and TAB3 as efficiently as TAK1 confirming our predictions based on just sequence similarity. The experiments designed would allow us to find out whether this binding has a functional impact on the IL-1 dependent signalling pathway.

PUBLICATIONS

Alvarez M, Altafaj X, Aranda S, de la Luna S "DYRK1A autophosphorylation on serine residue 520 modulates its kinase activity via 14-3-3 binding". Mol Biol Cell (in press)

de la Luna S, Estivill X "Cooperation to amplify gene dosage imbalance effects." Trends Mol Med, 12, 451-454 (2006)

GENES AND DISEASE

Associated Core Facility: Genotyping Unit

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in the human genome. A small fraction of this genetic variation is likely to explain the majority of the differences between individuals, including their predisposition to develop common human disorders, such as cardiovascular disease, hypertension, diabetes, asthma and cancer. SNP genotyping can be useful for genetic mapping, disease association studies, population genetics, and in other fields of research, including research in model organisms and trait selection for agricultural, cattle farming or aquaculture applications. Some of the genotyping technologies also enable to identify copy number variants (CNVs), to accurately characterize loss of heterozygousity (LOH) and/or to assess DNA methylation status. As a core facility we are committed to provide medium and high troughput genotyping services and analysis suport at established rates to scientists from the CRG, PRBB (UPF and IMIM) and external public and private institutions Our research activity is focused on the identification of genes that contribute to genetically complex disorders, in some cases arising from the involvement of the Genotyping Unit in collaborative projects

Group Leader:	**Xavier Estivill**
Unit Responsible:	Mònica Bayés
Postdoctoral Fellows:	Rafael de Cid Mònica Gratacòs Juan Ramón González
Technicians:	Carles Arribas Anna Brunet Cecília García Kristin Kristjansdottir Magda Montfort Anna Puig

SERVICES

The Genotyping Unit, supported by "Genoma España", through the National Genotyping Center (CeGen) provides support to scientists for genotyping projects in every aspect of research, from planning, DNA extraction, genotyping, data interpretation, through statistical analysis. The Unit offers custom, cost effective and flexible solutions for projects of any scale to both internal and external users, from public or private institutions. The Unit has produced more than 15 millions of genotypes during 2006.

At the CeGen Barcelona Node several genotyping and related services are available:

- Custom Genotyping by SNP*lex* (*Applied Biosystems*): genotyping of 24-48 SNPs selected by customer.
- Automated DNA extraction from blood or other tissues (*Chemagen*)
- DNA quantification using *Picogreen* (*Molecular Probes*)
- Whole Genome Amplification using *GenomiPhi* (*Amersham*)

Other genotyping services that can be provided for internal users and collaborators at the CRG Genotyping Unit:

- Mutation screening or SNP discovery through dHPLC (*Transgenomics*)
- Custom Genotyping by *Pyrosequencing* (*Biotage*): genotyping of 1-3 SNPs selected by the customer
- Custom Genotyping with *BeadArray* technology (*Illumina*): genotyping of 96-1536 SNPs selected by the customer
- Focused-content SNP Genotyping with *BeadArray* technology (*Illumina*):
  - LinkageIVb Panel: 5,861 SNPs distributed evenly across the human genome
  - Mouse LD Linkage Panel: 377 SNPs optimized for N2 and F2 mouse genetics crosses
  - Mouse MD Linkage Panel: 1,449 SNPs distributed evenly across the mouse genome
  - MHC Mapping Panel: 1,239 SNPs distributed evenly across the MHC region
  - Cancer SNP Panel: 1,421 SNPs in cancer genes
  - DNA Test Panel: 360 SNPs that may be used as genomic controls

Whole-genome genotyping with *Infinium* technology (*Illumina*):

- Human-1 *BeadChip*: 109,000 human SNPs, 70% of which are located in exons or within 10 kb of transcripts
- HumanHap300 *BeadChip*: 317,000 tagSNP markers derived from the International HapMap Project
- HumanHap550 *BeadChip*: 555,000 tag SNP markers derived from the International HapMap Project

All services are integrated with robust software tools for experimental design, management of data and analysis. Extensive quality control measures (both human and computational) let us further refine the quality of data.

COLLABORATIVE RESEARCH PROJECTS

Our research is focused on the identification of susceptibility genes and the interacting environmental exposures that contribute to genetically complex disorders/traits such as postpartum depression, autism, attention deficit and hyperactivity disorder, asthma, psoriasis, or behavioural and cognitive development in early childhood. Methodologies include traditional linkage analysis, sib-pair and affected-pedigree-member methods, case-control or family-based association studies, genome-wide scans and candidate gene analysis.

Other topics under investigation through collaborations with other Institutions are:

- The identification of the genetic basis of the variability in the response to methadone maintenance treatment (M Torrens, IMIM)
- Search for genetic variants involved in smoking cessation (JM Argimon, Fundació Gol i Gorina)
- Study of genes involved in the circadian rhythm in major depression (M Urretavizcaya, CSUB)
- Study of candidate genes in pathologic gambling (F Fernandez-Aranda, CSUB)
- Study of candidate genes involved in environmental-asthma interactions in European population (M Kogevinas, CREAL)
- Health impacts of long-term exposure to disinfection by-products in drinking water (Mark J Nieuwenhuijsen, CREAL)

- Finally, we involved in developing new statistical and bioinformatic tools for genotype data analysis and interpretation. In this regard, a new software package (*SNPassoc*, available at http://davinci.crg.es/estivill_lab/snpassoc) has been implemented.

PUBLICATIONS

Gonzalez JR, Wang W, Ballana E, Estivill X "A recessive Mendelian model to predict carrier probabilities of DFNB1 for non-syndromic deafness." Human Mutat, 27(11), 1135-42 (2006)

Castro-Giner F, Kauffmann F, de Cid R, Kogevinas M "Gene-environment interactions in asthma." Occup Environ Med, 63, 776-86 (2006)

Publications by core facility users

Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, Gonzalez JR, Gratacos M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME "Global variation in copy number in the human genome." Nature, 444 (7118), 444-54 (2006)

GENES AN

DISEASE


Ph
FALCON

BIOINFORMATICS AND GENOMICS

Coordinator: Roderic Guigó

The Bioinformatics and Genomics programme includes a number of research groups in which computational analysis plays an essential role to address relevant questions in genome research. Currently, the programme has two active groups in Genome Bioinformatics and Microarrays, but we are in the process of the expanding the programme with new groups

The two groups have been very active during the past year. A number of solid collaborations have been established between these groups and several experimental groups from other CRG programmes. Most of the programme's efforts in terms of hardware and personnel have been directed towards the Microarray facility, which we consider essential within the CRG structure, and which is now fully functional. We expect this trend to continue during the next year, while the new facilities are not available. In particular, we plan to strength the bioinformatics and statistics component of the Microarray group, partly through a more intimate collaboration between the Microarray and the Genome Bioinformatics groups.

Current structure of the programme:

- 2 Research Groups:
  - Bioinformatics and Genomics
    (Roderic Guigó, join group with GRIB (IMIM, UPF))
  - Genomic Analysis of Development and Disease
    (Lauro Sumoy)
- Associated Core Facility: Microarray Unit

BIOINFORMATICS AND GENOMICS

Bioinformatics and Genomics

Research in the Genome Bioinformatics group focuses in the problem of eukaryotic gene identification. Our group is involved in both the development of software for gene prediction in genome sequences, and in the investigation of the signals involved in gene specification. Our group has actively participated in the analysis of many eukaryotic genomes and it is leading one of the NIH funded ENCODE projects

GROUP STRUCTURE:

Group Leader:	**Roderic Guigó**
Postdoctoral Fellows:	Sarah Djebali (IMIM) Tyler Alioto Sylvain Foissac David Martin Christoforous Nikolau
Students:	Charles Chapple Hagen Tilgner Anna Kedsierska
Technicians:	Oscar González Julien Lagarde (IMIM) Francisco Câmara Sergi Beltran

RESEARCH PROJECTS

1. GENE PREDICTION

We are working in the development of geneid, an "ab initio" gene prediction program, and sgp a comparative gene finded. Geneid and sgp have been used in the annotation of many eukaryotic genomes. Recently geneid has been used in the annotation pipeline of *Paramecium tetraurelia* (Jaillon *et al.*, 2006), sequenced at the Genoscope, and with Genoscope we are also collaborating in the annotation of other genomes. We are also collaborating with the Broad Institute in the annotation of many fungal genomes, and have participated in the analysis and annotation of the genomes of the 12 *Drosophila* species recently sequenced. We have also been collaborating with the group of Stylianos Antonarakis, from the University of Geneva, in the analysis of the first sequences obtained in the heterochromatic fraction of the human genome

2. PREDICTION OF SELENOPROTEINS

Particularly difficult in eukaryotic genomes is the prediction of selenoprotein genes, because selenocysteine is specified by the UGA codon, normally an stop codon. Since year 2000 we have been developing computational methods for selenoprotein prediction. During the last years we have successfully used this methods to characterize mammalian selenoproteins (Castellano *et al.*, 2001, Kryukov *et al.*, 2003, Castellano *et al*, 2004). Recently, using comparative genomics methods we have discovered a novel selenoprotein families, whose phylogenetic distribution is challenging long standing assumptions about the taxonomic distribution of eukaryotic selenoproteins (Castellano *et al.*, 2006, see also figure 1).

3. SPLICING

In strong collaboration with the group of Juan Valcárcel, from the CRG's Gene Regulation programs we are investigating the mechanisms by means of which splice signals are recognized and processed. We are developing new methods to infer sequences that may play a role in the regulation of alternative splicing, and have been investigating the dynamics of the evolution of U12 introns. We have developed a database of U12 introns (Alioto, 2007).

4. RECOGNITION OF PROMOTER REGIONS

In collaboration with Xavier Messeguer from the Universitat Politècnica de Catalunya, we have initiated a research line on algorithmics for promoter recognition; in particular addressing the problem of comparing and characterizing the promoter regions of genes with similar expression patterns. This remains a challenging problem in sequence analysis, because often the promoter regions of co-expressed genes do not show discernible sequence conservation. In our approach, thus, we have not directly compared the nucleotide sequence of promoters.

Figure 1. Phylogenetic distribution of eukaryotic selenoproteins (from Castellano *et al.*, 2005)

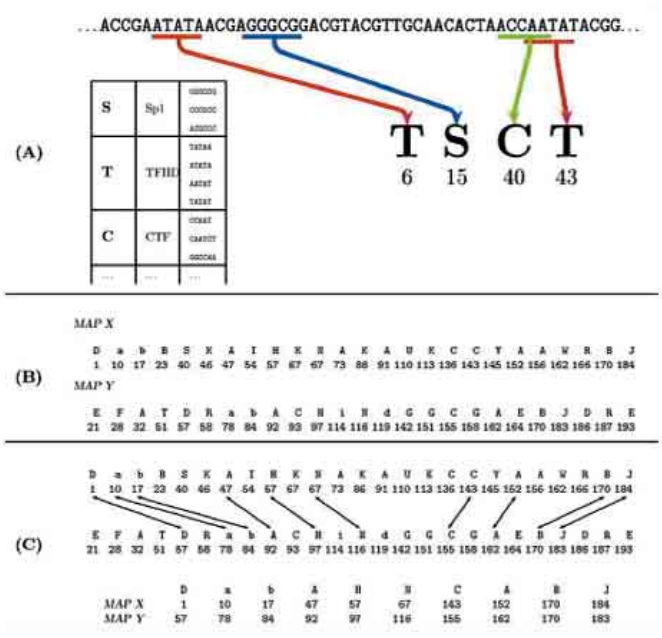


Figure 2. TF-Map alignment of promoter regions (Blanco *et al.*, 2006)

Instead, we have obtained predictions of transcription factor binding sites, annotated the predicted sites with the labels of the corresponding binding factors, and aligned the resulting sequences of labels—to which we refer here as transcription factor maps (TF-maps, see Figure 2). To obtain the global pair wise alignment of two TF-maps, we have adapted an algorithm initially developed to align restriction enzyme maps. We have optimized the parameters of the algorithm in a small, but well-curated, collection of human–mouse orthologous gene pairs. Results in this dataset, as well as in an independent much larger dataset from the CISRED database, indicate that TF-map alignments are able to uncover conserved regulatory elements, which cannot be detected by the typical sequence alignments (Blanco *et al.*, 2006).

5. ENCODE PROJECT

The National Human Genome Research Institute (NHGRI) launched a public research consortium named ENCODE, the Encyclopedia Of DNA Elements, in September 2003, to carry out a project to identify all functional elements in the human genome sequence (The ENCODE consortium, 2004). In its pilot phase the project is aiming to characterize all functional elements in 1% of the human genome. Within ENCODE, we are leading the GENCODE consortium with the goal of identifying all protein coding genes

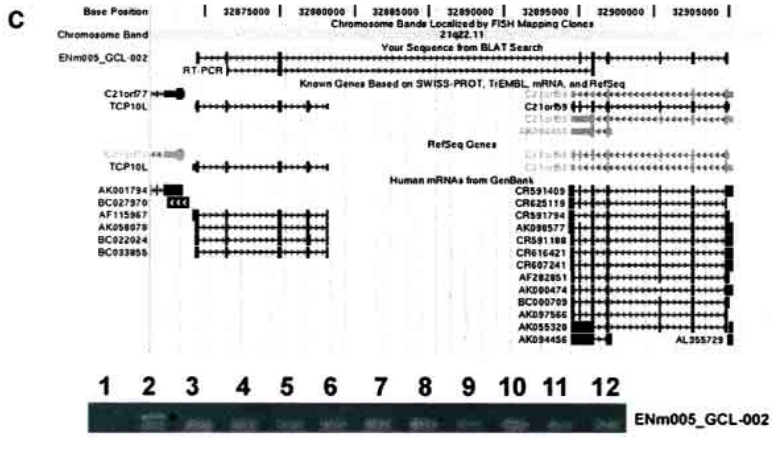


Figure 3. A computationally predicted chimera in one ENCODE regions verified by RT-PCR (Parra *et al.*, 2006)

in the ENCODE regions. A first version of this annotation was released in early 2005, and we organized EGASP a community experiment to assess the quality of this annotation. An special issue of Genome Biology has been published during year 2006 devoted specifically to EGASP (Reese and Guigó, eds).

Within the framework of the ENCODE project, we have been collaborating with the groups of Stylianos Antonarakis, from the University of Geneva, and Tom Gingeras, from Affymetrix, to exhaustively characterize the transcript diversity of protein coding loci. Towards that end, we have developed the RACEarray strategy. In such strategy, RACE products originated from primers anchored in exons from annotated protein coding genes are hybridized into high density genome tiling arrays, and sites of transcription specifically linked to the index exon are in this way uncovered. Such experiments are revealing as many novel exons as annotated ones (Denoeud *et al.*, in press). Particularly interested is the discovery of the high frequency with which chimerical transcripts between nearby genes are formed. These often keep the Open Reading Frame, and could encode novel protein sequences. We published in 2006 a preliminary study on this issue (Parra *et al.*, 2006, Figure 3)

PUBLICATIONS

Blanco E, Farré D, Albà MM, Messeguer X and Guigó R "ABS: a database of Annotated regulatory Binding Sites from orthologous promoters." Nucleic Acids Res, Jan 1, 34 (2006)

Mularoni L, Guigo R, Alba MM "Mutation patterns of amino acid tandem repeats in the human proteome." Genome Biol, 7(4), R33 (2006)

Blanco E, Messeguer X, Smith TF, Guigo R. "Transcription factor map alignment of promoter regions" PLoS Comput Biol, May, 2(5):e49 (2006)

Guigo R, Valcarcel J "Unweaving the meanings of messenger RNA sequences." Mol Cell, 23(2), 150-1 (2006)

Reese MG, Guigo R "EGASP: Introduction." Genome Biol, 7 Suppl 1, S1.1-3 (2006)

Guigo R, Flicek P, Abril JF, Reymond A, Lagarde J, Denoeud F, Antonarakis S, Ashburner M, Bajic VB, Birney E, Castelo R, Eyras E, Ucla C, Gingeras TR, Harrow J, Hubbard T, Lewis SE, Reese MG. "EGASP, The human ENCODE Genome Annotation Assessment Project." Genome Biol,7 Suppl 1,S2.1-31 (2006)

Harrow J, Denoeud F, Franklish A, Reymond A, Chen CK, Charast J, Lagarde J, Gilbert JG, Storey R, Swarbreck D, Rossier C, Ucla C, Hubbard T, Antonarakis SE, Guigo R. "GENCODE: producing a reference annotation for ENCODE." Genome Biol, 7 Suppl 1, S4.1-9 (2006)

Aury JM,Jaillon O,Duret L,NoelB, Jubin C,Porcel BM,Segurens B,Daubin V,Anthouard V,Aiach N,Arnaiz O,Billaut A,Beisson J,Blanc I,Bouhouche K,Camara F,Duharcourt S,Guigo R,Gogendeau D,Katinka M,Keller AM,Kissmehl R,Klotz C,Koll F,Le Mouel A,Lepere G,Malinsky S,Nowacki M,Nowak JK, Plattner H, Poulain J, Ruiz F, Serrano V, Zagulski M, Dessen P, Betermier M, Weissembach J, Scarpelli C, Schachter V, Sperling L, Meyer E, Cohen J, Wincker P. "Global trends of whole-genome duplications revealed by the ciliate Paramecium tetraurelia." Nature 444(7116), 171-8 (2006)

Parra G, Reymond A, Dabbouseh N, Dermitzakis ET, Castelo R, Thompson TM, Antonarakis SE, and Guigó R "Tandem chimerism as a means to increase protein complexity in the human genome." Genome Research, 19(8), 475-484 (2006)

BIOINFORMATICS AND GENOMICS

Genomic Analysis of Development and Disease

The group is interested in the study of gene regulation at the transcriptional level and genomic changes in a variety of processes including early embryonic development, differentiation of the central nervous system and cancer progression. Our goal is to use global genomic analysis tools to discover target and co-regulated genes affected under specific conditions, to understand the function of these newly characterized genes through inference from gene expression profile data, to correlate these with changes in transcription factor occupancy at gene promoter and chromatin modifications, and to understand large scale copy number variation through comparative genomic hybridization.

The use of microarray technologies has become a powerful tool to begin to understand regulatory gene networks. We plan to apply these to specific biological and clinical problems in our own research and in collaborative projects derived from working as the core microarray facility for PRBB.

GROUP STRUCTURE:

Group Leader:	**Lauro Sumoy**
Postdoctoral Fellow:	Franc Llorens
PhD student:	Susana Iraola
Bioinformatician:	Juanjo Lozano

RESEARCH PROJECTS

1. GENE REGULATORY NETWORK ANALYSIS

We continued our independent basic line of research centered on the functional analysis of the LRRN6A gene, which has derived in a larger project aiming at understanding gene regulatory networks from global gene expression and chromatin immunoprecipitation results, with a focus on the central nervous system. We are continuing to characterize this gene and to use molecular biology and genomics tools to study global gene regulatory mechanisms, with a focus on mammalian nervous system.

We will use natural variation under basal conditions as well as perturbation responses in response to treatment with growth factors, neurotrophins and myelin derived inhibitors of axonal regeneration and oligodendrocyte differentiation to study gene regulatory networks important for CNS development and function. We plan to generate our own datasets as well as gather data from public microarray repositories on gene expression and chromatin immunoprecipitation, and to develop tools for data extraction and integration that allow inferring functional relationships, signalling cascades and gene regulatory networks among genes, relating them to events relevant to the pathophysiology of the nervous system. We have begun a collaboration with Robert Castelo, UPF, to predict regulatory gene networks from microarray expression data.

2. MICROARRAY BENCHMARKING, PROCESS QUALITY CONTROL, STANDARDIZATION AND META-ANALYSIS

One of the main current challenges in microarray research is the comparison between different data sets. We intend to advance in the development of methods that allow inference of conserved patterns of gene co-regulation using meta-analysis methodologies. We plan to apply these to understand the signalling pathways affected during neuronal development and differentiation, and in the adult brain in response to pathological situations.

In addition, we plan to apply these novel developments to the comparison and mining of microarray datasets in public databases.

Through participation in several large scale projects involved in the study of diseases such as cancer or genomic disorders, we are developing new approaches to the study of gene expression profiles by use of cross-platform standardization, meta-analysis and multivariate methods. This should allow us to integrate already published datasets and information derived from our own microarray experiments.

3. MICROARRAY TECHNOLOGY DEVELOPMENT

Many of our technological research activities are focused on implementing new experimental applications. Many of these arise from the involvement of the microarray core facility in many different collaborative projects. We are committed to developing and optimizing procedures for the design, fabrication, hybridization, processing and analysis of data generated from two colour DNA microarrays.

We are involved in projects focusing on breast cancer (collaboration with Dr. Miguel Beato, CRG; Dr. Francesc Solé, Hospital del Mar-IMAS), genome structure variation in control population and genomic disorders mediated by homologous recombination events between segmental duplications (collaboration with Drs. Xavier Estivill, CRG, and Luis Perez Jurado, UPF), and variation in splicing (collaboration with Drs. Roderic Guigó and Juan Valcarcel, CRG). We are testing procedures for CHIP on chip to detect differences in binding of transcription factors and chromatin components to DNA (collaborations with Dr. Miguel Beato, CRG; Dr. Jorge Ferrer, IDIBAPS; Dr. Anna Bigas, IRO). Currently planned applications include detection of nucleosome scale changes on chromatin (collaboration with Dr. Miguel Beato, CRG) and of miRNA expression in mammalian tissues (collaboration with Dr. Yolanda Espinosa and Xavier Estivill, CRG).

4. MICROARRAY BIOINFORMATICS

We have set up automated image data acquisition, pre-processing, filtering, normalization and quality control Web based software for analysis of microarray experiments. We are implementing an Oracle based database for direct data browsing by service users. In the future, web tools for advanced data set

classification, clustering, grouping, discriminant, factor analysis and data mining purposes, already under development (Sanchez-Corbayo *et al*, 2003). This also includes capabilities for analysis of other types of microarrays data such as Affymetrix (Lozano and Kalko, 2006).

We are directly involved in providing support for the analysis of CGH data from large cancer datasets (collaborations with Dr. Paco Real, IMIM; Dr. Rosa Miró, UAB; Timothy Thomson, CID-CSIC; Pedro Fernández, Hosp. Clinic) and for annotation of alternative splicing microarrays (collaboration with Dr. Juan Valcarcel, CRG). We are also involved in a collaboration to detect expression markers of tolerance response to liver transplantation (with Dr. Alberto Sánchez-Fueyo, IDIBAPS).

PUBLICATIONS

Mengual L, Burset M, Ars E, Ribal MJ, Lozano JJ, Minana B, Sumoy L, Alcaraz A. "Partially degraded RNA from bladder washing is a suitable sample for studying gene expression profiles in bladder cancer." Eur Urol, 50(6), 1347-55 (2006)

Camps J, Armengol G, del Rey J, Lozano JJ, Vauhkonen H, Prat E, Egozcue J, Sumoy L, Knuutila S, Miro R "Genome-wide differences between microsatellite stable and unstable colorectal tumors." Carcinogenesis, 27(3), 419-28 (2006)

Lozano JJ, Kalko SG "AMarge: Automated Extensive Quality Assessment of Affymetrix chips." Appl Bioinformatics, 5(1), 45-7 (2006)

Sanchez-Carbayo M, Socci ND, Lozano JJ, Haab BB, Cordon-Cardo C "Profiling bladder cancer using targeted antibody arrays." Am J Pathol, 168(1), 93-103 (2006)

Associated Core Facility: Microarrays Unit

UNIT STRUCTURE

Senior Technician:	**Eva González**
Senior Technician:	Belen Miñana
Technician:	Ana Marina Mosquera
Bioinformatician:	(Open position)

Guest or associated members:

PhD student:	Mireia Vilardell (PRBB, L. Pérez Jurado – UPF)

As a core facility, the laboratory is mainly responsible for the experimental and bioinformatics aspects of different research projects that use microarrays. In addition, it provides microarray methodologies as a service at established rates to scientists from the CRG, PRBB (UPF, IMIM, CREAL, CMRB, CAT) and other external public and private institutions.

SERVICES

Services offered include: microarray probe selection and design, microarray probe preparation, microarray fabrication through contact spotting, RNA purification, quality control and amplification, RNA and DNA sample labelling, hybridization of microarrays and data processing and analysis. We have already used microarrays to study gene expression including whole genome arrays (yeast, human, rat, maize and mouse) and customized targeted small arrays (pancreas, breast cancer and neural). We also have used custom BAC arrays for comparative genomic hybridisation, and promoter arrays for chromatin immunoprecipitation on microarrays. The facility is also set up for optimal processing of in situ synthesized long oligonucleotide arrays (Agilent).

PUBLICATIONS BY CORE FACILITY USERS

Vicent GP, Ballare C, Nacht AS, Clausell J, Subtil-Rodriguez A, Quiles I, Jordan A, Beato M
"Induction of progesterone target genes requires activation of Erk and Msk kinases and phosphorylation of histone H3." Mol Cell, 24(3), 367-81 (2006)

[O I N F O R M A T I C
I D G E N O M I C S


CRG

CELL AND DEVELOPMENTAL BIOLOGY

Coordinator: Isabelle Vernos

The programme of Cell and Developmental Biology is one of the youngest programmes of the CRG. This year the programme inaugurated its quarters in the new PRBB building in November. Hernan Lopez-Schier who was recruited in 2005 as a junior group leader moved from The Rockefeller University in New York (USA) and joined the programme in the middle of the year to establish a group working on the development, cellular organization and regeneration of the auditory organ of the zebra fish.

One major goal in 2006 was to identify and attract a high profile scientist in the field to become the coordinator of the programme. We expect the coordinator to join the CRG next year and recruit additional groups to complete the programme.

Another important goal of the programme is the establishment of an Advanced Light Microscopy Unit that will become an essential resource for the programme and the CRG. This year, Timo Zimmermann was recruited to head the Unit. He will leave the ALMF at EMBL, Heidelberg (Germany) to join the CRG in January 2007. In addition, two confocal microscopes have been purchased and will be installed and running in the facility space in early 2007. Further light microscopy instrumentation will be acquired next year to provide a wide range of imaging possibilities.

Structure of the Programme:

Acting coordinator and Senior group:	Isabelle Vernos
Junior Group:	Hernan Lopez-Schier
Associated Core Facility:	Advanced Light Microscopy Unit (under construction)

CELL AND DEVELOPMENTAL BIOLOGY

Microtubule Function and Cell Division

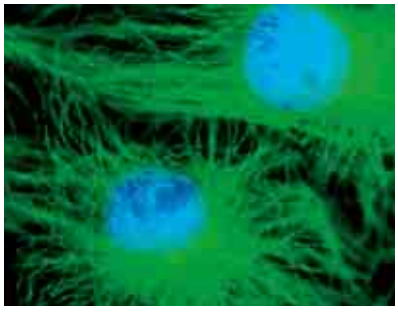
Research in my lab is directed at understanding the role of the microtubule network in cell organization and function. To address this question we study various microtubule-associated proteins (molecular motors and MAPs) and their regulators (kinases, phosphatases and the small GTPase Ran during M-phase). One major goal is to unravel how the self-organization of cellular components results in the morphogenesis of dynamic molecular machines. In the last few years, we have focused on two examples of self-organization: the morphogenesis of the Golgi apparatus in interphase and of the bipolar spindle in mitosis and meiosis.

Our favorite experimental system is the Xenopus egg extract system for studies on cell cycle progression and regulation, microtubule dynamics, spindle assembly and chromosome behaviour (Karsenti and Vernos, 2001). We combine it with the use of human tissue culture cells in which we validate some of the results obtained in egg extract and we study the role of microtubules and motors in membrane traffic and Golgi morphogenesis.

ISABELLE VERNOS HAS AN SENIOR ICREA GROUP LEADER POSITION.

GROUP STRUCTURE:

Group Leader:	**Isabelle Vernos**
Postdoctoral Fellows:	Laurent-Herve Perez (until July 2006) Teresa Sardon (since March 2006)
Students:	Vanessa Dos Reis Ferreira Isabel Peset Martin Schütz (since July 2006) David Vanneste
Technician:	Luis Bejarano



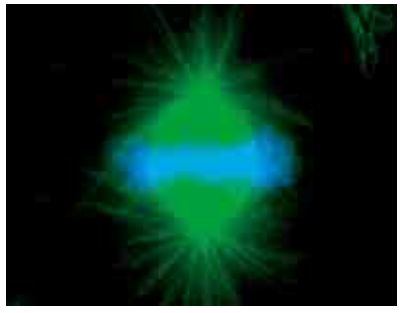


Figure 1. Microtubule organization in interphase (left) and in mitosis in HeLa cells. DNA is shown in blue, microtubules in green

RESEARCH PROJECTS

1. MECHANISM OF SPINDLE ASSEMBLY AND CHROMOSOME SEGREGATION

Cell division is characterized by the dramatic reorganization of the microtubule network into a spindle shaped apparatus that segregates the chromosomes into the two daughter cells. Spindle assembly and function relies on complex protein interaction networks that are finely regulated in time and in space. In addition to phosphorylation-dephosphorylation reactions, recent work has shown that the small GTPase Ran in its GTP bound form plays an important role in the spatial regulation of spindle assembly (Gruss and Vernos, 2004). One of the proteins regulated by RanGTP during M-phase is the nuclear protein TPX2 (Targeting Protein for Xklp2) (Wittmann *et al,* 1998). After being released from importins by RanGTP TPX2 triggers the nucleation of microtubules (Figure 2). We have shown that TPX2 activity is essential for spindle assembly both in Xenopus egg extracts and in HeLa cells therefore suggesting that in general spindle assembly may require a centrosome independent assembly of microtubules in the vicinity of the chromosomes (Gruss *et al.,* 2002; Wittmann *et al.,* 2000).

TPX2 has additional functions. It targets the kinesin-like protein Xklp2 to the spindle poles (Wittmann *et al,* 2000) and the kinase Aurora A to spindle microtubules. We have previously shown that this interaction is regulated by RanGTP and 'locks' the kinase into an active conformation (Bayliss *et al,* 2003; Bayliss *et al,* 2004). This could potentially be a mechanism by which the RanGTP regulatory network around chromosomes is translated into a phosphorylation network associated to the forming spindle. We are currently investigating these issues and trying to determine the role of Aurora A activation in spindle assembly and cell cycle progression.

1. Function/mechanism of RanGTP/TPX2 induced microtubule assembly

Although we have shown that TPX2 is essential for RanGTP induced microtubule assembly in M-phase we do not know the mechanism involved. We have found that the C-terminal domain of TPX2 is sufficient and we are currently characterizing it at the structural and functional levels. In addition, we know that other factor(s) are also involved and that at least one or more of them are also under RanGTP regulation. We are currently trying to identify these factor(s) and performing experiments to understand their respective role in this pathway.

2. Role of the Xenopus TACC family member Maskin in spindle assembly and microtubule dynamics

The Xenopus protein Maskin, previously identified and characterized in the context of its role in translational control during oocyte maturation, belongs to the TACC protein family. In other systems, members of this family have been shown to localize to centrosomes during mitosis and to play a role in microtubule stabilization. Last year we showed that Maskin plays an essential role for microtubule assembly from the centrosomes during M-phase and that both its localization and function are regulated by phosphorylation by the Aurora A kinase. We also obtained some evidence indicating that Maskin works in concert with XMAP215 to oppose the destabilizing activity of XKCM1, therefore promoting microtubule growth off the centrosome (Peset I. *et al,* 2005). Over the last year we have extended these studies by exami-

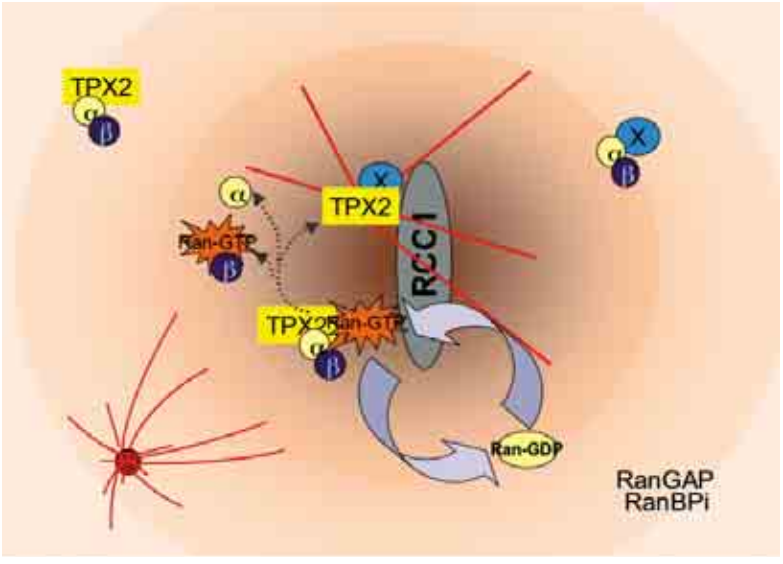


Figure 2. The localization of RCC1 to the chromosomes promotes the formation of a RanGTP gradient. TPX2 and others factors that are bound to importins in the cytoplasm are dissociated from them as the RanGTP concentration increases close to the chromosomes. TPX2 promotes then microtubule assembly, a process that is essential for spindle formation (Karsenti and Vernos, 2001)

ning the putative role of Maskin in the RanGTP dependent microtubule assembly pathway and the functional implications of its interaction with the Aurora A kinase.

3. Functional studies on chromosome-associated microtubule dependent motors during cell division

Chromatin undergoes dramatic changes during the cell cycle. As the cell enters into M-phase chromatin condenses into chromosomes and the nuclear envelope breaks down. As the mitotic spindle start to form, chromosomes establish dynamic interactions with the microtubules. These interactions play an active role in spindle formation and are responsible for the movement of chromosomes that lead to their alignment on the metaphase plate and their segregation during anaphase.

Some of these interactions are mediated by chromokinesins, kinesin-like proteins that localize to the chromosome arms during M-phase. We have previously identified two of them in the Xenopus system: Xklp1 (Vernos *et al.*, 1995; Walczak *et al.*, 1998) and Xkid, (Antonio *et al.*, 2000). To get a better understanding on Xklp1 function, we have examined the effect of Xklp1 depletion on spindle assembly in Xenopus egg extract. We found that in the absence of Xklp1 spindles form less efficiently and adopt a barrel-like shape due to an increase of the number of microtubules (Figure 3). Consistently an excess of Xklp1 in the egg extract resulted in the formation of spindles with reduced microtubule density. Similar results were obtained on centroso-

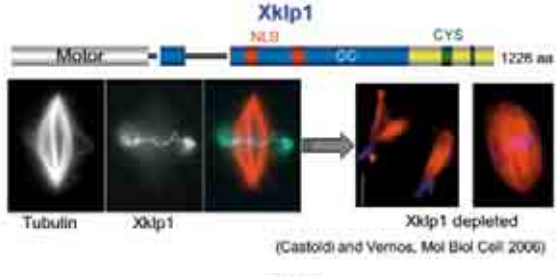


Figure 3.

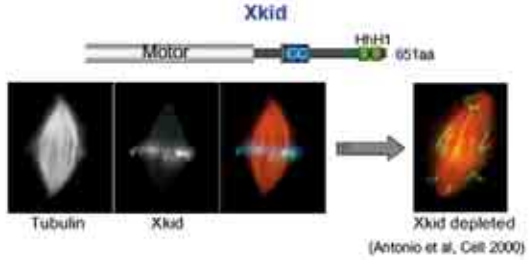


Figure 4. Xkid localizes to chromosome arms during M-phase. In Xenopus egg extracts chromosomes do not align on the metaphase plate in the absence of Xkid.

me nucleated microtubule asters (Castoldi M, Vernos I. Mol Biol Cell., 2006). These results and the study of the Xklp1 motor domain in vitro (Bringmann et al, 2004) indicate that Xklp1 has unique properties and plays an important role for spindle assembly and function in the Xenopus egg extract system.

We found previously that Xkid has a very different function. It is required for chromosome alignment on the metaphase plate (Figure 4) (Antonio *et al.*, 2000). In addition Xkid has a role in cell cycle progression during Xenopus oocyte meiotic maturation (Perez *et al*, 2002). We are currently performing experiments in oocytes and egg extract to try to understand the role of this molecular motor for meiotic cell cycle progression and its regulation by phosphorylation.

2. ROLE OF KINESIN-2 IN MEMBRANE TRAFFIC

In interphase, microtubules originating from the centrally located centrosome irradiate towards the cell periphery. They provide polarized tracks used by motors to move and position different intracellular components. These transport events define membrane traffic inside the cell. Kinesin-2 is a heterotrimer consisting of two closely related kinesin-like proteins that heterodimerize through a coiled coil and interact with a third non-motor subunit named KAP (Kinesin Associated Protein). Some time ago we showed that Xklp3, one of the two kinesin-like proteins forming the Xenopus heterotrimeric kinesin-2 complex, is required for traffic between the ER to the Golgi apparatus (Le Bot *et al*, 1998) but its precise role remained uncertain. Re-examining the role of Kinesin-2 in HeLa cells, last year we found that it is not an effector in rab6-induced redistribution events (Young J. *et al.*, 2005).

We have also examined kinesin-2 function using an RNA interference approach to downregulate the expression of KAP3, the non-motor subunit of kinesin-2, in HeLa cells. We found that KAP3 silencing results in the fragmentation of the Golgi apparatus (Figure 5) and a change in the steady-state localisation of the KDEL-receptor (KDEL-R). Using specific transport assays we have shown that the rate of anterograde secretory traffic is unaffected in these cells but that KDEL-R-dependent retrograde transport is strongly abrogated. These data strongly support a role for kinesin-2 in KDEL-R- / COPI-dependent retrograde transport pathway from the Golgi complex to the ER (Stauber. et al., Current Biology, 2006).

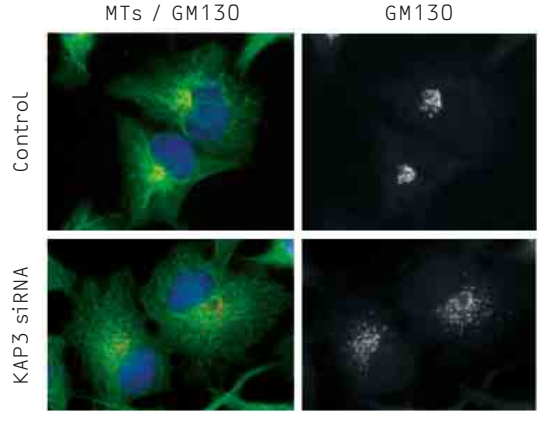


Figure 5.

PUBLICATIONS

Castoldi M. and I.Vernos "Chromokinesin Xklp1 contributes to the regulation of microtubule density and organization during spindle assembly." Mol Biol Cell, Mar, 17(3),1451-60 (2006) (*)

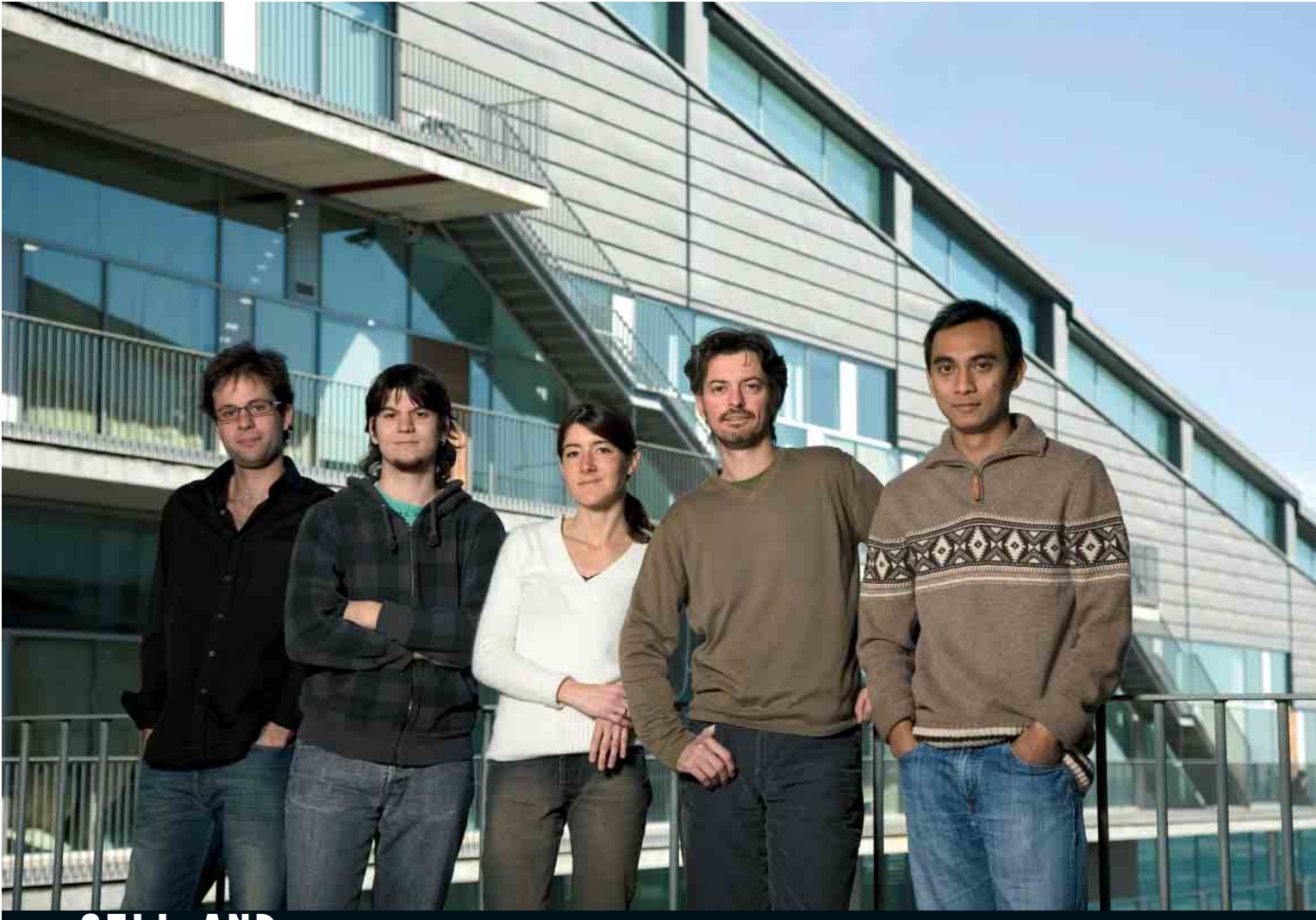
Christodoulou A., Lederer C.W., Surrey T., Vernos I., and N. Santama "Motor protein KIFC5A interacts with Nubp1 and Nubp2 and is implicated in the regulation of centrosome duplication" J Cell Sci, 119, 2035-2047 (2006) (*)

Brunet S., Zimmermann T., Reynaud E., Vernos I., E. Karsenti and R. Pepperkok "Detection and quantification of protein-microtubules interactions using GFP photo-conversion" Traffic, 7, 1283-1289 (2006)

Vernos I "Only one spindle, if you please..." Nat Cell Biol, 8(9), 901-2 (2006)

Tobias Stauber, Jeremy C. Simpson, Rainer Pepperkok and Isabelle Vernos "A role for Kinesin-2 in COPI-dependent recycling between the ER and Golgi complex" Current Biology, 16(22), 2245-51 (2006) (*)

(*) All these publications are the result of the work of Dr. Isabelle Vernos at the EMBL, Heidelberg, Germany



CELL AND DEVELOPMENTAL BIOLOGY

Sensory Cell Biology and Organogenesis

Research in my laboratory focuses on understanding the fundamental principles that govern the development of tissues and organs in vertebrates. We attempt to define the cellular and molecular bases underlying the acquisition and maintenance of tissue architecture, and its relationship to the function of sensory organs. We use the mechanosensory lateral line of the zebrafish (Danio rerio) (Figure 1) as a model system to study cell-fate specification, and the formation and remodelling of epithelial architecture during organ development and regeneration. For our studies, we employ cellular, genetic and molecular approaches and state-of-the-art optical imaging techniques, and are also developing methods to analyse in vivo the reinnervation of sensory cells during regeneration. In the long term, our studies should provide insight into how sensory organs develop and regenerate, and how their cellular organisation and function are maintained throughout life.

GROUP STRUCTURE:

Group Leader:	**Hernán López-Schier (since October 2006)**
Postdoctoral Fellows:	Mariana Muzzopappa (since October 2006)
Graduate Students:	Indra Wibowo (since October 2006) Filipe Pinto Texeira (since October 2006)

Figure 1. Birightfield image of a zebrafish at 5 days of age (left panel) and one of the same fish labelled with 4-Di-2-Asp (orange) to highlight the sensory hair cells of the lateral-line organ (right panel).

RESEARCH PROJECTS

1. CELLULAR RESPONSES TO POLARITY SIGNALS, IN PARTICULAR PLANAR CELL POLARITY

The coordinated orientation of polarised cells within the plane of an epithelium is termed planar cell polarity. The orientation of hair cells within the neuroepithelium of the inner ear represents a striking example of planar cell polarity in vertebrates. Directional deflections of apical mechanosensitive organelles (stereocilia), respectively open or close transduction channels to depolarise or hyperpolarise the hair cell's plasma membrane. The axis of morphological polarity of the stereocilia therefore corresponds to the direction of excitability of the hair cell, and bestows the organ with maximal sensitivity to mechanical stimuli. The senses of hearing and equilibrium thus rely on the exquisite precision with which hair cells are oriented across the sensory epithelium. In spite of its importance, we only have a very superficial knowledge of the mechanisms that control the planar polarisation of hair cells.

Some aquatic vertebrates sense directional water movements with the lateral-line system, a sensory organ closely related to the inner ear. This system comprises a stereotyped array of sensory clusters called neuromasts, each with a very simple organisation. A neuromast contains two types of peripheral supporting cells and a few centrally located hair cells innervated by afferent and efferent axons (Figure 2 left). Hair cells in neuromasts are polarised within the plane of the epithelium in a way comparable to that of the inner ear (Figure 2 right). The lateral-line organ of the zebrafish is thus ideally suited to investigate the mechanisms that control hair-cell planar polarisation.

2. SENSORY ORGAN GROWTH AND REGENERATION, WITH AN EMPHASIS ON EPITHELIAL REMODELLING AND INNERVATION

Historically, planar cell polarity has been studied in invertebrates on tissues that undergo polarisation during a very brief period, to eventually become fixed with negligible or non-existent plasticity, including lack of cellular proliferation, tissue remodelling or cell migration. Such tissues, consequently, will not undergo repair or regeneration after cell death or mechanical damage. Extensive genetic and molecular studies in *Drosophila* have shown that the establishment of planar polarity relies on the concerted activity of

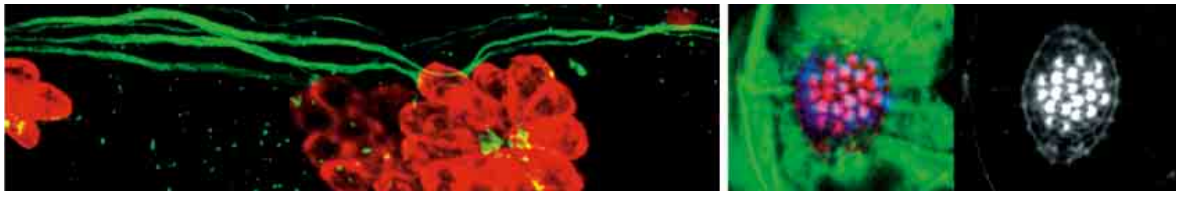


Figure 2. Neuromasts of the zebrafish posterior lateral-line organ contain sensory hair cells (red) that are innervated by efferent (not labelled) and afferent neurones (green) (left panel). Upon hair-cell regeneration, the neurones reinnervate the target and the organ regains anatomical and functional recovery within 48 hours after hair-cell damage. Hair bundles (stereocilia) in neuromasts are polarised within the plane of the epithelium along a single axis (right panel).

We have now identified a series of mutations that disrupt the establishment and maintenance of planar cell polarity in neuromasts. The combination of these strains with several of our multicolour transgenic animals will permit us to generate three- and four-dimensional images of living wild type and mutant specimens with great precision, and to track protein localisation patterns within seconds, or cellular behaviours over days. The combination of the genetic approaches afforded by the zebrafish with live imaging shall allow us to understand sensory-organ development, regeneration, and function in whole animals and at the single-cell level.

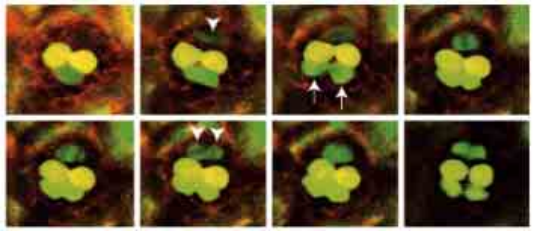


Figure 3. Timecourse analysis of hair-cell production. In a twelve-hour-long series of confocal images, labelling of an ET4 transgenic zebrafish with Texas Red-ceramide (red) reveals two GFP-positive mature hair cells (yellow). At the lower edge of the neuromast, a pair of immature hair cells (green; arrows in the third panel) separate over the course of three hours and become yellow as they mature. A hair-cell precursor meanwhile develops at the upper edge of the neuromast (arrowhead in the second panel). This precursor increases its green fluorescence and commences mitosis. The daughter cells (arrowheads in the sixth panel) eventually separate to form two hair cells. This time-lapse series indicate that hair cells develop in pairs along a single axis in neuromasts.

These studies shall provide insight into how organs develop and function throughout life, and also how they regenerate and re-innervate to recover function after damage. This not only represents a very interesting biological problem, but also is relevant to the successful application of therapies aimed to restore sensory function in humans, for aberrant repair would prevent the organ from performing properly.

many proteins. The cellular responses to polarity cues, especially in remodelling tissues are not understood. We are trying to define the cellular and molecular bases underlying the acquisition and maintenance of planar cell polarity, epithelial architecture and innervation in a vertebrate, and its relationship to organ function.

Sensory perception is a complex process that allows organisms to sample the environment and to react appropriately. Sensory dysfunction can thus be a major handicap that dramatically decreases the quality of life of the affected individual. All sensory modalities are liable to deteriorate during one's lifetime. Hearing deficits, for example, afflict more than 10% of the population in industrialized countries, including 0.1% of newborn children and 50% of those aged 80 years or over. Some sensory organs have an impressive capacity to recover after environmental insult, while others can lose function permanently. The inner ear is among the later: hearing loss owing to the degeneration or denervation of the mechanosensory hair cells is irreversible.

Although the search for a hair-cell progenitor resident in sensory epithelia has been pursued for over twenty years, to date there are no reports demonstrating the identification, or even the existence of such cell type. Our recent work has identified a hair-cell progenitor in neuromasts, which allows us the analysis of hair-cell development from its very outset. It also suggests the existence of a stem-cell population, and pinpoints its location within the neuromast (Figure 3).

Within the context of this research, we are also attempting to devise methods to follow every cells and complex tissue movements to reconstruct a digital organ *in vivo*.

3. SENSORY PERCEPTION AND INTEGRATION, AND SENSORY DYSFUNCTION

The zebrafish has relatively acute hearing, which the animal uses to school, capture prey and evade predators. We have identified a mutation that leads to profound deafness in the zebrafish. Hair cells develop normally and survive in adult homozygous mutant fish, but they are unable to transduce mechanical stimuli. This mutation appears to affect a very specific component of the mechanotransduction machinery in hair cells. We have recently identified the affected gene and begun to decipher its biological function in hearing and balance (unpublished results). We are also attempting to understand the mechanisms that govern the communication between sensory organs and the central nervous system in vertebrates using genetic, molecular and cell biological approaches. The knowledge gathered with these initial studies should permit the rational design of tests to evaluate the contributions of the relevant molecules, and will ultimately allow further progress into understanding how an animal responds to complex environmental stimuli.

PUBLICATIONS (*)

López-Schier H, Hudspeth AJ
"A two-step mechanism underlies the planar polarization of regenerating sensory hair cells."
Proc Natl Acad Sci USA, 103(49), 18615-20 (2006)

(*) All these publications are the result of the work of Dr. Hernan Lopez-Schier at The Rockefeller University, New York, USA

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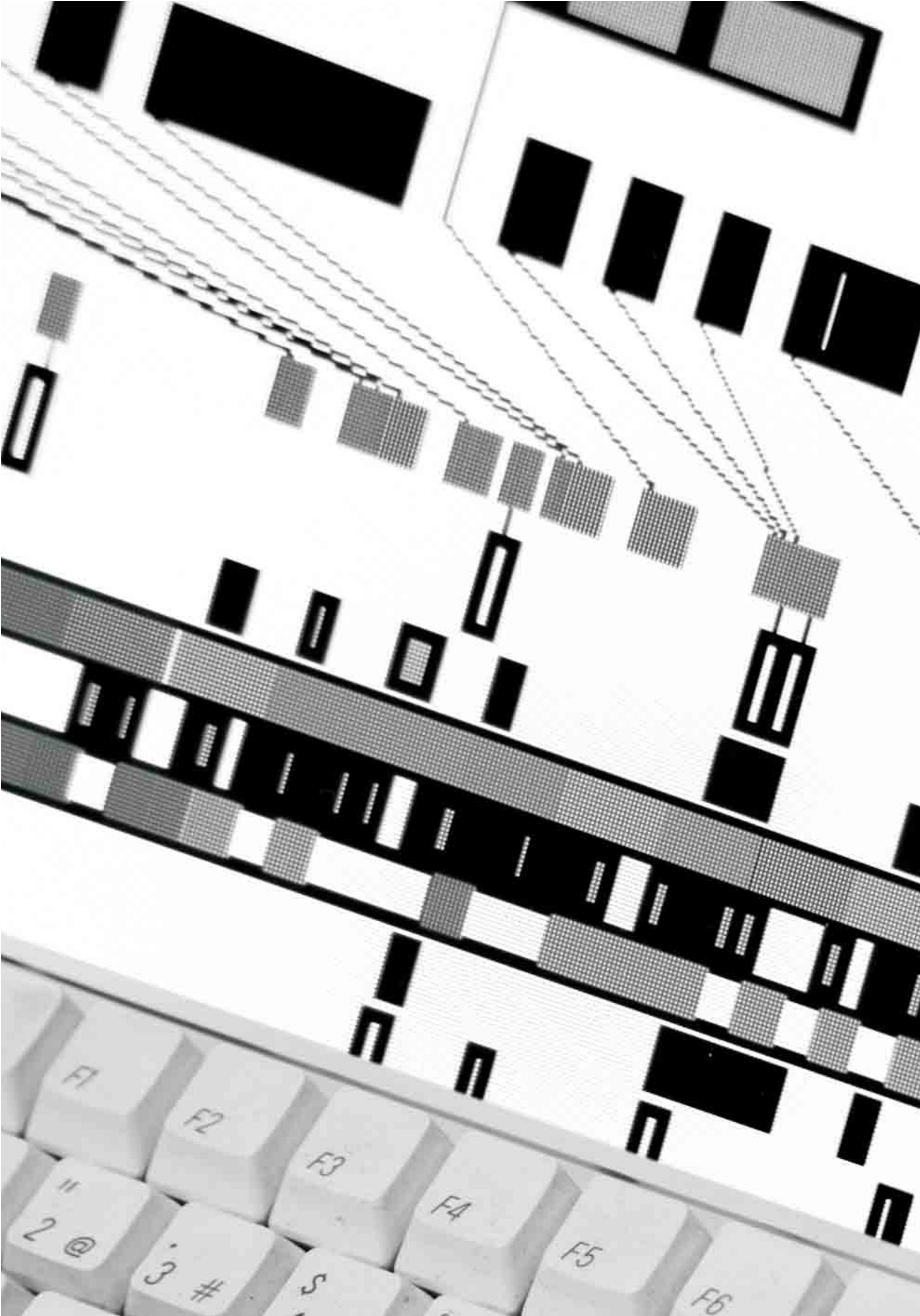
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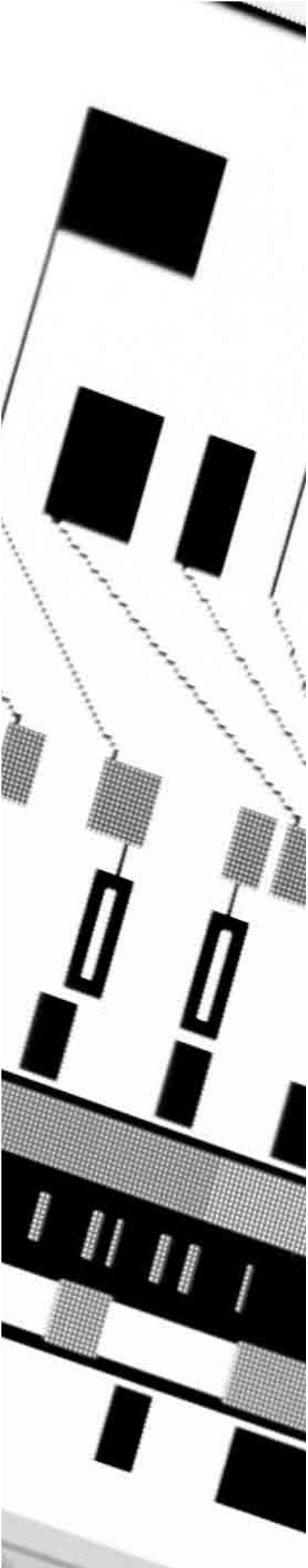


SYSTEMS BIOLOGY

Coordinator: Luis Serrano

Scientists have been in a quandary about definitions of Systems Biology for the past few years. These range from collections of physiological data with quantified molecular parts lists (e.g. genes, expression levels, localizations) to abstract mathematical modelling of biological processes. The scale at which Systems Biology focuses is also a matter of contention: A tiny protein can be a complicated biological system (we still don't know how it folds) as is obviously an entire ecosystem with thousands of species. The term "Systems Biology" will probably soften even further as it is now under the limelight and funding opportunities have to be taken seriously by very diverse scientific communities. Thus Systems Biology encompasses many different aspects starting with standardised data collection, archiving and management. This data then needs to be integrated to allow for comparative evaluation (comparative genomics and proteomics). Once that is done we need an idealized reconstruction of the experimental situation close to reality by using computer modeling. Based on this modeling exercise new experiments can be designed and new insights obtained. Finally experimental testing of the model closes the circle and feedbacks on the whole procedure. We expect that a systematic analysis of biological systems will allow new insights in human diseases.

In 2005 we recruited our first three group leaders: James Sharpe, Ben Lehner and Mark Isalan. Together they cover modelization, experimental analysis of developmental systems and the engineering and design of new gene networks (see below). Our aim for 2007 will be to hire two more groups to complete the programme.

Structure of the Programme:

Coordinator:	Luis Serrano
Senior Group:	James Sharpe
Junior Groups:	Ben Lehner
	Mark Isalan
Systems Manager:	Yann Dublanche
Responsible of Equipment:	Raul Gomez
Grant Manager:	Michela Bertero

SYSTEMS BIOLOGY

Design of Biological Systems

The group of Luis Serrano is aiming at a quantitative understanding of biological systems to an extent that would enable prediction of systemic features and with the hope to reach rational design and modify their behaviour. This applies to any system comprising biological components that is more than the mere sum of its components, or, in other words, the addition of the individual components results in systemic properties that could not be predicted by considering the components individually. By achieving this objective the group aims at new global understanding and treatment of human diseases in which the target will not be a single molecule but a network. For this purpose the group on one hand develops new software and theoretical approximations to understand complex systems and on the other performs experiments to validate the predictions.

LUIS SERRANO HAS A SENIOR ICREA GROUP LEADER POSITION.

GROUP STRUCTURE:

Group Leader:	**Luis Serrano**
Wet lab. Research Associate:	Christina Kiel
Postdoctoral Fellows:	Eva Yus Almer van der Sloot Raik Grünberg Tobias Maier Vicente Tur
Predoctoral Fellows:	Ronan Bourgeois Marc Güell
Technician:	Justine Leigh
Dry lab. Research Associate:	François Stricher
Postdoctoral Fellows:	Andreu Alibés Paolo Ribecca Emanuele Raineri
Sofware Engineer:	Patrick Herde
At EMBL:	Barbara di Ventura Alexandra Esteras

1.- SOFTWARE DEVELOPMENT FOR DESIGN OF BIOLOGICAL SYSTEMS

a) FoldX

Although we are still far from predicting the way a large protein folds, we have made significant progress in the past few years in been able to modify protein properties in a rational way. Thus from making proteins thermostable, to modify protein-protein interactions or to fully de novo design a protein, examples are abundant of the successes of protein design. The tool used in all cases are algorithms that using a rotamer library and an empirical force field are able to explore the sequence and conformational space of a protein in order to identify new amino acid combinations that will satisfy the required properties. Although advances need to be made in some aspects, like extending the success to protein-DNA and protein-RNA interactions, small molecule binding, protein dynamics, docking etc…, in many other aspects protein design is becoming a tool like PCR that can be used routinely in the lab. It is in many cases in its application were the science and the fun can be done. Using the different tools developed by the group (Fold-X, Tango, AmyScan), as well as other publishable available we have developed a web server (http://snpeffect.vib.be), that predicts the functional effects of SNPs in terms of posttranslational modifications, turnover, stability, subcellular localization, binding and aggregation. The server is open to public access.

Currently we are improving FoldX to incorporate backbone flexibility and refine the force field to predict protein-nucleic acid interactions.

b) SmartCell

SmartCell has been developed to be a general framework for modelling and simulation of diffusion-reaction networks in a whole-cell context. It supports localization and diffusion by using a mesoscopic stochastic reaction model (Ander *et al.*, 2004). The SmartCell package can handle any cell geometry, considers different cell compartments, allows localization of species, supports DNA transcription and translation, membrane diffusion and multistep reactions, as well as cell growth. Moreover, different temporal and spatial constraints can be applied to the model. A GUI interface that facilitates model making is also available. In this work we discuss limitations and advantages arising from the approach used in SmartCell and

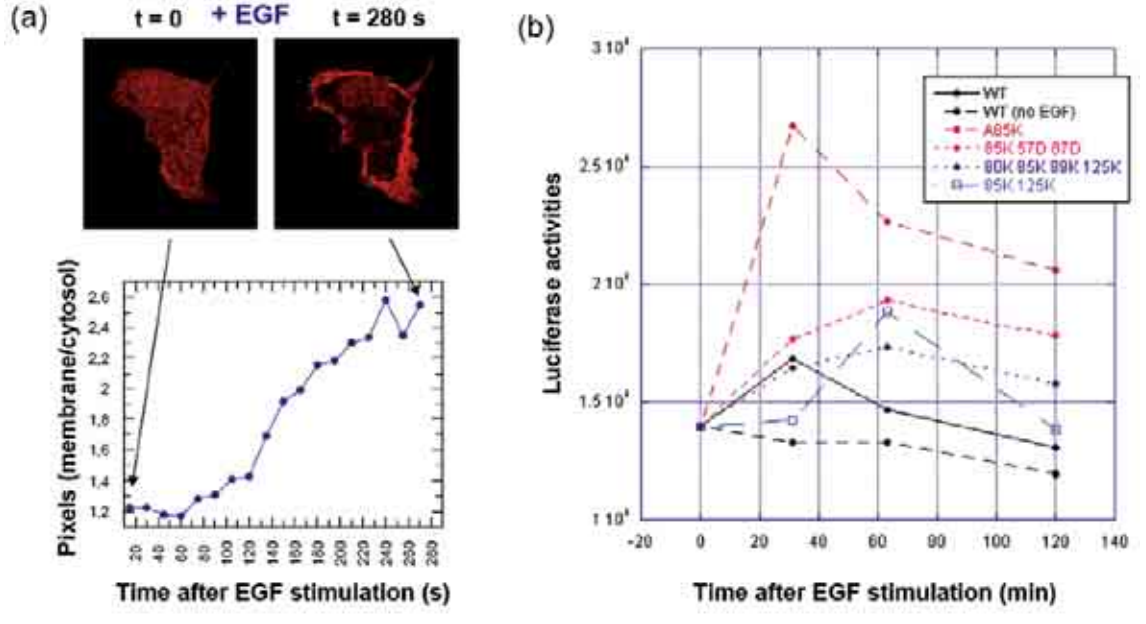


Figure 1. Preliminary results of the quantification of EGF signalling at two points of the pathway. Wildtype Ras-Raf complexes will be compared with Raf-mutant-Ras complexes with similar affinity, but changed association and dissociation rate constants. (a) Monitoring Raf translocation to the cell membrane (binding to active Ras-GTP) at different time points after EGF stimulation. (b) Quantification of gene expression (using a luciferase expression system) at different time points after EGF stimulation.

determine the impact of localization on the behaviour of simple well-defined networks, previously analyzed with differential equations. Our results show that this factor might play a major role in the response of networks and cannot be neglected in cell simulations. Recently we have increased the speed of calculation of SmartCell by a factor of 10 and also a new GUI which allows the user to analyze the output is available (http://smartcell.embl.de/).

The objective now is to integrate the different tools developed in the group and elsewhere in a single package that will be used to model cellular processes, design new networks and simulate the effect of drugs on cellular processes.

2.- EXPERIMENTAL ANALYSIS

SIGNAL TRANSDUCTION

a) Protein interaction networks.

One of the more challenging problems in biology is to determine the cellular protein interaction network. Using structural information we have drawn the network of interactions between 20 Ras subfamily proteins with 52 putative Ras binding domains. To validate this network we have cloned six not so well characterized Ras binding domains (RBD) and two Ras proteins (Rerg, DirasI). These, together with previously described RBD domains, Ras and Rap proteins have been analyzed in 70 pull-down experiments. Comparing our interaction network with these and previous pull-down experiments (total of 150 cases), shows very high accuracy for distinguishing between binders and non-binders (~0,80). Bioinformatics information is used to sort out those in vitro interactions that are more likely to be relevant in vivo. We proposed several new interactions between Ras family members and effector domains that are of relevance in understanding the physiological role of these proteins.

b) Importance of kinetic properties in signal transduction.

One important question in systems biology to answer is, whether the magnitude of cellular signal transduction is dependent on the affinity alone, or whether individual rate constants of association and dissociation are important as well. In order to address this question we have selected the EGF pathway. By using FoldX and following the rules of electrostatic steering, we have successfully designed mutants of the Ras-Raf kinase complex which have similar affinities, but altered association and dissociation rate constants. The mutants are currently analyzed in two cell biological assays (Fig. 1): (a) Monitoring the kinetics of Raf recruitment to the cell membrane upon stimulation with EGF. (b) Monitoring the time course of gene-expression.

c) Therapeutic molecules for treatment of solid tumours by modulating death receptor-mediated apoptosis

Current treatment of solid tumours has a varying degree of success, for certain types it is successful while for many others the degree of success is still disappointingly low. New therapeutic strategies and novel tumour selective anti-cancer agents are necessary in order to improve the treatment of solid tumours. Signalling pathways that control cellular proliferation and death are attractive targets for more tumour selective anti-cancer agents. Signal transduction by members of TNF ligand / TNF receptor family members provides such an attractive pathway for defining new targets for novel anti-cancer agents. Signalling (and derangement of signalling) by several members of the TNF ligand / TNF receptor family is implicated in the aetiology and pathology of solid tumours. Some members having a tumour-promoting role, others have a tumour inhibiting role whilst some have both a tumour proliferating or -inhibiting role. Previously, we developed receptor selective variants of the TNF ligand family member TRAIL; these variants could kill various types of (solid) tumour cells selectively by induction of apoptosis. Moreover, these variants proved to be more efficacious than native TRAIL in certain tumour types and these variants showed synergistic behaviour when combined with other chemotherapeutics or ionising radiation therapy. In the current project we will further investigate and characterise these receptor selective TRAIL variants pre-clinically in various solid-tumour models, both *in vitro* and *in vivo*, and we will define new treatment strategies with these variants in combination with already proven treatments. By use of

computational design and molecular evolution techniques novel receptor selective apoptosis-inducing agonists and cell-proliferation antagonists will be developed.

MYCOPLASMA

This project has two main objectives: a) the first complete global understanding of a living organism (M. pneumoniae) and b) its engineering to create a shuttle vector that could be modified InVitro and then transfected in human cells incorporating itself as a new cell organelle. Once inside the cell the living vector should couple its division to that of the cell host and be able to deliver proteins in the cell to correct human pathologies. M. pneumoniae is one of the smallest free-living bacteria that exist (~680 genes), does not have cell wall and can be introduced inside human cells. The project will benefit from a structural genomics project aimed at determining the structures of all M. pneumoniae proteins (http://www.strgen.org/proteome/), from existing extensive previous work (http://www.zmbh.uni-heidelberg.de/M_pneumoniae/genome/) and from the participation in the context of the CRG/EMBL partnerhips of a consortium involving the EMBL structural and computational biology programme. Currently all proteins of the organism are been tagged for pull down to identify all the protein complexes, electron microscopy is done on these complexes to obtain 3D shapes which will be fitted inside EM 3D tomograms of the bacteria at 40-50 A resolution. In our group we are going to do: a) The whole proteome in collaboration with Mathias Mann (MPI-Munich). b) We have designed a DNA array to analyze the expression of different genes under different conditions. c) We are going to do pull downs using the promoters as baits to find all TFs. d) We are going to analyze the metabolic fluxes in collaboration with M. Sattler (EMBL-Heidelberg). e) We are developing a system to allow homologous recombination. f) Finally we will integrate all the information inside our cell simulation tool, SmartCell (http://smartcell.embl.de/). While we compile all this information we will start to modify the organism in order to transfect human cell lines, engineer a regulation system of its division that will couple it to that of the host cells and develop a system able to secrete proteins inside the host. At the end of the project we hope to have a living shuttle vector that could be used as a living pill.

PUBLICATIONS

Arnould S, Chames P, et al. "Engineering of large numbers of highly specific homing endonucleases that induce recombination on novel DNA targets." J Mol Biol, 355(3), 443-58 (2006) (*)

de Lorenzo V, Serrano L, et al. "Synthetic biology: challenges ahead." Bioinformatics, 22(2), 127-8 (2006) (*)

Kempkens O, Medina E, et al. "Computer modelling in combination with in vitro studies reveals similar binding affinities of Drosophila Crumbs for the PDZ domains of Stardust and DmPar-6." Eur J Cell Biol (2006) (*)

Kiel C and Serrano L "The ubiquitin domain superfold: structure-based sequence alignments and characterization of binding epitopes." J Mol Biol, 355(4), 821-44 (2006) (*)

Musi V, Birdsall B, et al. "New approaches to high-throughput structure characterization of SH3 complexes: the example of Myosin-3 and Myosin-5 SH3 domains from S. cerevisiae." Protein Sci,15(4), 795-807 (2006) (*)

Rousseau F, Schymkowitz J et al. "Protein aggregation and amyloidosis: confusion of the kinds?" Curr Opin Struct Biol, 16(1), 118-26 (2006) (*)

Rousseau F, Serrano L, et al. "How evolutionary pressure against protein aggregation shaped chaperone specificity." J Mol Biol, 355(5), 1037-47 (2006) (*)

Dublanche Y, Michalodimitrakis K, Kummerer N, Foglierini M and Serrano L "Noise in transcription negative feedback loops: simulation and experimental analysis." Mol Syst Biol, 2, 41 (2006) (*)

Di Ventura B, Lemerle C, Michalodimitrakis K and Serrano L "From in vivo to in silico biology and back." Nature, 443, 527-533 (2006) (*)

Fernandez-Ballester G and Serrano L "Prediction of protein-protein interaction based on structure." Methods Mol Biol, 340, 207-234 (2006) (*)
Fernandez-Escamilla AM, Ventura S, Serrano L and Jimenez MA "Design and NMR conformational

study of a beta-sheet peptide based on Betanova and WW domains." Protein Sci, 15, 2278-2289 (2006) (*)

Rousseau F, Wilkinson H, Villanueva J, Serrano L, Schymkowitz JW and Itzhaki LS "Domain Swapping in p13suc1 Results in Formation of Native-like, Cytotoxic Aggregates." J Mol Biol, 363, 496-505 (2006) (*)

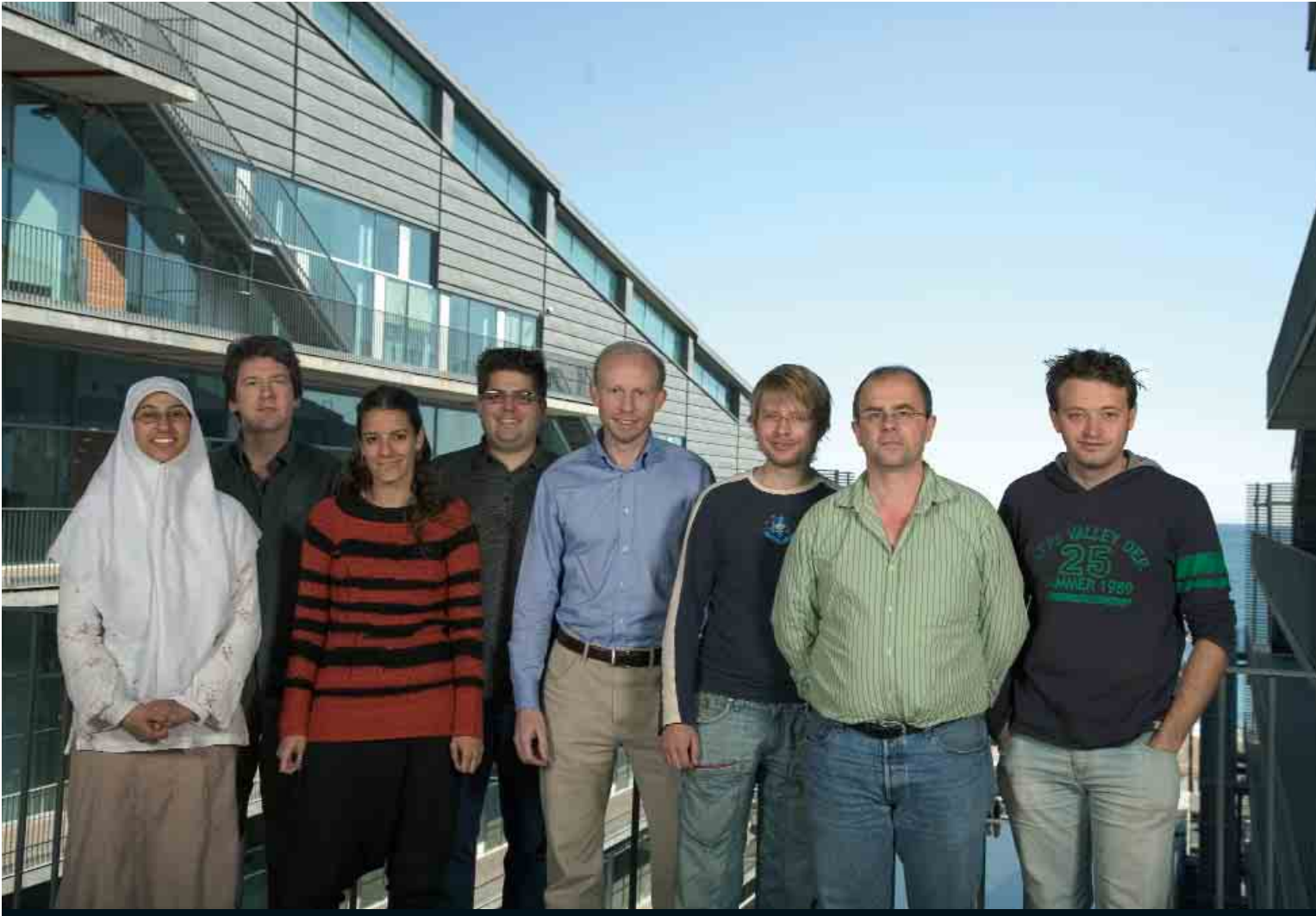
Sanchez IE, Tejero J, Gomez-Moreno C, Medina M and Serrano L "Point mutations in protein globular domains: contributions from function, stability and misfolding." J Mol Biol, 363, 422-432 (2006) (*)

Van der Sloot AM, Tur V, Szegezdi E, Mullally MM, Cool RH, Samali A, Serrano L and Quax WJ "Designed tumor necrosis factor-related apoptosis-inducing ligand variants initiating apoptosis exclusively via the DR5 receptor." Proc Natl Acad Sci U S A, 103, 8634-8639 (2006) (*)

Kempkens O, Medina E, Fernandez-Ballester G, Ozuyaman S, Le Bivic A, Serrano L, Knust E "Computer modelling in combination with in vitro studies reveals similar binding affinities of Drosophila Crumbs for the PDZ domains of Stardust and DmPar-6." Eur J Cell Biol, 85(8),753-67 (2006) (*)

Santori MI, Gonzalez C, Serrano L, Isalan M "Localized transfection with magnetic beads coated with PCR products and other nucleic acids." Nature Protocols, 1, 526-531 (2006)

(*) All these publications are the result of the work of Dr. Luis Serrano at the EMBL, Heidelberg, Germany



SYSTEMS BIOLOGY

Systems Analysis of Development

The primary goal of our group is to further our understanding of developmental biology, and in particular the vertebrate limb bud, by bringing together a diverse range of techniques from biology, physics, imaging and computer science. Rather than building abstract models we are basing all our simulations on high-quality quantitative empirical data.

Over the last few years we have therefore concentrated on developing new 3D and 4D imaging technologies which allow us to extract this information (such as gene expression data) from real biological systems. In particular, we developed the new technique called Optical Projection Tomography (OPT – Science 296:541, 2002), and one part of the lab focuses on improving this technology and working on 3D microscopy techniques in general. This part of the lab is not restricted to limb development, and one of our important lines of research is 3D imaging of pancreata from mouse models of diabetes (Nature Methods 4:31, 2007).

The larger part of the lab is now focusing on a systems biology approach for understanding limb development. This includes both experimental lab-work for analysing real limb buds (we use the mouse and the chick as model systems), and also the development of computational methods for integrating this data in multi-scale simulations.

JAMES SHARPE HAS A SENIOR ICREA GROUP LEADER POSITION.

GROUP STRUCTURE

Group Leader:	**James Sharpe**
Postdoctoral Fellows:	Jim Swoger Jean-François Colas Sahdia Raja
Students:	James Cotterell Bernd Boehm Michael Rautschka
Technician:	Laura Quintana (to start March 2007)

Figure 1. We have worked closely with the MRC to commercialise OPT technology and to design a user-friendly version of the scanner (pictured here).

RESEARCH PROJECTS

1. DEVELOPMENT OF 3D AND 4D MESOSCOPIC OPTICAL IMAGING TECHNOLOGIES.

Although OPT has already proven itself in a number of research fields, it is still a "new" technology and probably has not yet achieved its maximal potential. We are therefore continuing to develop OPT both in terms of hardware and software. Additionally, since the interests of the lab focus on small biological specimens (a few millimeters in diametre, sometimes labelled as "mesoscopic" imaging) we are exploring and developing other 3D imaging principles that could provide extra information for understanding embryo development.

2. OPT IMAGING FOR QUANTITATIVE ASSESSMENT OF MOUSE MODELS OF DIABETES

We have explored many new applications for OPT, and one of the most exciting is the ability, for the first time, to quantify the number of Islets of Langerhans in an intact adult mouse pancreas in a single scan. Until now this has been performed using the time-consuming approach of traditional histology (cutting hundreds of thin paraffin sections for each pancreas). By contrast, we have demonstrated that the speed of OPT makes it feasible to compare many pancreata in a single study (*Nature Methods* 4:31-33, 2007). Our lab has a grant from the JDRF (Juvanile Diabetes Research Foundation) to continue this research which is a close collaboration with the lab of Dr. Ulf Ahlgren in Umea, Sweden.

3. 4D TIME-LAPSE OPTICAL PROJECTION TOMOGRAPHY

Optical projection tomography has proven to be a powerful tool for developmental biologists. But so far OPT has been performed almost exclusively on *ex-vivo* specimens which have been optically cleared to

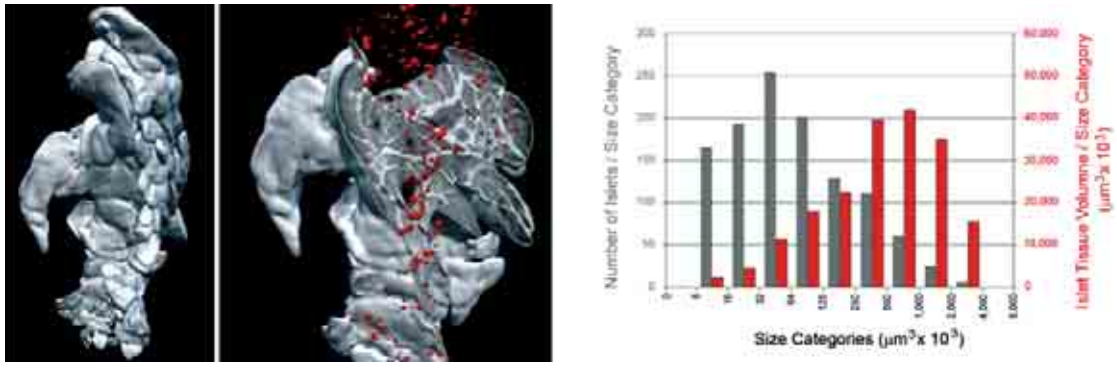


Figure 2. OPT scans of a whole adult mouse pancreas (left) which has been fluorescently-labelled with antibodies against insulin to highlight the 3D distribution of Islets of Langerhans (centre). This data can be morhometrically quantified to provide statistics on the volumetric size distribution (right).

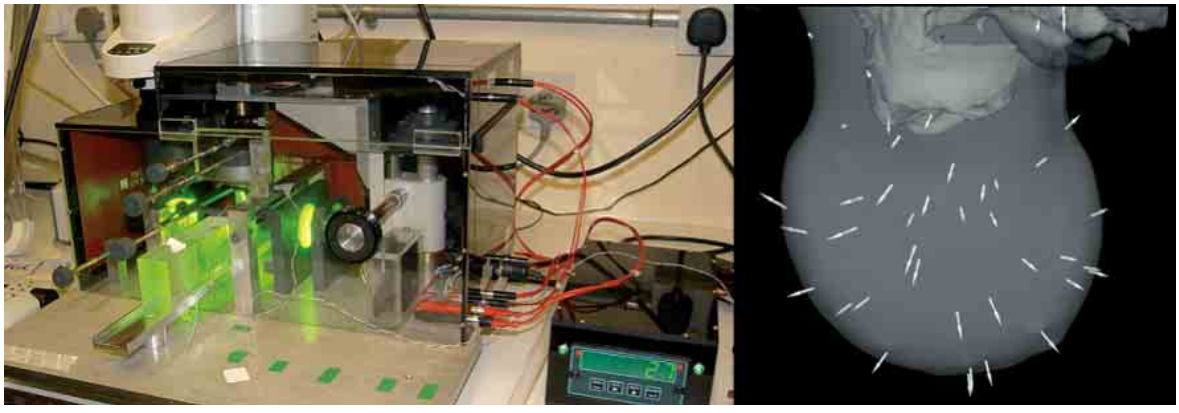


Figure 3. 4D OPT analysis of a cultured mouse limb bud. The ectoderm was labelled with fluorescent microspheres and these were tracked over time to provide a measurement of global tissue movements.

increase the quality of 3D images obtained. Building on the success of this new technology we are now exploring various improvements to allow the 4D imaging of the developing mouse limb bud in culture. This technique now provides us with some of the data on tissue movements that is essential for subsequent computer modelling. In the near future it should also provide data on dynamic gene expression patterns. Our lab has grants from the EU and HFSP (Human Frontiers Science Program) to develop different aspects of these improvements.

4. COMPUTER MODEL OF LIMB MECHANICS

We are exploring the ways in which cell behaviours combine with various physical models of tissue to generate the observed shape changes. In particular, it has become clear from early modelling results that explaining phenomena as apparently simple as how the limb bud becomes progressively dorso-ventrally flattened, may be more complicated than expected. We have created a finite-element model of limb development within which we are exploring different hypotheses. An essential aspect of this project is that we also perform labwork to generate our own empirical data for the simulation – the model therefore serves as a framework for combining different types of information. While various types of biological material have previously been mechanically modelled over a short time periods (for example stress analysis on bones and cartilage) a mathematical/physical description of 3D embryonic tissue displaying volumetric growth over a period of hours or days has not previously been achieved, and this is therefore one of the general goals of this project.

5. COMPUTER MODELS OF SPATIALLY-PATTERNING GENE NETWORKS

Enough is known about the genetics of limb development to be sure that it involves many signalling molecules (Shh, BMPs, FGFs, Wnts) and many transcription factors (Msx, Hox, Meis) which are wired together into a complex gene network. We aim to explore how these networks function within the computer model – both in control of the cell behaviours that govern the limb bud shape, and also in another famous patterning case: the spatial organisation of the skeletal elements.

For skeletal patterning, different patterning strategies have been proposed within the literature to explain the process, ranging from pure "Turing-type" reaction-diffusion models at one extreme, to morphogen gradient models on the other. We are exploring the ways that these different principles might work at the detailed level of gene regulatory networks in a 1D simulation of spatial patterning. This will soon be extended to a 3D simluation within the context of the correct limb bud shape, and we are therefore using OPT and confocal microscopy to gather accurate 3D expression data on genes thought to be involved in, for example, the initiation of mesenchymal condensation. Our goal then is to test existing hypotheses and explore new ideas about how this particular example of pattern formation is controlled

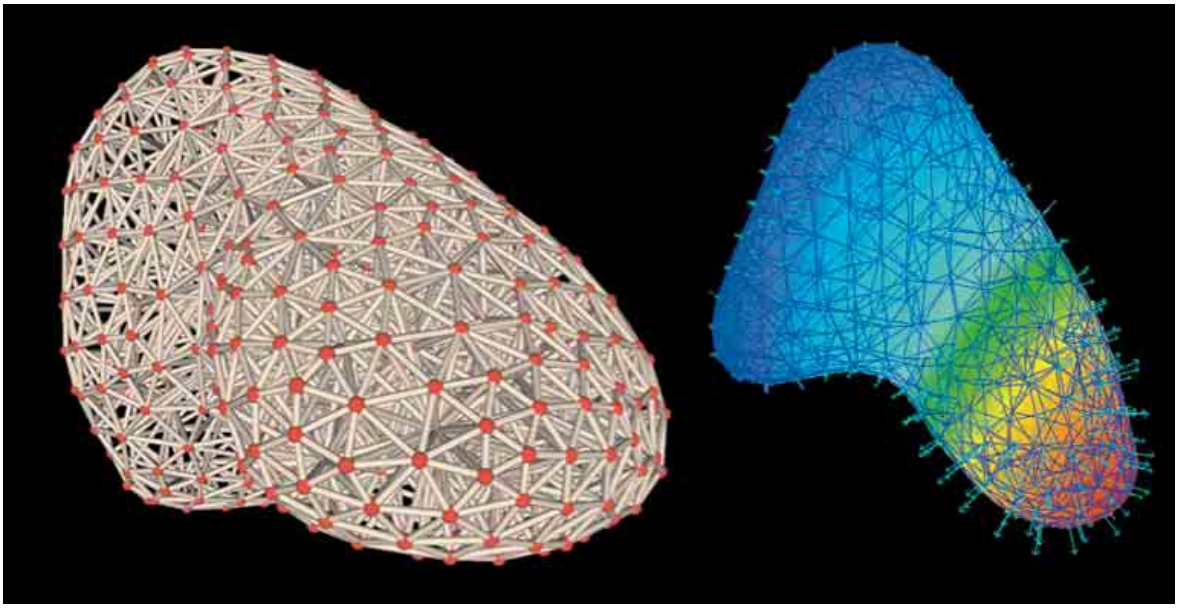


Figure 4. The finite-element model used for simulating limb development. The 3D shape is represented by a tetrahedral mesh (left), and different proliferation rates therefore cause a global movement of the tissue (right).

6. MORPHOMETRIC STAGING OF LIMB BUDS

Another important project in the lab relates to measurements of time. Almost all the gene expression data which exists about limb development has been recorded from *ex-vivo* analysis. However, the spatial patterns of certain genes (eg. *Sox9*) are extremely dynamic – apparently changing hour-by-hour. Knowing the age of a given limb bud should therefore be very important when comparing results, but the field has not had a convenient but accurate method for determining the stage of a limb.

Our lab has therefore created a new staging system which is based on morphometric measurements from the profile of the limb bud. It can determine the stage of a limb to a high temporal accuracy, and we have turned this method into a web-based java application that will soon be openly available to to the whole limb community. Researchers with jpg images of their limb buds will be able to log-on to our web-site and determine an accurate age of the specimen within a few minutes.

PUBLICATIONS (*)

Alanentalo T, Asayesh A, Morrison H, Lorén CE, Holmberg D, Sharpe J, Ahlgren U. "Tomographic molecular imaging and 3D quantification within adult mouse organs" Nature Methods, 4, 31-33 (2006)

Lee K, Avondo J, Morrison H, Blot L, Stark M, Sharpe J, Bangham A, Coen E. "Visualising Plant Development and Gene Expression in Three Dimensions Using Optical Projection Tomography." The Plant Cell, 10, 1105 (2006)

Asayesh A, Sharpe J, Watson R, Hecksher-Sorensen J, Hastie N, Hill R, Ahlgren. "Spleen versus pancreas: strict control of organ interrelationship revealed by analyses of Bapx1-/- mice." Genes and Development, 20, 2208-13 (2006)

(*) All these publications are the result of the work of Dr. James Sharpe at the MRC Human Genetics Unit, Edinburgh, UK

SYSTEMS BIOLOGY

Gene Network Engineering

We are interested in engineering synthetic gene networks to control gene expression in cells and to construct self-organising patterns, analogous to those used by organisms in morphogenesis and development. By transfecting cell populations with various gene networks, we hope to find the 'design principles' underlying why certain networks form particular structures or functions. We are exploiting this information to deliver genetic programs into cells to make them differentiate in the ways we desire.

The Group is divided into two subgroups, one of which is dedicated to making artificial DNA-binding domains (Figure 1) and the other which employs these technologies to synthesise artificial gene networks.

THIS GROUP IS PART OF THE EMBL/ CRG RESEARCH UNIT IN SYSTEMS BIOLOGY

GROUP STRUCTURE

Group Leader:	**Mark Isalan**
Postdoctoral Fellows:	Mireia Garriga Frank Herrmann Emmanuel Fajardo
Students:	Andreia Carvalho Marco Constante
Technician:	Phil Sanders

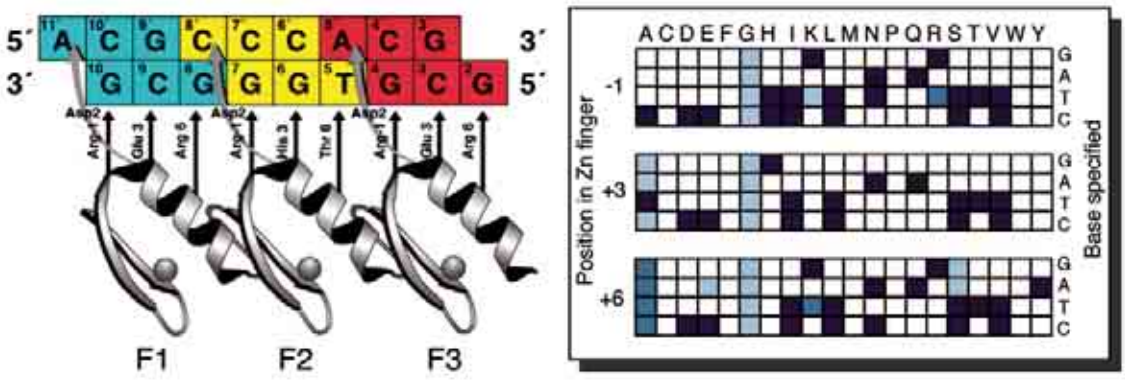


Figure 1. Zinc fingers recognise DNA in a modular way, making 1:1 amino acid-base contacts (arrows). They are therefore ideal for re-engineering. Changing single residues at certain helical positions (-1, +2, +3, +6) alters the DNA-binding specificity and this can be described be a protein-DNA recognition code (right). Because the code is not absolute, we routinely engineer zinc fingers by phage display (Isalan, M., Klug, A. & Choo, Y. *Nature Biotechnology*, 19, 656-60; 2001).

RESEARCH PROJECTS

1. ENGINEERING PATTERN FORMING GENE NETWORKS IN EUKARYOTIC CELLS.

Mireia Garriga, Phil Sanders, Marco Constante and Andreia Carvalho

We are looking at methods of designing self-organising patterns using mammalian cells as an engineering scaffold. To do this we are employing a technique that we recently developed: magnetic beads, coated with PCR products, can be used to transfect cells with gene network constructs, with spatio-temporal control (*Nature Methods* 2: 113-118 (2005); *Nature Protocols* 1, 526 - 531 (2006); Fig 2).

We are carrying out several pattern-engineering projects in parallel, corresponding to different scales of cellular organisation. These start from engineering localised gradients and patterns in single cells and move towards designing gene networks using diffusible factors that operate over fields of cells.

For example, we recently built some in vitro gene networks to create diffusing components to create expression patterns. ("Engineering gene networks to emulate Drosophila embryonic pattern formation", *PLoS Biology* 3(3) e64, 2005): in the Drosophila embryo, maternal morphogen gradients establish gap gene expression domain patterning along the anterior-posterior axis, through linkage with an elaborate gene network. To understand the evolution and behaviour of such systems better, it is important to establish the minimal determinants required for patterning and one approach is to attempt rebuild the system from the bottom-up, using reconstituted or artificial components.

We therefore engineered artificial transcription-translation networks that generate simple patterns, crudely analogous to the Drosophila gap gene system. The Drosophila syncytium was modelled using DNA-coated paramagnetic beads fixed by magnets in an artificial chamber, forming a gene expression network (Fig 3). Transient expression domain patterns were generated using various levels of network connectivity. An accompanying computer model for our system allowed us to search for parameter sets compatible with patterning.

The model suggested that simple diffusion may be too rapid for Drosophila-scale patterning, implying that sublocalisation, or ''trapping,'' is required. Second, we found that for pattern formation to occur under the conditions of our in vitro reaction-diffusion system, the activator molecules must propagate faster than the inhibitors. Third, adding controlled protease degradation to the system stabilized pattern formation over time.

This approach of reconstituting systems in order to test our understanding of them should be generally applicable to the study of any biological network with a spatial component.

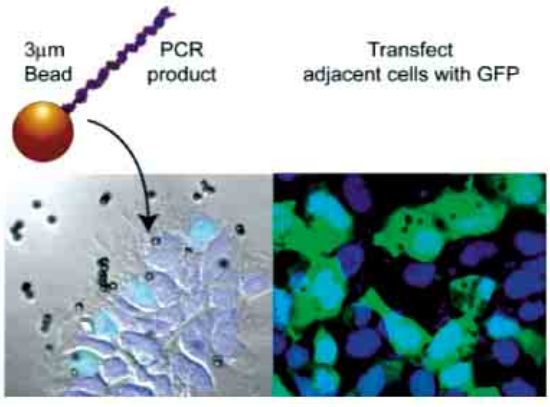


Figure 2. Our system for magnetically-defined transfection. A GFP-expressing PCR product is directed to cells using the paramagnetic bead as a scaffold. Note the multiple beads adjacent to transfected cells. This technique may be employed to engineer patterning gene networks in eukaryotic cells. The spatial control of transfection can be applied either to individual cells or to many cells in a culture.

2. SYNTHESISING ZINC FINGERS FOR GENE THERAPY AND GENE REPAIR

Frank Herrmann and Emmanuel Fajardo

As part of two EU-funded projects (Netsensor, EC Contract No. 012948 http://netsensor.embl.de/ and Integra, EC Contract No. FP6 - 29025) we are building a number of artificial sequence-specific DNA-binding proteins using our established protocol (Isalan, M., Klug, A. & Choo, Y. *Nature Biotechnology*, 19, 656-60; 2001). We will aim to develop upon the recent reports of endogenous gene repair using zinc finger nucleases (Bibikova *et al. Science* 300, 764, 2003; Urnov *et al., Nature* 435, 646-51, 2005; Fig 4).

The power of homologous recombination as a tool for genome engineering has been illustrated by the gene targeting strategies developed since the seminal work of Capecchi and Smithies group in the eighties (Capecchi 1989, *Science* 244(4910): 1288-92; Smithies 2001, *Nat Med* 7(10): 1083-6). However, the use of homologous recombination has long been limited by the low efficiencies observed in classical approaches. Use of strong selection and/or counter-selection procedures, sometimes associated with extensive screening have often been necessary to isolate a few bona fide gene targeting events in a sea of target cells.

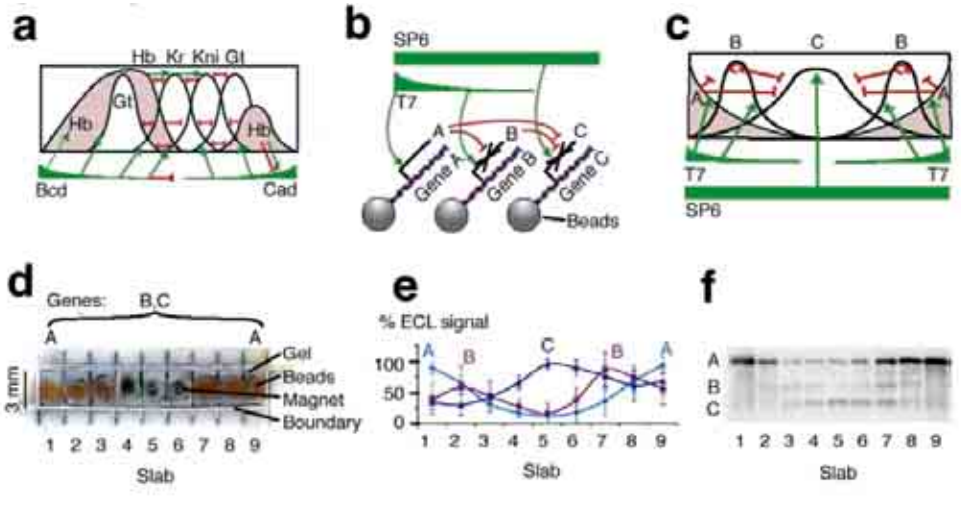


Figure 3. Gene circuits and chambers. **a**, Principal interactions in the Drosophila Gap gene network (Rivera-Pomar, R. & Jäckle, *Trends Genet.* 12, 478-483 (1996). Relative levels of and distributions of Hunchback (Hb), Giant (Gt), Kruppel (Kr), Knirps (Kni), Bicoid (Bcd) and Caudal (Cad) shown from anterior (left) to posterior (right). Green arrows indicate activation, red T-bars repression. **b**, Artificial gene network design, with transcription activators T7 and SP6 polymerases, and zinc finger repressors A, B and C. Genes are immobilised on paramagnetic beads and T7 forms a directional concentration gradient. **c**, Principle interactions in a simple designed network showing the desired patterning for proteins A, B and C. **d**, Transcription-translation chamber. Genes for repressor A are localised at the 'poles', whereas B and C are ubiquitous. Gel slabs 4-6 have been excised, exposing the magnets below, illustrating gel dissection for Western analysis. **e**, Normalised Western data for 4 replicate chambers, showing mean levels of A, B and C after 20 minutes (+/- 1 s.d.). **f**, Sample Western blot from the 4-replicate experiment.

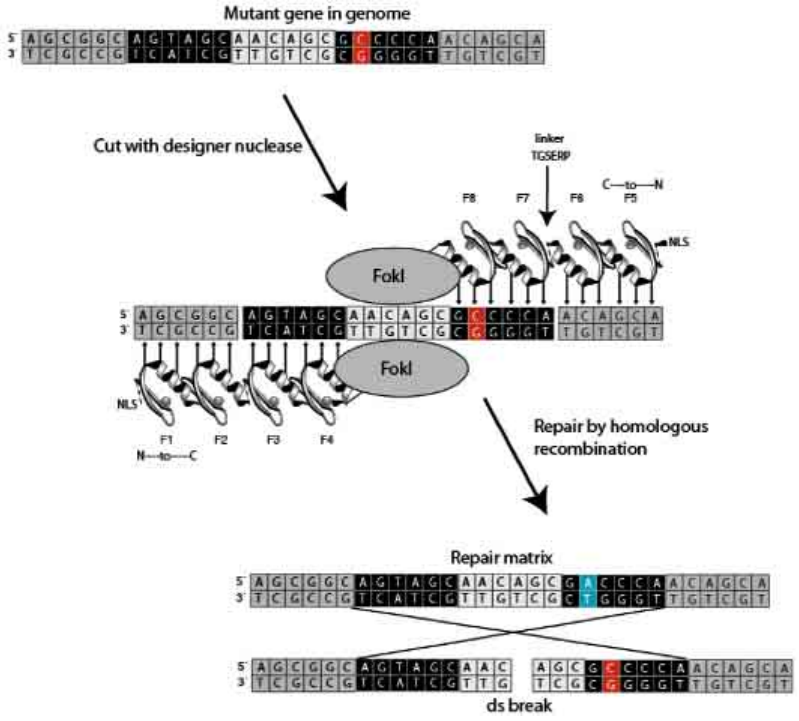


Figure 4. Mechanism of homologous gene repair by zinc finger nucleases. In our laboratory, we are designing and synthesising a number of different zinc finger proteins to target unique disease-related genes. By linking the fingers to FokI nuclease, a cut is made in the genome. A gene repair matrix (blue) then recombines into the genome to repair the mutant gene (red).

In the last decade, nuclease-induced homologous repair has emerged as the best way to reconcile the advantages of gene targeting with a workable level of efficiency (Rouet, Smih *et al.* 1994, *Proc Natl Acad Sci U S A* 91(13): 6064-8; Choulika, Perrin *et al.* 1995, *Mol Cell Biol* 15(4): 1968-73; Cohen-Tannoudji, Robine *et al.* 1998, *Mol Cell Biol* 18(3): 1444-8; Donoho, Jasin *et al.* 1998, *Mol Cell Biol* 18(7): 4070-8; Elliott, Richardson *et al.* 1998, *Mol Cell Biol* 18(1): 93-101). The nucleases used can be zinc finger nucleases or meganucleases; highly sequence specific endonucleases, able to cleave DNA in vivo at rare >12-18bp cleavage sites. Targeted DNA double-strand breaks are repaired by the endogenous maintenance systems of the cell, among which homologous repair is a major player (Paques and Haber 1999, *Microbiol Mol Biol Rev* 63(2): 349-404).

Nuclease-induced recombination is today an established technology, and hundreds of publications testify its robustness in various organisms and cell types. In addition, the design of novel artificial meganucleases with dedicated specific cleavage sites has open the door for rational genome engineering, and a wider use of the technology, for example in therapeutic applications (Smith, Bibikova *et al.* 2000, *Nucleic Acids Res* 28(17): 3361-9; Chevalier, Kortemme *et al.* 2002, *Mol Cell* 10(4): 895-905; Epinat, Arnould *et al.* 2003, *Nucleic Acids Res* 31(11): 2952-62). Compared to classical gene therapy, meganuclease-induced recombination should allow for a precise correction of a deficient gene instead of a mere complementation, thus bypassing the inherent problems of transgene misregulation, random deletorious insertion, and possible codominant effect of the deficient endogenous gene copy.

PUBLICATIONS

Isalan M "Construction of semi-randomized gene libraries with weighted oligonucleotide synthesis and PCR." Nature Protocols, 1, 468-475 (2006)

Santori MI, Gonzalez C, SerranoL and Isalan M "Localized transfection with magnetic beads coated with PCR products and other nucleic acids." Nature Protocols, 1, 526-531 (2006)

SYSTEMS BIOLOGY

Metazoan Systems (New Group December 2006)

The aim of our lab is to use computational and systematic experimental approaches to understand and predict the biology of multicellular animals. We are interested in fundamental questions of animal biology such as how mutations in genes interact to produce disease phenotypes, how we can predict gene function in the many different systems within an animal, and how animals evolve through changes in their regulatory networks.

Although we are primarily interested in human biology, we use C. elegans as a system for experimental analysis. Using C. elegans we can perform reverse genetic experiments at a throughput that is not possible in any other animal, and so can provide unique insights into the organisation of metazoan systems. This also means that we can extensively experimentally validate computational approaches in *C. elegans,* before applying these validated methods to human biology.

THIS GROUP IS PART OF THE EMBL/ CRG RESEARCH UNIT IN SYSTEMS BIOLOGY

GROUP STRUCTURE

Group Leader:	**Ben Lehner**
Postdoctoral Fellows:	Jennifer Semple
	Tanya Vavouri

RESEARCH PROJECTS

1. GENETIC INTERACTION NETWORKS AND HUMAN GENETIC DISEASE

Most hereditary diseases in humans are not caused by mutations in single genes, but by the combined effects of mutations in many different genes. However these synthetic effects are very difficult to identify from population studies, and we understand little about how mutations interact to produce phenotypes. Using RNA interference (RNAi) in *C. elegans* we can systematically test how combinations of mutations interact to produce synthetic phenotypes. The resulting genetic interaction networks can be used to identify novel modulators of important signalling pathways, but more importantly also provide global insights into how combinations of mutations interact to produce disease. For example we have proposed the concept of 'hub' genes in genetic interaction networks. Hub genes are genes that, when mutated, enhance the phenotypes resulting from mutations in many unrelated genes. 'Hub' genes may be important in many different, unrelated human diseases.

2. AN ANIMAL IN A NETWORK?

Is it possible to construct a single gene network that makes useful functional predictions for all of the different cells, tissues and processes in a complicated multicellular animal? In collaboration with the labs of Edward Marcotte (University of Texas, Austin) and Andrew Fraser (Sanger Institute) we are using *C. elegans* as a system to test whether this is possible. We are using a Bayesian framework to integrate many different types of data to link genes that are likely to be functionally related ('Wormnet'). We are then using reverse genetic analysis to systematically test the ability of Wormnet to predict loss-of-function phenotypes in many different systems within the worm. For example we have identified novel genes that suppress the effects of mutations in the Retinoblastoma tumour suppressor pathway, and have successfully predicted that the Dystrophin complex modulates EGF/Ras/MAPK signalling. The aim of this approach is to use *C. elegans* to experimentally test methods for gene function prediction, before then applying these validated methods to human biology.

3. REGULATORY NETWORKS AND THE EVOLUTION OF ANIMAL BODY PLANS

Genetic and functional networks can be used to understand and predict the relationship between the genotype and the phenotype of an organism. However we would also like to understand how gene networks are regulated

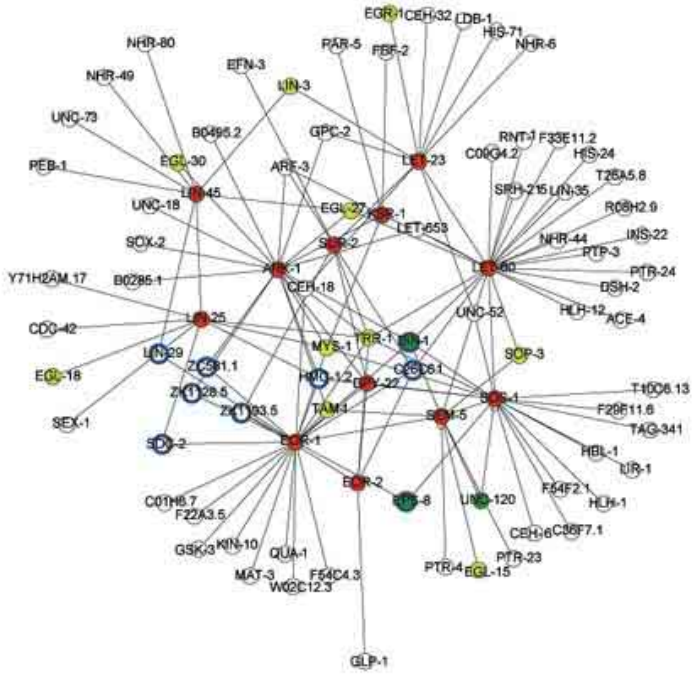


Figure 1. Modulators of EGF/Ras/MAPK signalling identified using synthetic genetic interaction screens.

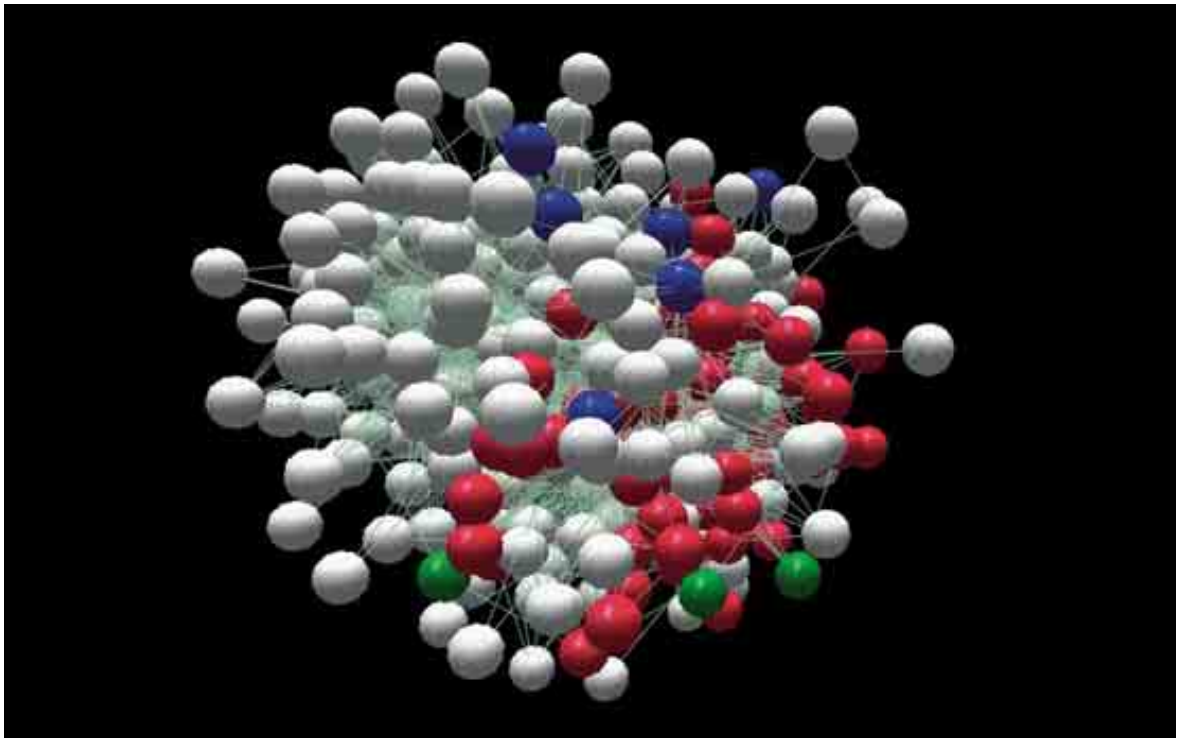


Figure 2. An animal as a network – *C. elegans* pictured as a network or interactions between ~400 functional modules of genes. Modules are coloured according to the predominant loss-of-funciton phenotypes of the genes they contain: red (nonviable), green (growth defective), blue (post-embryonic phenotypes), white (no visible phenotypes).

during the development of an animal, and how they evolve during evolution.

As a first step towards this, we have been using comparative genomics to understand the evolution of regulatory elements in animal genomes. We have first focussed on the most highly conserved noncoding elements (CNEs) in various animal genomes. CNEs represent a very unusual class of sequences that are extremely conserved within specific animal lineages yet are diverged beyond recognition between lineages. Most remarkably we have found that a core set of genes that regulate development is associated with CNEs across three animal groups (worms, flies and vertebrates). We propose that these CNEs reflect the parallel evolution of alternative enhancers for a common set of developmental regulatory genes in different animal groups. This re-wiring of gene regulatory networks containing key developmental coordinators was probably a driving force during the evolution of animal body plans. CNEs therefore may represent the genomic traces of these 'hard-wired' core gene regulatory networks that specify the development of each alternative animal body plan.

As a basis for further understanding the evolution of gene networks, we are now using both computational and experimental approaches to systematically define gene regulatory networks in a complex animal system.

PUBLICATIONS (*)

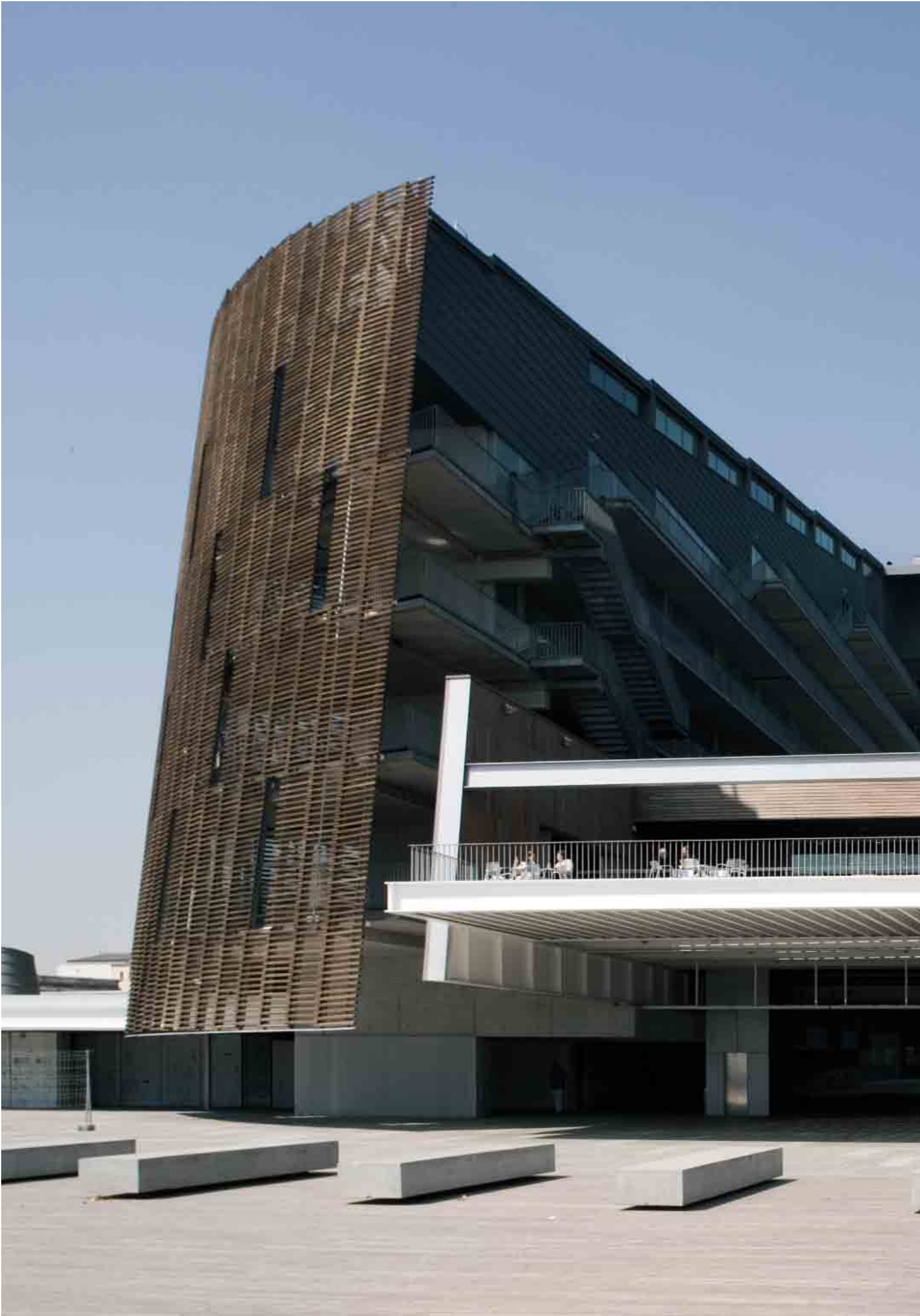
Tischler J, Lehner B, Chen N, Fraser AG. "Combinatorial RNA interference in *C. elegans* reveals that redundancy between gene duplicates can be maintained for more than 80 million years of evolution." Genome Biol, 7(8), R69 (2006)

Lehner B, Tischler J, Fraser AG. "RNAi screens in *C. elegans* in a 96-well liquid format and their application to the systematic identification of genetic interactions." Nature Protocols, 1(3),1617-1620 (2006)

Lehner B, Crombie C, Tischler J, Fortunato A, Fraser AG. "Systematic mapping of genetic interactions in *Caenorhabditis elegans* identifies common modifiers of diverse signaling pathways" Nat Genet, 38(8), 896-903 (2006)

Lehner B, Calixto A, Crombie C, Tischler J, Fortunato A, Chalfie M, Fraser AG. "Loss of LIN-35, the *Caenorhabditis elegans* ortholog of the tumor suppressor p105Rb, results in enhanced RNA interference." Genome Biol, 7(1), R4 (2006)

(*) All these publications are the result of the work of Dr. Ben Lehner at The Wellcome Trust Sanger Institute, Cambridge, UK



APPENDIX 1

V ANNUAL SYMPOSIUM OF THE CENTER FOR GENOMIC REGULATION

Systems Biology: A Cell in the Computer?

The V CRG Symposium was held on 15 and 16 December 2006, at the PRBB Auditorium in Barcelona. This edition was titled "SYSTEMS BIOLOGY: A CELL IN THE COMPUTER". The purpose of the symposium was joining a group of scientists, leaders in the field of Systems Biology, gathering from purely theoretical groups to groups working with topics about the application to health of Systems Biology.

All attendants agreed that the speakers selected were the best worldwide in their respective areas and according to the different sessions in which the symposium was divided. The topics presented, as well as the discussions afterwards were of high interest for the scientists from the CRG and the entire scientific community in the area of Barcelona, as well as for all the attendants coming from the rest of Spain and Europe. This fact shows the dissemination of the symposium was considerably successful, since scientists from different Spanish and European institutes were attracted to attend. On the other hand, it is very important to highlight that contacts amongst several of the speakers' groups were established, which in some cases have crystallized in collaboration projects that will be submitted to the 7th Framework Program of the European Union.

Sessions were structured by topics, in order to gather similar research groups. As mentioned above, all interesting topics of Systems Biology were covered. The symposium included excellent presentations, as for example the one by Ron Weiss, from Princeton University, in the States; Alfonso Martínez-Arias, from the University of Cambridge, in the United Kingdom; and Ricard V. Solé, from the University Pompeu Fabra, in Barcelona.

The symposium was announced at the CRG website and was disseminated amongst many scientific institutions around the world. Moreover, thanks to the symposium, some of the speakers appeared in the media. Therefore, we do believe the symposium contributed to increase the visibility of the CRG and Barcelona in this field and allowed offering a complete picture of this innovative research area. The high number of attendants (around 200), the level of the invited speakers and the discussions contributed to internationalize the scientific image of Barcelona, Catalonia and Spain.

We do believe the final result of this symposium was really interesting for the attendants, due to the relevance and prestige of experts in this area. The contents and format of the symposium worked as a forum, clearly suitable for the consecution of these objectives and for this reason, we consider it a great success.

V CRG Annual Symposium 15-16 December 2006

Systems Biology: A Cell in the Computer?

Organizer:

Luis Serrano

CRG

Centre de Regulació Genòmica

On-line registration at www.crg.es

Invited Speakers:

Bork, Peer
EMBL Heidelberg, Germany

Gardner, Timothy
Boston University, USA

Isalan, Mark
Centre for Genomic Regulation (CRG), Barcelona, Spain

Kaern, Mads
University of Ottawa, Canada

Kashtan, Nadav
Weizmann Institute of Science, Rehovot, Israel

Lemaire, Patrick
Developmental Biology Institute of Marseilles-Luminy, France

Lercher, Martin
University of Bath, United Kingdom

Martinez-Arias, Alfonso
University of Cambridge, United Kingdom

Sauer, Uwe
Institute for Molecular Systems Biology, ETH Zürich, Switzerland

Sharpe, James
Centre for Genomic Regulation, Barcelona, Spain

Solé, Ricard V.
Universitat Pompeu Fabra (UPF), Barcelona, Spain

Vidal, Marc
Dana-Farber Cancer Institute, Boston, USA

Weiss, Ron
Princeton University, USA

Centre for Genomic Regulation (CRG), PRBB Building, Dr. Aiguader 88, 08003 Barcelona (Spain) - Ph: +34 93 316 01 00

crea NOVARTIS prbb Parc Recerca Biomèdica

APPENDIX 2

CRG SEMINARS 2006

CRG SEMINARS

27/11/06
SARAH A. TEICHMANN (PRBB-CRG)
Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge, UK
"Evolution and Dynamics of Transcriptional Regulatory Networks"

17/11/06
WIM CRUSIO (PRBB-CRG)
Laboratoire de Neurosciences Cognitives, CNRS UMR 5106
"How smart is my mouse? The genetic dissection of memory systems in the mouse"

03/11/06
ANNE WILLIS (PRBB-CRG)
Chair of Cancer Cell Biology, RNA Biology Group, School of Pharmacy, The University of Nottingham, UK
"Regulation of gene expression by control of translation"

30/10/06
ALAIN KROL (PRBB-CRG)
Directeur Recherche CNRS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France
"A complex molecular machinery to specifically incorporate selenium into proteins important for health and disease"

24/10/06
PATRICK LEMAIRE (PRBB-CRG)
IBDML, Campus Luminy, Marseille, France
"Formation of neural tissue in Ciona intestinalis: combining embryology, functional genomics, bioinformatics and imaging"

09/10/06
JEFFREY ROSSEN (PRBB-CRG)
Professor, Dept. of Molecular & Cellular Biology, Baylor College of Medicine, Houston, Texas, USA
"Stem/progenitor cells in the etiology and treatment of breast cancer"

06/10/06
CARL-PHILIPP HEISENBERG (PRBB-CRG)
Group Leader, Max-Planck-Institute of Molecular Cell Biology and Genetics
"Molecular and cellular control of zebrafish gastrulation movements"

06/10/06
ADRIAN KRAINER (PRBB-CRG)
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA
"The splicing factor SF2/ASF is an oncoprotein"

05/10/06
PATRICK CRAMER (PRBB-CRG)
Managing Director, Gene Center, University of Munich (LMU), Munich, Germany
"Molecular bioimaging of gene transcription"

02/10/06
JAVIER MARTÍNEZ (PRBB-CRG)
IMP, Vienna, Austria
"RNA interference, tRNA splicing and mRNA 3' end formation, linked by a novel, human RNA kinase"

29/09/06
STEPHAN VAGNER
Institut Claudius Régaud, Toulouse, France
"Novel functions of splicing factors in 3'-end processing and translation"

07/09/06
PEER BORK
EMBL, Heidelberg, Germany
"Predicting biological function at different scales"

07/09/06
LUIS SERRANO
EMBL, Heidelberg, Germany
"Systems Biology and the Medicine of the 21st century"

14/07/06
VIVEK MALHOTRA
Professor of Cell & Developmental Biology, University of California San Diego, Center for Molecular Genetics, La Jolla, USA
"Cutting Golgi membranes to make transport carriers and to enter mitosis"

28/06/06
CEDRIC NOTREDAME
Research Investigator, Structural and Genetic Information, CNRS UPR 2589, Marseille, France
"Making multiple sequence alignments of sequences and structures with T-Coffee"

23/06/06
HAN G. BRUNNER
Affiliated Principal Investigator, Human Genetics, Nijmegen Center for Molecular Life Sciences, Nijmegen, The Netherlands
"Microdeletion syndromes, syndromes and genes"

09/06/06
MAARTEN VAN LOHUIZEN
The Netherlands Cancer Institute, Division of Molecular Genetics, Amsterdam, The Netherlands
"Polycomb repressors controlling stem cell fate: Implications for cancer and development"

02/06/06
SAVERIO MINUCCI
Group Leader, Dept. of Experimental Oncology, Istituto FIRC di Oncologia Molecolare, European Institute of Oncology, Milan, Italy
"Chromatin alterations in tumorigenesis"

29/05/06
MAREK MLODZIK
Professor, Dept. of Molecular, Cell & Developmental Biology, Mount Sinai School of Medicine (MSSM), New York, USA
"Regulation and specificity of Wnt/Frizzled signaling in planar cell polarity generation"

26/05/06
PAOLA BOVOLENTA
Instituto Cajal, CSIC, Madrid, Spain
"Sfrp1, a Wnt signalling component, with multiple functions in vertebrate eye development"

22/05/06
OLIVER RANDO
Genome Fellow, Bauer Center for Genomic Research, Harvard University, Cambridge, MA, USA
"Genome-scale characterization of chromatin structure in yeast: implications for regulatory complexity"

16/05/06
ALEX GREENWOOD
Postdoctoral Research Fellow, Institute of Molecular Virology, GSF-Research Center for Environment and Health, Munich, Germany
"From junk DNA to extinct genomes: Endogenous retroviruses and ancient DNA"

16/05/06
MARTIN FRITH
Joint postdoctoral position at the Institute for Molecular Bioscience, University of Queensland and the RIKEN Genomic Sciences Center, Australia/Japan
"Do genes exist? The complexity of the mammalian transcriptome"

16/05/06
JENS LAGERGREN
Professor, Stockholm Bioinformatics Center and Numerical Analysis and Computing Science, Royal Institute of Technology, Sweden
"Probabilistic analysis of gene families from multiple species: gene duplication, loss and lateral transfer"

16/05/06
PHILIPP KHAITOVICH
Postdoctoral fellow at the Max-Planck-Institute for Evolutionary Anthropology, Leipzig, Germany
"Gene Expression in Human Evolution"

10/05/06
MICHAEL KARIN
Distinguished Professor of Pharmacology, University of California, San Diego, La Jolla, USA
"The IKK complex and the control of Innate Immunity and Inflammation"

05/05/06
RONALD H.A. PLASTERK
The Hubrecht Laboratory, Utrecht, The Netherlands
"MicroRNAs in Animal Development"

28/04/06
NICOLA K. GRAY
Group leader, Chromosomes and Gene Expression, MRC Human Genetics Unit, Western General Hospital, Edinburgh, Scotland, UK
"Two related families of RNA-binding proteins that regulate translation during gametogenesis and early development."

24/04/06
TOM TULLIUS
Chemistry Department, Metcalf Center for Science and Engineering, Boston University, Boston, MA, USA
"Mapping the Structure of the Human Genome Using the Chemistry of the Hydroxyl Radical"

21/04/06
MELISSA J. MOORE
Howard Hughes Medical Institute, Dept. of Biochemistry, Brandeis University, Waltham, MA, USA
"The exon junction complex: master modulator of mRNA metabolism"

19/04/06
HARRY NOLLER
Professor of MCD Biology and Director of the Center for Molecular Biology of RNA, Sinsheimer Labs, University of California, Santa Cruz, CA, USA
"Ribosome Structure and the Mechanism of Translation"

07/04/06
SAKARI KAUPPINEN
Visiting Professor, Dept. Medical Biochemistry & Genetics, Univ. of Copenhagen, and Associate Director of MicroRNA Research, Santaris Pharma, Denmark
"MicroRNAs in animal development and mammalian brain"

31/03/06
CORNELIUS GROSS
Mouse Biology Unit, EMBL, Moterotondo (Rome), Italy
"Nature via Nurture: Studying Gene-Environment Interactions in the Mouse"

24/03/06
CLAUDIA BAGNI
Professor in Molecular Biology, Dept. of Biology, University of Rome "Tor Vergata", Rome, Italy
"What's new in the Fragile X Syndrome: from translational impairment to spine dysmorphogenesis"

17/03/06
VADIM GLADYSHEV
Charles Bessey Professor, Dept. of Biochemistry, Nebraska Redox Biology Center, University of Nebraska, Lincoln, NE, USA
"The selenoproteome"

15/03/06
BEN LEHNER
Sanger Institute Postdoctoral Fellow, Andrew Fraser Lab, The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK
"Systematic mapping of genetic interactions in C. elegans suggests a new paradigm for human genetic disease"

15/03/06
KAREN LIU
Postdoctoral advisor, Gerald R. Cabtree Lab, Stanford University Medical Center/HHMI, Stanford, California, USA
"Study signaling processes in development: small molecule regulation of GSK-3"

14/03/06
WOLFRAM LIEBERMEISTER
Post-doc at Kinetic Modeling Group, MPI for Molecular Genetics, Berlin, Germany
"Modelling and control of metabolic systems"

14/03/06
ANDREAS BEYER
Post-doc, Group Dr. Thomas Wilhelm, Leibniz-Institute for Age Research, Jena, Germany
"The route from a genome to proteins: Understanding expression regulation in yeast"

14/03/06
EDDA KLIPP
Junior Group Leader "Kinetic Modeling", Dept. Vertebrate Genomics, MPI for Molecular Genetics, and Berlin Center for Genome Based Bioinformatics
"Dynamic modeling of yeast cellular stress response"

09/03/06
JOHN MATTICK
Professor of Molecular Biology & ARC Federation Fellow, Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia
"The hidden layer of RNA regulatory networks in the evolution and development of complex organisms"

03/03/06
NORBERT B. GHYSELINCK
Directeur de Recherche au CNRS, IGBMC - UMR7104, Dept. of Physiological Genetics, Illkirch, France
"FIEx: a directional strategy for monitoring cre-mediated recombination at the cellular level in the mouse. Examples of conditional gene targeting..."

24/02/06
CHARLES LEE
Assistant Director of Cytogenetics, DF/HCC, Assistant Professor, Dept. Pathology, Brigham and Women's Hospital, Harvard Medical

School, Boston, USA
"Structural Variation in the Human Genome: Past, Present, and Future"

13/02/06
ANNALISA PASTORE
The National Institute for Medical Research, London, UK
"Towards a structural understanding of neurodegenerative diseases"

10/02/06
ANTONIO BALDINI
Professor, Pediatrics (Cardiology), Molecular & Human Genetics, Baylor College of Medicine, Feigin Center, Houston, USA
"Tbx1 in mouse development and human disease"

07/02/06
JEAN-CHRISTOPHE ANDRAU
Genomics Lab, Dept. of Physiological Chemistry, UMC Utrecht, Utrecht, The Netherlands
"Genome-wide location analysis reveal new functions for RNA Polymerase II and Mediator transcription complexes in actively dividing and quiescent cells"

07/02/06
RAMESH PILLAI
Post-doctoral research fellow, Witold Filipowicz's group, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
"MicroRNAs: Ribo-regulators of protein synthesis"

06/02/06
JERNEJ ULE
Postdoctoral Associate, Robert Darnell Lab, Rockefeller University, New York, USA
"A positional RNA code predicts alternative splicing regulation by Nova"

03/02/06
ERAN HALPERIN
International Computer Science Institute, Berkeley, CA, USA
"Tag SNP selection and haplotype reconstruction methods"

03/02/06
MARTINE SIMONELIG
Research Director at CNRS, Genetique du Developpement de la Drosophile, Institut de Genetique Humaine, Montpellier, France
"Translational control of maternal mRNAs by poly(A) tail length in Drosophila"

19/01/06
SALVADOR AZNAR-BENITAH
Keratinocyte Laboratory, London Research Institute, CR-UK, London, UK
"Role of Rho GTPases in epidermal stem cell homeostasis and neoplasia"

19/01/06
SÉBASTIEN HOLBERT
INRA-PII COMICS Team Tours, Tours, France
"Two hybrid technology for a large screen of Autism candidate genes"

19/01/06
BASSEM HASSAN
Professor & Group Leader, Laboratory of Neurogenetics, Dept. of Human Genetics-VIB4, VIB and University of Leuven School of Medicine, Leuven, Belgium
"Making, un-making and re-making neuronal circuits: adventures through a fly brain"

18/01/06
MICHAELA FRYE
Keratinocyte Laboratory, London Research Institute, CR-UK, London, UK
"Mechanisms regulating epidermal stem cell fate"

18/01/06
BERENIKA PLUSA
Wellcome Trust/Cancer Research UK, Gurdon Institute of Cancer and Developmental Biology, University of Cambridge, Cambridge, UK
"Cleavage in the mouse embryo: more than just making new cells"

18/01/06
FLORENCIA CAVODEASSI
Research Fellow, Dept. of Anatomy, University College London, London, UK
"Coordination of fate determination and morphogenesis during early eye development"

17/01/06
TIM NEWSOME
Cell Mobility Lab, London Research Institute, Cancer Research UK, London, UK
"Regulation of Actin Nucleation"

17/01/06
MONICA GOTTA
Professor, Institute of Biochemistry, ETH Zürich-Hönggerberg, Zürich, Switzerland
"Cell polarity and asymmetric cell division in C. elegans"

16/01/06
PETER ASKJAER
Independent "Ramón y Cajal" Researcheer, Cell Division Group, Parc Científic de Barcelona, Barcelona, Spain
"The nuclear envelope: formation of an essential barrier"

13/01/06
MARÍA BLASCO
Head, Molecular Oncology Program, Telomeres and Telomerase Group, Centro Nacional de Investigaciones Oncológicas (CNIO)
"Role of telomeres and telomerase in cancer and aging"

PROGRAMME SEMINARS 2006

GENE REGULATION

19/12/06
EVA ESTEBANEZ
Biochemistry & Biophysics University of California
Inhibitors of the androgen receptor-corregulator assembly and discovery of new allosterix regulatory surfaces

11/12/06
DR. FRANÇOIS XAVIER OGI
Ornex, France
Regulation of p53 by HRad23

24/11/06
AMY WOOD
Center for Biomolecular Science, Nottingham, UK
"Structural and Energetic Characterisation of allo-MHC restricted Tumour Antigen Targeting"

17/07/06
DR. STEPHANIE BOUE.
European Molecular Biology Laboratory-EMBL, Heidelberg, Germany
"Transcripts in Space and Time".

30/03/06
ANTOINE GRAINDORGE
Université de Rennes 1, CNRS, Dept. Expression Genetique et Développment Faculté de Medicine, Rennes, France
"Identification of post-transcriptionally regulated Xenopus tropicalis mRNAs during early development"

23/03/06
RAMESH PILLAI
Postdoctoral research fellow (in Prof. Witold Filipowicz's group), Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
"MicroRNAs: Ribo-regulators of protein synthesis"

10/02/06
MARIJA MIHAILOVIC
European Molecular Biology Laboratory, Gene Expression Unit, Cytoplasmic gene regulation and molecular medicine, Heidelberg, Germany
"Translational control of BACE-1 expression"

17/01/06
HENNING URLAUB
Max-Planck-Institute for Biophysical Chemistry, Bioanalytical Mass Spectrometry Group, Göttingen, Germany
" Mass spectrometry-based proteomics of protein-RNA particles"

DIFFERENTIATION & CANCER

04/12/06
HERVE LUCHE
University of Ulm, Germany
Lineage tracing and efficient Cre-mediated genome manipulation within the T-Cell compartment

01/12/10
ADRIAN BRACKEN
Biotech Research Innovation Centre, Copenhagen, Denmark
"Polycombs on Target Genes During Cell Fate Transitions and Cancer"

16/11/10
EVA GONZALEZ
Department of Cancer Biology, Amgen Inc., Seattle, USA
"RANK overexpression in the mouse mammary gland results in increased proliferation and tumorigenesis"

27/10/10
AGATA M. D´AGOSTINO
Post Doctoral Candidate, Epithelial Homeostasis and Cancer Group (D&C)
"Kaposi´s Sarcoma: An animal model to study KSHV oncogenesis"

24/10/10
Rakel Fernandez
Post-Doctoral Candidate, Epithelial Homeostasis and Cancer Group (D&C)
"Repression of SOX6 transcriptional activity by SUMO modification".

GENES & DISEASE

03/08/06
DR.JOHN VINCENT
The Centre for Addiction and Mental Health Assistant Professor, Dept of Psychiatry, and Institute of Medical Science.R30, Neurogenetics Section CAMH
"Cytogenetic and Genomic Analysis in Autism. Molecular analysis of 8 autism patients with cytogenetic abnormalities."

09/05/06
EDUARD SERRA
IRO - Institut de Recerca Oncològica, l'Hospitalet de Llobregat, Barcelona, Spain
"From yeast to man (and vice versa). About prediction, cell size and genetic variability."

31/01/06
MIGUEL ANGEL GENESTAR PUJANA
Institut Català d'Oncologia, Laboratori de Recerca Translacional, Barcelona, Spain
"A Breast Cancer Network Model Establishes a Molecular Link between BRCA1 and Centrosome Dysfunction."

BIOINFORMATICS & GENOMICS

21/12/06
XAVIER ROCA
Adrian R. Krainer Lab, Cold Spring Harbor Laboratory, USA
"Deciphering 5' splice-site selection"

18/12/06
VINCENT MIELE
Statistics & Genome Lab, CNRS (National Center for Scientifical Research) directed by Pr.Bernard Prum, France
"DNA physical properties predict nucleosome occupancy along genome sequences"

10/11/06
PAUL WAN
PhD. in the Children's Medical Research Institute, Australia.CMRI is affiliated with the University of Sydney
"Bioinformatic Characterisation of the Human 3' Splice Site"

09/10/06
DAVID MARTIN
Bioinformatics Postdoc, Center for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada
"Bioinformatics approaches to transcriptional regulation and functional gene annotation"

19/09/06
SHANNAN HO SUI
University of British Columbia, Centre for Molecular Medicine and Therapeutics, Vancouver, Canada
"Identification of regulatory programs in sets of co-expressed genes"

14/07/06
HEATHER TRUMBOWER
Center for Biomolecular Science & Engineering, Santa Cruz, CA , USA
"Variation Resources in the UCSC Genome Browser" "A Roadmap to the UCSC Genome Browser Source Code"

12/07/06
SARAH DJEBALI
Dyogen Lab, CNRS UMR854,Ecole Normale Supérieure & IBISC Lab, CNRS FRE2873, Université d'Evry Val d'Essonne, Paris, France
"Exogean: a formal framework for annotating protein-coding genes in eukaryotic genomic DNA"

29/06/06
XAVIER DE LA CRUZ
Institut de Recerca Biomèdica de Barcelona, IRB-PCB, Barcelona, Spain
"SPLASH - A simple procedure for the annotation of alternative splicing events at the protein level"

10/05/06
CARLOS MANCHADO PERDIGUERO
Especialista en sistemas, ROCHE Diagnostics
"Introduction to Real Time quantitative PCR"

29/03/06
YANN FILAUDEAU / JUAN CRUZ CIGUDOSA
Agilent Technologies / CNIO Madrid
"CGH technology workshop"

25/01/06
GABRIEL VALIENTE
Department of Software Research Group on Algorithms, Bioinformatics, Complexity, and Formal Methods Technical University of Catalonia, Barcelona, Spain
"Perfect Phylogeny under Mutation and Constrained Recombination"

19/01/06
BERND NEUFELD
AMAXA GMBH
"NucleofecciónTM: Transporte eficiente de DNA y siRNA al interior celular"

CELL & DEVELOPMENTAL BIOLOGY

25/10/06
ZEYNEP OEKTEN
Stanford University (USA) and Ludwig-Maximillians University, Munich (Germany)
"Single molecule studies of myosin VI and myosin V motors".

17/10/06
MARKUS EHRAT.
Managing Director, Zeptosens.
"Protein Arrays: High throughput protein quantification from total cell lysates".

08/06/06
KURT HERRENKNECHT.
Evotec technologies GmbH
"Opera: The new generation confocal microplate imaging reader."

27/04/06
ALVARO CREVENNA
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.
"Kinesin: to cooperate one needs to be flexible."

13/03/06
NEIL VINCENT.
BD BIOSCIENCES
"BDPPathway: True Confocal Real-time Imaging System"

09/02/06
SYLVAIN MEUNIER
Institut Louis Bugnard
"Translokin and intracellular trafficking of FGF2"

SYSTEMS BIOLOGY

17/03/06
CARSTON HORN
European Molecular Biology Laboratory - EMBL, Germany
"Design and evaluation of zinc finger nucleases".

APPENDIX 3

GRANTS

THE GRANTS THAT THE CRG OBTAINED FROM 1ST JANUARY TO 31ST DECEMBER 2006 ARE THE FOLLOWING:

ORGANISM	AMOUNT (euros)
MINISTERIO DE EDUCACION Y CIENCIA	4.157.810,63
EUROPEAN COMMISSION	2.319.372,24
FUNDACION DESARROLLO INVESTIGACION GENOMICA Y PROT	951.997,90
MINISTERIO DE SANIDAD Y CONSUMO-FIS	521.296,52
AGAUR - AGENCIA GESTIO D'AJUTS UNIVERSITARIS	246.954,36
MUSCULAR DYSTROPHY ASSOCIATION	123.979,10
FONDATION JEROME LEJEUNE	102.000,00
HUMAN FRONTIERS SCIENCE	97.941,00
NOVARTIS FARMACEUTICA S.A.	80.000,00
FUNDACIO MARATO TV3	74.854,27
MINISTÉRIO DA CIÉNCIA E DO ENSINO SUPERIOR	37.500,00
FUNDACIÓN DE INVESTIGACIÓN MÉDICA MUTUA MADRILEÑA	31.900,00
ASSOCIATION FRANÇAISE CONTRE LES MYOPATHIES AFM	30.000,00
CENTRE D'INNOVACIÓ I DESENVOLUPAMENT EMPRESARIAL	30.000,00
FUNDACIO LA CAIXA	25.000,00
JDRF - JUVENILE DIABETES RESEARCH FOUNDATION	22.227,22
INTAS	20.000,00
FUNDACIO IMIM	13.756,80
ALMIRALL PRODESFARMA S.A.	12.000,00
FUNDACION MARCELINO BOTIN	12.000,00
FUNDACION BBVA	12.000,00
SANOFI-AVENTIS, S.A.U.	12.000,00
VWR INTERNATIONAL EUROLAB, S.L.	9.152,00
GRANTS (MISCELLANEOUS)	23.828,33
CAIXA CATALUNYA	6.000,00
CONTRATAS Y OBRAS EMPRESA CONSTRUCTORA, SA	6.000,00
GRUPO FERRER INERNACIONAL, SA	6.000,00
LABORATORIOS DR. ESTEVE, SA	6.000,00
PROUS SCIENCE	6.000,00
SARTORIUS, S.A.	4.310,34
HUCOA-ERLÖSS, S.A.	2.069,93
TOTAL AMOUNT	9.003.950,64

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