CRG
Centre de Regulació Genòmica

Centre for Genomic Regulation
Annual Report 2008

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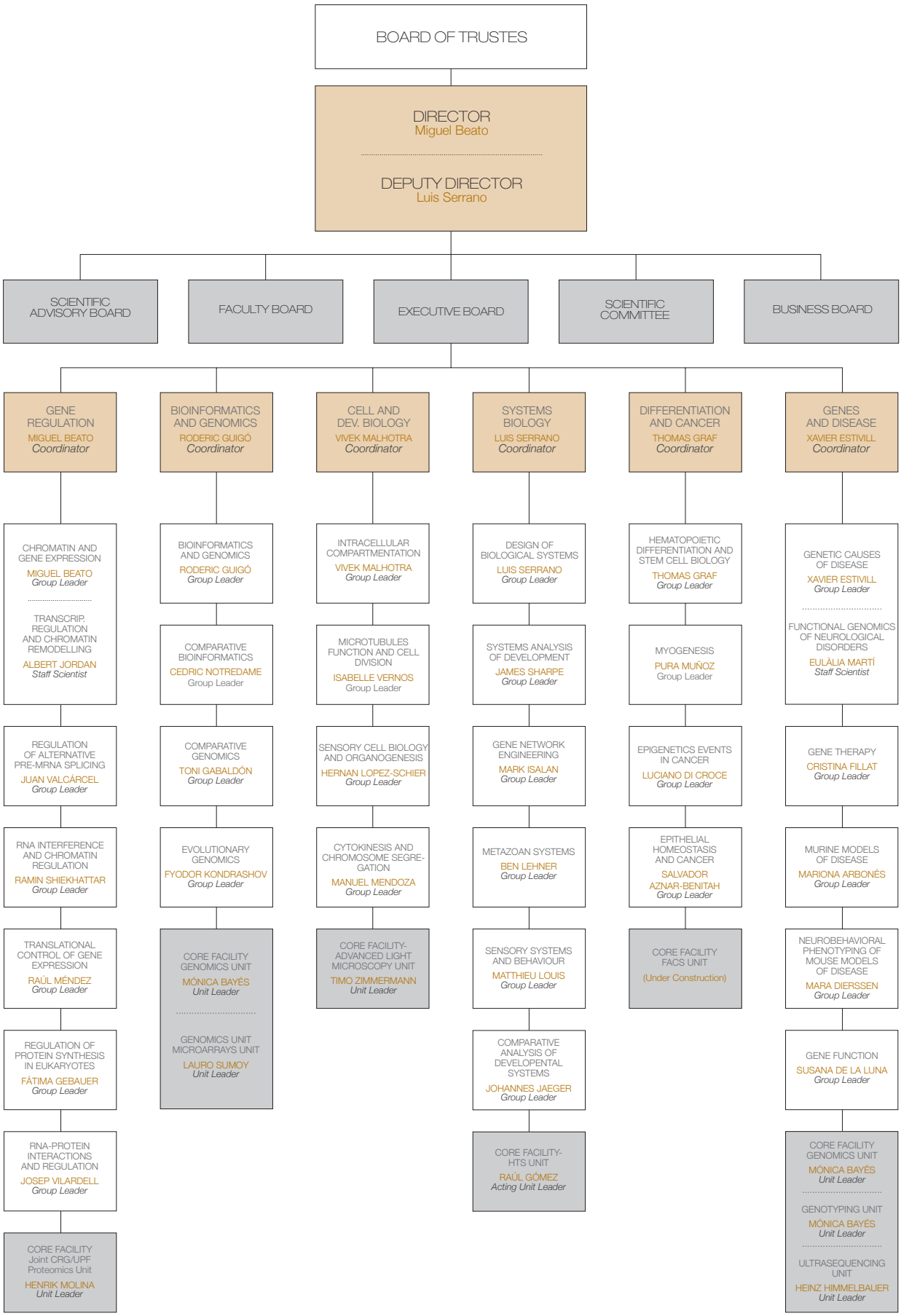
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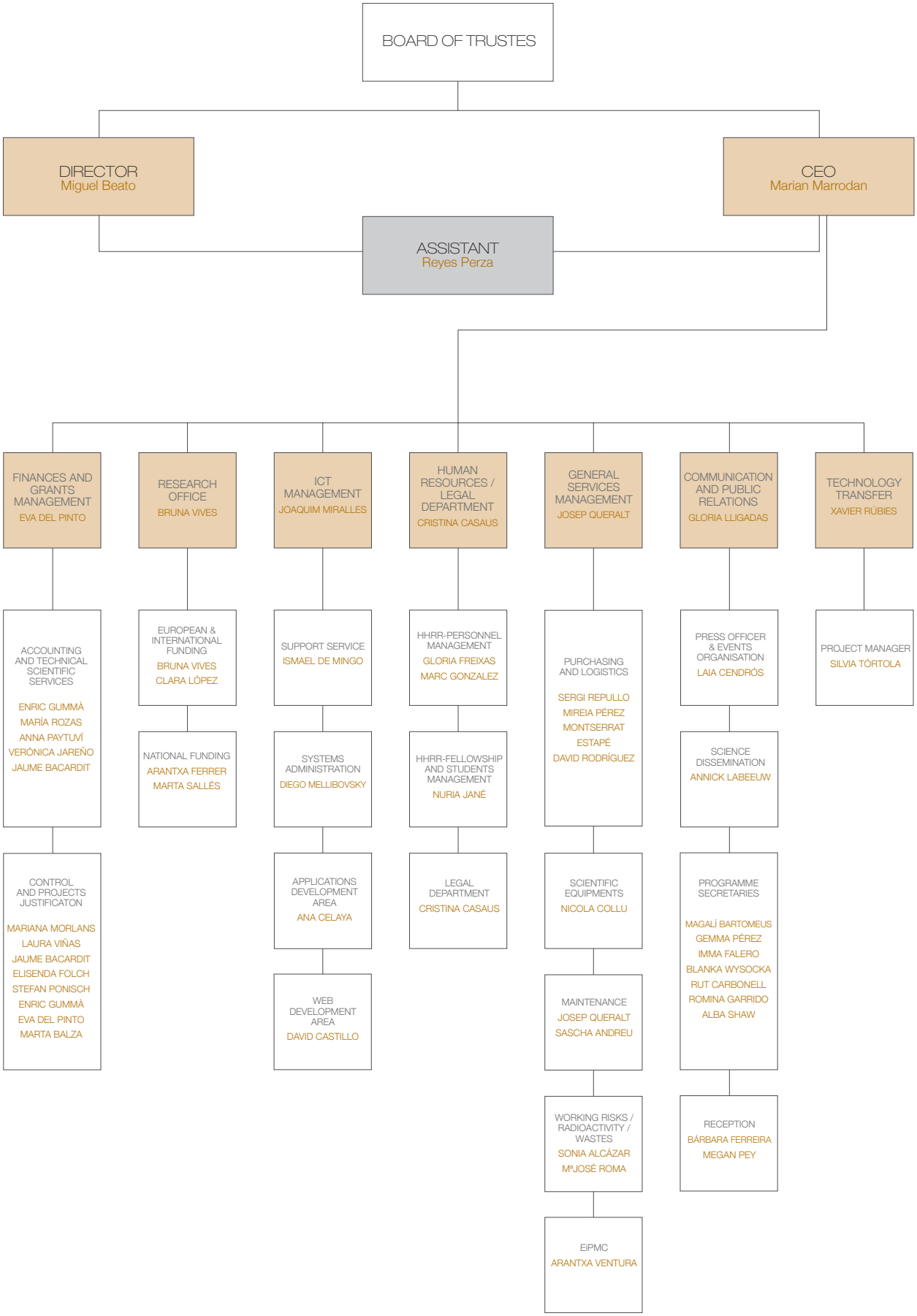
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YEAR RETROSPECT

by the Director of the CRG: Miguel Beato

Last year the CRG continued the consolidation of its six scientific programmes and launched a rapid development of the core facilities, especially genomics and proteomics. The most important event in 2008 was the approval by the Board of Trustees of the Strategic Plan for the next 5 years. The plan envisages a 10% annual growth of our public budget and two different time schedules for the development of the centre depending on an eventual increased participation of the central government in our core budget. Another important decision was the approval by the Leading Board of the PRBB of the use of additional 600 m2 in the ground floor for the further development of the PRBB common Core Facilities for Genomics, Proteomics and Bioinformatics that will be mainly run by the CEXS/UPF and the CRG.

In the spring we interviewed 30 candidates for the first 10 positions of the International PhD programme generously financed by the "la Caixa" Foundation. The positions were filled with excellent students from many different nationalities, who joined the program in the fall.

As in the past years, the CRG organized its Annual Symposium in the fall of 2008. This 7th edition, coordinated by the Cell and Developmental Biology Programme, focused on "Mechanisms Regulating Cell Growth and Division" and was a great success of attendance and scientific quality. Another interesting scientific meeting organized by CRG scientists was the "First EMBO Conference on the Molecular and Cellular Mechanisms regulating Skeletal Muscle Development and Regeneration", held in September in Sant Feliu de Guixols. At the national level there were three events related to science and society co-organized by CRG scientists, "Down Syndrome, a multidisciplinary perspective", "Brain Fair 2008" and the "First Open Doors Day of the PRBB", that were very successful. Graduate students and young postdocs organized the second "CRG Young Researchers Symposium" in December. Finally, in collaboration with the programme "Youth and Science" of the Caixa Catalunya Foundation, young CRG scientists guided 12 high school students during two weeks through a series of experiments in molecular biology and genomics with the objective to promote scientific career choices.

As scientific achievements we have to underline the three young group leaders of the CRG that have negotiated and signed with the ERC the conditions for the use of their Starting Grants. Luis Serrano has been elected for one of the ERC Advanced Grants.

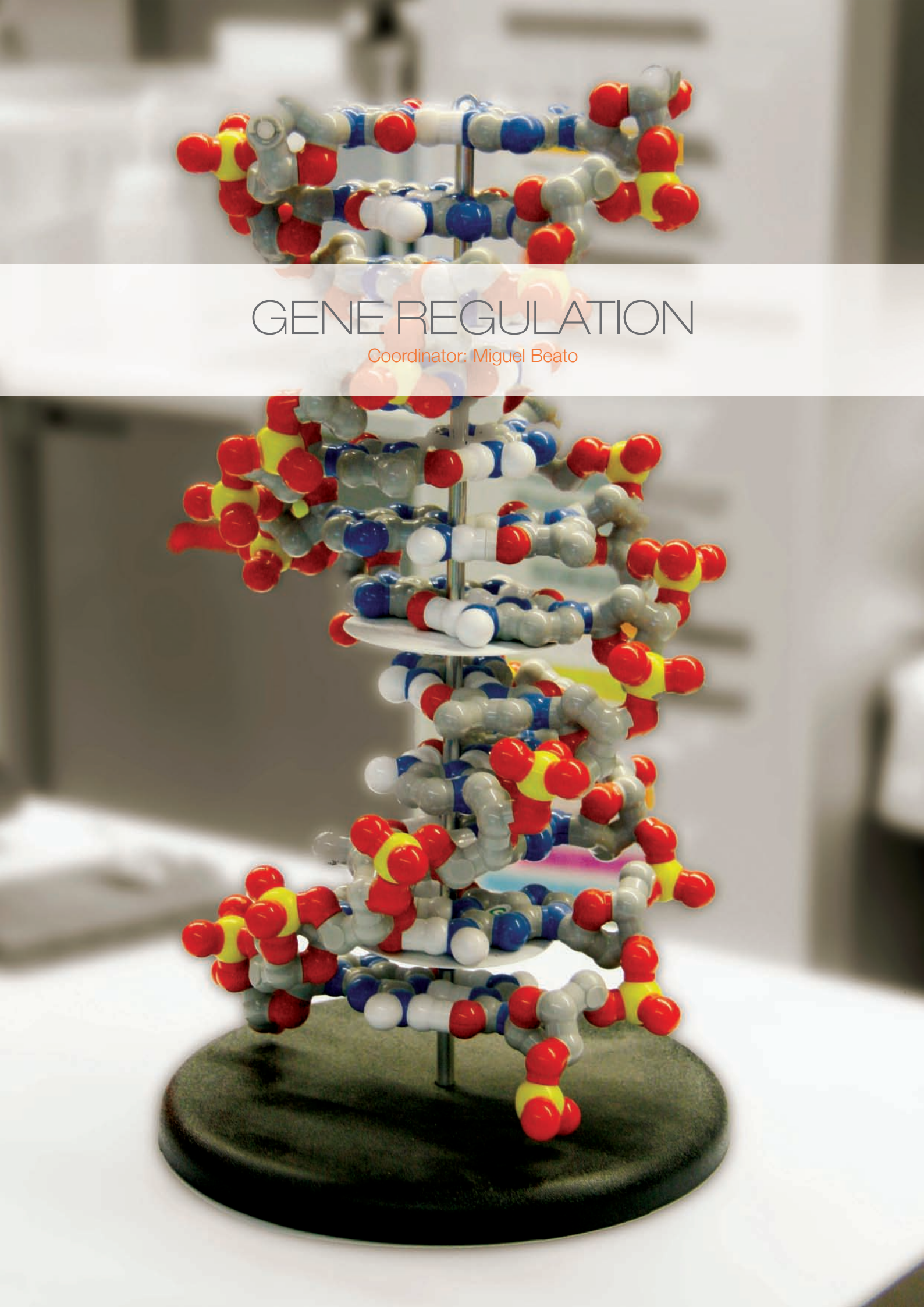
In terms of the development of the scientific programs, two new groups have started in the EMBL/CRG Systems Biology Research Unit, which has reached its final size of four groups. Two new groups have been recruited to the Cell and Developmental Biology programme, including the coordinator Vivek Malhotra from the University of California in San Diego, and another two groups have been recruited to the Bioinformatics and Genomics programme.

The Core Facilities have been strengthened by the acquisition of state of the art instruments for microscopy, DNA sequencing, quantitative proteomics, and medium throughput screening coupled to a fluorescence microscope to select for cellular phenotypes. We have also created a histopathology service, and have recruited a Head of the Deep Sequencing Facility and a Head of the Proteomics Facility, which is run in cooperation with the University Pompeu Fabra. To coordinate all the Core Facilities we made an offer to Doris Meder from the MPI in Dresden, who will start early 2009.

At the end of 2008 there were 296 scientists working at the CRG; 28 Group Leaders, 5 Unit Heads, 8 Staff Scientists, 92 postdoctoral fellows, 80 PhD students and 83 technicians. Over 50% of the group leaders, postdocs and graduate students are foreigners.

The number and quality of the papers published by the CRG scientists continue to increase as do the number and quality of the seminars by external speakers. In 2008 142 were published or in press in international journals with an average impact factor of 9,177, and 146 seminars were held by invited speakers.

With the objective to serve the growing needs of the CRG, the administration team has continued to evolve expanding the research office, the technology transfer unit and the science and society area within the communication and public relations department. All together researchers and administration are contributing to make the CRG an attractive place for young scientists around the world.



GENE REGULATION

Coordinator: Miguel Beato

Gene Regulation Programme

In 2008 the programme has continued its consolidation with the development of the proteomics facility in collaboration with the Department of Experimental and Health Sciences (CEXS) of the UPF. The common facility is implanted in spaces from the UPF in the third floor of the PRBB and incorporates the already existing equipment and personnel from the CEXS/UPF and new equipment, in particular a new LTQ Orbitrap machine for quantitative proteomics, and personnel hired by the CRG. Henrik Molina has been hired as head of the facility, which has been providing advanced services since September 2008.

Following the recommendations of the review panel the programme has increased the institutional support for Raúl Méndez, who published three key papers on translational control of gene expression. These papers have been selected to highlight the contribution of the Gene Regulation programme to the scientific output of the centre.

The structure of the programme at the end of 2008 was

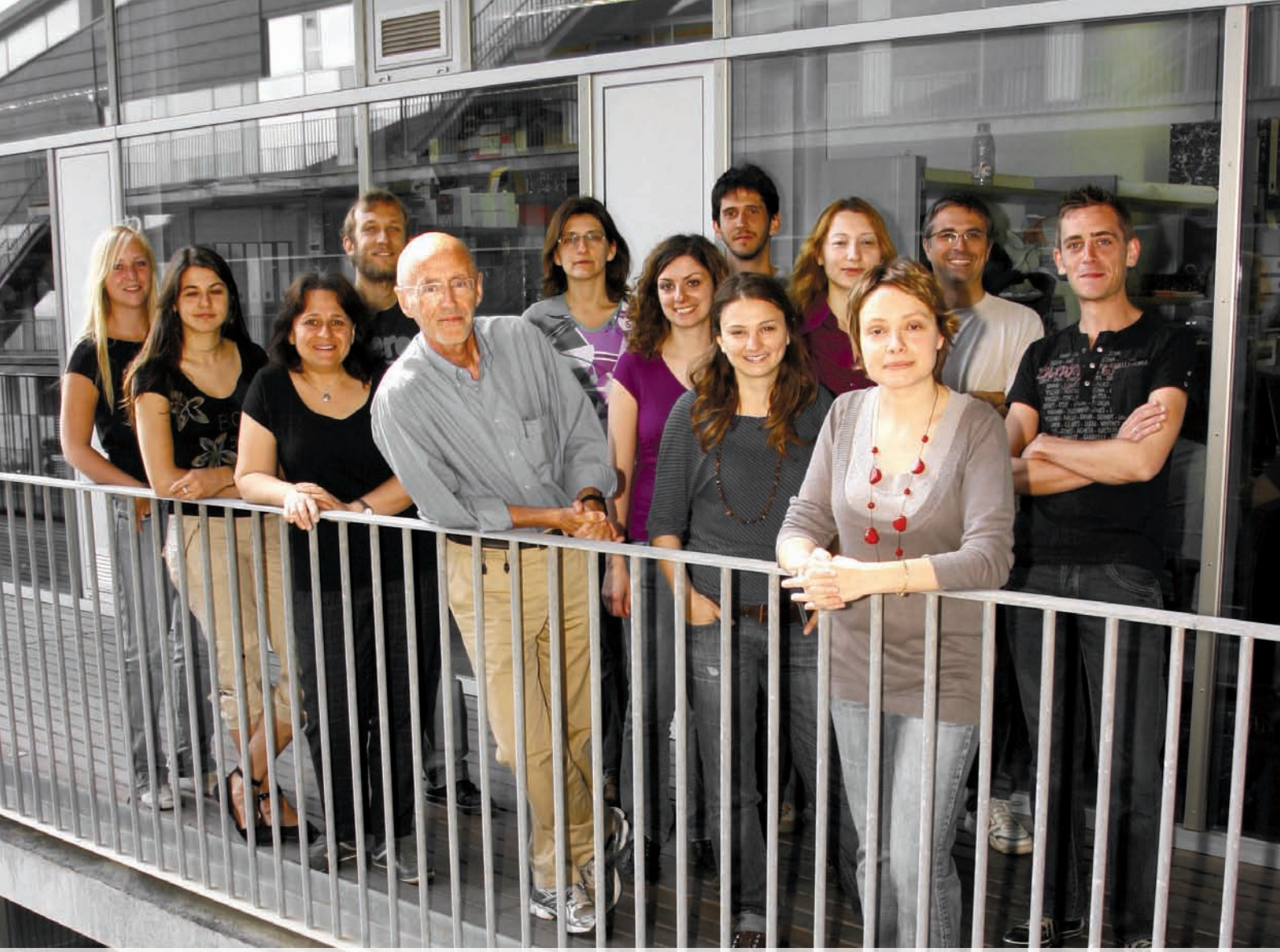
6 Research groups:

> Chromatin and Gene Expression (Miguel Beato, coordinator)
> 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)

Programme´s Secretary: Imma Falero

Associated Core Facility: Proteomics (Henrik Molina)

Numbers: 30 postdocs, 27 students, 9 technicians and support personnel.



GENE REGULATION

Group structure:

Group: Chromatin and Gene Expression

Group Leader:	Miguel Beato
Postdoctoral Fellows:	Cecilia Ballaré, Francois Le Dily, Guillermo Vicent (Staff Scientist since November), Roni Wright
PhD Students:	Verónica Calvo, Michael Wierer, Diana Reyes, Roser Zaurin
Technician/s:	Jofre Font, Silvina Nacht
Visitors:	Ang Li, Houston, USA (till September), Patricia Saragüeta, CONICET, Argentina

Subgroup: Transcriptional Regulation and Chromatin Remodelling (till October 2008)

Staff Scientist:	Albert Jordan, subgroup leader
PhD Students:	Edurne Gallastegui, Lluís Millán-Ariño, Mónica Sancho
Visiting student:	Erika Diani

SUMMARY

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. To tackle these questions we study gene regulation by steroid hormones in breast and endometrial cancer cells. More specifically, attention is focused on 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. Given the relevance of positioned nucleosome for hormonal regulation we are also studying the topological information of the double helix that directs the location of histone octamers and their response to ATP-dependent chromatin remodelling complexes. On a more practical line, we try to use this knowledge to decipher the role of steroid hormones in breast and endometrial cancer cell proliferation and differentiation.

RESEARCH PROJECTS

1. Global analysis of signalling pathways, protein complexes and chromatin changes involved hormonal gene regulation

C. Ballaré, J. Font, F. Le Dily, D. Reyes, M. Wierer, R. Wright, R. Zaurin

Progesterone controls proliferation and gene expression in breast cancer cells, where it activates 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). Activated Erk moves to the nucleus, where it phosphorylates PR and Msk1. Activation of Erk and Msk1 is essential for the proliferative response of breast cancer cell lines (Ballaré et al Mol Cell Biol 23, 1994-2008, 2003) as well as for progestin induction of MMTV and other target genes (Vicent et al Mol Cell 27, 367-81,2006). Presently we are studying the mechanism by which Msk1controls cell proliferation in response to estrogens or progesterone in order to develop selective strategies to interfere with the proliferative effect of estrogens and progesterone.

In collaboration with Belen Miñana, Juanjo Lozano and Lauro Sumoy from the Microarray Unit we have performed gene profiling studies and ChIP-on-chip experiments in breast cancer cell lines to study the response 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. We use selective chemical inhibitors and siRNA specific for various kinases (Erk, Msk, Akt, JAK/STAT, CDK2) combined with transcriptome analysis in response to hormones (Figure 1). Using quantitative proteomics (SILAC) in breast cancer cells carrying tagged PR we have initiated the analysis of the PR interactome in order to identify the different complexes involved in hormonal regulation via various signalling pathways. These results along with results from inihibiting ER activation and protein synthesis are integrated in dynamic network, which should serve to identify relevant nodes connecting various signalling pathways with regulation of different gene cohorts.

In collaboration with Christophoros Nikolau and Roderic Guigo from the Bioinformatics and Genomic programme (B&G) we have develop an algorithm for predicting nucleosome positioning in the human genome and are currently validating the predictions using tiling microarray and massive nucleosome sequencing. We have analyzing a selection of 40 hormone responsive promoters by high resolution ChIP-on-chip to define their nucleosomal structure before and after hormone induction, and have identified several PR binding sites within 10 kb flanking the transcription initiation site. We are correlating these findings with posttranslational modifications of core histones and binding of other transcription factors and chromatin remodelling complexes. Currently these results are being extended to the whole genome of breast cancer cells by massive parallel sequencing to determine the total number of PR binding sites and the positions of nucleosomes before and after hormone addition. A correlation between nucleosome occupancy and the transition between introns and exons has already been identified (Tilgner et al, submitted).

Using in vivo and in vitro approaches, including atomic force microscopy (AFM), we have investigated the role of histone H1 subtypes and their modifications in the regulation of chromatin spacing and chromatin compaction as well as in the control of chromatin remodelling by ATP-dependent complexes (Clausell et al, submitted).

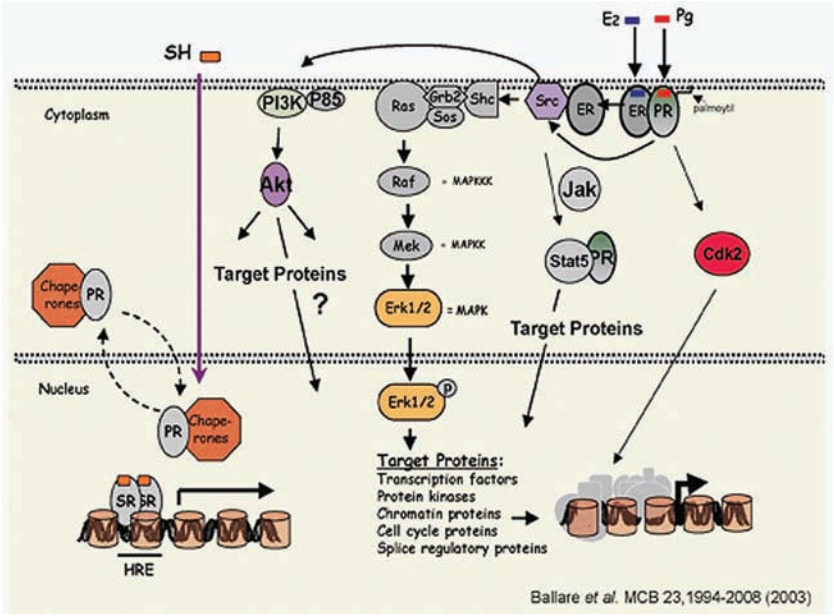


Figure 1. *Crosstalk between PR and ER in the inner side of the cell membrane. In addition to the direct activation of PR followed by homodimerization and binding to HREs in chromatin, progesterone can induce the activation of a preformed ER-PR complex attached to the cell membrane via palmoitylation, leading to interaction of ER with c-Src and activation of the mitogenic Ras > Raf > Mek > Erk1/2 cascade. In the nucleus Erk1/2 can phosphorylate transcription factors, cell cycle proteins and chromatin proteins, directly or via activation of downstream kinases. One of those, Msk1, phosphorylates histone H3 at S10 and initiates activation of some target promoters. PR can also activate c-Src directly, and c-Src can activate the PI3K/Akt pathway and the Jak/Stat5 pathway leading to regulation of other target genes. PR can also interact with Cdk2 leading to phsophorylation of histone H1. Blocking the different signalling pathways with specific inhibitors followed by expressi*

2. Regulation of MMTV transcription in the chromatin context

G. Vicent, R. Zaurin, S. Nacht, J. Font

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 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 phosphorylates histone H3 at S10. This leads to dissociation of a repressive complex containing HP1γ 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) (Figure 2). 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).

In 2007 we have demonstrated a key role of the BAF chromatin remodelling complex and the PCAF histone acetyltransferase in hormonal activation of MMTV. Upon hormone addition, BAF is recruited to the promoter by PR, and depleting the BAF complex leads to inhibition of hormonal induction. BAF binding to the MMTV promoter is stabilized by PCAF mediated acetylation of lysine 14 of histone H3. BAF mediated remodelling leads to displacement of H2/H2B dimers and facilitates NF1 access and binding of additional PR to the central HREs on the remodelled nucleosome (Figure 2). The new PR molecules bring additional BAF complexes to the promoter and lead to full activation (Vicent et al, submitted).

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 H1 isoforms and their phosphorylation by Cdk2 on the remodelling and transcription of MMTV chromatin.

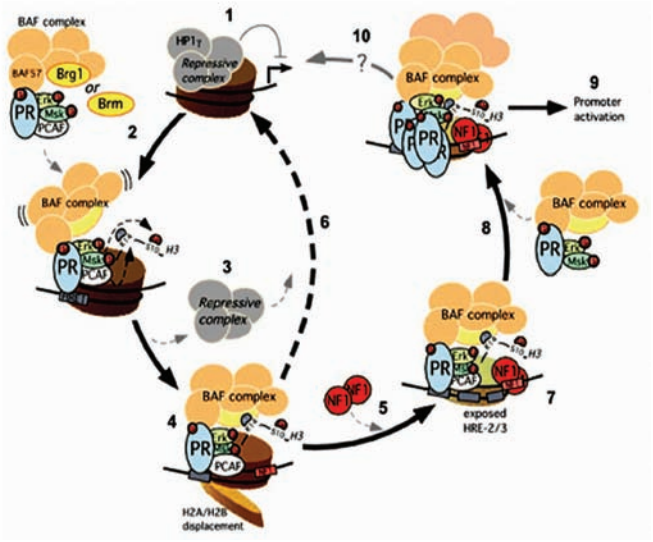


Figure 2. *The MMTV promoter nucleosome B is maintained in a repressive state by bindng of a repressive complex containing HP1γ (1). A quaternary complex pPR/pErk/pMsk1/PCAF, possibly including BAF, binds to the exposed HRE1 on the MMTV nucleosome and phosphorylates H3 at S10 and acetylates H3 at K14 (2). This leads to displacement of a repressive complex (3), as a prerequisite for the stable binding of BAF via an interaction with H3K14ac (4). The BAF complex catalyzes the ejection of H2A/H2B dimers, enabling NF1 binding. In the absence of NF1 this remodelled nucleosome may revert to the closed conformation (6). Binding of NF1 (5) stabilizes the open nucleosome conformation (7) and facilitates binding of further PR molecules and BAF remodelling complexes to the internal HREs (8). Subsequently, PR recruits transcriptional co-activators and the transcription initiation complex, leading to promoter activatio (9). We do not know how the activated promoter nucle*

3. Role of steroid hormones in breast cancer and endometrial physiology

C. Ballaré, V. Calvo, D. Reyes

In collaboration with the Departments of Pathology and Oncology of the Hospital del Mar and with the microarray unit of the CRG, we are studying the gene networks regulated by estrogens and progestins in primary tumour material from breast cancer patients. In the meantime 108 samples have been analyzed on a microarray platform containing over 800 cDNAs relevant for breast cancer and hormone action. A comparison of the gene clusters affected in each tumour sample with the results obtained in breast cancer cell lines (see point 1 above) should permit to classify the tumours according to the perturbed signalling pathways. A correlation of this molecular description with clinical and histological data will allow to establish diagnostic and prognostic markers for future cancer management (Miñana et al, in preparation). We are also directly investigating the mechanism of the mutual inhibitory relationship between BRCA1 and PR function.

In collaboration with the group of Patricia Saragüeta, CONICET Buenos Aires, we found that picomolar concentrations of progestins induce proliferation of endometrial stromal cells via activation of a crosstalk of PR with ERß and the mitogenic kinase cascades (Vallejo et al Mol Endocrin 2005). In 2007 we have studied how blood serum induces the decidual differentiation of these cells, a physiological example of trans-differentiation (Maschi et al, submitted). We plan to compare these results with the changes in gene expression detected during decidualization of UIII cells induced by the combination of estrogens and progestins.

In a screen for genes that bypass a p53-mediated cell cycle arrest, we identified the small GTPase Rem2 as a suppressor of p19ARF expression that promotes endothelial cell proliferation and angiogenesis and is under steroid hormone control (Bierings et al 2008).

Subgroup: Transcriptional Regulation and Chromatin Remodelling

1. Transcriptional regulation and chromatin remodelling of hormone responsive promoters.

A. Jordan, Ll. Millán, A. Subtil

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). Microarray experiments have been performed to define the subsets of genes affected on its response to hormone by the different PR defects. A majority of genes require an intact PR, able to interact with DNA and to activate kinase cascades, pinpointing the importance of cross-talk between PR modes of action (Quiles et al, in press).

We have also performed the characterization of the progesterone-responsive 11ß-HSD type 2 human promoter. Two distinct regions of this promoter recruit PR upon hormone addition: a proximal region where PR interacts directly with DNA, and a distal region containing STAT5A binding sites. This last region is essential for the hormone responsiveness of the promoter. Hormone treatment activates the JAK/STAT pathway and STAT5A transcription factor recruits PR, co-activators and the RNA polymerase to the distal region. Along with promoter activation, non-coding RNAs are synthesized from upstream, which nature and functionality is being investigated. In addition, we are performing chromatin immunoprecipitation (ChIP) to study histone modifications, as well as the composition of associated chromatin remodelling complexes and transcriptional complexes (Subtil-Rodríguez et al, 2008).

2. Role of linker histone H1 variants in chromatin and transcription.

A. Jordan, E. Diani, M. Sancho

At least six histone H1 variants exist in somatic mammalian cells that bind to the linker DNA and stabilize the nucleosome particle contributing to higher order chromatin compaction. In addition, H1 seems to be actively involved in the regulation of gene expression. However, it is not well known whether the different variants have distinct roles or if they regulate specific promoters. We have explored this by inducible shRNA-mediated knock-down of each of the H1 variants in a human breast cancer cell line. Rapid inhibition of each H1 variant was not compensated for by changes of expression of other variants. Microarray experiments have shown a different subset of genes to be altered in each H1 knock-down. Interestingly, H1.2 depletion caused specific effects such as a cell cycle G1-phase arrest, the repressed expression of a number of cell cycle genes, and decreased global nucleosome spacing. On its side, H1.4 depletion caused cell death in T47D cells, providing the first evidence of the essential role of an H1 variant for survival in a human cell type. Thus, specific phenotypes are observed in breast cancer cells depleted of individual histone H1 variants, supporting the theory that distinct roles exist for the linker histone variants (Sancho et al, 2008).

3. Influence of chromatin at the integration site on the transcriptional activity of the HIV promoter.

A. Jordan, E. Gallastegui

HIV integrates at a multitude of sites without any clear preference in the human genome. Little is known about the cell elements mediating the repressive role of the 5'HIV tramscribed region (5'HIV-TR). An analysis of this phenomenon in Saccharomyces cerevisiae, performed in collaboration with the group of Sebastián Chávez in Seville, showed a critical role played by FACT, Spt6, and Chd1, proteins so far associated with chromatin assembly and disassembly during ongoing transcription. This group of factors plays a role in HIV-1 postintegration latency in human cells as shown by depleting the corresponding human orthologs with shRNAs. These results indicate that chromatin reassembly factors participate in the establishment of the equilibrium between activation and repression of HIV-1 when it integrates into the human genome, and they open the possibility of considering these factors as therapeutic targets of HIV-1 latency (Vanti et al PLoS Genet, in press).

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Mol Endocrinol, In press.

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

Juan Valcárcel has a Senior ICREA Group Leader position.

Group Structure:

Group Leader: Juan Valcárcel

Staff scientist: Sophie Bonnal

Postdoctoral Fellows: Elias Bechara (since November 2008), Claudia Ben-Dov, Britta Hartmann (until May 2008) Maria Paola Paronetto (since April 2008), Veronica Raker, Joao Tavanez

Students: Mafalda Araujo (until May 2008), Anna Corrionero, Camilla Ianonne (since December 2008) Nuria Majos (until September 2008), Juan Ramón Tejedor (since February 2008)

Technicians: Belén Miñana, Elisabet Muñoz

GENE REGULATION

SUMMARY

Alternative splicing of mRNA precursors allows the production of multiple mRNAs from a single gene and thus provides rich opportunities for the regulation of gene expression. Our group is interested in the molecular mechanisms that cells use to generate distinct complements of alternatively spliced mRNAs in different biological situations. During 2008 we have made progress in understanding the impact of the activation of signal transduction pathways on alternative splicing; how a single nucleotide difference between two closely related genes leads to differential processing which is at the basis of the genetic disease spinal muscular atrophy; and how the protein RBM5, encoded by a putative tumor suppressor gene, regulates alternative splicing of the apoptotic receptor Fas.

RESEARCH PROJECTS

1. Impact of signalling pathways on alternative splicing in Drosophila cells

We carried out a genome-wide analysis of changes in alternative splicing induced by activation of two distinct signaling pathways, insulin and wingless, in Drosophila cells in culture using splicing-sensitive microarrays. We found that more than 150 genes show alternative splicing changes induced by insulin and more than 50 genes display regulated splicing upon wingless activation. About 40% of the genes showing changes in alternative splicing also show regulation of mRNA levels, suggesting distinct but also significantly overlapping programs of transcriptional and post-transcriptional regulation. Distinct functional sets of genes are regulated by each pathway and a significant overlap is observed between functional categories of genes regulated transcriptionally and at the level of alternative splicing. For example, functions related to carbohydrate metabolism are enriched among genes regulated by insulin, while cellular signaling genes are enriched by activation of the wingless pathway, suggesting that alternative splicing can provide a novel molecular mechanism for crosstalk between different signaling pathways. Our data support the notion that signaling cascades trigger pathway-specific and biologically coherent regulatory programs of alternative splicing regulation.

2. Mechanism of 3' splice site recognition in spinal muscular atrophy genes

Spinal Muscular Atrophy is a prevalent genetic disease caused by mutation of the SMN1 gene, which encodes the SMN protein involved in assembly of small nuclear ribonucleoprotein (snRNP) complexes. A paralog of SMN1, SMN2, cannot provide adequate levels of functional SMN because exon 7 is skipped in a significant fraction of the mature transcripts. A C to T transition located at position 6 of exon 7 is critical for the difference in exon skipping between SMN1 and SMN2. We have found that this nucleotide difference results in increased crosslinking of the splicing factor U2AF65 with the 3' splice site of SMN1 intron 6 in HeLa nuclear extract. U2 snRNP association, which is facilitated by U2AF, is also more efficient on SMN1 than on SMN2, particularly under conditions of competition, suggesting more effective use of limiting factors. Two trans-acting factors implicated in SMN regulation, SF2/ASF and hnRNP A1, promote and repress, respectively, U2 snRNP recruitment to both RNAs. The effects on U2 binding correlate with changes in U2AF65 crosslinking in a transcript- and regulatory factor-specific manner, indicating that both U2AF binding and other steps of U2 snRNP recruitment can be control points in SMN splicing regulation (Figure 1).

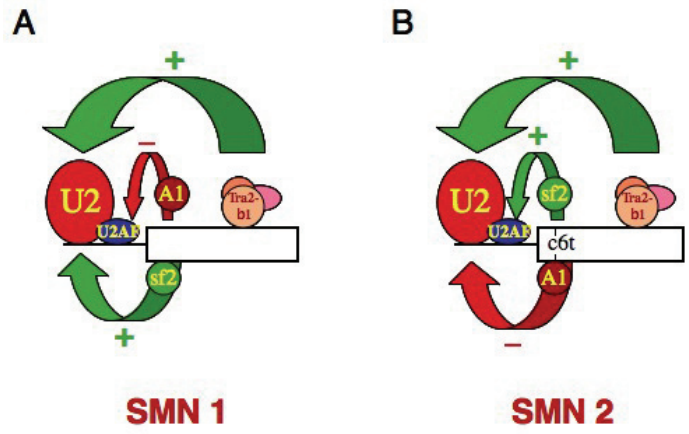

Figure 1. *Mechanism of 3' splice site recognition in SMN genes. A single nucleotide difference in position 6 of exon 7 between SMN1 and SMN2 genes causes extensive exon skipping in SMN2 transcripts and therefore the production of a non-functional protein which cannot provide enough SMN protein when SMN1 is mutated in patients with Spinal Muscular Atrophy, one of the most prevalent genetic diseases. The substitution of C by T at position 6 not only affects the assembly of SF2 and hnRNP A1, factors with antagonistic effects on 3' splice site recognition, but also the mechanisms by which these factors mediate their effects. Tight binding of SF2 to SMN1 transcripts and of hnRNP A1 to SMN2 transcripts enhance or inhibit, respectively, stable assembly of U2 snRNP. The same factors target the binding of U2AF, the previous step in U2 assembly, when acting through the other RNA substrate. These results illustrate how a single nucleotide change which does not overlap with splice sites can however change the balance of activities that determine exon recognition.*

3. Mechanism of Fas alternative splicing regulation by RBM5

The exon 6 of Fas pre-mRNA can be alternatively spliced to generate mRNAs encoding proteins with antagonistic functions in the control of programmed cell death. RBM5, also known as Luca-15 or H37 is a gene frequently inactivated in lung cancers and overexpressed in breast tumors.. We have found that RBM5 is a component of complexes involved in 3' splice site recognition and regulates alternative splicing of apoptosis-related genes, including the Fas receptor, switching between isoforms with antagonistic functions in programmed cell death. In contrast with classical mechanisms of splicing regulation, RBM5 does not affect early events of splice site recognition that lead to Fas exon 6 definition. Instead, RBM5 inhibits the transition between pre-spliceosomal complexes assembled around exon 6 to mature spliceosomes assembled on the flanking introns. It also promotes sequence-specific pairing of the distal splice sites (Figure 2). These findings suggest that multiple steps in the complex process of spliceosome assembly are targets of regulation to achieve splice site selection.

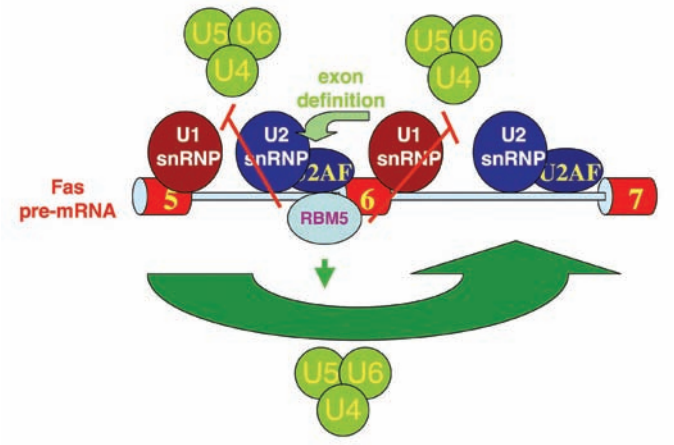

Figure 2. *Mechanism of Fas alternative splicing regulation by the splicing regulator RBM5. RBM5 inhibits splicing of Fas introns 5 and 6 by blocking incorporation of the U4/5/6 tri-snRNP on pre-spliceosomal complexes assembled in the introns flanking exon 6. In addition, RBM5 promotes pairing between the distal sites, thus acting as a selector of splice site pairing after exon definition. These findings illustrate how splicing regulators can modulate late events in the process of spliceosome assembly to influence splice site selection, thus opening the complex mechanisms of splicing complex formation as targets of regulation.*

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Curr Opin Cell Biol, In press.

GENE REGULATION

Group: RNA interference and Chromatin Regulation
Ramin Shiekhattar has a Senior ICREA Group Leader position.

Group Structure:

Group Leader: Ramin Shiekhattar

Staff Scientist: Glenda Harris

Postdoctoral Researchers: Ildem Akerman, David Baillat, Malte Beringer, Clement Carre, Klaus Fortschegger Alessandro Gardini, Fan Lai, Frederic Tort, Laure Weill

Predoctoral Researcher: Dimitra Saviolaki

Technician: Albane Lamy de la Chapelle, Leonor Avila

SUMMARY

We are interested to understand the mechanisms by which protein-coding genes are silenced and how disruption of negative regulation may lead to neoplasia. Such genomic repression is achieved at both transcriptional and post-transcriptional levels. Recent evidence has implicated changes in chromatin structure as an important mechanism in gene regulation. Moreover, besides a classic role for proteins in mediating transcriptional and post-transcriptional effects, it has become clear that non-coding RNAs play an integral role in silencing through fine-tuning gene expression patterns during development and differentiation. We believe that this "signaling by RNA" is an emerging field of research that will uncover novel pathways in genome organization and regulation. In the past few years my laboratory has employed a biochemical approach to uncover a number of key factors that mediate transcriptional repression through regulation of chromatin structure, or post-transcriptional silencing via non-coding RNAs. We will continue our studies through a detailed structure/function analysis of these factors in regulation of genomic silencing and extend our experiments to elucidate the role these factors in the genesis of cancer.

RESEARCH PROJECTS

1. Transcriptional regulation through chromatin modifying complexes

Using biochemical approaches my laboratory has identified novel co-repressor complexes (SMRT-complex; LSD1-containing complexes; JARID1d/Ring6a) that mediate tissue and gene-specific transcriptional repression through modification of chromatin structure. These studies have been instrumental in understand mechanisms by which nuclear hormone receptors and tissue-specific transcriptional repressors (such as the neuronal silencer, REST), mediate their biological functions – these activities include remodelling of nucleosomes and lysine demethylation of histones. More recently, our studies have identified an intimate connection between the polycomb repressive group of proteins and histone demethylases (Figure 1) that underscore their cooperation in maintaining gene expression patterns.

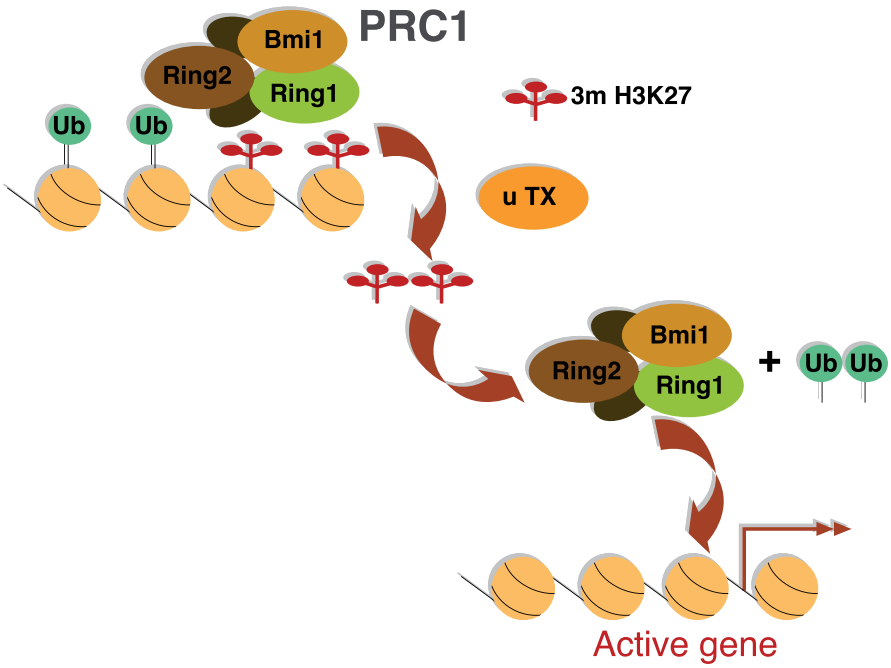

Figure 1.
UTX a histone demethylase specific for histone H3 lysine 27 increase the occupancy of polycomb complex PRC1 on target promoters

2. Transcriptional and post-transcriptional regulation by non-coding RNAs

My laboratory has also identified a pathway by which small non-coding RNAs, termed microRNAs (miRNAs), are synthesized and their mechanism in mediating post-transcriptional silencing in human cells. We identified two distinct RNase III-containing complexes, Drosha/DGCR8 and Dicer/TRBP, which mediate the stepwise processing of primary miRNAs to mature miRNAs. The last step of miRNA processing by Dicer/TRBP involves the incorporation of mature miRNA into a larger complex composed of Argonaute 2 (Figure 2), the subunits of 60S ribosome and the ribosome anti-association factor eIF6. Our studies demonstrate a key role for eIF6 (through ribosome disruption) as the mediator of miRNA inhibitory function.

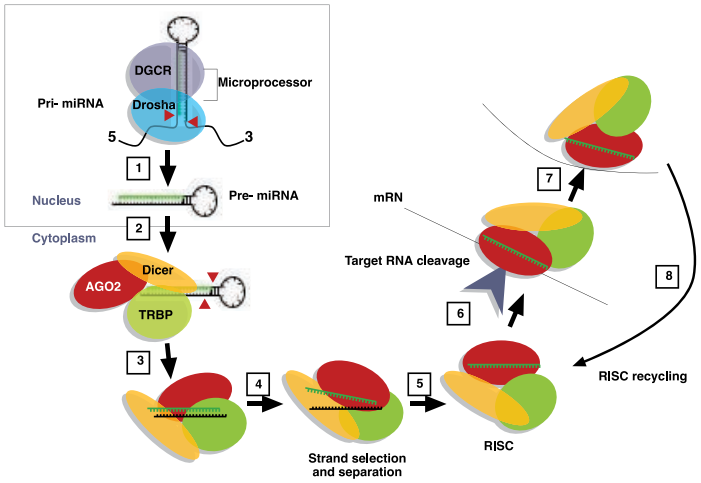

Figure 2.
Steps of microRNA biogenesis and RNA-induced silencing complex (RISC) loading designated by numbers 1 through 8.

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Di Croce L, Shiekhattar R.
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(*) This publication is the result of the work of Dr. Ramin Shiekhattar at the Wistar Institute, Philadelphia, USA.

GENE REGULATION

Group: RNA-Protein Interactions and Regulation

Group Structure:

Group Leader:	Josep Vilardell
Postdoctoral Researcher:	Maria Camats
PhD Student:	Markus Meyer
“Leonardo” Student:	Valeria Giangarrà
Practice Student:	Mercè Guzman
Visiting Student:	Bernardo Cuenca

SUMMARY

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 study the mecanisms that control the selection of 3' splice sites in yeast. A third project is a joint effort with Susana-Rodriguez Navarro (Principe Felipe, Valencia), centered on the possibility of exon-definition mechanisms in yeast, which would offer a powerful tool to study a complex problem.
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.

RESEARCH PROJECTS

1. Genetic screen to select mutants in regulation of splicing

Mercè Guzman, Maria Camats

RPL30, one of the best understood models of splicing regulation in Saccharomyces, encodes the ribosomal protein L30. Through 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.

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 STO1, 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 is related to a remodelling of the interaction between the intron and U1 snRNP before U2 binding.

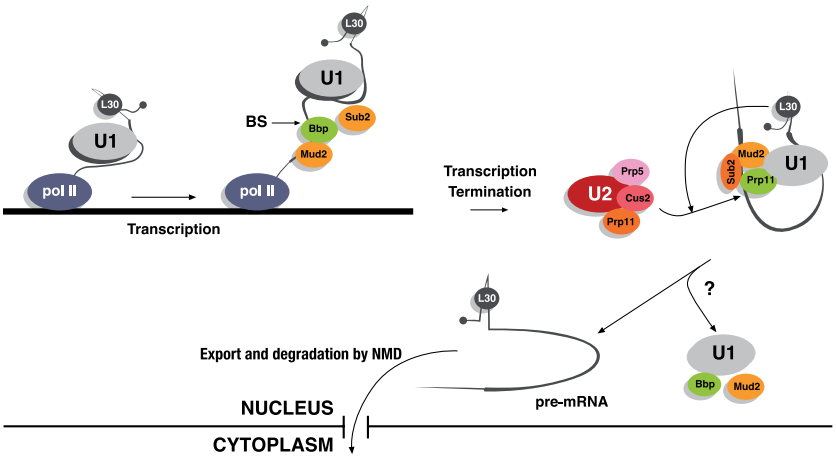
The relevance of this system of regulation, evidenced by our biochemical dissection, makes it relevant the continuation of this genetic approach. In addition to new screenings, we are asking the possible role of mutations in factors like BBP, Mud2, Prp5, or Sub2.

2. Biochemical analysis of the mechanism of RPL30 control of splicing.

Maria Camats, Markus Meyer

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. Several in vitro and in vivo approaches produced data suggesting that this system of regulation follows a novel strategy, based in interfering with the rearrangements that occur during spliceosome assembly. 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. Some of the pre-spliceosomal components show altered ChIP profiles during regulation, and we are investigating the relevance of these findings.

Figure 1.
Regulation of RPL30 splicing by L30. L30 binds to the RPL30 nascent transcript; however, initial intron recognition is not affected. Inhibition of splicing occurs because the L30-containing nascent spliceosome fails to undergo a necessary remodelling that is required for the joining of U2 snRNP. Eventually the "failed" pre-spliceosome will be disassembled and the pre-mRNA exported to the cytoplasm. There is will be degraded by NMD since it contains premature stop codons.



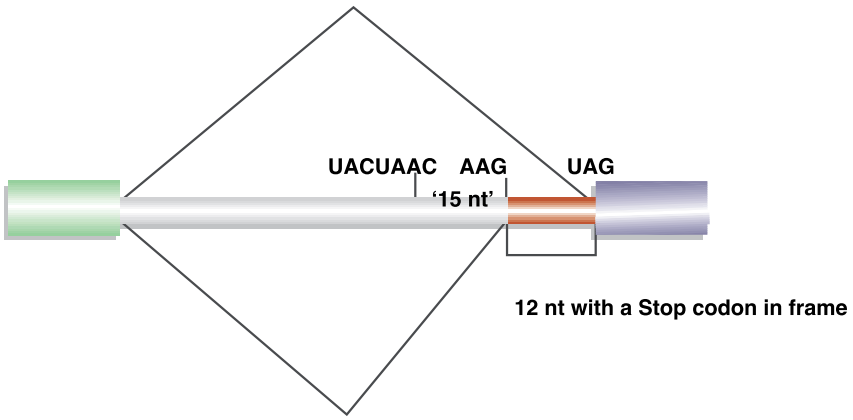
3. Other S. cerevisiae genes with regulated splicing

Markus Meyer, Valeria Giangarrà

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 3' 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.

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, and we are studying splicing and 3'SS usage in splicing in several conditions, including meiosis, mutations in splicing factors, and several stresses (collaboration with Francesc Posas, UPF).

Moreover, we have found that in several yeast introns the RNA structure plays an important role on 3'SS choice, and we are investigating whether this role is under some control by the cell.

In addition to the microarray approaches, we have started using deep-sequencing techniques, in collaboration with the group of Roderic Guigó. This new approach offers us considerable advantages, especially regarding the identification of new spliced forms.

4. Exon definition in yeast ?

Bernardo Cuenca

In a joint effort with Susana-Rodríguez-Navarro (Instituto Príncipe Felipe, Valencia), we are studying the splicing of the gene SUS1 (Fig. 3), involved in transcription and RNA export. This gene contains two introns, and some spliced forms show exon 2 skipping. We are investigating the mechanisms involved in the splicing of this gene; with an interest in asking, in vitro and in vivo, whether the mechanisms involved in exon skipping in metazoans are also having a role in this phenomenon.

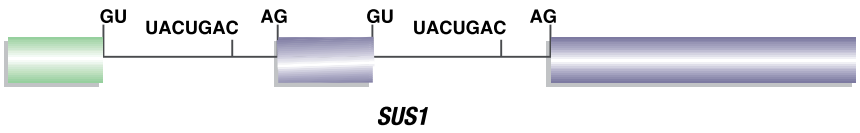


Figure 3.
Genomic structure of the SUS1 gene.

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Brief Funct Genomic Proteomic, In press.

GENE REGULATION

Group: Regulation of protein synthesis in eukaryotes

Group Structure:

Group Leader: Fátima Gebauer

Lab manager: Olga Coll

Postdoctoral Researchers: Rafael Cuesta, Marija Mihailovic, Antoine Graindorge

Students: Solenn Patalano, Aida Martínez, Ana Villalba, Cristina Militti

Technician: Elisabeth Muñoz

SUMMARY

We are interested in the regulation of mRNA translation by RNA-binding proteins and microRNAs. This type of regulation is widely used in biology to modulate processes such as metabolism, cell differentiation, embryonic patterning or synaptic transmission. We wish to understand the molecular mechanisms of translational control and the way that translational regulators are connected with general cell function.

RESEARCH PROJECTS

1. Translational control of dosage compensation

X-chromosome dosage compensation is the process that equalizes the expression of X-linked genes in males (XY) and females (XX). Appropriate dosage compensation is essential for viability and is initiated early during embryonic development. In Drosophila, dosage compensation is achieved by hypertranscription of the male X chromosome, and is repressed in female flies by the female-specific RNA binding protein Sex-lethal (SXL). SXL inhibits the translation of msl-2 mRNA, which encodes a rate-limiting component of the dosage compensation complex (DCC). In order to repress translation, SXL recruits the protein UNR to bind to the 3' UTR of msl-2. UNR is a conserved cytoplasmic protein present in both male and female flies. While in females it is required to repress dosage compensation, in males it displays the opposite function: it is required for the assembly of the DCC on the X chromosome (Figure 1). This function of UNR is independent of translational regulation of known DCC components. The opposite, sex-specific functions of UNR underlie the versatility of RNA binding proteins in the control of gene expression.

2. Translational regulation of early embryonic patterning

The antero-posterior and dorso-ventral patterning systems in Drosophila heavily depend on translational control. Expression of the key morphogens Bicoid and Toll is activated at precise times in development by a process called cytoplasmic polyadenylation. The sequences and factors regulating cytoplasmic polyadenylation in Drosophila are largely unknown. We have used a cell-free cytoplasmic polyadenylation/translation system to study the translational regulation of toll mRNA and have identified novel cytoplasmic polyadenylation elements. We are currently trying to isolate the machinery binding to these elements by RNA-affinity chromatography.

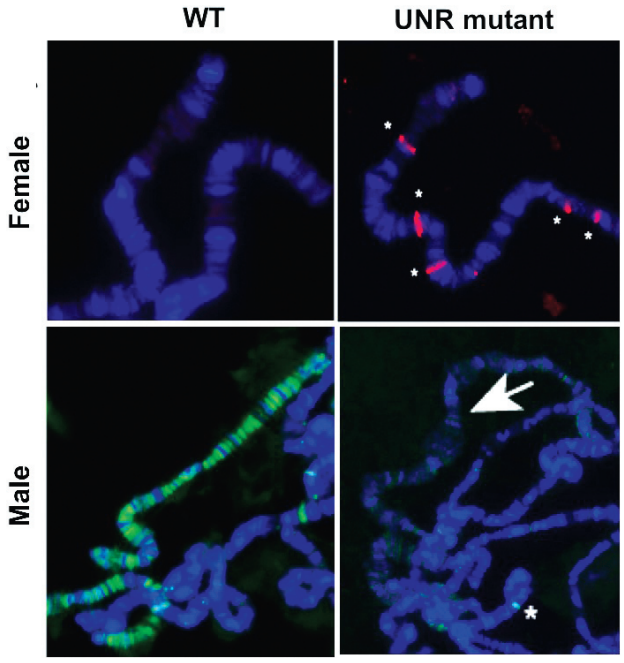

Figure 1.
Double, sex-specific roles of UNR in dosage compensation. The DCC is stained in red (females) or green (males). Mutation of UNR leads to the appearance of the DCC on the female X chromosome, and the dissappearance of the DCC from the male X chromosome.

3. Regulation of p27kip mRNA translation

p27kip (p27) is a tumor suppressor and cell cycle inhibitor whose modulation is essential for appropriate cell proliferation and differentiation. One of the mechanisms that dictate the levels of p27 is the translational regulation of its mRNA. We have found that, contrary to previous reports, p27 mRNA is translated via a cap-dependent mechanism in Hela and HL60 cells. Cap-dependent translation of p27 is regulated by the microRNA miR-181a in HL60 cells, and is necessary for differentiation of these cells into monocytes.

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GENE REGULATION

Group: Translational Control of Gene Expression

Group structure:

Group Leader: Raul Méndez

Postdoctoral Researchers: Isabel Novoa (Ramón y Cajal-awarded), Maria Piqué, David Pineda (Juan de la Cierva awarded), Carolina Segura

Students: Carolina Eliscovich (Graduate Student, PhD. June 2008), Eulalia Belloc (Graduate Student, PhD. December 2008), Ana Igea (Graduate Student), Alessio Bava (Graduate Student)

Technician: Javier Gallego

SUMMARY

The primary interest of our group is to understand the molecular mechanisms that dictate the temporal and spatial translational control of specific mRNAs during cell cycle progression and early embryonic development. Meiotic progression and early development are programmed, at least in part, by maternally inherited mRNAs. These mRNAs are not translated en masse at any one time, or even at any one place; rather, their translation is specifically regulated by sequences located at the 3´-untranslated region (3´-UTR) of the mRNA and their binding proteins. Cytoplasmic polyadenylation is one of the most important mechanisms for regulating translation during meiotic progression and is directly controled by the RNA-binding protein CPEB. The work of our group focuses on four questions in the area of translational regulation by cytoplasmic changes in the poly(A) tail length of mRNAs encoding for factors that drive cell cycle progression: 1) Genome-wide identification of the mRNAs that are regulated by cytoplasmic changes in their poly(A) tail length; 2) Determination of the configuration of cis-acting elements that define the temporal and spatial translational regulation by CPEB; 3) Role of the localized CPE-mediated translational regulation in meiotic progression; and 4) Identification of the cell cycle-related events regulated by the CPEB familly of proteins.

RESEARCH PROJECTS

1. Determination of the 3'-UTR features that define the timing of cytoplasmic polyadenylation and the silencing of an mRNA.

The analysis of the polyadenylation state of the endogenous cyclin mRNAs during meiosis, the capability of numerous 3'-UTR variants to direct translational repression and subsequent cytoplasmic polyadenylation and translational activation, as well as the analysis of the trans-acting factors assembled on specific cis-acting elements, allowed us to define a combinatorial code that can be used to predict the translational behavior of CPE-containing mRNAs (Figure 1). We have translated these rules into regular expressions, to define qualitative and quantitatively whether a given mRNA could be a target for cytoplasmatic polyadenylation control, and performed a computational analysis, identifying hundreds of mRNAs potentially regulated by CPEB, mainly related to cell cycle and cell differentiation, but also to other biological events such as chromosome segregation, synaptic stimulation, embryonic polarity or even implicated in angiogenesis and tumor development.

We are performing a proteomic analysis of the trans-acting factors recruited by the different arrangements of cis-acting elements, defined in our combinatorial code for CPE-mediated translational control, to determine the molecular mechanism that define the phase-specific translational regulation of the different mRNA subpopulations.

2. Sequential waves of polyadenylation and deadenylation drive meiotic progression.

We have found an additional mechanism, overlaying the above-described rules, controlling the phase-specific deadenylation of a subset of CPE-regulated mRNAs (Figure 1). Our results show that an "early" wave of cytoplasmic polyadenylation activates a negative feedback loop, which opposes CPEB activity on mRNAs containing both CPEs and AREs, by activating the synthesis of C3H-4, which in turn recruits the CCR4-deadenylase complex to the ARE-containing mRNAs. These sequential waves of polyadenylation and deadenylation define a circuit of mRNA specific translational regulation that drives meiotic progression. We are following our research on the biological function of C3H-4 in two main directions. First, determining the regulation of C3H-4 by post-translational modifications. Second, performing a genome-wide screening for C3H-4 and CPEB-regulated mRNAs and define their meiotic functions.

3. Cytoplasmic polyadenylation role in the mitotic spindle formation and chromosome segregation during cell division

To determine the relevance of localized CPE-mediated translational control during meiotic progression, we have shown that spindle-localized translational activation, by cytoplasmic polyadenylation, is essential to complete the first meiotic division and also for chromosome segregation in Xenopus oocytes (Figure 2). We are performing functional screenings to identify in a comprehensive manner the mRNAs locally translated by CPEB during spindle formation/maintenance in oocytes and somatic cells.

4. Meiotic translational control by other members of the CPEB family of proteins:

Other than the above mentioned CPEB1, the CPEB family of proteins is composed of other three members. We are working on the mechanisms of translational regulation by other members of the CPEB family of proteins, identifying their target mRNAs and their biological functions in cell cycle control.

5. Translational control of mitotic cell cycle and cancer:

Using the knowledge acquired in Xenopus oocytes, we are trying to determine whether cytoplasmic polyadenylation also regulates cell cycle progression in somatic cells. We are also studying its regulation in normal and tumoral tissues and the physiological relevance of CPEB(s) overexpression in tumor development.

PUBLICATIONS

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"A combinatorial code for CPE-mediated translational control".
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Nature, 452(7190):1017-21 (2008).

Elíscovich C, Peset I, Vernos I and Méndez R.
"Spindle-localized CPE-mediated translation controls meiotic chromosome segregation"
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Belloc E, Piqué M and Méndez R.
"Sequential waves of polyadenylation and deadenylation define a translation circuit that drives meiotic progression".
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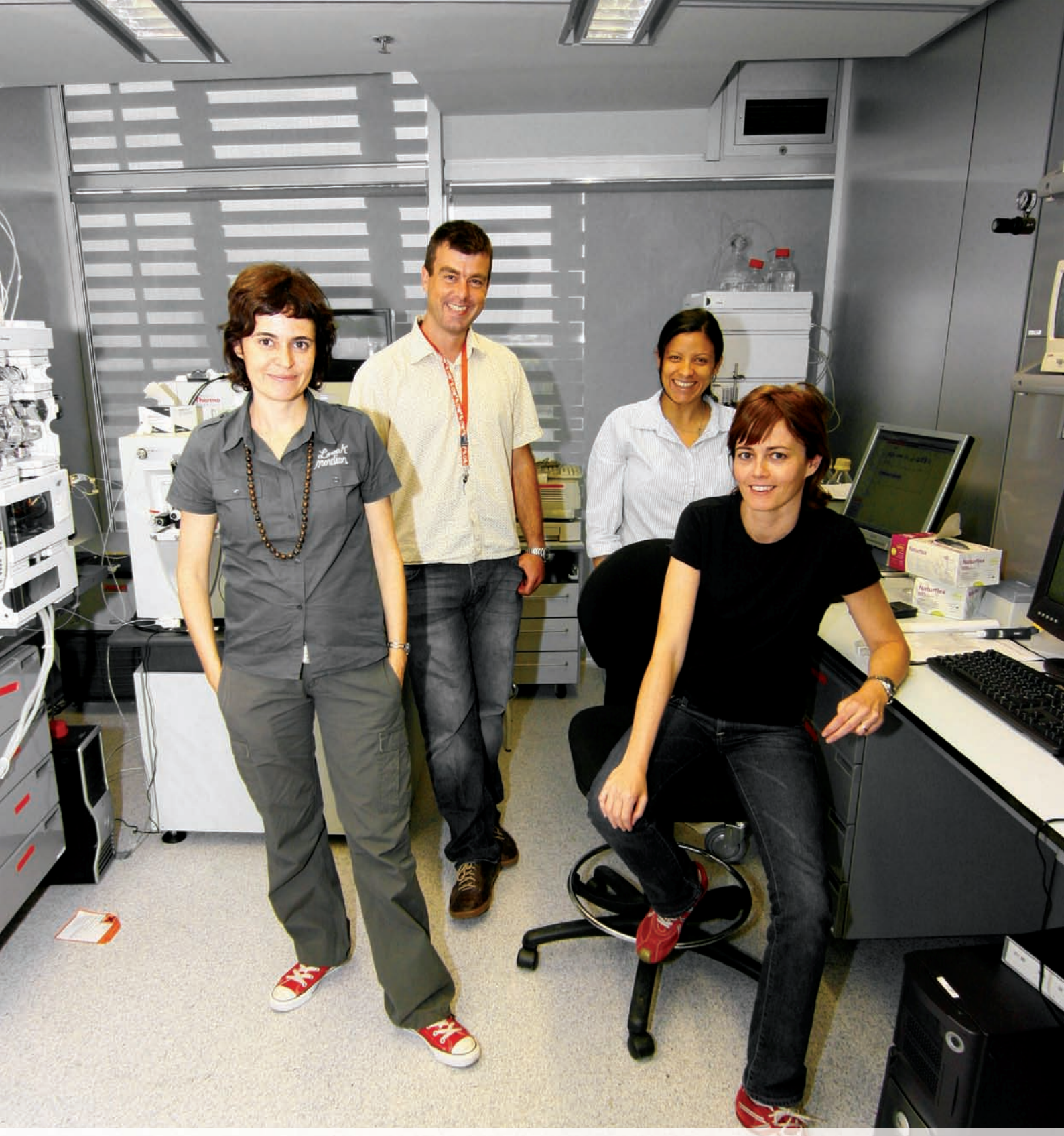


GENE REGULATION

Associated Core Facility: Joint CRG/UPF Proteomics Unit

Unit Structure:

Unit Leader: Henrik Molina, PhD (CRG)

Staff Scientists: Cristina Chivas, PhD, (UPF), Eva Borras, PhD (UPF), Carolina de la Torre, PhD (UPF)

SUMMARY

Proteomics: A genome is defined as an organism's genetic material. The proteome is defined as the complete set of proteins that can be expressed by the genome of an organism.

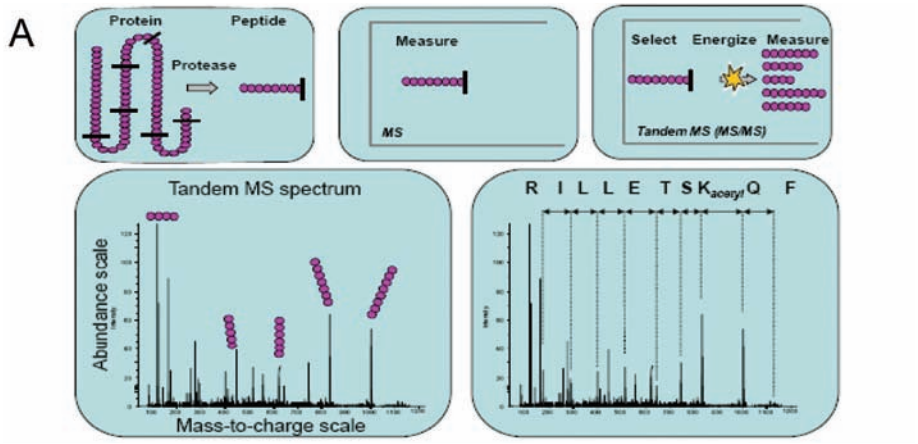
The identity between the genome of a chimps and humans is over 99%. Yet, at the organism level, the differences are drastic and cannot simply be accounted for by the genomic differences alone. Instead, the proteome which are the products of the genes - gene products – accounts for the majority of differences in the phenotype. While the genome is rather confined and static the proteome is dynamic.

Proteomics is a discipline that covers the analysis of expression, localizations, functions, and interactions of the proteome. Where genomics (study of an organism with regard to its genome) to some extend can predict gene products, proteomics allows one to actually identify and measure the proteins in time and in space. Proteomics is thus a very broad area that pertain the study of proteins on a larger scale to elucidate:

I) How genes are combined to give rise to alternative gene products (splicing) or isoforms
II) Where specific proteins are expressed?
III) When they are expressed,
IV) How proteins interact (protein-protein interactions),
V) How proteins are being modified (post-translational modifications), and
VI) The dynamics of proteins with respect to stimuli or other cellular states.

It is safe to say that mass spectrometry has been the driving force for proteomics, and that proteomics has been driving the development of mass spectrometry. Today, close to half of all proteomics studies somehow lean on mass spectrometry.

Figure 1A shows in a schematic form how tandem mass spectrometry typically is applied in the identification of proteins.



EQUIPMENT OF THE JOINT UPF/CRG PROTEOMICS CORE FACILITY.

Currently, the Joint UPF/CRG Proteomics have three mass spectrometric platforms: MALDI-Time-of-Flight, quadrupole Time-of-Flight and a state-of-the-art hybrid ion trap Orbitrap. In addition to mass spectrometers, the facility also houses 1D and 2D gel electrophoresis (proteins) and liquid chromatography (proteins and peptides) based fractionations. The latter techniques are needed for separation of complex samples.

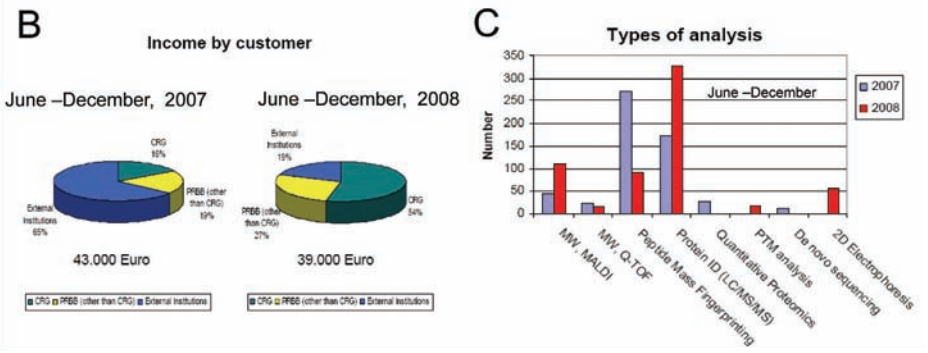
SERVICES TYPE OF OPERATIONS OFFERED

I) Identifications of proteins by either peptide mass finger printing (PMF) or liquid chromatography coupled and tandem mass spectrometry (LC-MS/MS)
II) Determination of molecular mass of molecules.
III) Phosphorylation enrichments and analysis by LC-MS/MS
IV) Quantitation of proteins
V) Chromatographic separation of peptides and proteins
VI) 1D and 2D gel electrophoresis

The users and their samples can be divided into three groups: I) hands-off users that have experience, II) users with samples that requires a more project-type approach and III) hands-on users that are using selected equipment of the Joint UPF/CRG Proteomics Core Facility. Group I) is my far the largest group. However, more and more project are classified as group II) which is a time consuming group.

STATISTICS

The income of the Proteomic Core for 2008 (June 1 to December 1) is approximately the same as for 2007. However, a grand difference is that income originating from CRG customers has tripled, while income from external customers has dropped likewise. Incomes and numbers of samples for 2nd half of 2008 and 2009 are shown in **Figure 1B and 1C.**



AFFILIATIONS

The Joint UPF/CRG Proteomics Core Facility is a member of ProteoRed, the Spanish Proteomics Network.

DIFFERENTIATION AND CANCER

Coordinator: Thomas Graf

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

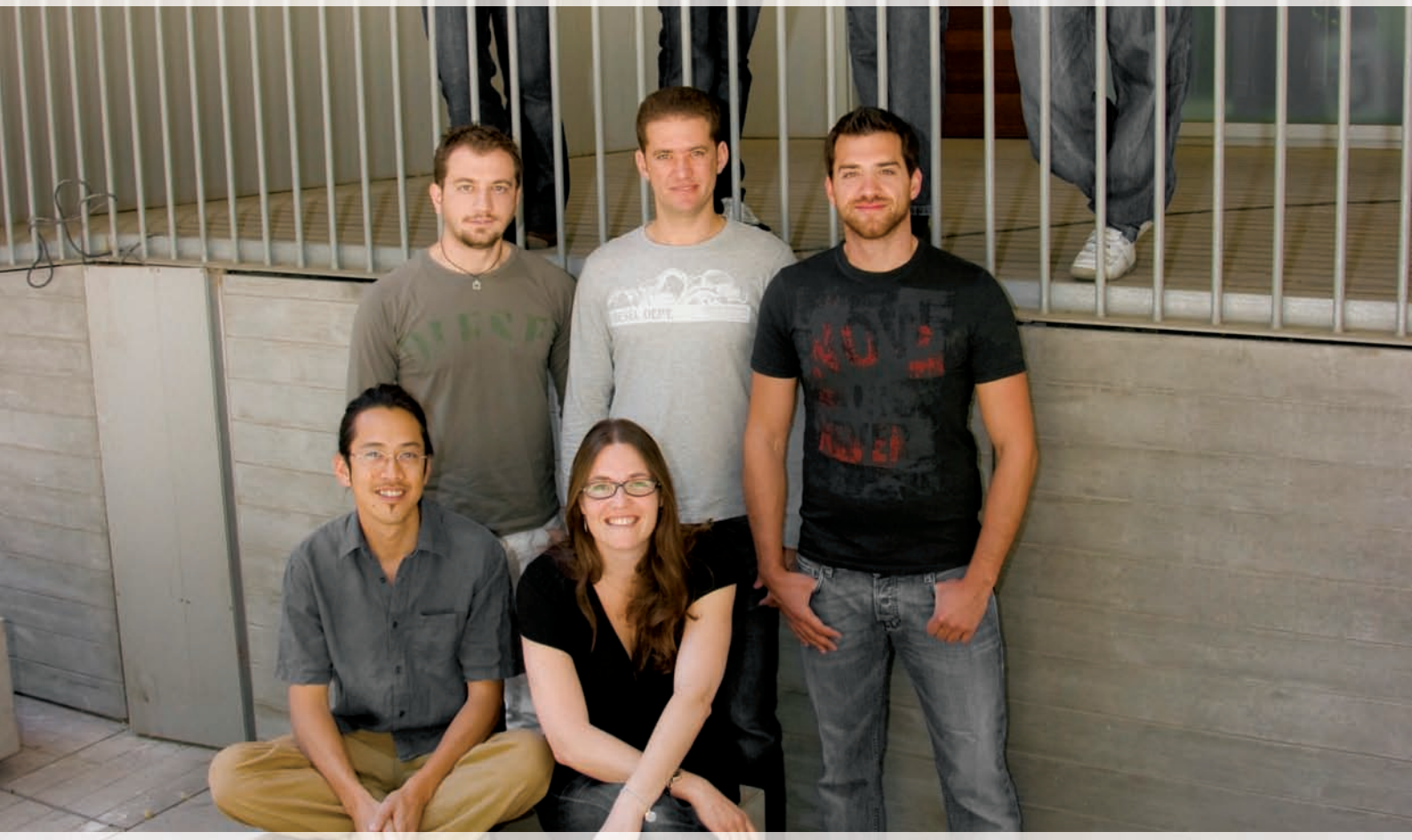
I. Transcription factors and signaling pathways in the development, regeneration and function of muscle cells (Pura Muñoz-Canoves)
II. Epigenetic events in PML-RAR induced leukemia (Luciano Di Croce)
III. Hematopoietic cell differentiation and reprogramming (Thomas Graf)
IV. Epithelial stem cells in the skin and cancer (Salvador Aznar Benitah)

Associated services: Histopathology and FACS Units

During this year Pura Muñoz decided to leave the CRG and join the Pompeu Fabra University to which she moved at the end of the year. The good news is that she has only moved three floors below, and so we hope that we will maintain close relationships. The Group Leaders of the Differentiation and Cancer Programmme thank Pura for her tremendous engagement and work in helping to set up the programme.

We have recently hired a new group leader, William Keyes. His main interest is in the relationship between cellular senescence and cancer of the skin, as well as the role of p53 pathway proteins in epidermal stem cells of the skin. Like the other groups of the programme he works with mammalian cell cultures as well as with mice. He will increase our critical mass in stem cell research and also use shared technologies such as mouse genetics, epigenetic analyses, flow cytometry, fluorescence microscopy, and histopathology. William is at Cold Spring Harbor Laboratory and will join the CRG in the spring of 2009. The programme has also an opening for a Senior Scientist, to replace Pura Muñoz, and we are in the process of conducting interviews with candidates.

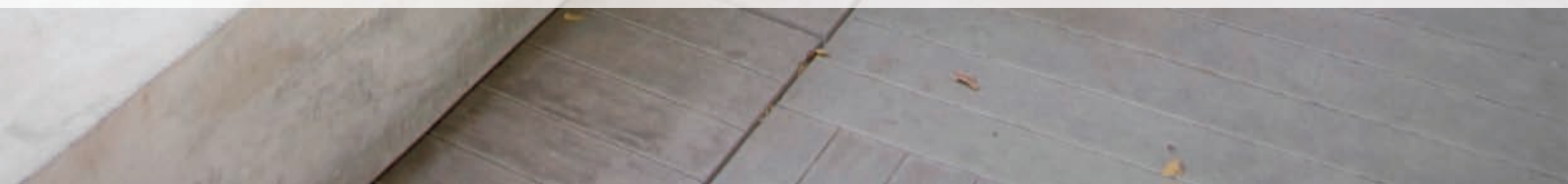
DIFFERENTIATION AND CANCER



Group: Hematopoietic Differentiation and Stem Cell Biology
Thomas Graf is an ICREA Professor.

Group structure:

Group Leader:	Thomas Graf
Postdoctoral Fellows:	Florencio Varas (FIS, CRG), Maribel Parra Bola (Ramon y Cajal), Sabrina Desbordes (Juan de la Cierva), Thien-Phong Vu Manh (HEROIC), Christos Gekas (as of Nov 2008, EMBO)
PhD Students:	Lars Bussmann (DAAD, CRG), Alessandro DiTullio (Leonardo, CRG)
Technicians:	Luisa Irene de Andrés (CRG), Vanessa Chigancas (Plan Nacional), Jose Francisco Infante (CRG)



SUMMARY

The laboratory's main interests are transcription factor-induced transdifferentiation of hematopoietic cells, the role of transcription factors in blood cell differentiation and the biology of hematopietic stem cells.

RESEARCH PROJECTS

1. Mechanisms of reprogramming B lineage cells into macrophages

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, and how these two lineages become established and maintained. To answer this question, we perturb transcription factor networks in committed hematopoietic cells by enforced transcription factor expression.

In earlier work 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 the direct inhibition of the CD19 regulator, Pax5 by C/EBPalpha. That the induced changes are due to a true transdifferentiation and not to the selection of inadvertent myeloid contaminants in the cells examined could be shown by lineage tracing experiments. In addition, the macrophages generated exhibited immunoglobulin rearrangements, again unambiguously showing their B cell origin. A disadvantage of the model was that only a maximum of about 65% of the B cells could be transdifferentiated. To circumvent this problem, we have developed an inducible system that consists of a fusion between C/EBPalpha and the estrogen hormone binding domain. Pre-B cells infected with this construct and treated with beta estradiol are induced to differentiate at an essentially 100% efficiency into cells closely resembling macrophages. This permits to study homogeneous populations of cells that are gradually reprogrammed.

2. Do the C/EBP alpha expressing pre-B cells go back in differentiation before they re-differentiate?

A question of fundamental interest about the mechanism of the induced reprogramming of differentiated cells into another cell type is whether the cells de-differentiate before they are able to re-differentiate into a new cell type. For example, they may have to reset their chromatin organization and perhaps establish an expression profile resembling that of an earlier progenitor. If this is the case, the cells should transiently acquire the phenotype of a multipotent progenitor, such as when Pax5 is inactivated in B cells, as described by Busslinger's lab. We have addressed this question by treating pre-B cells containing C/EBPalphaER for 3, 12, 48 and 120 hours with the inducer, and generating Affymetrix 430.2 expression arrays. Then the data were compared to the expression profiles of various normal progenitors. The rationale was that if the cells retro-differentiate they should transiently express markers of earlier progenitors, such as that of lymphoid myeloid precursors, hematopoietic stem cells or even embryonic stem cells.

Analysis of our expresson arrays revealed no transient activation of ES cell marker genes, including Oct4 or Sox2. Next we compared them to Affymetrix expression arrays of multipotent hematopoietic progenitors. These were obtained through a collaboration with Robert Mansson (University of Lund, Sweden) and consisted of data for long term hematopoietic stem cells (LT-HSC), short term HSC, lymphoid myeloid primed progenitors (LMPPs), common lymphoid progenitors (CLPs), pre-granulocyte macrophage progenitors (pre-GMPs) and GMPs. Unsupervised hierachical clustering did not show any similarities between the intermediate stages of pre-B cells in the process of transdifferentiation and the various hematopoietic progenitors. We then determined the gene expression signature for the different progenitors and asked whether there is a selective upregulation of these genes during transdifferentiation above the background of statistical variations in gene expression. Our results showed that there was no significant upregulation of HSC, LMPP or CLP genes, but that a subset of GMP specific genes was upregulated transiently relatively late in the transdifferentiation process (2 days after induction). These genes became downregulated in artificial, 5 day induced macrophages and were also not expressed in normal macrophages. From these observations we conclude that the C/EBP alpha induced transdifferentiation of pre-B cells into macrophages is a direct process, leading to the formation of myeloid cells without

passing through the stage of a multipotent progenitor. The reason why pre-B cells first activate GMP specific genes but then differentiate in macrophages but not granulocytes, a cell type closely related to macrophages and whose formation requires C/EBPa, needs to be investigated.

3. Do the C/EBP alpha induced cells become stably committed to a macrophage fate once the inducer is withdrawn?

A key feature of cell reprogramming and transdifferentiation is that the induced change in phenotype becomes stable, even when the transcription factor(s) is no longer active. This is important as otherwise it would be still possible that the changes seen merely reflect the activation of downstream targets of the transcription factor. To test this we studied a transdifferentiation system that we recently developed, and that consists of a B cell line expressing C/EBPalphaER, called C10. This line can be induced to transdifferentiate at a 100% efficiency after a 2 to 3 day treatment with beta estradiol. Characterization of 3-day induced C10 cells showed that they are large, granulated, adherent, polarized (Fig.1) and exhibit an increase in their migratory activity as seen by time-lapse microscopy. During the process the cells also become phagocytic and down and upregulate thousands of genes, including most of the known B cell and macrophage functional markers. Remarkably, they also exhibit a reciprocal regulation of B cell and macrophage associated transcription factor genes, showing that the regulatory networks of the two cell types can be disassembled and re-assembled again. Using this system we asked what happens when the cells are exposed for limited times to the inducer, washed to become inducer-free, and cultured again. Our results showed that a 24 to 48 hour treatment with beta estradiol is sufficient to commit most or all of the cells, respectively. This demonstrates that C/EBPa indeed induces a true transdifferentiation of B cells into macrophages. Interestingly, a 6-hour treatment of the cells with the inducer leads to a transient upregulation of Mac-1 but not to a CD19 downregulation. These observations support the idea, developed in our earlier work, that activation of the macrophage program is, at least initially, independent of the extinction of the B cell program. Mechanistically this is probably because C/EBP alpha, together with endogenous PU.1, can rapidly activate macrophage genes and that to obtain a persistent extinction of B cell genes the level of Pax5 protein must fall below a certain threshold, something which requires relatively long exposure times with activated C/EBP alpha. Using C10 cells we are currently study changes in epigenetic modifications, requirement for cell division and other basic questions that address the mechanism of transdifferentiation in a well defined model system.

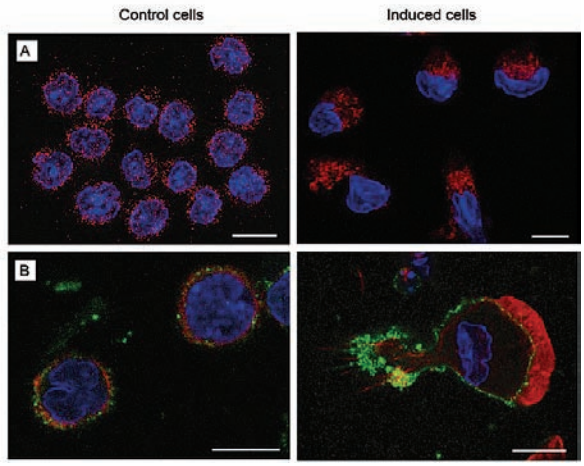


Fig.1.
Changes in cell morphology during C/EBPa induced transdifferentiation of C10 A, staining of Golgi apparatus with antibodies against mannosidase II (red) and nuclei with DAPI (blue). B, staining of cells with antibodies to cell surface antigens (green), with phalloidin-Alexa Fluor 568 for F actin (red) and with DAPI for nuclei (blue). The green fluorescence in the control cells represents CD19 antigen; the green fluorescence in the induced cell represents Mac-1 antigen. Left panels: uninduced cells; right panels cells induced for 3 days. Bars, 10 um.

4. Four transcription factors are sufficient to reprogram fibroblasts into pluripotent cells

Several laboratories have reported the reprogramming of mouse and human fibroblasts into pluripotent ES -like cells, using retroviruses carrying the Oct4, Sox2, Klf4 and Myc transcription factor genes. In these experiments the frequency of reprogramming was typically less than 0.1% of the infected cells, raising the possibility that retroviral integrations activate an endogenous gene(s) additionally required to induce reprogramming. If a putative fifth factor could be identified, this should make reprogramming much more efficient. To study this question, we collaborated with Konrad Hochedlinger (Harvard), who provided us with the DNA of a number of iPS cell clones. To identify insertion sites we LM-PCR amplified DNA fragments from six fibroblast derived iPS cell clones and in a first round of experiments performed bacterial cloning. After realizing that this approach was not saturating we used high throughput sequencing to exhaustively map the retroviral integration sites. Seventy-nine integration sites were identified that mapped to gene transcription units or that were within gene rich areas. No common integration site was detected between the six iPS clones studied. Moreover, bioinformatic analyses, conducted in collaboration with Cedric Notredame (CRG), revealed no enrichment for a common network or pathway. We concluded that the transcription factors Oct4, Sx2, Klf4 and Myc are sufficient to induce the cell reprogramming and propose that the observed low reprogramming sequences commonly observed have alternative explanations. Since our study was completed two other laboratories showed that it is possible to reprogram cells with the four factors in the absence of genetic modifications of the host cells, reaching essentially the same conclusion.

5. The role of CD41 in hematopoietic stem cells

Hematopoietic stem cells (HSCs) are widely used for clinical purposes to regenerate the hematopoietic system and there is an intense interest to understand their biology. One of the still poorly explored areas is which cell surface determinants are important for their development, homing and mobilization. The earliest cell surface marker of HSCs is the CD41 antigen, corresponding to the alpha 2b chain (CD61) that associates with the beta 3 chain to form a functional integrin. However, although CD41 (as well as CD61) can be detected on HSCs, the role of the integrin in these cells remained elusive. We have previously generated a knockout mouse lacking the alpha 2b locus, and have now studied the HSCs of these mice. Preliminary data showed that they exhibit a reduction in the number of long term repopulating HSCs and a concomitant increase in short term HSCs. These mice are currently being studied in attempts to determine the mechanism by which CD41 determines the ratio between the two types of stem cells.

PUBLICATIONS

Feng R, Desbordes SC, Xie H, Tillo ES, Pixley F, Stanley ER and Graf T.
"PU.1 and C/EBPalpha/beta convert fibroblasts into macrophage-like cells."
Proc Nat Ac Sci USA, 105:6057-6062 (2008).

Graf T.
"Immunology: blood lines redrawn."
Nature, 452:702-703 (2008).

Graf T.
"Lymphoid myeloid lineage specification."
Semin Immunol, 20:205-206 (2008).

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"B young again."
Immunity, 28:606-608 (2008).

Graf T and Stadtfeld M.
"Heterogeneity of embryonic and adult stem cells."
Cell Stem Cell, 3:480-483 (2008).

Varas F, Stadtfeld M, De Andres-Aguayo L, Maherali N, di Tullio A, Pantano L, Notredame C, Hochedlinger K and Graf T.
"Fibroblast derived induced pluripotent stem cells show no common retroviral vector insertions."
Stem Cells, 2008 Nov 13. [Epub ahead of print].

DIFFERENTIATION AND CANCER

Group:	Myogenesis
Group structure:	
Group Leader:	Pura Muñoz Cánoves
Ramón y Cajal Investigator:	Antonio Serrano
Postdoctoral Fellows:	Eusebio Perdiguero, Mónica Zamora, Esther Ardite, Roberta De Mori
PhD Students:	Vanessa Ruíz-Bonilla, Berta Vidal, Pedro Souza-Victor, Elisa de Lorenzo
Technicians:	Mercè Jardí, Vera Lukesova

SUMMARY

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. The 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. 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)/fibrinolytic system in skeletal muscle regeneration (after injury or in inherited myophinopathies) and in fibrosis development. Finally, we aim to analyze the mechanisms involved in the regulation of adult physiological muscle growth. In summary, our laboratory is pursuing three main lines of research:

I. Control of muscle-specific gene transcription by p38 MAP kinases.
II. Role of fibrin in fibrosis development during muscle dystrophy progression.
III. Molecular mechanisms regulating adult skeletal myofiber growth.

RESEARCH PROJECTS

1. Control of myogenesis 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 p38 MAP kinase signalling pathway plays a pivotal role in myogenesis by controlling muscle-specific genetranscription. However, the role of this pathway (and the relative contribution of the different p38 isoforms) in muscle function in vivo remains unknown.

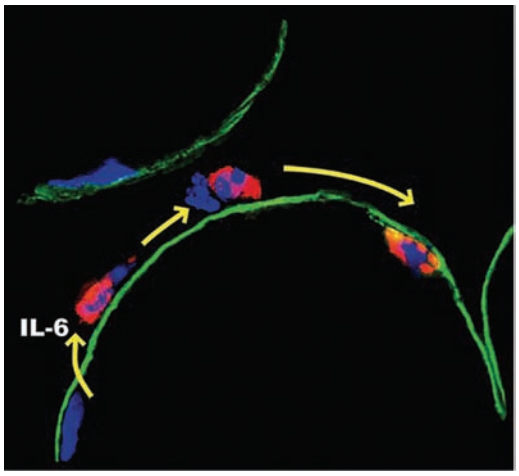
- p38alpha (but not p38beta, p38gamma or p38delta) is necessary for myogenesis. p38alpha controls myoblast proliferation both in vitro as in neonatal muscle. Indeed, myoblasts derived from p38alpha-deficient mice show persistent proliferation, and a subsequent block in myoblast differentiation. This 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. Furthermore, our results demonstrate that p38beta, p38gamma and p38delta are dispensable for injury-inducuced skeletal muscle regeneration in vivo.

2. Role of fibrin in fibrosis development during muscle dystrophy progression.

Our results have demonstrated that fibrinogen, through induction of IL-1b, drives the synthesis of TGFbeta by mdx macrophages, which in turn, induces collagen production in mdx fibroblasts. Fibrinogen-produced TGFbeta further amplifies collagen accumulation through activation of pro-fibrotic alternatively activated macrophages. These data unveil a pro-fibrotic role of fibrinogen deposition in muscle dystrophy by inducing TGFbeta production by alternatively activated macrophages, and suggest for the first time the implication of alternatively activated macrophages in dystrophic muscle fibrosis.

3. Molecular mechanisms involved in the regulation of adult skeletal muscle growth.

Skeletal muscles adapt to increasing workload by augmenting their fiber size, through yet poorly understood mechanisms. We identified identified the cytokine IL-6 as an essential regulator of satellite cell-mediated hypertrophic muscle growth. IL-6 is locally and transiently produced by growing myofibers and associated satellite cells (muscle stem cells), and genetic loss of IL-6 blunted muscle hypertrophy in vivo. IL-6 deficiency abrogated satellite cell proliferation and myonuclei accretion to the preexisting myofiber, by impairing STAT3 activation and expression of its target gene cyclin D1.



Muscle-released IL-6 is necessary for incorporation of adult muscle stem cells during skeletal muscle growth. The composition is based on confocal images of a growing muscle section labeled for myofiber membrane (green), muscle stem cell (red) and nuclei (blue). Illustration by E. Perdiguero and A.L. Serrano.

PUBLICATIONS

Vidal B, Serrano AL, Tjwa M, Suelves M, Ardite E, De Mori R, Baeza B, Martínez de Lagrán M, Ruiz-Bonilla V, Jardí M, Gherardi R; Degen J, Christov C, Dierssen M, Dewerchin M, Carmeliet P, Muñoz-Cánoves P.
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"Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy."
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Ruiz-Bonilla V, Perdiguero E, Gresh L, Serrano AL, Zamora M, Sousa-Victor P, Jardí M, Wagner EF, Muñoz-Cánoves P.
"Efficient adult skeletal muscle regeneration in mice deficient in p38beta, p38gamma and p38delta MAP kinases."
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Serrano AL, Jardi M, Suelves M, Klotman PE, Muñoz-Cánoves P.
"HIV-1 transgenic expression in mice induces selective atrophy of fase-glycolytic skeletal muscle fibers."
Front Biosci, 13:2797-805 (2008).

Book Chapters

Perdiguero E, Muñoz-Cánoves P.
"Transcriptional regulation by the p38 MAPK signaling pathway in mammalian cells."
In: Stress-Activated Protein Kinases. Top Curr Genet, 20:51-79 (2008).

DIFFERENTIATION AND CANCER

Group: Epigenetics events in cancer

Luciano Di Croce has an ICREA Group Leader position.

Group structure:

Group Leader: Luciano Di Croce

Postdoctoral: Marcus Buschbeck, Holger Richly, Celia Jeronimo, Elisabeth Simboeck, Luciana Rocha Viegas, Martin Lange

PhD Students: Santiago Demajo, Sophia Teichmann, Iris Uribesalgo Micás, Joana Ribeiro

Visiting PhD Student: Paola Pisano

Technician: Arantxa Gutierrez

SUMMARY

Understanding the genetic basis of cancers has been a topic of intense research, and hundreds of gene mutations have been identified that can cause carcinogenesis. However, in the past few years, increasing evidence has suggested that mutations are not the only genetic changes that lead to cancer. Indeed, perturbations of chromatin structure and of other epigenetic mechanisms can cause inappropriate gene expression and genomic instability, resulting in cellular transformation and malignant outgrowth.

Our research investigation is focused on understanding the role of several protein complexes that are involved in chromatin dynamics and metabolism, which when altered could participate in the establishment and maintenance of the aberrant silencing of tumor suppressor genes during transformation. Our results suggested that the Nucleosome Remodelling and Deacetylase complex (NuRD), Polycomb group of proteins (PcG) and the histone variant macroH2A are - with different timing and kinetics - involved in setting up an altered chromatin structure with aberrant gene silencing in acute promyelocytic leukemia (APL). We believe that the results of our research will lead to the identification of new biological tools with a potential impact on cancer therapeutic intervention.

RESEARCH PROJECTS

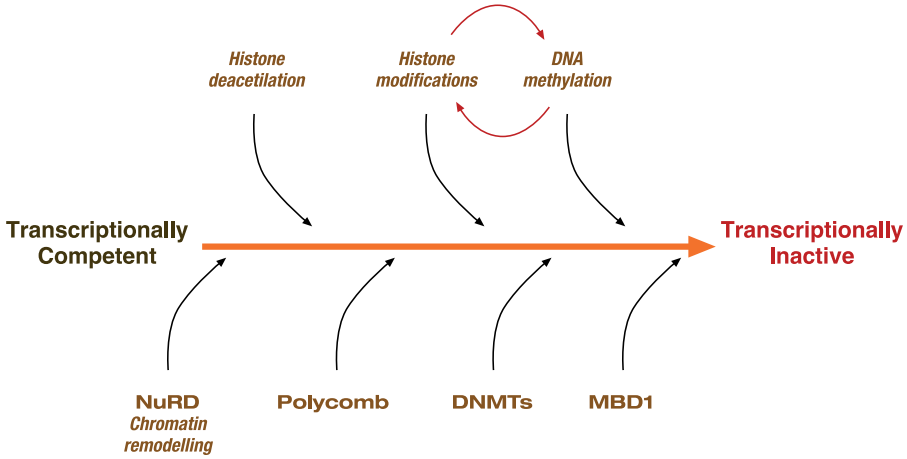
1. Polycomb and cancer

Polycomb repressive complex 2 has been strongly implicated in cancer development, but to date mechanistic insight into the function of PRC2 in cancer cells is lacking. In addition, in mammalian cells, it is not well understood how PRC2 is targeted to promoter regions. Using as paradigm the oncogenic transcription factor PML-RARa, we have investigated the role of Polycomb group proteins in the establishment and maintenance of the aberrant silencing of tumor suppressor genes during transformation induced by the leukemia-associated PML-RARa fusion protein. We show that in leukemic cells knockdown of SUZ12, a key component of Polycomb repressive complex 2 (PRC2), reverts not only histone modification but also induces DNA de-methylation of PML-RARa target genes. This results in promoter re-activation and granulocytic differentiation. Importantly, the epigenetic alterations caused by PML-RARa can be reverted by retinoic acid treatment of primary blasts from leukemic patients. Our results demonstrate that the direct targeting of Polycomb group proteins by an oncogene plays a key role during carcinogenesis.

2. Biochemical link between epigenetic marks and chromatin remodelling

In plants, as in mammals, mutations in SNF2-like DNA helicases/ATPases were shown to affect not only chromatin structure but also global methylation patterns, suggesting a potential functional link between chromatin structure and epigenetic marks. The SNF2-like ATPase containing nucleosome remodelling and deacetylase corepressor complex (NuRD) is involved in gene transcriptional repression and chromatin remodelling. As mentioned above PML-RARa represses target genes through recruitment of DNMTs and Polycomb complex.

We have recently investigated a direct role of the NuRD complex in aberrant gene repression and transmission of epigenetic repressive marks in acute promyelocytic leukemia (APL). Our results indicate that PML-RARa binds and recruits NuRD to target genes, including to the tumor-suppressor gene RARß2. In turn, the NuRD complex facilities Polycomb binding and histone methylation at lysine 27. Retinoic acid treatment, which is often used for patients at the early phase of the disease, reduced the promoter occupancy of the NuRD complex. Knock-down of the NuRD complex in leukemic cells not only prevented histone deacetylation and chromatin compaction, but also impaired DNA and histone methylation as well as stable silencing, thus favouring cellular differentiation. These results unveil an important role for NuRD in the establishment of altered epigenetic marks in APL, demonstrating an essential link between chromatin structure and epigenetics in leukemogenesis.

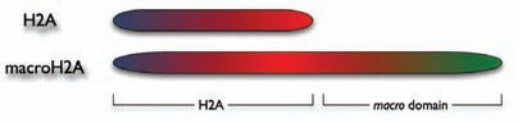


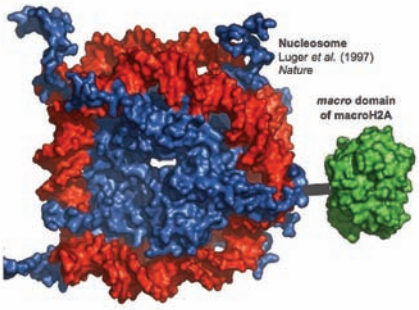
3. macroH2A and gene silencing

The most extensive histone modification is the complete exchange of canonical histones for variant ones. Among all known histone variants, the so-called macroH2A is the one that is the most divergent from its canonical histone and is the least understood in its function. In addition to a homologous histone domain, macroH2A possesses a large C-terminal domain of unknown function – the macro domain.

The H2A domain of macroH2A histones is ~65% identical to that of the conventional H2A.

The early observation that macroH2A is enriched on the inactive X chromosome and centrosomes suggested an involvement in gene repression and heterochromatinization. Biochemical studies using synthetic templates have indicated that macroH2A-containing nucleosomes are structurally different in the vicinity of the dyad axis, and this correlates with the inability of transcription factors to bind to DNA sites inserted nearby. These observations suggest that incorporation of macroH2A into nucleosomes could confer an epigenetic mark for gene repression. However, there are no known gene targets for macroH2A-dependent transcriptional repression, and no evidence for the mechanisms by which macroH2A could be recruited to specific genes and repress transcription in vivo.





Our ChIP-on-chip analysis, using Agilent microarray, shows that macroH2A is deposited in the regulatory regions of tumor suppressor genes and genes regulated by Polycomb group in an isoform-specific fashion. We are studying the role of macroH2A in transcriptional regulation of these genes during cell fate decisions and cancer development.

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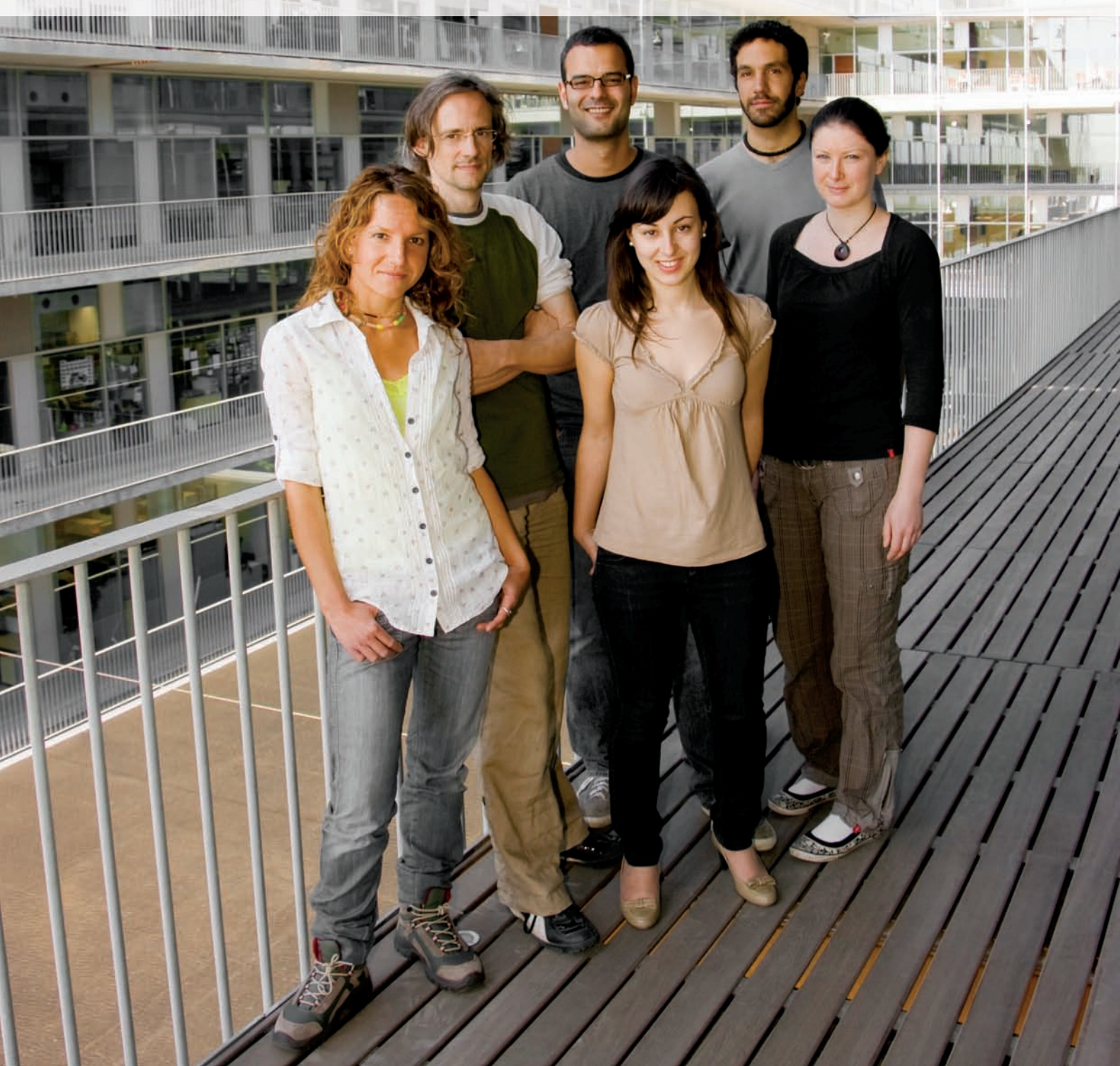
DIFFERENTIATION AND CANCER

Group: Epithelial Homeostasis and Cancer

Salvador Aznar Benitah has a Junior ICREA Group Leader position.

Group structure:

Group Leader:	Salvador Aznar Benitah
Postdoctoral:	Lluís Riera
PhD Students:	Peggy Janich, Nuno Silva
Technicians:	Bernd Kuebler, Gloria Pascual
Masters Student:	Lara de Llobet



SUMMARY

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.

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/Rac1flox/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/Rac1flox/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).

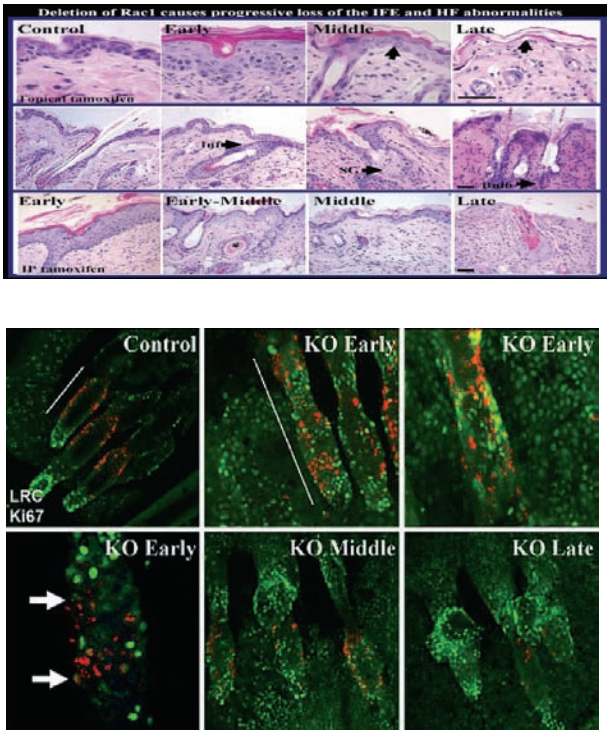


Figure 1
Inducible deletion of Rac1 in adult epidermis of K14CreER/Rac1flox mice. Upper Panel: Loss of Rac1 causes an early hyperproliferative phase (Early) followed by irreversible terminal differentiation (Middle). Ultimately, the epidermis looses its self-renewal capacity and only remnants of the 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).

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 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 kept in a stem cell state or a transit amplifying state. Since the Rac pathway is important for balancing epidermal homeostasis, in addition, we are using a proteomic approach to identify proteins that interact with members of this signalling cascade. We are combining these two approaches, microarray data and proteomics, to identify new players relevant for different aspects of epidermal stem cell behaviour. Selected targets are being validated using cellular and molecular biology tools with primary cultures, as well as with our in vivo mouse models.

3. Studying novel pathways relevant to epidermal and squamous 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.

In collaboration with the Hospital del Mar we are obtaining samples of SCCs (fresh live tissue, and blocks for immunohistological analysis) at different stages of tumour progression. Squamous cell carcinomas are the most diagnosed types of tumours in western countries with poor prognosis when developed in the oral cavitiy. We are analyzing the status of the different pathways studied in the lab with respect to their possible role in squamous cancer stem cells and validating the results using our mouse models.

PUBLICATIONS

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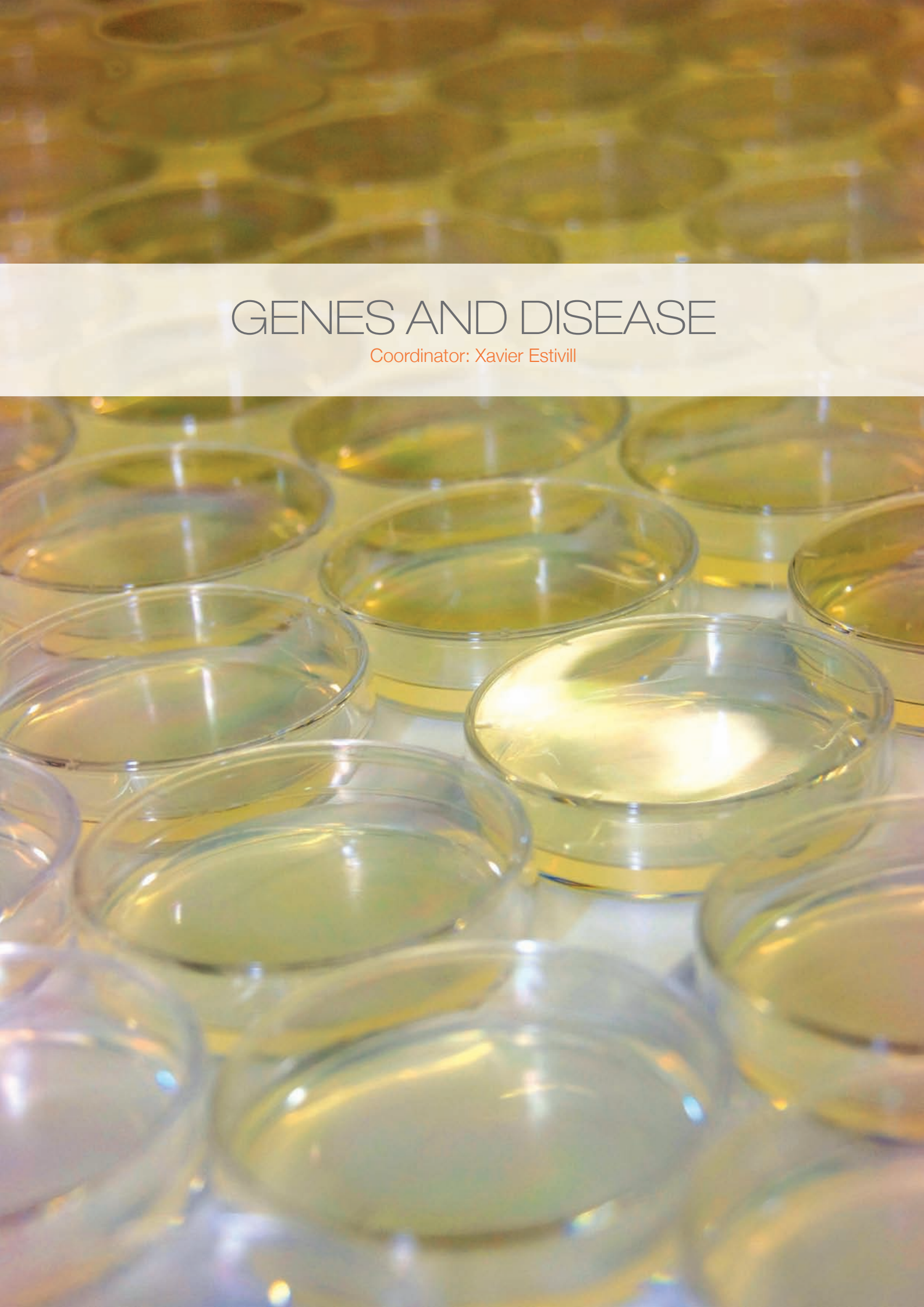
GENES AND DISEASE

Coordinator: Xavier Estivill



The mission of the Genes and Disease Programme is to perform research on the molecular basis of human disease, from the discovery of genes involved in common human disorders to the development of preventive and therapeutic strategies. The Programme combines large-scale experimental approaches with advanced genetic strategies to elucidate biological determinants of common human disease.

Efforts of the Genes and Disease Programme researchers are focused on analysing sequence and genomic variants of the human genome that could participate in the predisposition to disease. Specific collective work within the Programme is focused on understanding the function of genes with potential implications in mental retardation and neurodegeneration, by using cellular and animal models. Programme researchers use murine models to develop therapeutic approaches that could correct features involved in mental retardation, anxiety disorders and cancer.

Translational research is a primary concern of many Genes and Disease members. To this aim, the Programme Group Leaders are part of the Rare Disorders and Epidemiology and Public Health Biomedical Research Networks, of the Spanish Medical Research Council, and other biomedical research networks at the regional, national or international levels. The programme has currently five research groups and will expand with new groups in the field of medical systems biology in the next years. An associated core facility on genomics is under the Genes and Disease Programme, with a special emphasis on high-throughput genotyping and massive sequencing.

Current Structure of the Programme:

Research Groups:

> Murine Models of Disease (Mariona Arbonés)
> Neurobehavioral Analysis (Mara Dierssen)
> Genetic Causes of Disease (Xavier Estivill)
> Gene Therapy (Cristina Fillat)
> Gene Function (Susana de la Luna)

Associated Core Facility:

> Genomics Unit (Mònica Bayés)
    > Genotyping Unit (Mònica Bayés)
    > Ultrasequencing Unit (Heinz Himmelbauer)

GENES AND DISEASE

Group: Genetic Causes of Disease

Group Structure:

Group Leader: Xavier Estivill

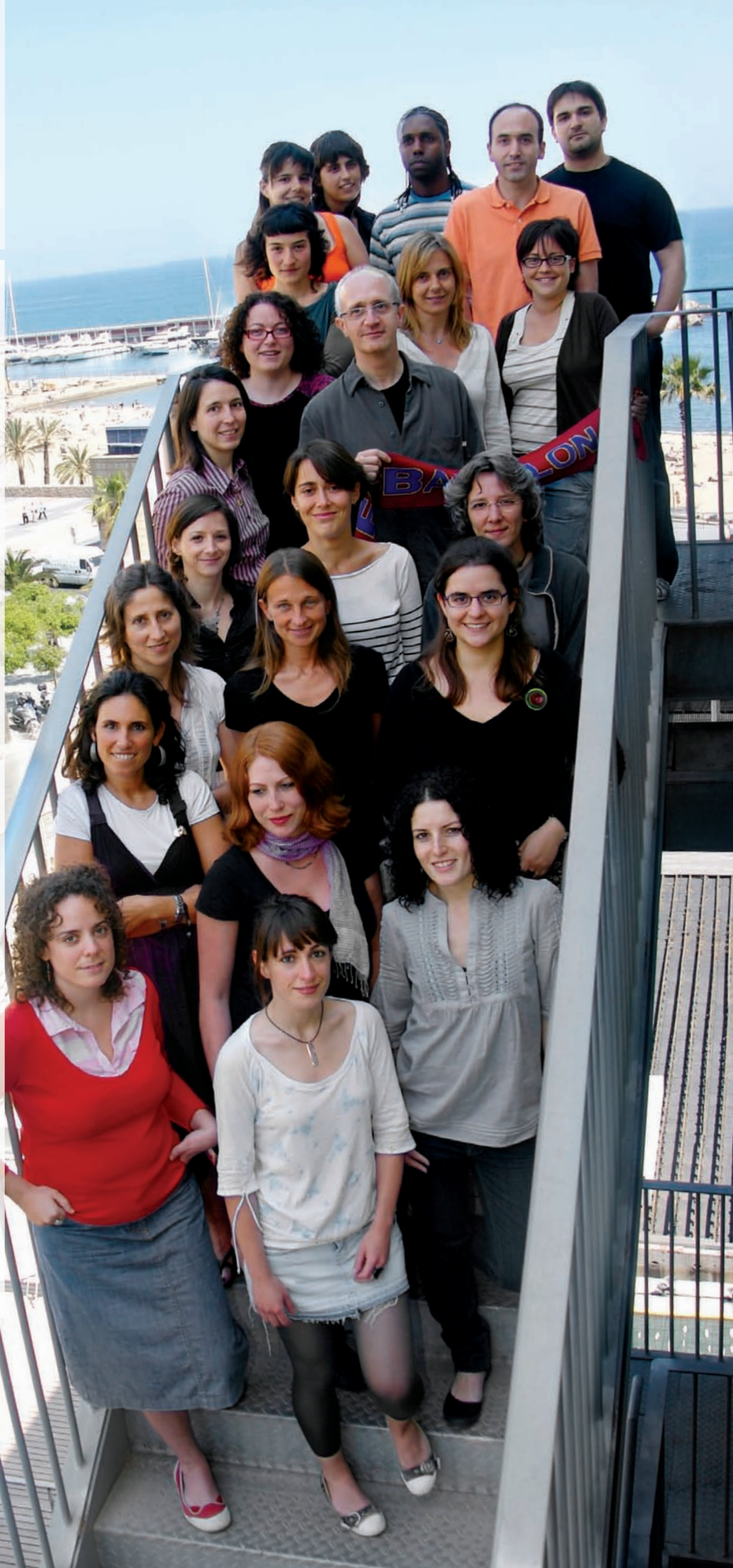
Staff Scientist: Eulàlia Martí

Scientific Officer: Veronique Blanc *(since September 2008)*, Joanne Gartlon *(February – June 2008)*

Postdoctoral Fellows: Lluís Armengol *(until December 2008)*, Mariona Bustamante, Mónica Bañez-Coronel, Mario Cáceres *(Ramón y Cajal)*, Yolanda Espinosa *(Ramón y Cajal) (until December 2008)*, Mònica Gratacòs *(CIBERESP)*, Kelly Rabionet *(Ramón y Cajal)*, Eva Riveira *(CIBERESP)*

PhD Students: Johanna Aigner *(since July 2008)*, Laia Bassaganyas *(since September 2008)*, Nina Bosch *(until February 2008)*, Elisa Docampo, Monica Guidi *(until March 2008)*, Susana Iraola, Alexander Martínez *(since October 2008)*, Elisabet Mateu *(since July 2008)*, Elena Miñones, Margarita Muiños, Lorena Pantano, Raquel Rubio *(since May 2008)*, Ester Saus

Technicians: Anna Carreras, Georgia Escaramís *(CIBERESP)*, Manel García, Birgit Kagerbauer, Marta Morell Silvia Porta *(CIBERESP)*, Sergi Villatoro



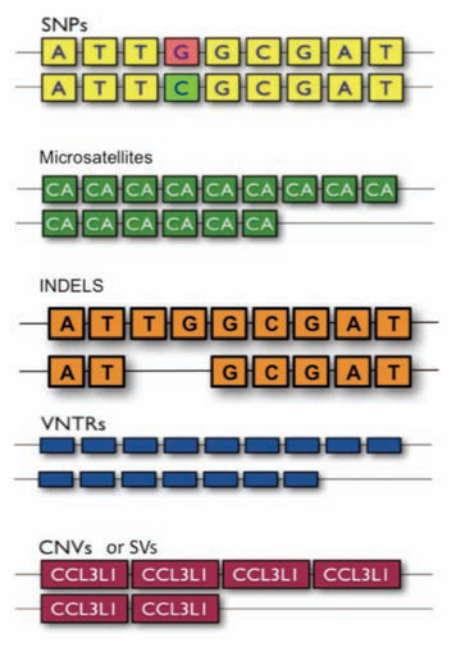
SUMMARY

We are interested on how the variability of the human genome at the nucleotide and structural levels contributes to common human disease. The availability of sequence information of the genomes of several individuals has provided the view that genetic variability is much larger than initial estimations. Genomic variability has different forms, from nucleotide changes, commonly referred as single nucleotide polymorphisms (SNPs) to structural variations (copy number variants or CNVs, and insertion/deletion (in-del) variants). Our work thus aims to understand how these variants influence common human disorders. We are also interested in the study of epigenetic modifications and small non-coding RNA pathways, as susceptibility factors in complex disorders. We are approaching these questions through the use of genotyping platforms that explore SNPs and CNVs and use deep sequencing for the characterisation and analysis of small non-coding RNAs. Functional studies are being used to address the consequences of the genetic and epigenetic changes in cell physiology and pathology. The study of a wide range of disorders in which the interaction between genes and environment seems to play an important role in the development of the disease status is a major focus of our research.

RESEARCH PROJECTS

1. Copy number variants, segmental duplications and genome structure

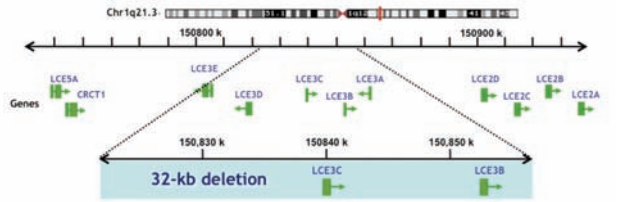
Large-scale segmental duplications (SDs) have played an important role in hominoid evolution and can be hotspots for non-allelic homologous recombination leading to deletion, duplication, inversion or translocation. Many of these SDs coincide with structural variations or copy number variants (CNVs) of the human genome (Figure 1). We are studying the genomic organisation of regions with structural variation in samples from different ethnic groups. We are also evaluating the nature of sequences contained in SDs and CNVs, including repetitive elements, transposable sequences and non-coding RNAs. Ongoing efforts will involve the evaluation of piRNAs in structural variations and their role in protecting the DNA sequence during important phases of the cell life.



2. Structural variations and human disease

After several successful genome-wide association (GWA) studies searching for genetic variants involved in common human disorders, it has become evident that a large proportion of the genetic component of human traits and disease does not reside in SNP variability. The attention of many investigators is now focusing on structural variation. We are systematically performing comparative genomic hybridization (CGH) experiments and we are using multiple technologies to further analyse SDs and CNVs at the genome scale for several common disorders. CNVs with specific profiles for several common disorders

are being investigated. Genes located within sequences containing structural variants are object of major attention by the group. Disorders that are studied include schizophrenia, stroke, psoriasis, multiple sclerosis and asthma. We have made specific progress with psoriasis which is a chronic disorder of the skin affecting most ethnic groups, but with the highest prevalence (3%) in northern European populations. We have identified several CNVs that are associated with psoriasis. One of them is within the epidermal differentiation complex, in a region of chromosome 1q21 (Figure 2), previously known to be linked to psoriasis. A deletion of two genes (LCE3B and LCE3C) has been found to be significantly more common in psoriasis than in unaffected subjects. The lack of these two genes, highly expressed in the injured epidemis, brings skin barrier alteration as a new player in psoriasis susceptibility.

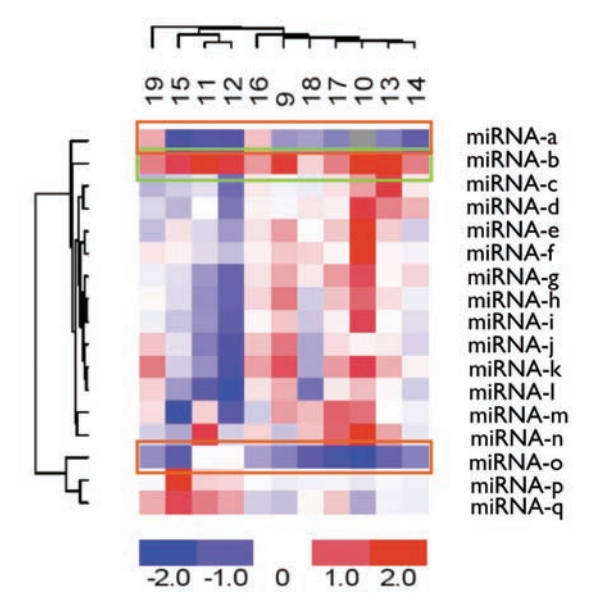


3. Genetic variants associated to psychiatric disorders

We have analyzed several psychiatric diagnostic categories (substance-abuse, anxiety, eating, psychotic and mood disorders) for potential functional SNPs in genes involved in neurotransmission and/or neurodevelopment. We found that multiple SNPs are strongly associated with several disorders. SNPs in BDKRB2 were associated with panic disorder, substance abuse, bipolar disorder, obsessive-compulsive disorder and major depression. Moreover, we have identified SNPs in the genomic regions of NGF, NTRK1, BDNF, NTRK2, NTF3, NTRK3, CNTF, CNTFR, NT4-5 and p75 for case-control or family-based association studies in patients with anxiety disorders, schizophrenia, eating disorders, affective disorder and substance abuse disorder. For eating disorders, we found that association with NGFB and NTRK3, in addition to the previous reported findings for BDNF and NTRK2, points to neurotrophin signalling genes as susceptibility factors for eating disorders. For obsessive-compulsive disorder we have genotyped tagging SNPs covering the BDNF and NTRK2 regions in patients and control subjects.
A significant association with an NTRK2 intronic SNP was identified. A protective haplotype in NTRK2 was also detected in female patients. Additionally, Haplotype analysis revealed a significant association between obsessive-compulsive disorder and a protective haplotype located toward the 5' of the BDNF gene containing the functional valine (Val)66-to-(Met) variant. These findings support a role for the BDNF/NTRK2 signalling pathway in genetic susceptibility to obsessive-compulsive disorder.

4. Functional genomics of neurological disorders

Small non-coding RNAs are involved in the guidance of diverse types of gene regulation, typically resulting in reduced expression of target genes. MicroRNA (miRNA) are a major class of non-coding RNAs. They are ubiquitously expressed and are believed to regulate most biological processes in a tissue- and temporal-specific manner. They are key players in the development of the central nervous system. miRNAs also play a role in mature neurons, participating in synaptic plasticity and neuronal survival. The group is exploring the contribution of small silencing RNA pathways in the aetiology and progression of neurodegenerative disorders, including Huntington's disease and Parkinson's disease. We have used Illumina/Solexa deep sequencing to analyse and characterize small non-coding RNAs in different affected and non-affected brain areas of individuals with these disorders (Figure 3). The majority of reads corresponded to previously annotated miRNAs. Our most recent results suggest that miRNAs signatures may contribute to the maintenance of the transcriptome in the different brain areas. Our studies highlight that, in addition to a strong miRNA expression deregulation in highly affected brain areas, some miRNAs are already deregulated at early stages of the disease. Therefore, selective miRNA should be considered as early markers for neuronal dysfunction. In agreement, functional studies have revealed that modifications in the expression of selective miRNAs are detrimental for neuronal cells. Thus, early deregulation in the expression of specific miRNAs may contribute to the progression of the neurodegenerative disease. The characterization of small RNA expression profiles in neurodegenerative disorders should provide important clues about the fine regulation of a plethora of genes that are involved in cell-specific survival of key neurones of brain regions that lead to disease.



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"Are MYO1C and MYO1F associated with hearing loss?"
Biochim Biophys Acta, Epub 2008 Nov 5.

PATENTS

Inventors: Xavier Estivill, Mònica Gratacós, Mònica Bayés, Rafael de Cid y Eulalia Martí
Title: *Procedimiento y kit para la determinación de predisposición, determinación de un riesgo a desarrollar o el diagnóstico de un trastorno mental*
Application form No: ES P200800057
Priority country: Nacional
Priority date: 03/01/2008
Companies that are licensing it: not being licensed

ENTERPRISES

Vat No: B64900459
Date: 25/06/2008
Name: *Quantitative Genomic Medicine Laboratories, SL (qGenomics) (http://www.qgenomics.com/index.php)*
Address: Dr. Aiguader, 88 - 08003 Barcelona

GENES AND DISEASE

Group: Gene therapy

Group structure:

Group Leader: Cristina Fillat

Postdoctoral Felows: Xavier Altafaj, Meritxell Huch

PhD Students: Jon Ortiz, Laura Garcia, Daniel Abate Daga, Anabel Jose

Masters: Xavier Bofill de Ros

Technicians: Núria Andreu

SUMMARY

Gene therapy is a molecular medicine 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 animal 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 developing optimal gene therapy approaches for pancreatic cancer. 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. Pharmacokinetic and pharmacodynamic studies are being conducted to evaluate the therapeutic response in preclinical mouse models.

Gene therapy has a great potential in complex diseases as a powerful genetic tool to contribute to the identification of the functional role of specific genes and to the identification of potential gene-targets for therapy. Understanding complex phenotypes in Down syndrome is being tested by mouse phenotype correction, as consequence of particular genetic interventions by gene therapy approaches.

RESEARCH PROJECTS

1. Pancreatic cancer

Pancreatic cancer is the fourth cause of cancer deaths in industrialized countries. This neoplasia has a very bad prognosis mainly due to the late diagnosis and inefficient current therapies. The group has been involved in the past few years in exploring the feasibility of gene directed enzyme prodrug therapy in pancreatic cancer. These systems are based on the principle that a gene encoding an enzyme that is not naturally expressed in the host is first introduced into the tumor cells, followed by the administration of a prodrug that will only be activated in the tumor by the expressed enzyme. Studies carried out by the group on tumor cell sensitivity to the Herpes Simplex Thymidine Kinase (TK)/ganciclovir (GCV) gene prodrug therapy and on the gap-junction mediated bystander effect, have shown the potential of the treatment for pancreatic cancer. However to produce a sustained therapy every tumor cell should be affected directly or indirectly by the therapeutic effect of the transduced gene. Current vectors reach a very limited amount of cells in a tumor, even by direct intratumoral injection, compromising the outcome of the therapy. Interestingly, replication-competent viruses with preferential replication in cancer cells can multiply and disseminate through the tumor mass can compensate this limitation. A gene-virotherapy approach developed by the group and based on the arming of replicative adenoviruses with TK proved to boost the potency of adenoviruses when TK was expressed at the late phase. The deletion of viral functions that are dispensable in tumor cells or the transcriptional control of key viral genes using promoters that are active in tumor cells are two strategies to confer selective replication. Both strategies are explored in the research group. In this direction, they have demonstrated that keratin 7, a keratin expressed in simple epithelia has a restricted expression, while mantains a selective expression in ductal cells of the pancreas. These data supports the notion that the keratin 7 promoter is a strong candidate to target transgenes to ductal cells of the pancreas and can provide ductal pancreatic tumor selectivity to replicative competent adenoviruses. The incorporation of DNA elements regulated by the tumor microenvironment, and cell type specific regulators are currently being tested to provide with enhanced selectivity to these viruses.

The group is also interested in the understanding of the basic mechanisms of tumor cell killing induced by the TK/GCV systems and in the definition of the genetic signatures that can sensitize pancreatic tumors to these particular therapies.

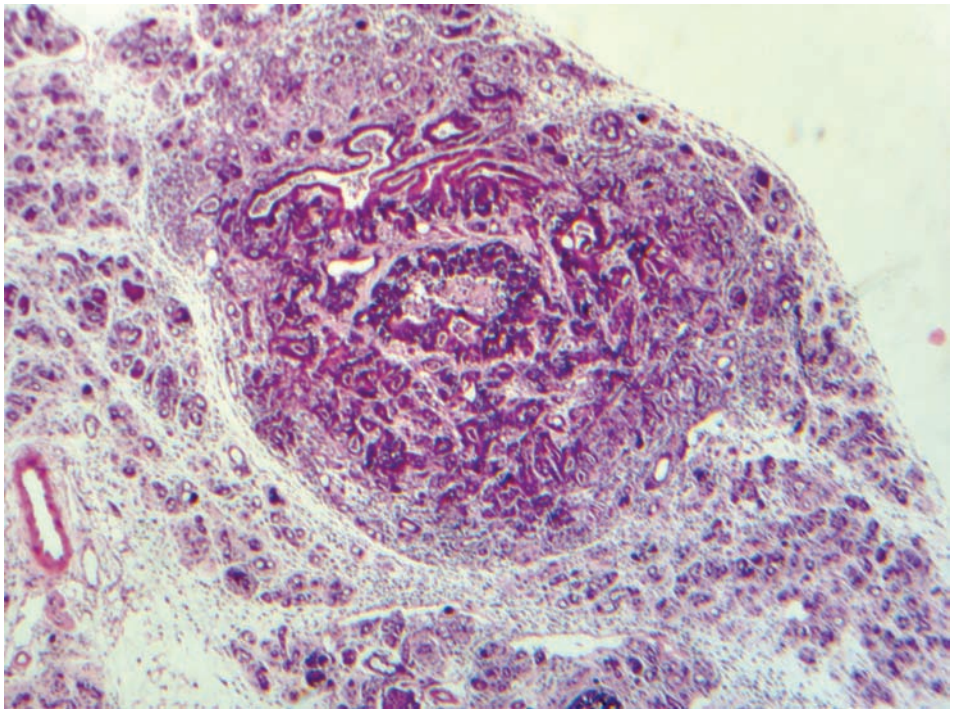

Figure 1.
Pancreatic tumor in a mouse model.

2. Down Syndrome

Down syndrome (DS) is a complex multi-system disorder, with a wide range of physical features, health and development problems, resulting from an extra copy of the human chromosome 21 gene HSA21. Although HSA21 contains more than three hundred genes, it has been hypothesized that the excess of specific target genes (dosage-sensitive) will have a major impact on the DS phenotype. To better understand the contribution of these specific genes, we have engineer a novel approach that takes advantage of the current knowledge from the existing mouse models. This approach is based on the rescue of a phenotype in a mouse model by the viral delivery of shRNA sequences targeting a candidate gene. A proof of principle approach has been undertaken by normalizing the expression of the Dyrk1A dosage sensitive gene in the mouse model of overexpression TgDyrk1A, and showed that AAVshDyrk1A delivery in the striatum of adult TgDyrk1A mice reverses motor impairment. This approach was devoid of toxicity and highlights Dyrk1A phenotypic dependence. Current studies evaluate the consequences of normalizing Dyrk1A in a trisomic context. Although we do not completely understand the molecular mechanisms by which the normalization of Dyrk1A can reverse such phenotypes, work form the laboratory has shown some molecular alterations in TgDyrk1A mice, such as altered expression on the transcription factor REST and an upregulation of the NMDA receptor subunits, associated to a prolonged decay on NMDA calcium transient in the cerebellum. Whether any of these pathways is involved in the phenotype rescue remains to be elucidated.

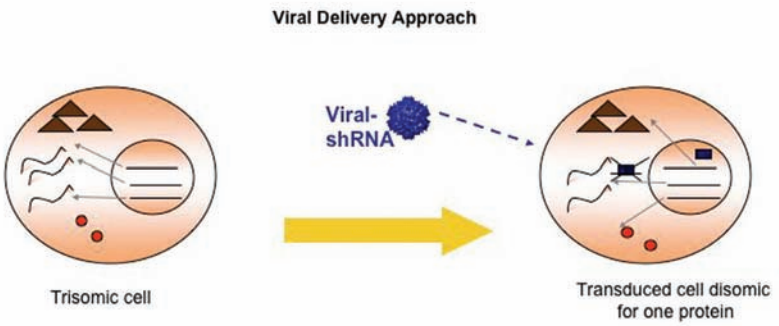

Figure 2.
Phenotype rescue by viralshRNA delivery

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Revista Internacional Síndrome de Down, 12:34-40 (2008).

GENES AND DISEASE

Group: Murine models of disease

Group structure:

Group Leader: Mariona Arbonés

Graduate students: Elisa Balducci (PhD), Ariadna Laguna (PhD), Sonia Najas (Master, since September)

Postdoctoral fellows: María José Barallobre, Ulrike Brandt-Bohne,

Technician: Erika Ramírez

SUMMARY

The ability of a human being to perform complex tasks relies on the correct formation of neuronal circuits. For this to happen, the many cell types of a mature brain have to be generated at the correct numbers and then differentiate properly during development, a complex process that is exquisitely regulated in time and space. Mutations disturbing the division mode of neuronal precursor cells, cell fate acquisition, differentiation, or natural cell death are likely to cause alterations in brain connectivity, which can result in neurological diseases and mental retardation.

The overall goal of the laboratory is to investigate the in vivo function of genes involved in diseases affecting the central nervous system (CNS) using the mouse as a model system. We aim to provide genetic and functional insights into the pathological events occurring in developmental gene-dosage diseases such as Down syndrome, William-Beuren syndrome and microcephaly associated to monosomy-21. A strong emphasis has been made during the last years in trying to elucidate the role of a human chromosome-21 kinase, DYRK1A, in CNS development. To this end, we have used different CNS structures, including the retina, the cerebral cortex and the adult brain subependymal zone (SEZ), as models to study neurogenesis and neuronal differentiation.

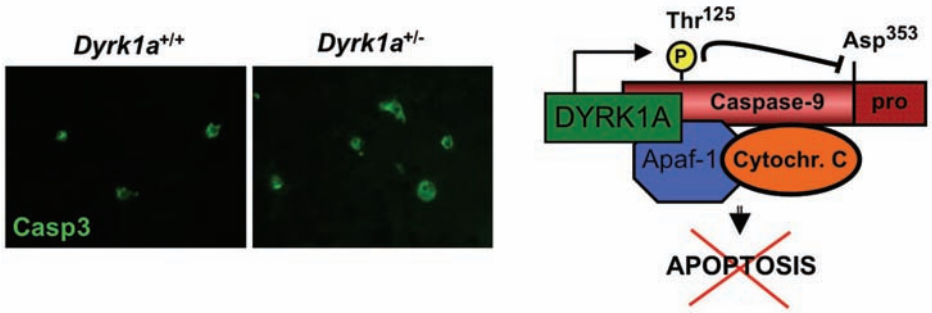
Our studies have demonstrated that a correct dosage of DYRK1A during development is required for the production of appropriate numbers of different CNS cell types and, in the adult, for maintaining brain cellular homeostasis.

RESEARCH PROJECTS

1. Retina development

Because of its accessibility and relatively low cellular complexity compared with the brain, the retina has long been used as a model system by which to study CNS development. Retina cells are generated from a pool of common precursors, the retina progenitor cells, that exit the cell cycle and acquire a specific cell fate in a precise spatial and temporal order. Another crucial event during retina development is the correct execution of programmed cell death; cells are produced in excess during neurogenesis and are later eliminated in a series of ordered apoptotic waves.

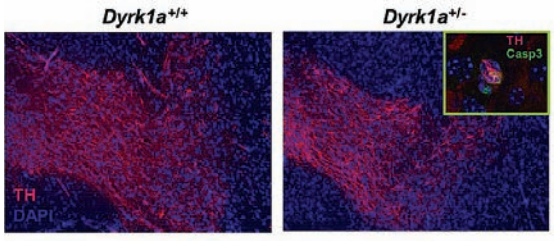
To provide some insights about the function of DYRK1A in CNS development we have analyzed the impact of DYRK1A gene dosage variations in the retina of gain- and loss-of function Dyrk1a mutant mice. Our results have shown that DYRK1A is a negative regulator of the intrinsic apoptotic pathway in the developing retina. We have provided evidence that changes in Dyrk1a gene dosage alter the cellularity of inner retina layers and result in severe functional alterations. We have show that DYRK1A does not affect the proliferation or specification of retina progenitor cells, but rather regulates the number of cells that die by apoptosis. We demonstrate that DYRK1A phosphorylates caspase-9 on threonine residue 125, and that this phosphorylation event is crucial to protect retina cells from apoptotic cell death.



Micrographs showing an increased number of apoptotic (Casp3+) cells in Dyrk1a mutant retinas. DYRK1A phosphorylates Thr125 in caspase-9 and this phosphorylation event prevents caspase-9 cleavage and subsequent activation of the intrinsic cell death pathway.

2. Dopaminergic neurons

Ventral midbrain dopaminergic (mDA) neurons control extrapyramidal movement and a variety of cognitive functions, such as motivation, drive, addiction and reward behaviours. The selective loss of mDA neurons, or perturbations to the neural circuits established by these neurons, such as the nigrostriatal and mesolimbic pathways, have been implicated in several neurodegenerative conditions, including Parkinson's diseases.
We have shown in the pass that decreased Dyrk1a dosage in the mouse leads to disturbed DA circuits and to motor, cognitive and emotional dysfunctions that resembled those in Parkinson disease or schizophrenia. The analysis of Dyrk1a mutant mice at different developmental stages has indicated that DYRK1A is dispensable for generation and specification of mDA neurons. Similarly to what we previously found in the retina, our experiments showed that DYRK1A is a pro-survival factor of differentiating mDA neurons. Our interest now is to find out 1/ the molecular mechanism by which DYRK1A protects mDA neurons from developmental cell death and 2/ if DYRK1A is acting as a Pro-survival factor also in mature mDA neurons.



Coronal sections showing a decreased number of DA neurons (expressing the enzyme tyrosine hydroxylase, TH) in the ventral midbrain of Dyrk1A deficient mice. The insert shows a cell expressing both TH and the active form of caspase-3 (Casp3). Nuclei are stained with DAPI.

3. Cortex development

The neocortex is the most complex region of the mammalian nervous system and is responsible for performing the most sophisticated cognitive and perceptual functions. It arises from the dorsal wall of the embryonic forebrain through repeated divisions by progenitor cells residing in the inner part of the neuroepithelium, the ventricular zone (VZ). These progenitor cells are, in many aspects, similar to the progenitor cells in adult brain SEZ.
We recently demonstrated that DYRK1A regulates self-renewal and cell fate decisions in adult SEZ neuronal stem cells in response to EGF. Base on this data and in the altered cytoarchitecture presented by gain- and loss-of function adult Dyrk1a mutant mice, we hypothesise that changes in DYRK1A gene-dosage affects cortical development resulting in the disruption of cortical circuits and cognitive dysfunction. We are now generating new mouse models that will allow us to test this hypothesis.

PUBLICATIONS

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GENES AND DISEASE

Group: Neurobehavioral phenotyping of mouse models of disease

Group structure:

Group Leader:	Mara Dierssen
Predoctoral Students:	Carla Obradors, Susanna Molas, Davide D'Amico, Meritxell Pons
Postdoctoral fellows:	Xavier Gallego Moreno, Monica Joana Pinto Do Santos,
Technician:	María Martínez de Lagrán Cabredo, Nuno Vasconcelos
Specialized Technician in phenotyping assay:	Ignasi Sahún Abizanda, Jerome MacDonald
Visiting Predoctoral Fellow:	Garikoitz Azcona (end May 2008)
Visiting Sabbatical Professor:	John Crabbe

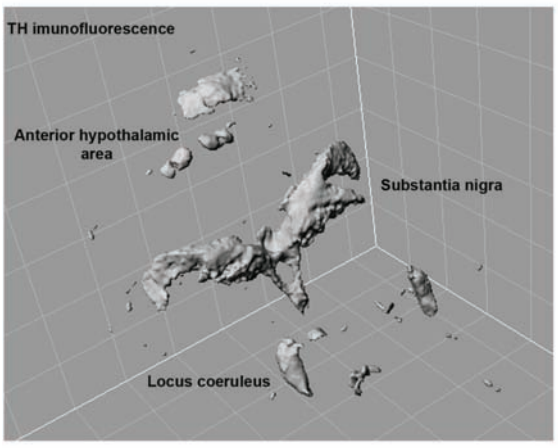
SUMMARY

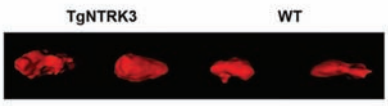
The overall goal of our research is to understand the role of putative candidate genes for human complex genetic diseases that affect cognitive systems, using genetically modified mouse models as a critical resource in the characterization of genes of biological importance, and in the dissection of the pathogenesis of neuropsychiatric and neurological disorders. We thereby obtain better knowledge of the genetic substrates regulating the expression of complex behavioral traits.

RESEARCH PROJECTS

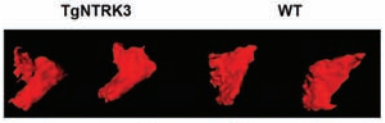
1. Down syndrome

The first research line of our group is aimed at dissecting the genetic architecture of mental retardation. During the last year we have contributed to characterize the functional and structural organization of the cognitive networks in DS models and the mechanistic aspects underlying the neuropathology. We focus on the fine dissection of different forms of learning and memory and other cognitive domains using innovative experimental paradigms and tracking tools (Coll. P. Verschure, Barcelona) and three dimensional analysis of the structural and gene-expression profiles using optical tomography techniques (Coll. J. Sharpe, Barcelona). We will also initiate the dissection of the molecular mechanisms affecting neuronal network formation in primary cortical and hippocampal cultures, analyzing genetically-driven alterations in dendritic structure and the possible involvement of Rho signaling and actin cytoskeleton (Coll. G. Ramakers, Amsterdam). However, one important question is the extent of the autonomous versus non-autonomous cellular effects of the trisomy 21. To answer this question we aimed at elucidating the epigenetic contributions to the functional and structural anomalies of cortical neurons, by generating mice with chimeric cortices in which neuronal precursor cells of trisomic or transgenic (DS) genotypes are under the influence of euploid host neurons (Coll. S. Martínez, Alicante). Also we aim to dissect the impact of gene imbalance of particular chromosomal regions in the pathogenesis DS and monosomy 21 using a panel of aneuploid and double transgenic mouse models, in an “in vivo” library strategy (Coll. Y. Herault, Orleans, Y. Groner, Tel-Aviv). We finally explore if environmental complexity ameliorates motor, cognitive and emotional functions with stable consequences on the neuronal organization of perception and cognition and impact on the organization of the perceptual and cognitive systems using an interactive synthetic environmental enrichment scenario to assess whether synthetic enrichment and augmented feedback is effective in promoting cognition in DS (Coll. P. Verschure, Barcelona).





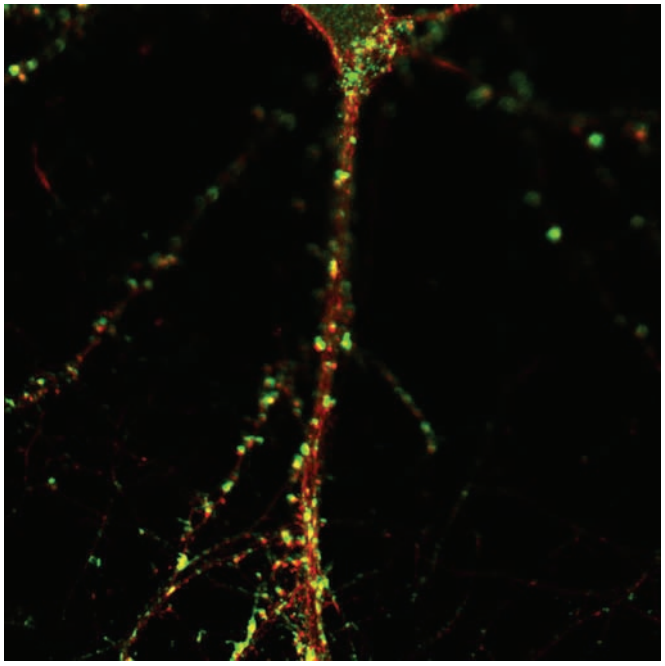
Anterior hypothalamic area



Locus coeruleus

2. Pathogenetic and molecular mechanisms involved in anxiety disorders

A second, more recent research line (Coll. X. Estivill, Barcelona) is aimed at identifying genetic causative and vulnerability factors underlying anxiety-related behavior that could predict the onset of panic disorder. We have identified several candidate genes and evaluated the functional and structural consequences of their dysregulation using genetically modified mouse models (Dierssen et al 2006, Sahun et al 2006, 2007, Amador-Arjona et al submitted; Gallego et al submitted and in preparation). Recurring memories of fearful or traumatic events result in inappropriate fear and in panic attacks in patients suffering anxiety disorders. We study the pathogenetic mechanisms that 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 such as stress and drug abuse disorders. The next step is to get insight into the specific aspects related to fear memories (M. Do Santos, in preparation): the modulation of persistence of amygdala- and hippocampus-dependent fear memory, by varying hippocampal involvement with contextual pre-exposure and contextual blocking, background and foreground context conditioning and trace conditioning protocols; the modulation of memory persistence in training conditions with different salience, by comparing recent, remote and far remote fear memory and the contribution of different candidate genes to the sensitivity to panicogenic/panicolitic agents.



3. Phenotype Ontology Project

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. 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. We are developing phenotypic descriptors that will allow the creation of phenotypic annotation.

PUBLICATIONS

Giralt A, Rodrigo T, Martin ED, González JR, Ceña V, Dierssen M, Canals JM, Alberch J.
"Brain Derived Neurotrophic Factor modulates the severity of cognitive alterations induced by mutant huntingtin through changes in phospholipaseCγ activity and glutamate receptor expression."
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Mercader JM, Lozano JJ, Sumoy L, Dierssen M, Visa J, Gratacos M, Estivill X.
"Hypothalamus transcriptome profile suggests an anorexia-cachexia syndrome in the anx/anx mouse model."
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"Association of NTRK3 and interaction with NGF suggest an altered cross-regulation of the neurotrophin signaling pathway in eating disorders."
Human Mol Genet, 17(9):1234-44 (2008).

Cheon MS, Dierssen M, Kim, SH, Lubec G.
"Protein expression of BACE1, BACE2 and APP in Down syndrome brains."
Amino Acids, 35(2): 339-43 (2008).

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Colección: Neurocientíficos españoles (CSIC)

GENES AND DISEASE

Group: Gene function

Susana de la Luna has an ICREA Group Leader position.

Group structure:

Group Leader: Susana de la Luna

Postdoctoral: Sergi Aranda

PhD Students: Eulàlia Salichs, Krisztina Arató, Chiara di Vona

Technicians: Alicia Raya

SUMMARY

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 not only help 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 studies the functional roles of several HSA21 genes.

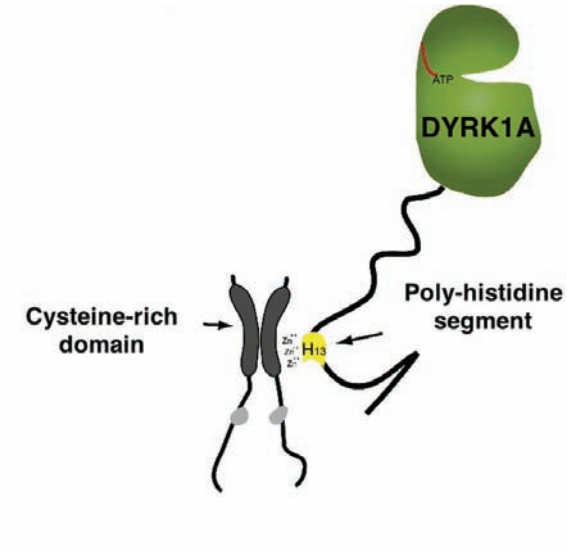
RESEARCH PROJECTS

1. DYRK1A: a crossroads for signal transduction pathways

Sergi Aranda, Krisztina Arató, Chiara di Vona

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 for investigation. Firstly, we regard the phenotypes shown by transgenic mice in which the gene is either overexpressed or has been deleted to be very interesting, and secondly, 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.

In that context, we have identified Sprouty2 as a substrate of DYRK1A. Sprouty proteins are well known antagonists of receptor tyrosine kinases (RTKs) signalling. DYRK1A interacts with and regulates the phosphorylation status of Sprouty2. We have identified Thr75 on Sprouty2 as a DYRK1A phosphorylation site in vitro and in vivo. This site is functional, since its mutation enhanced the repressive function of Sprouty2 on fibroblast growth factor (FGF)-induced Erk signaling. Further supporting the idea of a functional interaction, DYRK1A and Sprouty2 are present in protein complexes in mouse brain, where their expression overlaps in several structures. Moreover, both proteins copurify with the synaptic plasma membrane fraction of a crude synaptosomal preparation and colocalize in growth cones, pointing to a role in nerve terminals. Our results suggest, therefore, that DYRK1A positively regulates FGF–mitogen-activated protein kinase signaling by phosphorylation-dependent impairment of the inhibitory activity of Sprouty2.



Other putative DYRK1A interacting-proteins are being explored. These include not only substrates for DYRK1A (downstream targets), but also molecules that can act as effectors and thus, represent upstream modulators of DYRK1A in signalling cascades. For instance, we have identified caspase-9 as a novel substrate of DYRK1A. Phosphorylation of caspase-9 at residue threonine 125 occurs both in vitro and in vivo, and may have a role in the pro-survival activities of DYRK1A.

2. Functional impact of homopolymeric histidine repeats

Eulália Salichs

Based on our findings that the histidine (His)-rich segment present within the DYRK1A carboxy-end is a signal for targeting to the subnuclear compartment known as nuclear speckles or splicing factor compartment, we have started a new research line aimed to find out the functional impact of homopolymeric histidine repeats. This research line is carried out in collaboration with Mar Albá (ICREA-IMIM, Barcelona).

Single amino acid repeats, also known as homopolymeric tracts, are very common in eukaryotes and between 18-20% of proteins in the human genome contain such repetitive sequences. Although most of them are thought to be functionally neutral, recent evidence suggests they may play important functional or structural roles. Indeed, several studies have demonstrated that there is a strong correlation between genes associated with human diseases and genes encoding proteins with amino acid runs. Among homopolymeric tracts, histidine repeats are relatively rare in eukaryotes. The physicochemical properties of histidine make it a versatile amino acid that can fulfill different roles, influencing protein conformation and enzymatic activity. Nevertheless, there is still no clear function associated to histidine homopeptides.

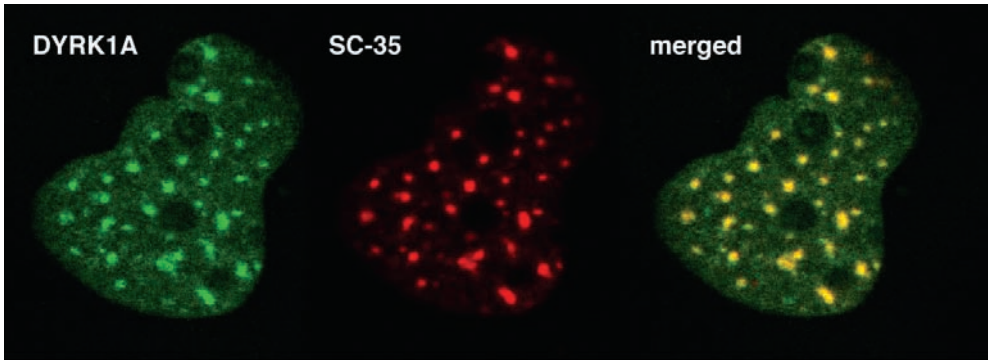


Figure 2.
DYRK1A accumulates in nuclear speckles (or splicing factor compartment) via its homopolymeric histidine repeat.

PUBLICATIONS

Laguna A, Aranda S, Barallobre MJ, Barhoum R, Fernandez E, Fotaki V, Delabar JM, de la Luna S, de la Villa P, Arbonés ML.
"The protein kinase DYRK1A regulates caspase-9 mediated apoptosis during retina development."
Dev Cell, 15(6):841-853 (2008).

Aranda S, Alvarez M, Turró S, Laguna A, de la Luna S.
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Mol Cell Biol, 28(19):5899-5911 (2008).

GENES AND DISEASE

The CRG Genomics Core Facility was established in 2008 integrating the existing Genotyping, Microarrays and the new Ultrasequencing Units and therefore becoming one of the largest genomic platforms in Spain. The facility has experienced staff including two Unit leaders, three project managers, nine wet lab technicians, and four bioinformaticists. Its mission is to facilitate interdisciplinary genomics research and training, with the premise of sharing resources and expertise among the different Units.

Since its foundation, the CRG has made significant investments in Genomics, including the purchase of two next generation sequencing instruments in 2008. Several high-throughput technologies have been implemented to assess differential expression, inter-individual genetic variation, microRNA discovery, genome sequencing, targeted resequencing, epigenetic profiling and identification of binding sites of DNA or RNA-associated proteins.

The CRG Genomics Core Facility has currently a very broad user base, with customers from all Reasearch Programmes and most Research Groups.

Associated Core Facility: Genomics Unit
Genotyping Unit

Unit Structure:
Unit Leader: Mònica Bayés

Project Manager: Magda Montfort

Technicians: Carles Arribas, Cecília García, Kristin Kristjansdottir, Sílvia Carbonell
Josiane Wyniger, Anna Puig

SUMMARY

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in the human genome. A 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, diabetes, asthma and cancer, and their differences in response to drugs. Other goals of SNP research include population genetics and trait selection for agricultural, cattle farming or aquaculture applications. Some of the genotyping technologies also enable to accurately characterized copy number variants (CNVs), loss of heterozygousity (LOH) and DNA methylation status. Our research activity, in some cases arising from the involvement of the Genotyping Unit in collaborative projects, is focused on the identification of genes that contribute to genetically complex disorders.

SERVICES

The Genotyping Unit, supported by "Genoma España" through the National Genotyping Centre (CeGen, www.cegen.org) (Barcelona Node Director Xavier Estivill, Genes and Disease Program), provides support to PRBB users and external users from public or private institutions for genotyping projects in every aspect of research, from experiment design, DNA extraction, genotyping, data interpretation, through to statistical analysis.

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

1. Automated DNA extraction from blood or other tissues (Chemagen)

2. DNA quantification using Picogreen (Molecular Probes)

3. Whole Genome Amplification using GenomiPhi (Amersham)

4. Custom Genotyping by SNPlex (Applied Biosystems):
genotyping of 24-48 SNPs selected by customer.

5. Custom Genotyping by Veracode (Illumina) (NEW):
genotyping of 48-384 SNPs selected by customer.

6. Custom Genotyping with GoldenGate technology (Illumina):
genotyping of 96-1,536 SNPs selected by the customer

7. Focused-content SNP Genotyping with GoldenGate technology on all catalogue Illumina products: African American admixture panel, DNA test panel, Cancer SNP panel and MHC panel set, Human Linkage V panel, Mouse LD Linkage panel and Mouse MD Linkage panel

8. Focused-content SNP Genotyping with Infinium technology on all catalogue Illumina products: Human1Mduo (NEW), Human660wquad (NEW), HumanHap370quad, HumanLinkage-12 (NEW), Bovinesnp50, Caninesnp20 and Equinesnp50 (NEW).

9. DNA methylation profiling using GoldenGate or Infinium technology on custom designed arrays and all catalogue Illumina products: HumanMethylation27 (NEW) and Methylation Cancer Panel I.

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

During 2008, the Unit has extracted 3,300 DNA samples from blood, and has produced 1.7 million genotypes with SNPlex, 225,000 genotypes using Veracode and has processed 350 arrays through Infinium technology.

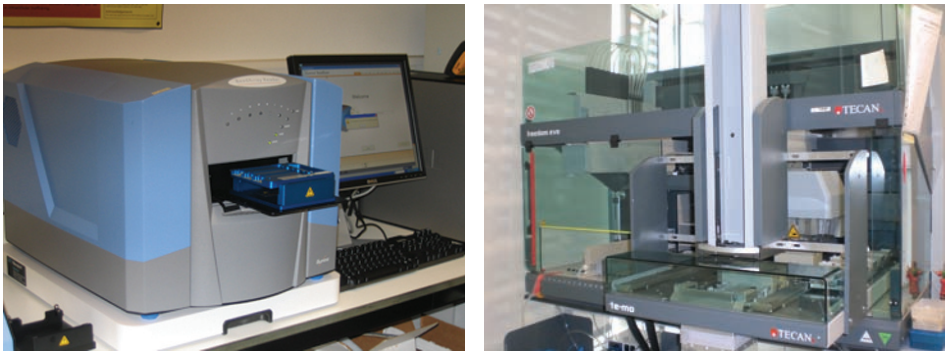

Figure 1. *The facility is equipped with state-of-the art instruments including high resolution scanners and liquid handling robots*

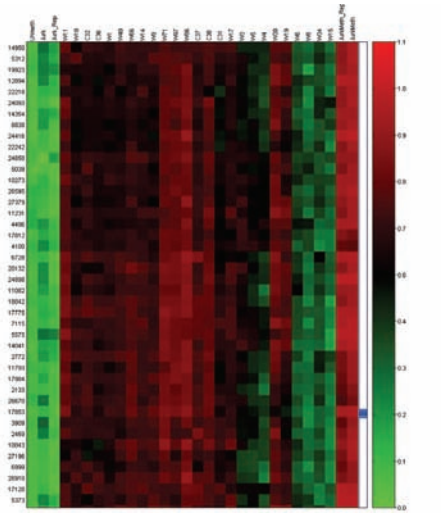

Figure 2. *Methylation profiles obtained with the HumanMethylation27 Beadchip Green, black and red colors correspond to low, medium and high methylation levels, respectively.*

PUBLICATIONS BY UNIT USERS

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Genetics, 180(2):1009-21 (2008).

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"Genetic susceptibility to obsessive-compulsive hoarding: the contribution of neurotrophic tyrosine kinase receptor type 3 gene."
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"Association study of 10 genes encoding neurotrophic factors and their receptors in adult and child attention-deficit/hyperactivity disorder."
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Mercader JM, Saus E, Agüera Z, Bayés M et al.
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Hum Mol Genet, 17(9):1234-44 (2008).

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de Cid R, Fonseca F, Gratacòs M, Gutierrez F, Martín-Santos R, Estivill X, Torrens M.
"BDNF variability in opioid addicts and response to methadone treatment: preliminary findings".
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Genes Immun, 9(4):279-88 (2008).

GENES AND DISEASE

Associated Core Facility: Genomics Unit
Ultrasequencing Unit

Unit Leader: Heinz Himmelbauer

Laboratory Technicians: Ana Vivancos, Ester Castillo, Anna Menoyo, Maik Zehnsdorf (GABI-FUTURE)

Bioinformaticians: Matthew Ingham, Debayan Datta, Robert Kofler (GABI-FUTURE), Juliane Dohm (GABI-FUTURE)

PhD student: Andrè Minoche (GABI-FUTURE)

SUMMARY

The Sanger sequencing technology has remained the gold standard for DNA sequence determination for almost thirty years. Despite many improvements on the original technology that have boosted both read length and throughput, Sanger-based sequencing is still a costly enterprise. The sequencing field has experienced a major shift by the introduction of innovative, high-throughput sequencing technologies developed by 454/Roche and Solexa/Illumina. The CRG Ultrasequencing Unit was established end of 2007 by Mònica Bayés and has been under the direction of Heinz Himmelbauer since his appointment as Unit Leader in June 2008. The Unit is presently equipped with one Illumina Genome Analyzer II sequencer, and one Roche 454 FLX sequencing instrument. Service is provided to the CRG, PRBB, and external users.

SERVICES

Next generation sequencing has paved the way for answering a plethora of different questions related to biology and biomedicine. While the majority of users of the Ultrasequencing Unit is from the CRG and from the PRBB, the service is also open to external users. The protocols currently in use reflect the needs of researchers at the CRG and include ChIP-Seq, RIP-Seq, digital gene expression profiling, miRNA discovery and expression analysis, amplicon sequencing, re-sequencing and de novo sequencing. The Illumina and 454 platforms currently established in the Ultrasequencing Unit complement each other very well. While Illumina sequencing generates large numbers of short reads per run (up to 8 x 10 million reads per run, 36-50mer reads), 454 datasets are smaller (up to 1 million reads per run with Titanium chemistry, reads > 400 nt). Thus, Solexa is the ideal platform for e.g. ChIP-Seq and miRNA detection experiments, while 454 is more suitable for projects that benefit from the information that longer reads entail, e.g. de novo sequencing and transcriptome characterization.

The services offered to users include the preparation of samples ready for sequencing on the GA II and the 454–FLX instruments, and performing the sequencing runs. Data are further processed and made available to the users (sequence files, quality files, alignment files).

RESEARCH PROJECTS

The research of the Ultrasequencing Unit focuses on the development of new procedures, both in the lab, and for data analysis. Towards this end, collaborations with several groups at the CRG have been established. Together with Luis Serrano's group, protocols for strand specific transcriptome sequencing are under development. MiRNA expression is studied together with Thomas Graf's and Ramin Shiekhattar's groups. Deep sequencing as a tool to identify mutations in yeast is pursued together with Josep Vilardell. With funding provided by the German Ministry for Education and Research (BMBF) in the context of the GABI-FUTURE program, we are presently sequencing the genome of sugar beet (Beta vulgaris), using next generation sequencing technology. At a size the quarter of the human genome, this project enables us to establish and test the workflows required to process and analyse sequence data from an organism without an available reference genome sequence.

PUBLICATIONS

Lange C, Holtgräwe D, Schulz B, Weisshaar B, Himmelbauer H.
"Construction and characterization of a sugar beet (Beta vulgaris) fosmid library."
Genome, 51(11): 948-51 (2008). (*)

Weichenhan D, Traut W, Göngrich C, Himmelbauer H, Busch L, Monyer H, Winking H.
"A mouse translocation associated with Caspr5-2 disruption and perinatal lethality."
Mamm Genome, 19(10-12):675-86 (2008). (*)

Schmidt S, Richter M, Montag D, Sartorius T, Gawlik V, Hennige AM, Scherneck S, Himmelbauer H, Lutz SZ, Augustin R, Kluge R, Ruth P, Joost HG, Schürmann A.
"Neuronal functions, feeding behavior, and energy balance in Slc2a3+/- mice."
Am J Physiol Endocrinol Metab, 295(5):E1084-94 (2008). (*)

Iwanami N, Higuchi T, Sasano Y, Fujiwara T, Hoa VQ, Okada M, Talukder SR, Kunimatsu S, Li J, Saito F, Bhattacharya C, Matin A, Sasaki T, Shimizu N, Mitani H, Himmelbauer H, Momoi A, Kondoh H, Furutani-Seiki M, Takahama Y.
"WDR55 is a nucleolar modulator of ribosomal RNA synthesis, cell cycle progression, and teleost organ development."
PLoS Genet, 4(8):e1000171 (2008). (*)

Dohm JC, Lange C, Reinhardt R, Himmelbauer H.
"Haplotype divergence in Beta vulgaris and microsynteny with sequenced plant genomes."
Plant J, Epub 2008 Aug 29. (*)

Tsend-Ayush E, Dodge N, Mohr J, Casey A, Himmelbauer H, Kremitzki CL, Schatzkamer K, Graves T, Warren WC, Grützner F.
"Higher-order genome organization in platypus and chicken sperm and repositioning of sex chromosomes during mammalian evolution."
Chromosoma, Epub 2008 Aug 26. (*)

Dohm JC, Lottaz C, Borodina T, Himmelbauer H.
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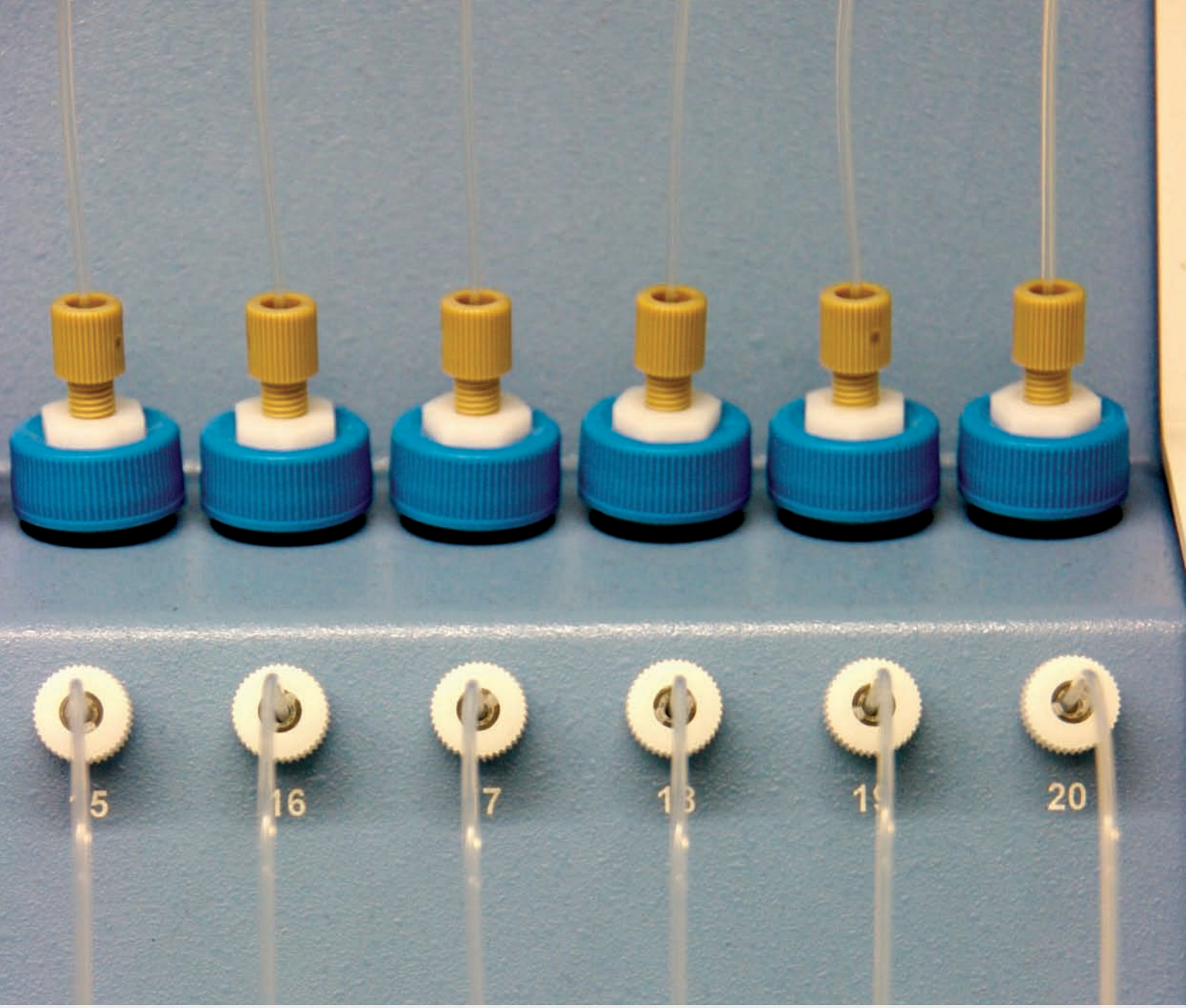
Mao L, Hartl D, Nolden T, Koppelstätter A, Klose J, Himmelbauer H, Zabel C. *"Pronounced alterations of cellular metabolism and structure due to hyper- or hypo-osmosis."*
J Proteome Res, 7(9):3968-83 (2008). (*)

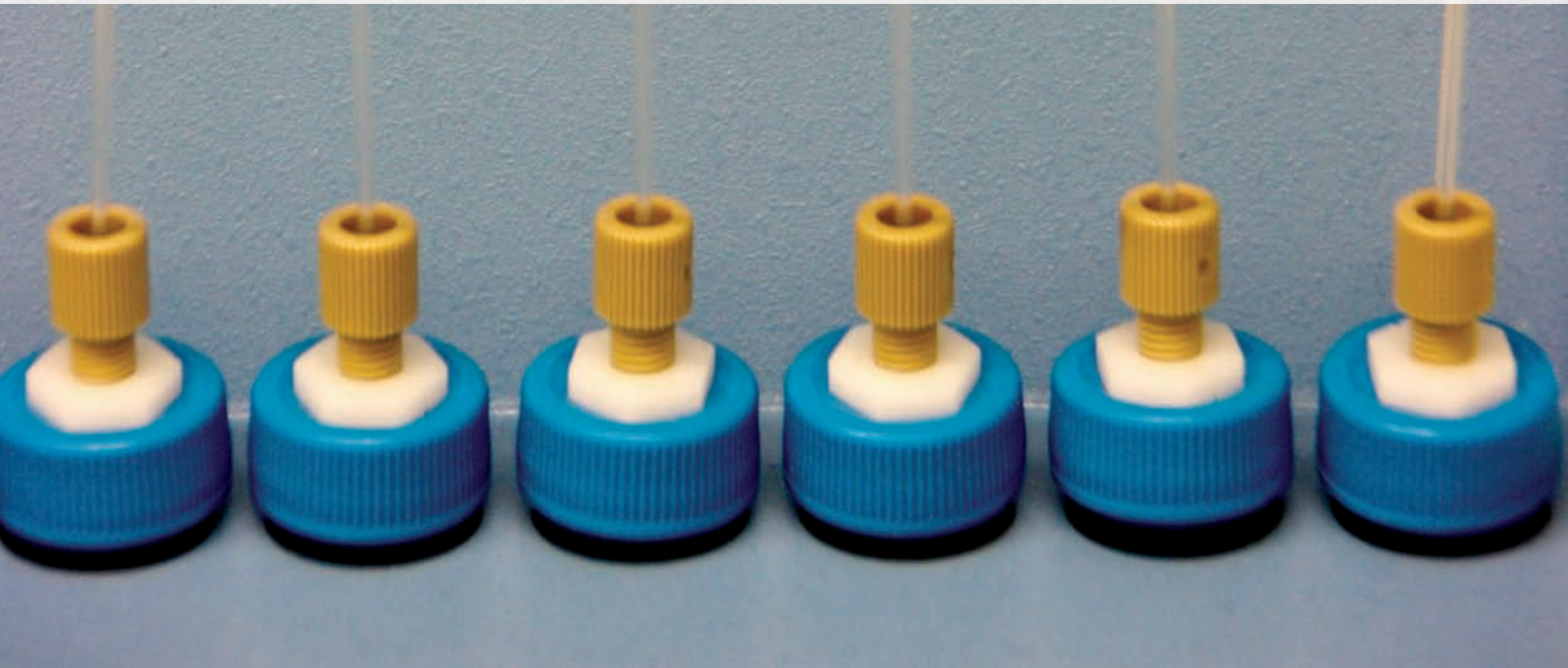
Rosenkranz R, Borodina T, Lehrach H, Himmelbauer H.
"Characterizing the mouse ES cell transcriptome with Illumina sequencing."
Genomics, 92(4):187-94 (2008). (*)

(*) All these publications are the result of the work of Dr. Heinz Himmelbauer at the Max Planck Institute for Molecular Genetics, Berlin, Germany.



BIOINFORMATICS AND GENOMICS

Coordinator: Roderic Guigó



The Bioinformatics and Genomics programme includes a number of research groups, which use computational analysis in genome research. Currently, the programme includes four groups, those lead by Roderic Guigó, Cedric Notredame, Toni Gabaldón, and Fyodor Kondrashov. Toni and Fyodor joined the program during year 2008. The programme is expected to grow with one additional group in year 2009.

In addition to carry out their own research agenda, the groups have established a number of solid collaborations with several experimental groups from other CRG programmes. The program is also taken active leadership in the delineation of the newly established CRG genomic unit, which will provides service in ultrasequencing, microarrays, genotyping, and bioinformatics, as well as in the design and implementation of the CRG's computing scientific network.

Current structure of the programme:

4 Research Groups:

> Genome Bioinformatics (Roderic Guigó)
> Comparative Bioinformatics (Cédric Notredame)
> Comparative Genomics (Toni Gabaldón). Since September 2008
> Evolutionary Genomics (Fyodor Kondrashov). Since November 2008

Associated Core Facility:

> Genomics Unit
    > Microarrays Unit (Lauro Sumoy)

BIOINFORMATICS AND GENOMICS

Group: Bioinformatics and Genomics

Group structure:

Group Leader:	Roderic Guigó
Postdoctoral Fellows:	Sarah Djebali, Tyler Alioto, Sylvain Foissac, David Martin, Christoforous Nikolau Maria José Truco, Vincent Lacroix, Michael Sammeth, Pedro Gabriel Dias Ferreira Thomas Derrien, Paolo Ribeca
PhD Students:	Charles Chapple (IMIM), Hagen Tilgner, Anna Kedzierska, Marco Mariotti
Master Students:	Sonja Althammer
Technicians:	Oscar González, Julien Lagarde, Francisco Câmara, Colin Kingswood, Judith Flo

SUMMARY

Research in the Genome Bioinformatics group focuses in the development and application of methods to identify functional domains in genomic sequences, with emphasis in protein coding genes and their splice variants. 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 in involved in NIH funded ENCODE project

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. During 2008, we have developed geneid as a "combiner", that is, a program that combines many inputs to obtain more accurate predictions (Alioto et al., 2008). When compared with other existing tools, in the N-GASP community experiment (Coghlan et al., 2008) Geneid ranked among the most accurate programs (Figure 1) . We have been collaborating with Genoscope, and with the Broad Institute, among other institutions, in using geneid and sgp in annotation of many eukaryotic genomes. During year 2008 we have collaborated in the annotation of a number of genomes, including that of cow, where we have lead the experimental verification group, and of the pea aphid.

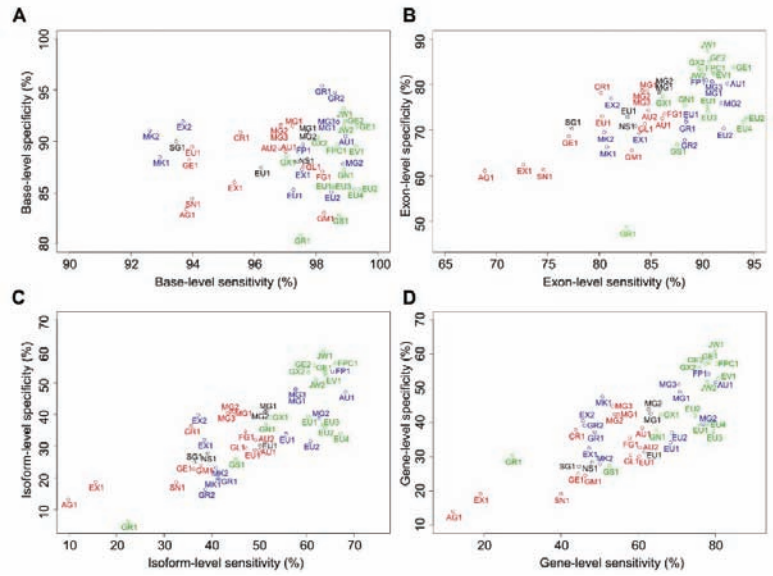


Figure 1.
Accuracy of the submitted gene sets. Plots of the specificity against sensitivity of the submitted gene sets, at the base level (A), exon level (B), isoform level (C) and gene level (D). The submitted gene sets are coloured by nGASP category, with ab initio (category 1) gene sets in red, gene-finders that used multi-genome alignments (category 2) in black, gene-finders that used transcript/protein alignments (category 3) in blue, and combiners (category 4) in green. The gene sets are labelled as follows: AU: AUGUSTUS, MG: MGENE, CR: CRAIG, AG: Agene, EU: EUGENE, FPC: Fgenesh++C, FP: Fgenesh++, FG: Fgenesh, GE: GeneID, GM: GeneMark.hmm, GX: GENOMIX, GS: GESECA, GN: GLEAN, GL: GlimmerHMM, GR: Gramene, JW: JIGSAW, MK: MAKER (using SNAP), MG: MGENE, NS: N-SCAN, SG: SGP2, SN: SNAP, EX: ExonHunter, EV: Evigan. (from Coghlan et al., 2008).

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, Castellano et al., 2006). Through the comparative analysis of the 12 drosophila genomes, we discovered that Drosophila willistoni lacks selenoprotein genes—the first animal known to lack selenoproteins. During this year we have investigated the fate of selenoproteins in a number of insect genomes, and found that selenoproteins have been lost in a number of insect species, probably owing to the relaxation across the entire taxa of the selective constraints acting to maintain selenoprotein genes.

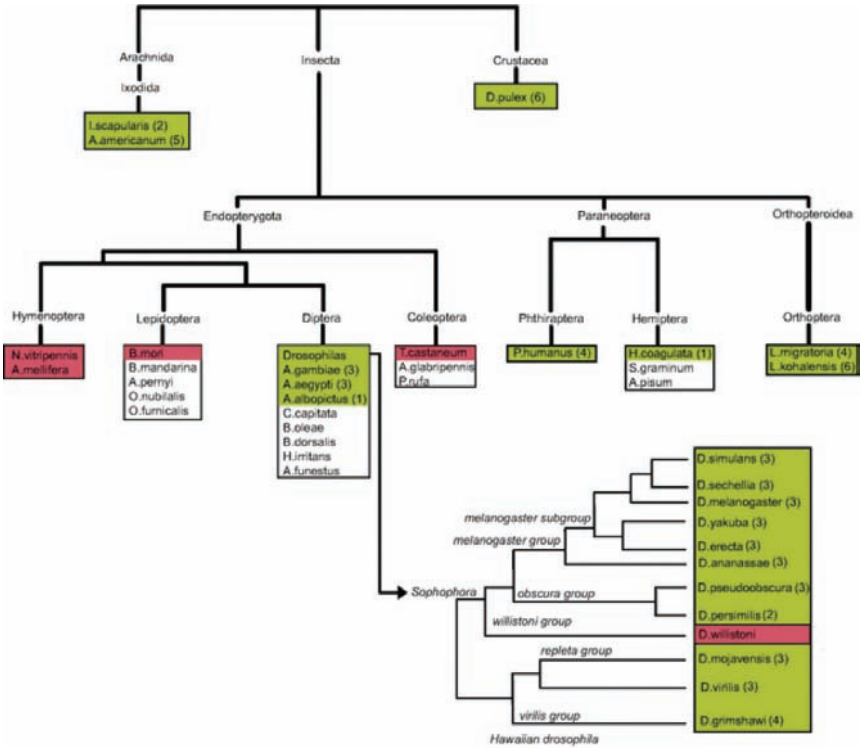


Figure 2.
Selenoprotein extinction in arthropoda. Species whose genomes do not code for selenoprotein genes are shown in red. Sec-encoding species are shown in green with the number of selenoproteins found in each genome in parentheses next to its name. Species for which the available data was inconclusive are shown in white (from Chapple and Guigó, 2008).

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.

4. Nucleosome positioning

The search of sequence prerequisites for nucleosome positioning has been a long-standing problem at the intersection of chromatin structure and gene regulation. With the use of structural information directly related to the primary DNA sequence we propose a new property of natural nucleosome forming sequences, which is inherently related to their intrinsic curvature and its symmetry. A measure of this property has been introduced and a corresponding method validated against novel high quality datasets of human nucleosomes, obtained through close collaboration with CRG's Chromatin and Gene Expression Group (Miguel Beato). Based on this symmetrical curvature property, we have implemented a computational ab initio method for nucleosome positioning prediction and have shown this method to be more efficient than previously published ab initio methods aiming at the same goal. The aforementioned method is already being applied in a number of analyses related to nucleosomal patterns of regions of interest in various organisms. The results have contributed to the formulation of the "key nucleosome" concept.

5. Nucleosome positioning and splicing

We have found evidence that strongly suggests a direct involvement of chromatin structure in RNA splicing. We have analyzed high throughput sequencing data recently produced by Schones et al. (2008) on positioning of nucleosomes in resting and activated human CD4+ T cells. Our analyses show evidence for stable nucleosome occupancy within internal exons of human genes, a pattern absent in pseudoexons (non-repetitive intronic sequences flanked by strong splice sites), which in contrast exhibit a weak

nucleosome depletion (Figure 3). Remarkably, this pattern depends on the strength of the splice sites. Indeed, the nucleosome peak is accentuated within exons with weak splice sites, while in the exons with strong splice sites, as well as in pseudoexons, a region of stable nucleosome occupancy is observed upstream of the acceptor sites. As a result, pseudoexons with strong splice sites—in which splicing does not occur despite the strength of the sites—show a pattern of nucleosome occupancy which is the mirror image of that observed on exons with weak sites—in which splicing occurs despite the weakness of the sites (Figure 3). These observations strongly suggest that positioning of nucleosomes influences RNA splicing.

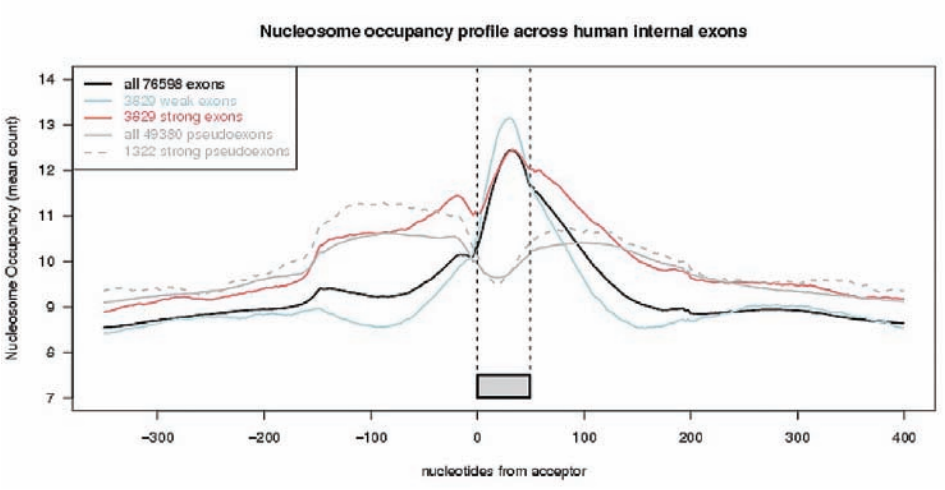


Figure 3.
Nucleosome occupancy profile across internal human exons in resting CD4+ T cells. Upstream and Downstream from the idealized exon (3' acceptor site at position 0), we plot the average number of nucleosome reads per nucleotide position. Within the exon, we have binned nucleosome reads in 50 intervals, irrespective of the length of the exon, and we plot the average number of reads in a 6bp window centered in the midpoint of the interval. In black, the profile for all internal exons, in light read, for the subset of exons with combined donor and acceptor score among the top 5%, in light blue, for the subset of exons with combined score among the bottom 5%, in solid grey for a set of intronic pseudoexons, and in dashed grey for the subset of pseudoexons with a combined score higher than the 90% percentil e of real exons.

6. ENCODE Project

We are part of the NHGRI funded ENCODE project. During the pilot phase of ENCODE, we lead one of the research groups. In the scaling phase of ENCODE we participate in the grants lead by Tom Gingeras (from Cold Spring Harbour Laboratory), where we are responsible for the bioinformatics, and by Tim Hubbard (from the Sanger Institute), where we contribute to build the reference annotation of the human genome. We are also leading the RNA analysis working group. A manuscript describing the results after the first phase of the scaling project will be ready by the end of this year.

7. Methods to characterize the cell's transcriptome

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 (Djebali et al., 2008). 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., 2007). We have further used this strategy to "normalize" the relative abundance of alternative splicing transcripts prior to random clone selection for sequencing (see Figure 2)

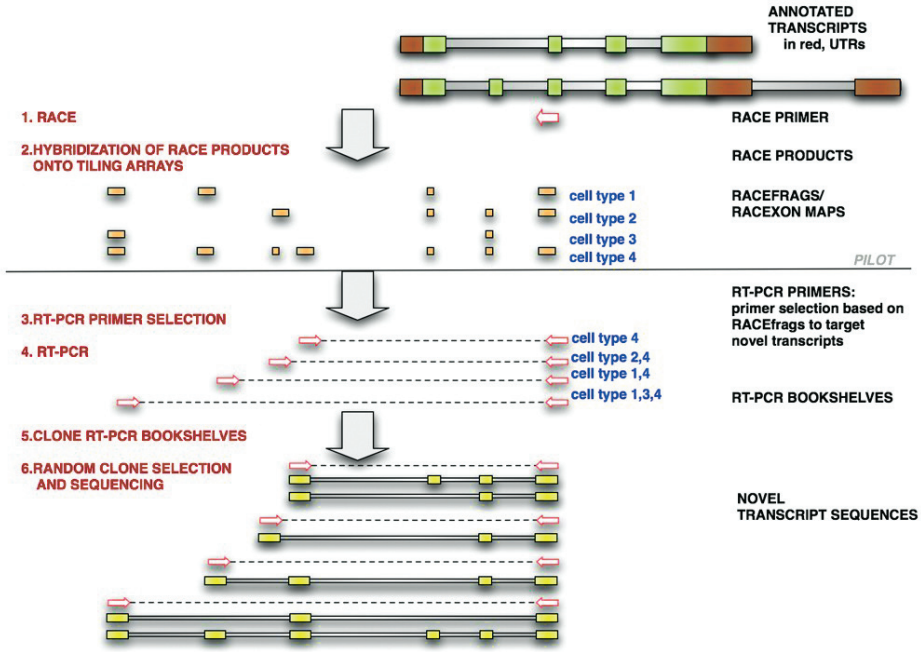


Figure 4. *Strategy for comprehensive characterization of novel isoforms from annotated genes. 1. RACE (5', 3' or both) is performed with primers from one or more annotated exons of known loci. 2. The RACE product are hybridized into a tiling array. 3. The detected sites of transcription (RACEfrags) are used to design RT-PCR primers. Primers are designed only on RACEfrags corresponding to previously undetected exons. 4. One RT-PCR reaction is performed for each primer in a novel RACEfrag, using the original RACE primer as the second primer. 5. Each RT-PCR reaction is cloned separately into a mini-pool (bookshelf). 6 Clones are randomly selected from the RT-PCR mini-pools and sequence (adapted from Djebali et al., 2008).*

Recently, we have started a research line on the development of methods to infer alternative transcript abundance from the short reads produced by the Next Generation Sequencing Instruments (Lacroix et al., 2008)

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BIOINFORMATICS AND GENOMICS

Group: Comparative Bioinformatics

Group structure:

Group Leader: Cédric Notredame

Postdoctoral Fellows: Ionas Erb, Emmanuele Raineri, Matthias Zytnicki,

Students: Lorena Pantano, Jia-Ming Chang, Giovanni Bussoti, Carten Kemena, Meritxell Oliva

Technicians: Emmanuel Beaudoing

SUMMARY

The main focus of the group is the development of novel algorithms for the comparison of multiple biological sequences. Multiple comparisons have the advantage of precisely revealing evolutionary traces, thus allowing the identification of functional constraints imposed on the evolution of biological entities. Most comparisons are currently carried out on the basis of sequence similarity. Our goal is to extend this scope by allowing comparisons based on any relevant biological signal such as sequence homology, structural similarity, genomic structure, functional similarity and more generally any signal that may be identified within biological sequences. Using such heterogeneous signals serves two complementary purposes: (i) producing better models that take advantage of the evolutionary resilience, (ii) improving our understanding of the evolutionary processes that leads to the diversification of biological features. We develop these novel methods in close collaboration with experimental groups [1] and make them available through an international network of web severs: www.tcoffee.org.

RESEARCH PROJECTS

1. T-Coffee: Development of template based multiple sequence alignments

Over the last years we have pioneered the development of a novel kind of multiple sequence alignments known as template based sequence alignments. The principle is relatively new and has marked an important shift in the field of multiple sequence alignments. While most methods tend to merely exploit the information contained in the provided sequences, template based methods couple the multiple sequence alignment with a either a database search or a structural prediction that enriches the information content of the sequence. After focusing on the combination of multiple protein structures and multiple sequences protein sequences, we have now started exploring the alignment of structured non coding RNAs. For this purpose we have developed a novel approach able to incorporate RNA secondary structural prediction within the computation of a multiple sequence alignment. The resulting algorithm (R-Coffee) is one of the most accurate RNA multiple sequence aligner currently available (as judged by benchmarking on BraliBase)[2, 3]. R-Coffee is the result of a collaboration with the lab of Des Higgins and the Swiss Institute of Bioinformatics.

In order to improve the use of T-Coffee we are also putting major effort into improving the T-Coffee underlying algorithm. This project is carried out in collaboration with the lab of Knut Reinert (Berlin Free University) and has recently result in some significant improvement of the T-Coffee algorithm [4].

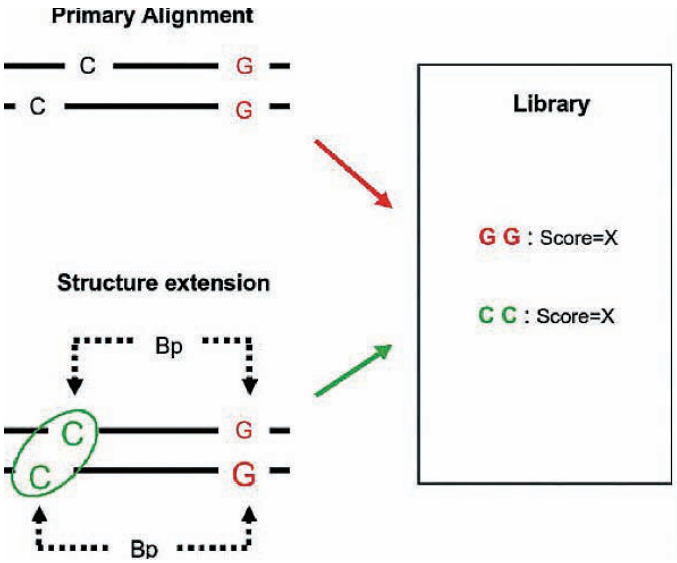

Figure 1.
Novel Library extension algorithm implemented in R-Coffee

2. Longitudinal Studies

Methods developed in bioinformatics for sequence analysis can in theory be applied to any type of strings exhibiting properties similar to those encountered in biological sequences. These may include strings resulting from experimental observation acquired through continuous monitoring of a phenomenon developing across time through a limited number of alternative states. In order to test this hypothesis we have collaborated with a group of sociologist from Lausanne University specialized in the compilation of life trajectories through longitudinal studies. Under this protocol, trajectories are coded as sequences made of pre-defined alphabet indicating various situations. We have used these sequences to train substitution matrices similar to those used in bioinformatics [5]. We have now initiated a new line of research in collaboration with Maria Derssen (CRG) in order to apply this methodology to animal models

3. Multiple Sequence Alignment Servers

Thanks to a partnership with the Swiss Institute of Bioinformatics, we are currently running a very powerful multiple alignment server mirrored by several international institutions (EBI, Cornell, CNRS, SIB). Altogether all the mirrors receive close to 20.000 hits a month originating from an average of 90 countries. In 2008 the various publications describing either the server of the original algorithms have received more than 400 citations. The latest addition to this popular service has been the R-Coffee alignment mode.

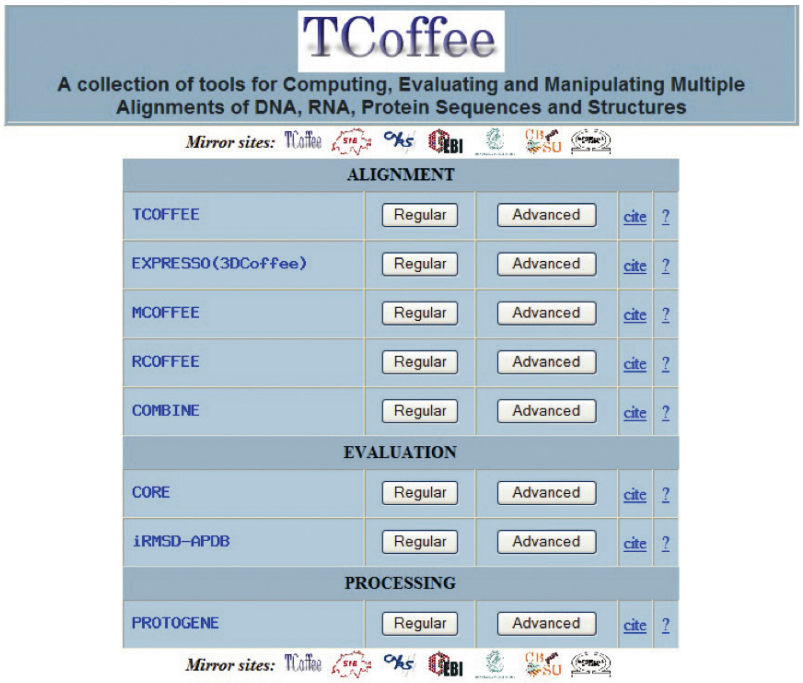

Figure 2.
The T-Coffee web server (http://www.tcoffee.org)

PUBLICATIONS

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BIOINFORMATICS AND GENOMICS

Group: Comparative Genomics (since September 2008)

Group structure:

Group Leader: Toni Gabaldón

Postdoctoral Fellows: Jaime Huerta-Cepas

Students: Salvador Capella-Gutiérrez

Technicians: Marina Marcet-Houben, Javier Díez-Pérez

SUMMARY

Our research interests are focused around the use of comparative genomics and phylogenomics to study the origin, evolution and function of complex biological systems. This includes understanding how specific biochemical pathways, protein complexes or cellular organelles emerged and evolved as well as using this evolutionary information to gain insight into their function. Through collaborations with experimental groups we apply comparative genomics to discover new mechanisms and genes involved in interesting processes, especially those of clinical relevance (see lines of research). On the technical side, our work often involves the development of new bioinformatics tools and algorithms that we make available to the community.

RESEARCH PROJECTS

1. Discovery of new genes involved in mitochondrial disease

Mitochondria play a central role in the cellular metabolism and the impairment of many mitochondrial proteins leads to disease. The list of such diseases is continuously growing and includes Parkinson´s, Alzheimer´s and Huntington´s diseases. Despite recent advances, the molecular basis of many mitochondrial diseases is yet to be understood and there is growing need to identify disease-causing genes and to unravel their functions. Recently, proteomics analyses have identified a large set of proteins that function inside the mitochondrion, the so-called mitochondrial proteome. Most of these proteins are not functionally characterized and it is expected that many of them may be involved in mitochondrial diseases. Moreover, other mitochondrial proteomic sets are being characterized in different species facilitating an evolutionary analysis of the mitochondrial system. The aim of this research line is to integrate different types of data and automatically combine them in order to facilitate the identification and functional characterization of mitochondrial disease-related genes.

2. Comparative genomics of fungal pathogens

Fungal infections constitute an ever-growing and significant medical problem. Diseases caused by such pathogens range from simple toe nail infections, to life-threatening systemic mycoses in patients with impaired immune systems. The molecular mechanisms driving invasion of mammalian hosts by fungal pathogens poses many scientifically challenging problems, which are as yet little understood. Candida glabrata is an emerging pathogen that represents the second most common cause of fungal infection. C. glabrata is evolutionarily closer to non-pathogenic Saccharomyces species than to other pathogen Candida species and uses different virulence mechanisms. Therefore, the problem of elucidating the mechanism for pathogenesis of Candida glabrata, as proposed here, can be approached with an evolutionary perspective by detecting specific adaptations in the C. glabrata genome that may explain why, in contrast to its closest relatives, is able to infect the human host. The present research line will be developed in the context of a international consortium (FunPath).

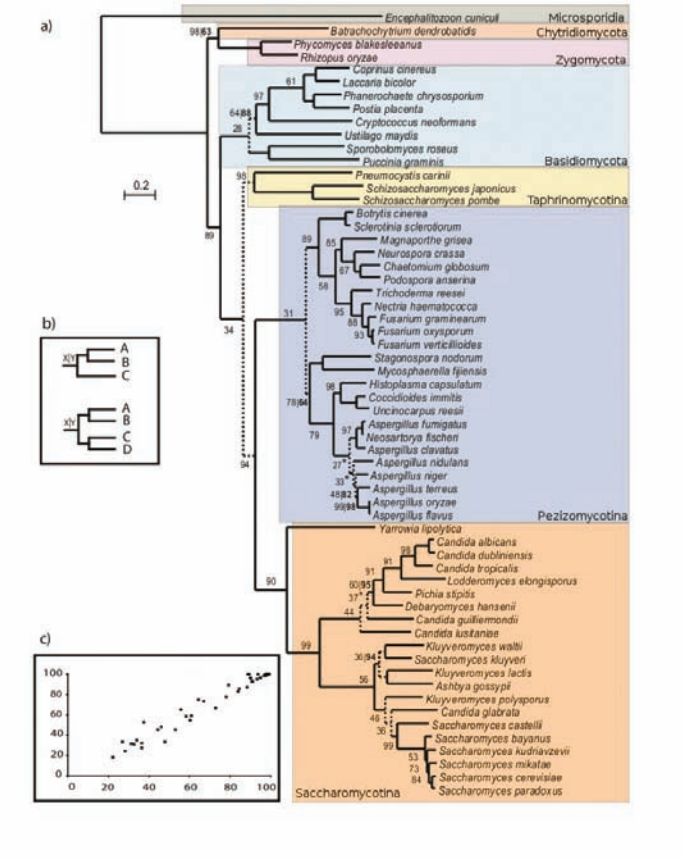

Figure 1.
Fungal tree of life including all species with completely sequenced genomes. The topology is based in the concatenated alignment of 69 widespread protein and the support is based on both a bootstrap analysis and the comparison with thousands of single-gene trees (Marcet-Houben and Gabaldón 2009)

3. Comparative genomics of apoptosis and other programmed cell deaths.

Programmed cell-death is a central biological process that in eukaryotes it evolved into different complex pathways. It acts as a control mechanism of homeostasis in cell number and is triggered by the onset of a coordinated biochemical cascade of events, a process called apoptosis in metazoan species. Although much progress has been made in the recent years, its origins and evolutions remain to be resolved. The aim of this project is to trace the evolution of the cell-death pathways in eukaryotes and to unravel the evolutionary relationship between caspases and mitochondria, which play a central role in the caspases-dependent apoptotic process. This aim includes tracing the origin of key components of the apoptotic pathways in order to ascertain their eukaryotic or endosymbiotic origin and to determine the evolutionary period in which they were recruited to function in cell-death pathways. Furthermore we will use genomic-context techniques to discover proteins that evolved coordinately with known apoptotic components. Such predictions will be tested experimentally. This project is performed in collaboration with the lab of Dr. Cristina Muñoz-Pinedo (IDIBELL, Barcelona) and is funded through as AICR grant.

4. Phylogenomics and genome evolution

In the genomic era it has been possible to move from the evolutionary analysis of single protein families (phylogenetics) to that of complete genomes and proteomes (phylogenomics). To achieve this transition new tools have been developed that allow the large-scale reconstruction of thousands of phylogenetic trees in an automatic way. This computerization of the whole process of tree construction often involves the use of standard parameters and conditions for all tree families, inevitably resulting in poor or incorrect phylogenies in many cases. Moreover, interpreting such type of complex data poses many difficulties and does require the development of novel algorithms, tools, forms of representing the data and even new semantics and concepts. We combine the development of original algorithms to treat phylogenomic data with its application to gain knowledge on problems of biological relevance. In particular we are interested in developing post-processing methods to interpret sequence alignments and phylogenetic trees in a large-scale and to mine such data to find evidence for functional interactions between proteins.

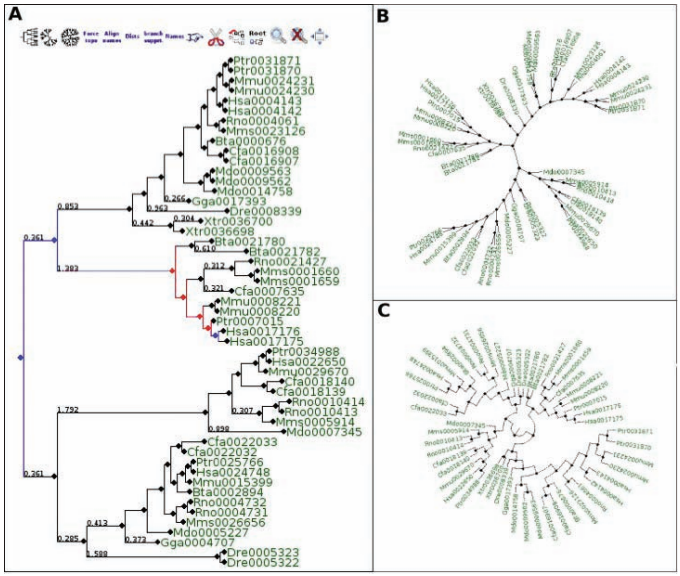

Figure 2.
Screenshots of trees stored in PhylomeDB (http://phylomedb.org) our database for complete phylomes. All visualizations are created by in-house programs. Part A, shows a mapping of speciation and duplication events used to automatically detect orthology. (Huerta-Cepas et al. 2008).

5. Evolution of the eukaryotic cell

Every eukaryotic organism shows a high level of sub-cellular compartmentalization that is significantly more intricate than the most complex prokaryotic cell. How such degree of complexity came to be is still not fully understood. In this context, endo-symbiotic events with bacterial organisms have been proposed to be the source of a number of organelles including mitochondria, chloroplasts and peroxisomes. Only recently, it has been possible to contrast these hypotheses with the growing availability of completely sequenced genomes and organellar proteomic data. We use large-scale evolutionary analyses to investigate the origin and evolution two most widespread organelles for which an endosymbiotic origin has been proposed: mitochondria and peroxisomes.

PUBLICATIONS

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(*) All these publications result from our work at the Centro de Investigación Príncipe Felipe, Valencia, Spain.

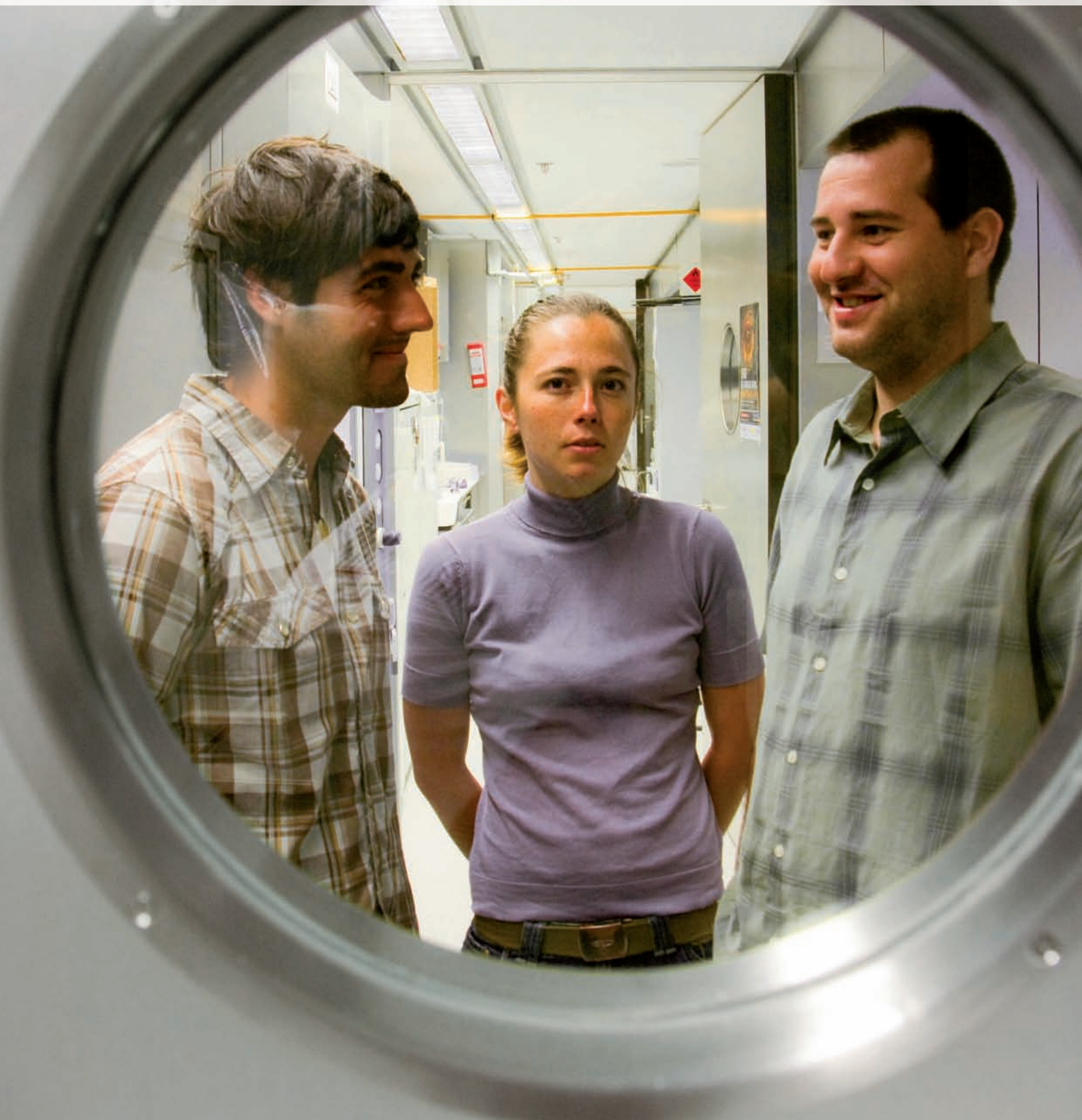
BIOINFORMATICS AND GENOMICS

Group Evolutionary Genomics (since December 2008)

Group structure:

Group Leader: Fyodor Kondrashov

Research Assistants: Margarita Meer, Inna Povolotskaya, Michael Breen



SUMMARY

Evolutionary thought is pertinent to all aspects of biological or biomedical sciences. Similarly, discoveries in molecular and systems biology provide a deeper understanding of the mechanisms involved in the origin and continued evolution of living things. Our laboratory attempts to strengthen the connection between evolution and other areas of biology by working on the interface of several disciplines. At the present time our work is restricted to the application of computational and bioinformatical tools to study any publically-available genome-wide data.

We make no categorical distinction in our choice of the organism of study, nor do we exclude particular evolutionary mechanisms from our interests. However, since our work relies almost exclusively on the available data many of our studies use the available data from humans and model organisms. In the next several years we hope to complement our computational inferences with our own data from an experimental section of our growing laboratory. At the CRG we are starting to build a new research programme, expanding on our previous efforts and creating new lines of inquiry.

RESEARCH PROJECTS

1. Evolution of complex systems

Evolution is thought of as a rejection of deleterious variants and the promotion of beneficial changes, with slight deviation due to genetic drift. Thus, the common and convenient perception is that evolution can be understood by revealing the impact of individual mutations on fitness. However, due to the interactions between different parts of a molecule or even between different molecules in genetic networks the fitness impact of one mutation can greatly depend on the genetic context in which this mutation occurs.

A change in fitness due to interaction between different loci is called epistasis. While recognised as being important for our understanding of many evolutionary phenomena, such as the evolution of sex, the prevalence of epistasis or the molecular mechanisms that could be responsible for its emergence in natural systems remain poorly understood. One of the main goals of our laboratory is to describe and discover the molecular and selective mechanisms of epistatic interactions in complex biological systems.

Currently, we are investigating the emergence of epistasis in the selection regime of intron length evolution of Drosophila melanogaster. We are able to show that a simple, non-epistatic fitness function for intron length cannot lead to the observed distribution of intron lengths in the genome of D. melanogaster. The effort to analytically solve for the epistatic fitness function in this species is ongoing.

Biological complexity per se is a difficult trait to define strictly; however, when comparing two different systems the distinction between the simple and the complex can be intuitively obvious. For instance, the organization of a genome without any gene overlap is inherently simpler than that of a genome that contains overlapping genes. We used this observation to study the evolution of gene organizational complexity in metazoan genomes. We were able to show that such organizational complexity is increasing independently in multiple genomes. However, this increase of organizational complexity appears to proceed without any influence of selection. Our study was the first to directly test the selective regimes of the evolution of complexity in metazoan systems.

2. Compensatory evolution

Perhaps the simplest form of epistasis is found in compensatory interactions within the structure of a single molecule. For example, in an RNA stem structure, the interacting Watson-Crick complementary pairs depend on each-other to maintain the structure and high fitness. Likewise, the fitness of a substitution in one of the two complementary sites in a RNA stem structure depends on the nucleotide of the other site.

By studying the evolution of complementary sites in mammalian mitochondrial tRNAs we hope to be able to elucidate the evolutionary mechanisms that play a leading role in their compensatory evolution. From the point of view of selection, there are three main mechanisms that may be driving compensatory evolution, for example the evolution of complementary sites from an AU Watson-Crick pair to a GC pair through a GU intermediate, whereby the path of evolution is AU -> GU -> GC. Firstly, the evolution can be entirely neutral, such that all three nucleotide pairs have equal fitness. Secondly, such evolution can beneficial, whereby the fitness of AU and GU pairs is equal, but the fitness of GC is higher than the other two possibilities. Finally, evolution can proceed through a path of disruptive fitness, whereby the fitness of the intermediate GU nucleotide pair is substantially lower than of the other two variants. Conventional wisdom upholds that the former, neutral mechanism is the main force behind compensatory evolution, while our preliminary data indicate that the latter may be the culprit.

PUBLICATIONS

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Assis R, Kondrashov AS, Koonin EV, Kondrashov FA.
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Schmidt S, Gerasimova A, Kondrashov FA, Adzuhbei IA, Kondrashov AS, Sunyaev S.
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Book chapter

Kondrashov FA.
"Gene dosage and duplication."
Eds. Dittmar K, Liberles D. In: Evolution after gene duplication. Wiley Press, In press. (*)

(*) All these publications are the result of the work of Fyodor Kondrashov at the University of California at San Diego, USA.

BIOINFORMATICS AND GENOMICS

Associated Core Facility: Genomics Unit
Microarrays Unit

Unit Structure:

Unit Leader:	Lauro Sumoy
Senior Technician:	Anna Ferrer
Technicians:	Heidi Mattlin, Maria Aguilar
Bioinformaticians:	Xavier Pastor, Manuela Hummel

SUMMARY

As a core facility, the laboratory provides microarray methodologies as a service at established rates to scientists from the CRG, other PRBB institutions and other external public and private institutions. It has also been responsible for the experimental and bioinformatics aspects of different collaborative research projects that use microarray technology.

SERVICES

Services offered include: sample quality control and amplification, RNA and DNA sample labelling, hybridization of microarrays and data processing and analysis, as well as array design and fabrication by spotting. The facility is set up for optimal processing of in situ synthesized long oligonucleotide arrays (Agilent, Illumina and Exiqon).

The Microarray Unit offers applications including:
> mRNA expression profiling on spotted cDNA and Agilent microarrays, and Illumina bead arrays.
> miRNA expression profiling on Exiqon and Agilent microarrays.
> array based comparative genomic hybridization (aCGH) on spotted BAC and Agilent microarrays.
> chromatin immunopreciptation on array (CHIP on chip) on spotted promoter, CpG island and Agilent microarrays.

During 2008 we have made use primarily of predesigned commercial microarrays from several species (yeast, human, rat, mouse). Using Agilent sure spot ink jet printing technologies, we have also processed some in situ synthesized oligonucleotide arrays designed in house that are specific for Caenorhabditis elegans, Caenorhabditis briggsae, Mycoplasma pneumoniae, predicted human non-coding RNAs and breast cancer related gene promoters. In addition, we have continued to use BAC arrays tiling the entire human X chromosome and custom designs covering regions flanked by segmental duplications and pericentromeric regions that has served as prototype for the CRG spinoff company qGenomics.

Automated image data acquisition, pre-processing, quality control, filtering, normalization and advanced data analysis (clustering, grouping, discriminant, factor analysis and data mining) is done using in house developed web tools.

Finally, besides updating procedures for the different services offered, the laboratory has tested a common set of samples processed with several microarray platforms and Solexa based next generation sequencing in order to validate these platforms and establish protocols for inter-platform comparison, genome scale data validation and data integration.

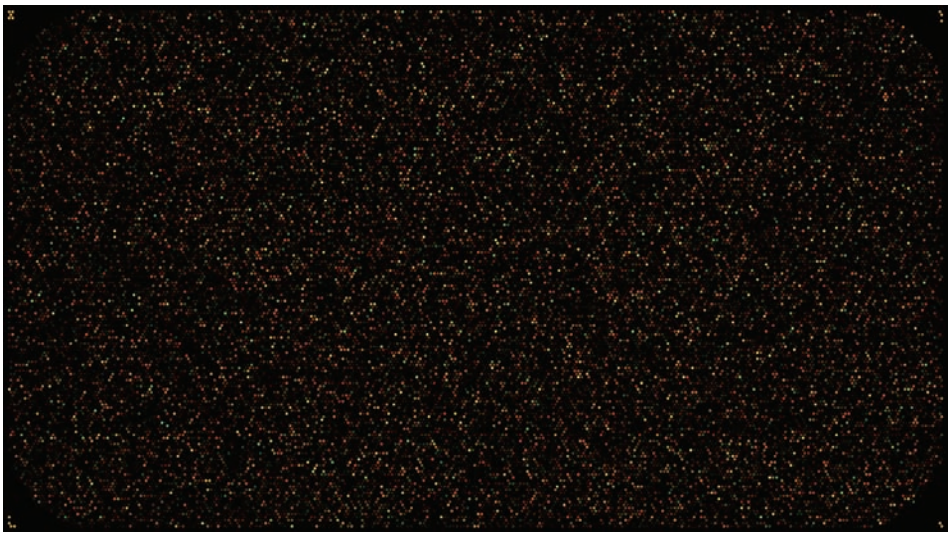

Fig 1.
Agilent high resolution scanner at the Microarray Unit.

Fig 2.
Two-color fluorescent scan of a human expression microarray containing more than 41,000+ unique human genes and transcripts.

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CRG

CELL AND DEVELOPMENTAL BIOLOGY

Coordinator: Vivek Malhotra

The programme of Cell and Developmental Biology is rapidly growing to its full capacity. We have succeeded in recruiting a new group leader, Jerome Solon from EMBL, who will arrive to the CRG in September, 2009. Jerome is interested in the biomechanics of morphogenesis. Specifically, he is interested in the mechanism of changes in tissue morphology and migration of cells during embryogenesis.

The arrival of Jerome will strengthen our overall interest in regulation of cell and developmental biology. One additional group leader will be recruited to compliment the existing expertise in the program during 2009.

Structure of the Programme:

Coordinator:	Vivek Malhotra
Senior Group	Isabelle Vernos
Junior Group:	Hernan Lopez-Schier
Junior Group:	Manuel Mendoza
Advanced Light Microscopy Facility	Timo Zimmermann

CELL AND DEVELOPMENTAL BIOLOGY

Group: Intracellular compartmentation
Vivek Malhotra has a Senior ICREA Group Leader position.

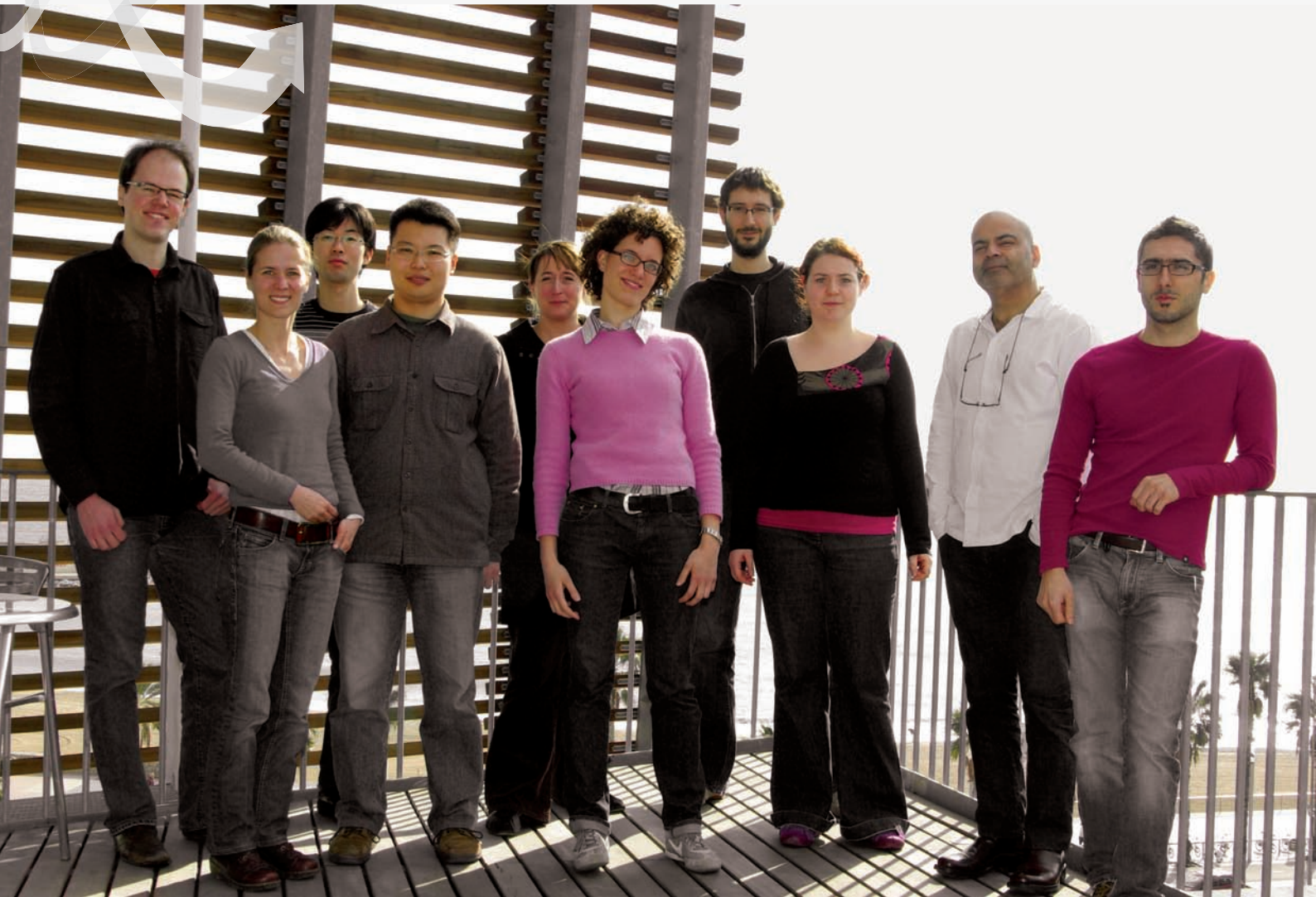
Group Structure:

Group Leader: Vivek Malhotra

Postdoctoral Fellows: Kota Saito, Yuichi Wakana, Juan Duran, Julia VonBlume, Sandra Mitrovic, Josse VanGalen, Felix Campelo

Visiting student: Elena Forlanelli

Technician: Anne-Marie Alleaume



SUMMARY

We are interested in the mechanism of protein secretion and biogenesis of Golgi membranes.

RESEARCH PROJECTS

1. Membrane fission

Yuichi Kawana, Felix Campleo and Anne-Marie Alleume

The Golgi apparatus is composed of cisternae (flat membranes) that are stacked and kept near the centrioles in mammalian cells. Why such unique organization and spatial location? How is this organization regulated during protein transport? The secret here is membrane fission (cutting) that is necessary to generate transport carriers. Membrane fission must be regulated to generate transport carriers commensurate with cargo size, prevent formation of empty carriers or complete conversion of Golgi into small vesicles, during protein secretion? We asked a simple question whether there were chemicals that would vesiculate Golgi membranes by uncontrolled activation of membrane fission. Identification of such a compound would make the process of membrane fission amenable to molecular analysis

Fluorescence microscopy was used to screen 600 sponge metabolites and a compound called Ilimaquinone (IQ) was identified based on its property to specifically vesiculate Golgi membranes into small 60-90 nm size vesicles (Takizawa et al., Cell 1993). IQ mediated Golgi vesiculation was reconstituted in vitro, which revealed the involvement of trimeric G protein subunits ßg. This was the first clear documentation of the involvement of trimeric G proteins in membrane fission (Jamora et a., Cell. 1997). Soon thereafter we found that Gbg activated a serine/threonine protein kinase called PKD (Jamora et al., Cell. 1999). The next obvious question was to test whether PKD is required for formation of transport carriers from Golgi membranes during protein secretion. This was indeed the case and we found that inhibition of PKD blocked secretion of proteins, interestingly and specifically, from Golgi to the cell surface. Under these conditions, cargo destined to the cell surface was packed into transport carriers, but the carriers failed to undergo cutting (fission) and grew, consequently, into large tubules. This was the first key component, a protein kinase, identified to be essential for regulating events leading to the biogenesis of a cargo filled Golgi to cell surface transport carrier (Liljedahl et al., Cell. 2001). How is PKD recruited to the Golgi membranes? We found that diacylglycerol was required for the recruitment of PKD to the Golgi membranes (Baron and Malhotra, Science. 2001). DAG was known to be required for protein secretion in yeast but our findings placed its requirement specifically in the events leading to membrane fission. Soon thereafter, we realized that there were three different isoforms of PKD in the mammalian cells and surprisingly, all were required for Golgi to cell surface transport (Yeaman et al., Nature Cell Biology. 2004). Moreover, all three forms were found to be specific for the trafficking of only those proteins that were destined to the basolateral cell surface. Apically targeted proteins did not require the activity of PKD. Our most recent findings suggest that all mammalian cells contain 2 of the 3 isoforms and these two isoforms form homo (1-1; 2-2, for example) and heterodimers (1-2, for example) and the formation of the dimers do not require DAG (Bossard et al., Journal of Cell Biology. 2007). There are numerous reports now on the involvement of PKD in protein secretion but the challenge is to understand the molecular mechanism of downstream events. PKD has been shown to activate a lipid kinase called PI4KIIIb, which converts phophatidylinositol (PI) into phophatidylinositol 4-phosphate (PI4P). PI4P is required for Golgi to cell surface transport. We suggest that local production of PI4P through a PKD dependent activation of PI4KIIIb recruits specific effectors that are required for membrane fission (Klaus Pfizenmeir and colleagues. Germany). PKD also phosphorylates a ceramide transfer protein called CERT. Phosphorylation of CERT by PKD prevents the attachment of the former to the Golgi membranes (Klaus Pfzemmeir and colleagues. Germany). Ceramide can be used to generate DAG and we suggest that this event occurs once the transport carriers are formed to prevent further production of DAG. This might be necessary to prevent uncontrolled production of vesicles (Bard and Malhotra, Ann Rev Cell and Dev Biol.2006). We have identified a novel protein called Yusukin (after a postdoc who identified it), which is a substrate of PKD and required for Golgi to cell surface transport. Clearly, a number of PKD substrates are now in hand but the only way forward to a mechanistic understanding of membrane fission is to reconstitute this process with pure components. We have reconstituted the budding of TGN to cell surface specific transport carriers in permeabilized mammalian cells. This assay is being used to test identify new components required for the formation of transport carriers, test the function of the components identified thus far, and to isolate TGN to cell surface transport carriers. The purified transport carriers will be analyzed to determine their lipid and protein composition.

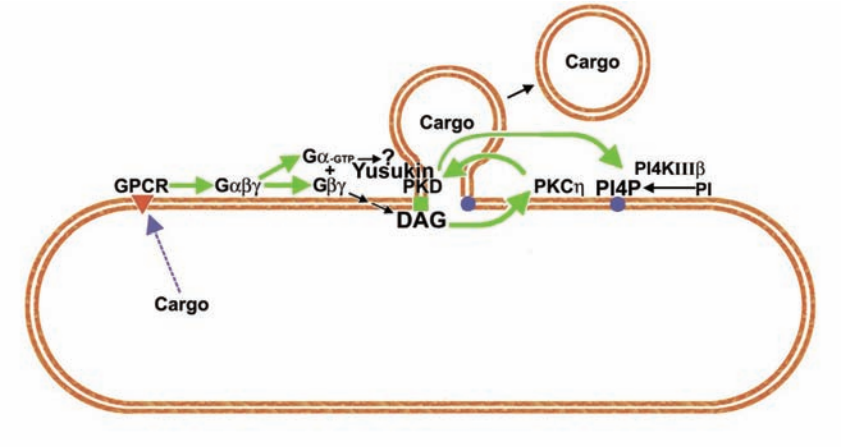


Figure 1.
Regulation of membrane fission by PKD. A trimeric G-protein is required for the recruitment and activation of PKD. PKD regulates the activity of a number of components to assemble a fission machine, which is used to cut cargo filled transport carriers from the TGN.

2. New protein transport components

Kota Saito

A genome wide screen was carried out to identify new components required for protein secretion. From the 22,000 genes tested, 110 gene products were identified to be essential for secretion. This contained 22 previously known components. The rest were cloned, tagged and expressed to identify the intracellular location of the cognate proteins. 20 genes products were further selected, based on their localization to compartments of the secretory pathway (Bard et al., 2006. Nature). The new genes of interest are called TANGO for Transport And Golgi Organization. TANGO1, 5 and a previously identified gene called twinstar are being further characterized for their roles in protein transport and Golgi organization.

TANGO1 is required for loading cargo into transport carriers at the Endoplasmic Reticulum (ER) exit sites (Kota et al., Cell in press). Our aim is to understand the mechanism by which TANGO loads cargo without entering the transport carrier itself.

3. Unconventional secretion.

Juan Duran and Josse VanGalen

Some of the interleukins, fibroblast growth factors (FGF), inhibitor of macrophage migration (MIF), Galectins etc., are secreted from cells without entering the ER-Golgi pathway. These secreted proteins are key players in the immune response, cell growth, angiogenesis, but the mechanism of their release from cells remains mysterious. We have found that the Golgi associated protein called GRASP in dictyostelium discoidium is required for secretion of a protein called AcbA. AcbA, like the proteins mentioned above, lacks a signal sequence necessary for targeting to the ER, and is secreted unconventionally (Kinseth et al., Cell. 2007). Mammalian cells contain 2 GRASPs, GRASP55 and GRASP65. We are testing the involvement of these proteins in unconventional secretion in mammalian cells.

4. Inheritance of Golgi membranes during cell division.

Sandra Mitrovic

How are Golgi membranes partitioned into daughter cells during cell division? 3 major findings from our laboratory on this issue of fundamental importance follow. 1, The Golgi membranes retain their identity throughout the cell cycle and do not relocate to the ER their delivery into daughter cells (Pecot and Malhotra, Cell 2004 and 2006). 2, Golgi membranes undergo a change in their overall organization, which is mediated by a specialized MAPK pathway (Acharya et al., Cell. 1998) and polo like kinase (Sutterlin et al., PNAS. 2001). 3, A change in golgi organization is required for entry of cell into mitosis (Sutterlin et al., Cell. 2004). In vitro assay reconstituting Golgi fragmentation by mitotic cytosol and system wide SiRNA are being used to identify new components involved in regulating Golgi organization. We are particularly interested in components involved in connecting Golgi membranes with the centrioles, the substrates of MAPK and polo-like kinase on the Golgi membranes and the mechanism regulating the shape and size of Golgi cisternae.

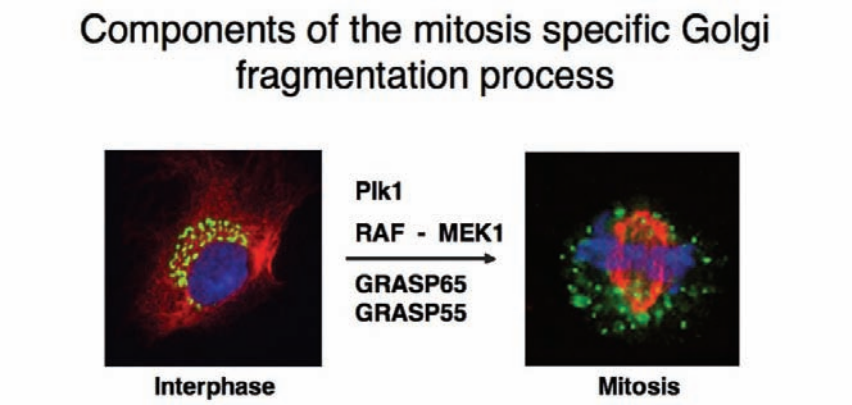


Figure 2.
Mechanism of Golgi fragmentation during mitosis. Fragmentation of Golgi membranes (in green) by mitotic cytosol is reconstituted in vitro. This assay has revealed the involvement of plk, Raf-MEK1 pathway, and the Golgi associated proteins GRASP55 and GRASP65.

5. A Golgi organization specific cell cycle checkpoint.

Julia Von Blume

Inhibiting changes in Golgi organization prevents entry of cells into mitosis (Sutterlin et al., Cell. 2004). Thus a mechanism exists to monitor organizational changes in Golgi and if there is any defect, entry of cells into mitosis is blocked. What is the molecular mechanism of this Golgi specific checkpoint? How is this event coordinated with other check points that are activated upon DNA damage and defective spindle dynamics? A combination of in vitro approaches and system wide SiRNA is being used to identify the Golgi specific cell cycle checkpoint.

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(*) All these publications are the result of the work of Dr. Vivek Malhotra at the University of California in San Diego, La Jolla, USA.

CELL AND DEVELOPMENTAL BIOLOGY

Group: Microtubule function and cell division

Isabelle Vernos is a Research professor from ICREA.

Group structure:

Group Leader:	Isabelle Vernos
Postdoctoral Fellows:	Teresa Sardon, Sylvain Meunier, Roser Pinyol
Students:	Vanessa Dos Reis Ferreira, Isabel Peset, Martin Schütz, David Vanneste, Antonios Lioutas
Technician:	Luis Bejarano, María Sanz, Nuria Mallol (since May 2008)

SUMMARY

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 2007, we have focused on the morphogenesis of the the bipolar spindle in mitosis and meiosis.

Our favourite 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.

RESEARCH PROJECTS

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 rely 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). To understand the molecular mechanism underlying cell division we study the process of microtubule nucleation and stabilization during M-phase and the role of molecular motors in bipolar spindle assembly and chromosome movements.

1. Pathways for microtubule assembly during M-phase

The centrosome is the major site for microtubule nucleation in animal cells and its activity is finely regulated during the cell cycle. In late G2 and prophase, the pericentriolar material expands by recruiting additional components, such as the γ-tubulin-ring complex and as a result the MT nucleation activity of the centrosome increases. Concomitantly after nuclear envelope breakdown, a centrosome independent pathway relying on a RanGTP gradient triggers MT nucleation in the vicinity of the condensed chromatin. We want to understand the molecular mechanisms underlying these two pathways and how they are regulated during the cell cycle.

- In the context of a collaborative project involving several research groups (Centrosome 3D) we are continuing our efforts to understand the activity and regulation of the centrosome for driving microtubule nucleation and stabilization during cell division.
- Relatively little is known on the molecular mechanism underlying the RanGTP dependent pathway for MT nucleation and stabilization. We have previously identified TPX2 as being essential for this pathway and for spindle assembly both in mitosis and meiosis (Gruss and Vernos, 2004; Gruss et al., 2002; Wittmann et al., 2000). To unravel the molecular mechanism underlying the RanGTP dependent pathway for MT assembly in M-phase, we continue our studies on TPX2 and its interaction partners and we aim at identifying the other RanGTP regulated factors involved in the same pathway. This year in collaboration with two groups working in plants we have shown that TPX2 has conserved functions in animals and plants (Vos et al, 2008; Evrard et al, 2009)
- We have previously shown that the Xenopus TACC family member, Maskin, plays an essential role for microtubule growth 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 (Peset I. et al, 2005). This year we have determined the role of Maskin in the RanGTP dependent microtubule assembly pathway (Sardon et al, 2008) and we have started to examine the functional implications of its interaction with the Aurora A kinase.

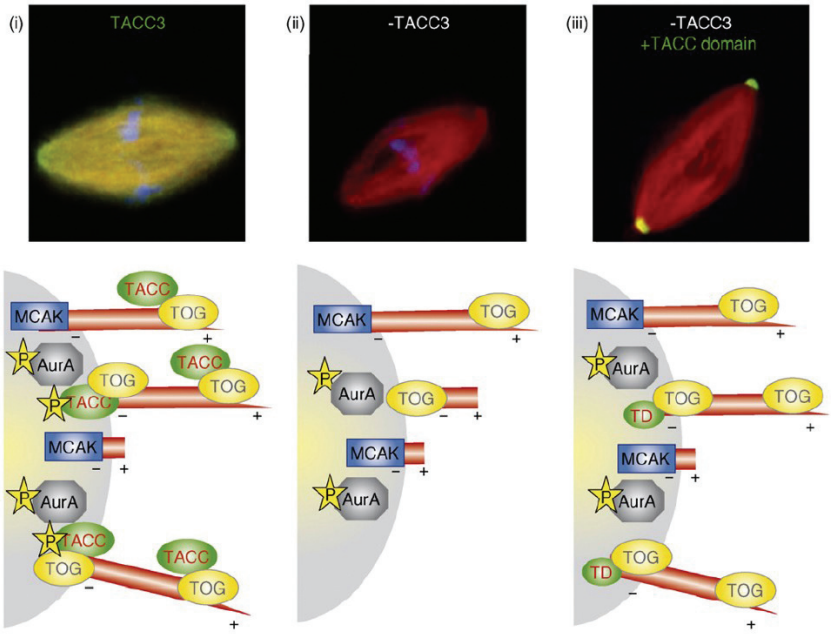

Figure 1:
Localisation of TACC3 in spindles assembled in Xenopus egg extract under different conditions (left control, middle in TACC3 depleted extract and right in depleted extract supplemented with a GFP-TACC domain). The lower part of the figure depicts some models for microtubule elongation from the centrosome in the three different conditions. (from Peset and Vernos, 2008)

2. Regulation of spindle assembly by the kinase Aurora A

The Aurora A kinase has been implicated in several important processes including centrosome maturation during G2, mitotic entry, centrosome separation and bipolar spindle assembly. However its precise role is still relatively unclear. We have previously shown that Aurora A interacts with TPX2 in a RanGTP dependent manner resulting in kinase activation and that it interacts with Maskin and regulates its function through phosphorylation. Using the egg extract system we have studied the function of Aurora A during spindle assembly by dissecting the different pathways involved in MT nucleation and stabilization. We found that Aurora A works though different mechanisms to regulate MT assembly during mitosis, ensuring bipolar spindle formation (Sardon et al, 2008). In collaboration with the group of Prof Giannis (Leipzig, Germany) we have identified and characterized novel inhibitors of the Aurora A quinase that could be useful for basic and applied research (Sardon et al, 2009).

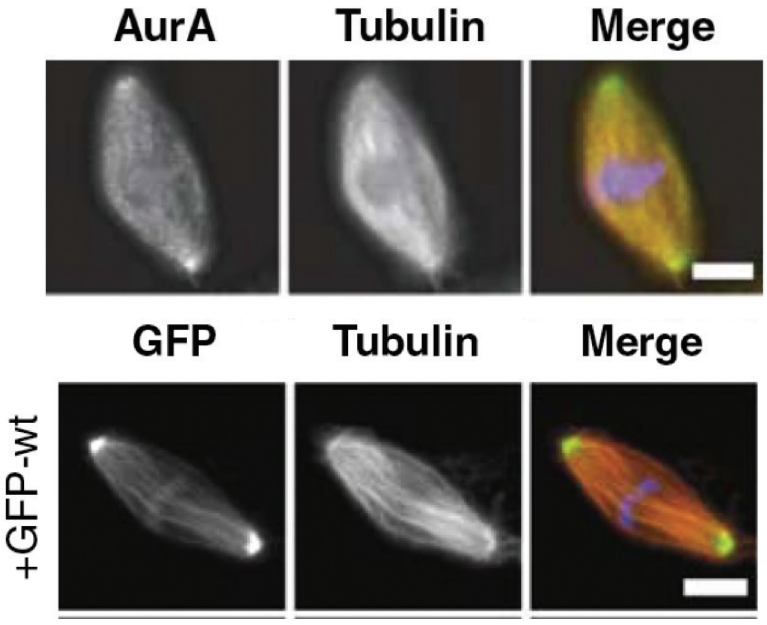

Figure 2:
Immunofluorescence images of spindles assembled in Xenopus egg extracts showing Aurora A (left, in green) localized all along the spindle. Microtubules are in red (Tubulin) and DNA in blue in the merge image.
In the lower pannel the localization of recombinant GFP-Aurora A added to the egg extract is shown. (from Sardon et al, 2008)

3. Role of molecular motors in spindle assembly and chromosome movements

The spindle is a dynamic assembly of microtubules and associated proteins including MAPs and molecular motors. A main functional characteristic involves dynamic interactions between chromosomes and the microtubules. These interactions play an active role in spindle formation and power chromosome movements leading to their alignment on the metaphase plate and their segregation during anaphase. Some of these interactions are mediated by 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. Xkid is required for chromosome alignment on the metaphase plate (Figure 2) (Antonio et al., 2000) and plays a role in cell cycle progression during Xenopus oocyte meiotic maturation (Perez et al, 2002). We are continuing our efforts to unravel how phosphorylation regulates Xkid activity during mitosis and meiosis. We are also trying to understand the role of hklp2 during spindle assembly in human tissue culture cells.

PUBLICATIONS

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"Development and Biological Evaluation of a novel Aurora A Kinase inhibitor."
ChemBioChem, In press.

CELL AND DEVELOPMENTAL BIOLOGY

Group: Sensory Cell Biology and Organogenesis

Group structure:

Group Leader: Hernán López-Schier (Ramón y Cajal fellow)

Postdoctoral Fellows: Mariana Muzzopappa (EMBO postdoctoral fellow), Adele Faucherre (Marie Curie postdoctoral fellow), Jean Pierre Baudoin (FRM postdoctoral fellow)

Graduate Students: Indra Wibowo, Filipe Pinto Teixeira, Jesús Pujol Martí

Technician: Andrea Durán

SUMMARY

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.

RESEARCH PROJECTS

1. Cellular responses to polarity signals, in particular planar cell polarity

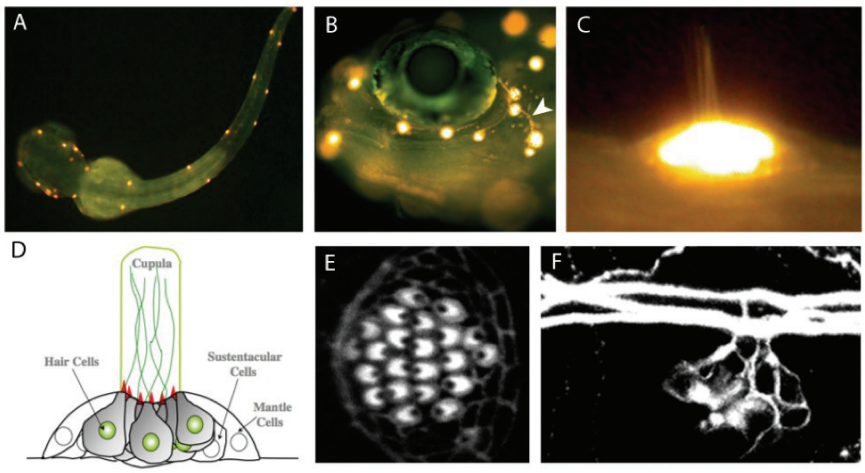
We use the mechanosensory lateral line of the zebrafish (Danio rerio) as a model system to study cell-fate specification, and the formation and remodelling of epithelial architecture during organ development and regeneration (Figure 1). For our studies, we employ cellular, genetic and molecular approaches 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.

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 (Figure 1 A-C). A neuromast contains two types of peripheral supporting cells and a few centrally located hair cells innervated by afferent and efferent axons (Figure 1 D-F). Hair cells in neuromasts are polarised within the plane of the epithelium in a way comparable to that of the inner ear (Figure 1 E). The lateral-line organ of the zebrafish is thus ideally suited to investigate the mechanisms that control hair-cell planar polarisation.

Figure 1

Low magnification view of a living zebrafish larva whose hair cells in the lateral line were labelled with the fluorescent vital dye DiAsp (bright orange). It shows the superficial and systematic distribution of neuromasts along the anterior (head) and posterior (trunk and tail) lateral-line systems. B: Higher magnification of head neuromasts revealing the transynaptic transport of DiAsp from hair cells to afferent nerves (white arrowhead). C: The highest magnification of a neuromast labeled with DiAsp shows a core of hair cells and their apical-projecting hair bundles. D: Scheme of a neuromast with its constituent cells. Neurons are not depicted. E: The orientation (planar polarity) of hair cells is evident in this neuromast whose actin-rich hair bundles were stained with phalloidin. The kinocilium (not stained) appears as a black hole giving the hair bundle its horseshoe appearance. F: Axonal arbor of two afferent neurons branching off the lateralis nerve below a neuromast. Each axonal branch makes a single button ending on each hair cell (not shown).



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.

2. Sensory organ 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 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 (Figure 1 F).

3. Sensory-organ growth and regeneration, with an emphasis on epithelial remodelling

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. 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 (Figure 2).

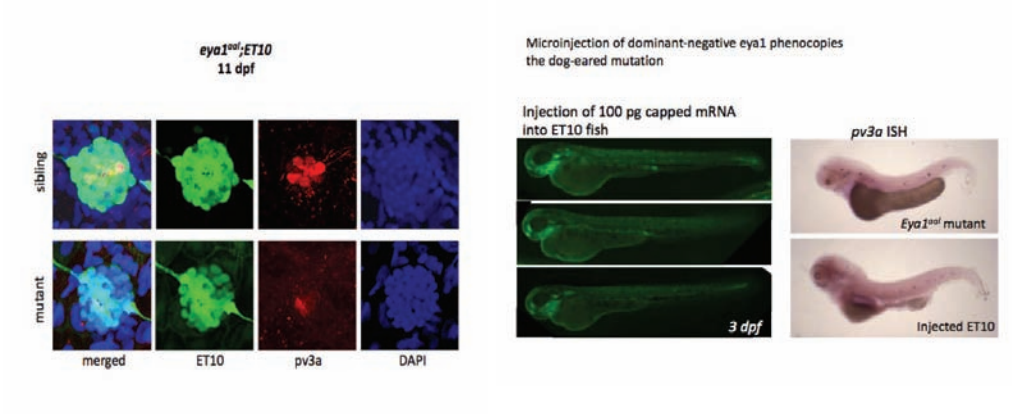


Figure 2: *High magnification view of wild type and eya1/dog eared zebrafish larvae whose hair cells in the lateral line were labelled with PV3a (red). Fish are transgenics for ET10, which expresses the GFP in supporting cells (green). Blue is DAPI staining. It shows that hair cells degenerate in eya1/dog eared. B: Low magnification of zebrafish of the ET10 line expressing Eya1DN. It phenocopies the eya1/dog eared mutation.*

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.

PUBLICATIONS

Faucherre A, Pujol-Martí J, Kawakami K and López-Schier H.
"Afferent neurons of the zebrafish lateral line are strict selectors of hair-cell orientation".
PLoS ONE, In press.

CELL AND DEVELOPMENTAL BIOLOGY

Group: Coordination of cytokinesis with chromosome segregation (since July 2008)

Group structure:

Group leader:	Manuel Mendoza (from July 2008)
PhD student:	Gabriel Neurohr (from Nov. 2008)
Technician:	Trinidad Sanmartin (from Oct. 2008)
Visiting student:	Basil Greber (until Dec. 2008)

SUMMARY

Living cells have a fascinating ability to generate complex and dynamic internal structures. Nowhere is this property more evident than during mitosis and cytokinesis: in a very short time (often of the order of a few minutes) cells alter their shape, duplicate and partition their internal components, and divide into two apparently identical halves. These dramatic morphological changes need to be carefully coordinated with each other in space and time. To learn more about the principles underlying this coordination, we focus on the events at the end of the cell cycle: chromosome segregation and cytokinesis in the budding yeast, Saccharomyces cerevisiae.

RESEARCH PROJECTS

1. Mechanisms of chromosome segregation sensing by the NoCut checkpoint

Cell division is completed through partition of the cytoplasm by ingression of the cleavage furrow, and the subsequent cleavage of the cell membrane into two during abscission. At the same time, the replicated chromosomes are segregated to opposite ends of the cell. Whereas cleavage furrow ingression usually proceeds concomitantly with poleward movement of the chromosomes, abscission must take place exclusively after the last pair of sister chromatids have been pulled out of the cleavage plane. In budding yeast and human cells, a checkpoint known as NoCut delays completion of cytokinesis when chromosome segregation is impaired. Inactivation of NoCut leads to premature abscission, and late-segregating chromosomes are trapped and (at least in yeast) cut by the cytokinesis machinery.

The Chromosome Passenger Complex (CPC) component Aurora B kinase (Ipl1 in yeast) plays a central role in the NoCut checkpoint (Fig. 1). The CPC localizes to the spindle midzone during anaphase, and our results indicate that it is activated by the presence of acetylated chromatin around the midzone. Ipl1 activity mediates the translocation of two anillin-related proteins, called Boi1 and Boi2, to the site of cytokinesis during anaphase, where they inhibit cytokinesis until chromosomes are cleared from the cleavage plane.

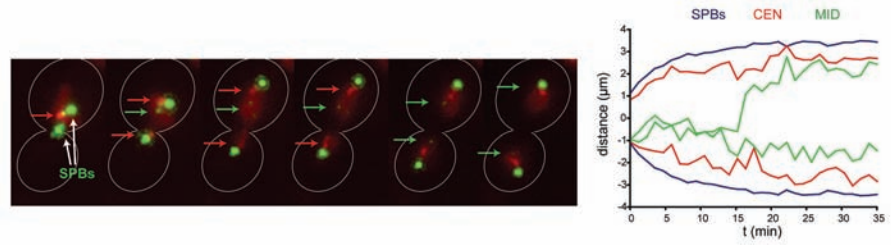


Fig. 1. *Model of the NoCut pathway. (A) In early anaphase, chromosomes (depicted in purple) are in contact with the CPC, which binds to spindle midzone microtubules (in orange). Inset: the CPC subunits Ipl1 and Sli15 are depicted in red and blue, respectively. Midzone-bound Ipl1 is kept active, probably through interaction with chromatin-associated factors (in green) which require histone acetylation (orange circles) to activate the CPC. As a result, Boi1 and Boi2 localize to the bud cortex, where they inhibit abscission. (B) When chromosomes are segregated away from the midzone, the CPC is no longer activated by chromatin and the NoCut signal is turned off; Boi1 and Boi2 leave the bud neck, and abscission (represented by red triangles) ensues.*

We want to determine the molecular mechanism by which lagging chromosomes activate the CPC, and how this signal is transduced to the plasma membrane to inhibit cytokinesis. Central to this effort will be the detailed characterization of novel NoCut components, which we have already identified through genetic screens.

2. Coordination of chromosome length with spindle elongation

We are also interested in other mechanisms ensuring robust chromosome segregation. The function of the mitotic spindle is to separate sister chromatids away from each other. When the length of the anaphase spindle reaches twice the length of the longest chromosome arm, chromosome segregation is complete. Are there mechanisms that allow the cell to adjust the size of the anaphase spindle and/or anaphase dynamics, to the length of chromosome arms? We are addressing this question through manipulation of chromosome arm length in yeast cells, and analysis of mitotic and cytokinetic events by live cell microscopy in normal and mutant strains (Fig. 2).

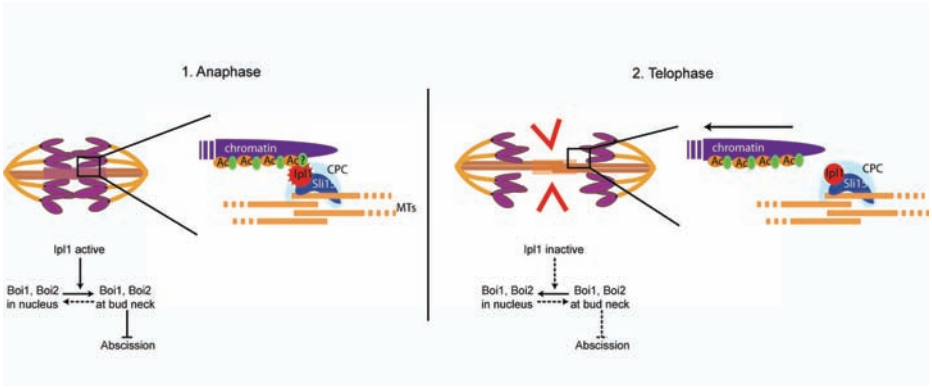


Fig. 2. *Analysis of chromosome segregation in living yeast cells. Spindle pole bodies are labeled with GFP (bright green dots) to track spindle elongation during anaphase. mRFP and GFP protein fusions allow tracking of the centromere (CEN) and middle of the right arm (MID) of chromosome IV, seen as dimmer red and green dots, respectively. The dynamics of the marked loci, relative to the mid-position of the spindle, is quantified in the graph. Note that CEN sequences segregate earlier than MID sequences, as indicated by arrows. Images by Gabriel Neurohr.*

PUBLICATIONS

Mendoza M, Norden C, Durrer K, Rauter H, Uhlmann F and Barral Y.
"A mechanism for chromosome segregation sensing by the NoCut checkpoint."
Nat Cell Biol, In press. (*)

Mendoza M and Barral Y.
"Cytokinesis: Keeping Ring and Membrane Together."
Curr Biol, 18(11):R479-R480. Review. (2008). (*)

Mendoza M and Barral Y.
"Co-ordination of cytokinesis with chromosome segregation."
Biochem Soc Trans, 36(Pt 3):387-90. Review. (2008). (*)

(*) All these publications are the result of the work of Dr. Manuel Mendoza at the Institute of Biochemistry, Biology Department, ETH Zurich, Zurich, Switzerland.

CELL AND DEVELOPMENTAL BIOLOGY

Associated Core Facility: Advanced Light Microscopy Unit

Unit structure:

Head of Facility: Timo Zimmermann

Microscopy Specialist: Raquel Garcia Olivas

SUMMARY

The Advanced Light Microscopy Unit (ALMU) of the CRG serves as a core facility for high-end light microscopy for CRG researchers. A range of instruments with unique capabilities covers the spectrum of advanced imaging applications from automated screening over fast in-vivo imaging to the sensitive detection of very faint signals. The staff of the facility provides advice in the initial experiment planning, training of the researchers on the instruments and assistance with the subsequent data analysis. It is the aim of the facility to provide a link for the biological questions of researchers to the capabilities of advanced light microscopy at the organismic, cellular and molecular level. Methods available in the facility include optical sectioning, spectral imaging, in-vivo timelapse imaging, Fluorescence Resonance Energy Transfer (FRET) detection, Fluorescence Recovery After Photobleaching (FRAP), Total Internal Reflection Fluorescence (TIRF) Microscopy as well as image analysis, particle tracking and 3D rendering.

By the end of its second year of operation, the ALMU has finished its initial installation phase. All available slots are filled with a variety of high-end microscopes. The unit is used regularly by researchers from all CRG programs in experiments ranging from immunofluorescence imaging of fixed samples to timelapse observations spanning several days.

FACILITY OVERVIEW

In 2008, the Advanced Light Microscopy Unit continued to extend the range of available imaging methods through the installation of three additional microscope systems.a

For the observation of big samples and for the dissection of samples for subsequent imaging on other facility microscopes, the Olympus MVX 10 Macrozoom fluorescence macroscope was installed at the beginning of the year. As its optics make it very suitable for the imaging even of weak fluorescence signals, it is additionally equipped with a CCD camera that can be operated in color as well as monochrome modes and that can provide up to 12 Megapixel image resolution.

In September, a fast confocal microscope especially suitable for in-vivo imaging was installed. The Andor Revolution XD microscope uses spinning disk technology for very fast confocal image acquisition and electron-multiplying CCD technology (EM-CCD) for highly sensitive detection of weak signals. This system is additionally equipped with a photobleaching/photoactivation device for fast FRAP experiments, an illumination coupling for TIRF microscopy and an additional EM-CCD camera for TIRF imaging. All these functions use the same set of lasers and can be freely combined, e.g. for FRAP experiments in combination with TIRF illumination. At the time of installation, this was the first Andor system of such a configuration.

In November, the installation of an upright Leica TCS SP5 spectral confocal microscope marked the filling of the last available instrument slot in the facility and thus the end of the initial two-year installation phase of the unit. This system is the first of its kind delivered to Spain as it contains the new fixed stage architecture of Leica's upright microscopes. This makes it very suitable for combinations of microscopy and physiology measurements as well as for whole animal imaging.

Both confocal microscopes were acquired through the Acciones Complementarios (k) of the MICINN. In November, the ALMU also applied successfully for an additional technician position through the Contratos de Apoyo ISCIII program.

An additional image processing workstation was set up together with Hernan Lopez Schier's group in the facility, so that now two stations with Imaris software for high-end image processing are available. Mark Isalan's group installed an Eppendorf micro-dissection device on the Zeiss Cell Observer system in the unit that can now be used in conjunction with the Eppendorf micro-injector.

Among the six available microscope systems in the unit, no two are identical in their features. Because of this, a wide range of microscopy applications can be covered. However, most applications can be performed on at least two systems. This redundancy ensures that experiment planning is not impaired by the limited availability of a single system (Fig. 1).

The usage time of the facility in 2008 was approx. 10.000 hours. In the second half of 2008, 62 CRG researchers from all CRG programs except Bioinformatics used the microscopes in the unit. This constitutes an increase of users by 35% compared to the months before. A monthly average of 30 users from 14 CRG groups uses the instruments for six sessions per month. The average daily usage of the microscopes amounts to 8.8 hours (data based on three instruments that could be analysed throughout the whole year). A significant proportion of the usage time consists of overnight in-vivo timelapse experiments.

During the year, the ALMU staff has participated in teaching in masters courses of the UPF, as well as in microscopy courses in other Barcelona universities and in international microscopy courses in other countries.

Regular training courses in microscopy for CRG researchers were started in January by providing four-day courses in six parallels for 36 participants. The course was organized in conjunction with microscopy application specialists from Leica Spain. The second such course is currently being prepared.

In February, a four-day specialist course for Fluorescence Resonance Energy Transfer (FRET) and Fluorescence Recovery After Photobleaching (FRAP) techniques was organized in conjunction with Leica International for five researchers from Norway.

In April, Leica Spain installed their new macroconfocal TCS LSI for two weeks for evaluation and testing by CRG researchers.

In May, Carl Zeiss AG Spain used the CRG as the venue for the Southern Spain product launch of its latest confocal microscope, the LSM 710. This system was once more installed for a more thorough evaluation for one month in December.

The microscope companies Leica and Andor used their instruments in the facility for equipment demonstrations in the last year.

PUBLICATIONS

Duran JM, Kinseth M, Bossard C, Rose DW, Polishchuk R, Wu CC, Yates J, Zimmermann T and Malhotra V.
"The role of GRASP55 in Golgi fragmentation and entry of cells into mitosis."
Mol Biol Cell, 19(6): 2579-87 (2008).

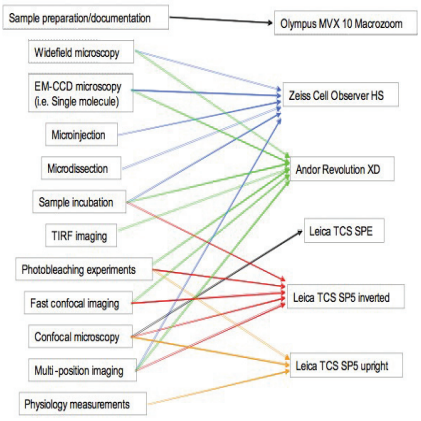


Fig. 1:
Distribution of imaging applications on the microscopes of the Advanced Light Microscopy Unit.

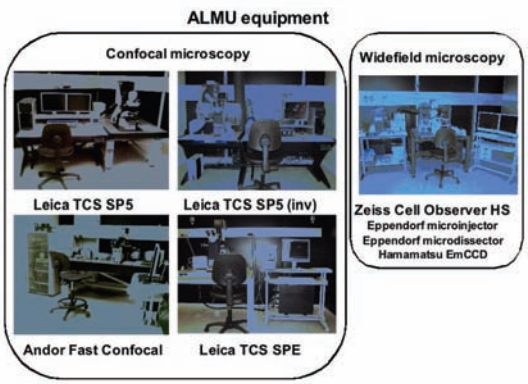


Fig. 2:
Imaging equipment in the Advanced Light Microscopy Unit.

SYSTEMS BIOLOGY

Coordinator: Luis Serrano

In 2008, the programme has finished its group leader recruiting. By hiring two promising young group leaders, Matthieu Louis and Johanes Jaeger, we have successfully filled up all the available slots and we are on cruise speed. This year has seen also the development of the high throughput screening facility, installed in a P2 lab with a fully authomatized robot coupled to a screening microscope, plate reader and PCR machine. This equipment will be used for doing all kind of high throughput assays going from simple ELISA studies to full RNAi and chemical compounds screening. Also this facility will play a strategic role in the CRG transversal project of Cell reprogramming.

An important success for our programme was the successful application to the first ERC round of young investigators. 9000 applicants from all over Europe applied to it and only 5 % were selected. Two of these candidates are young PIs in our programme, Mark Isalan and Ben Lehner.

Structure of the Programme:

Coordinator	Luis Serrano
Senior Group	James Sharpe
Junior Groups	Ben Lehner, Mark Isalan, Matthieu Louis, Johannes Jaeger

Associated Technical and Core Facility services:

Systems Manager	Yann Dublanche
Grant Manager	Michela Bertero
Responsible of the HTS Unit	Raúl Gomez

SYSTEMS BIOLOGY

Group: Design of Biological Systems

Luis Serrano has a Senior ICREA Group Leader position.

Group Structure:

Group Leader:	Luis Serrano
Staff Scientist:	Christina Kiel
Staff Scientist:	François Stricher
Postdoctoral Fellows:	Tobias Maier, Raik Grünberg, Eva Yus, Almer Van der Sloot, Emanuele Raineri, Paolo Ribeca, Alejandro Nadra, Andreu Alibés, Kostas Michalodimitrakis, Cedrik Magis
Students:	Marc Güell, Ronan Burgeois, Anne Campagna, Judith Wodke
Technician:	Justine Leigh, Sira Martinez

SUMMARY

Our group is interested in the rational engineering of living systems (ranging from gene networks to organisms). For this purpose we use a combination of tools that involve software for protein design and simulations of networks and experimental approaches. Our approach is based on first understanding a system and then engineers it to obtain the properties we want. Our philosophy is also whenever possible identifying the possible practical applications for human health and biotechnology of our work.

RESEARCH PROJECTS

1. Quantitative understanding of M. pneumoniae

The idea of harnessing living organisms for treating human diseases is not new and has been in fiction books since a long time ago. So far the majority of the living vectors used in human therapy are viruses which have the disadvantage of the limited number of genes and networks that can contain. Bacteria have the advantage of allowing the cloning of complex networks and the possibility of making a large plethora of compounds either naturally or through careful re-design. One of the main limitations for the use of bacteria to treat human diseases is their complexity, the existence of a cell wall that difficult the communication with the target cells, the lack of control over its growth and the immune response that will elicit on its target. Ideally one would like to have a very small bacterium (of the size of a mitochondria), with no cell wall, which could be grown “In Vitro”, could be genetically manipulated, for which we will have enough data to allow a complete understanding of its behavior and which could live as a human cell parasite. Such a microorganism could in principle be used as a living vector in which genes of interests, or networks producing organic molecules of medical relevance, could be introduced under “In Vitro” conditions and then inoculated either on extracted human cells or in the organism, and then become a new organelle in the host. Once the living vector enters inside the host cells it could then produce and secrete into the host proteins which will be needed to correct a genetic disease, or drugs needed by the patient. Putting it into engineering terms, the living vector will be alike a processor which will have a complicated set of instructions and circuits but will only communicate with the host through input and output outlets. Thus the processor could be reprogrammed but the interface with the hosting cell will remain the same. For some particular applications it will not be needed to integrate the bacteria as an organelle, but rather have it inside the cell for a limited amount of time to achieve its goal and then eliminate it by antibiotic treatment for example.

In order to achieve the above goals we need to understand in excruciating detail the Biology of the target bacterium as well as how to interface with the host cell cycle (Systems biology aspect). Then we need to have the engineering tools (network design, protein design, simulations …) in order to modify the target bacterium to behave like an organelle once inside the cell (Synthetic biology aspect). Thus this project has two objectives;

a) Obtain a complete quantitative understanding of a free-living organism (a bacterium in this case).

b) Engineer the bacterium to enter into a mammalian cell line, adapt to the host so as to keep a fixed number of bacteria per host, respond to the host environment and secrete into the host proteins or organic molecules that will provide missing functionalities.

c) Signal Transduction and disease.

2. Cancer therapy

The efficacy of current treatments for some types of solid tumours is disappointingly poor. Thus, new therapies using novel tumour-selective anti-cancer agents are necessary. A major aim of anti-tumour therapies is to inhibit proliferation and induce death of tumour cells without affecting normal cells. In this regard, members of TNF ligand/receptor family are of interest since they regulate both apoptosis and cell proliferation. One TNF family member, TRAIL, is of particular interest since it selectively induces death of tumour cells without affecting normal cells. Currently, TRAIL and TRAIL-specific antibodies are being investigated as anti-cancer agents. However, one drawback to their efficacy is that they bind to multiple receptors, not all of which transduce an apoptotic signal. Previously, we developed DR5 receptor-selective TRAIL variants, which are potent inducers of apoptosis in various tumour cells, are more efficacious than native TRAIL, and display synergistic effect in combination with other chemotherapy treatments or radiotherapy.

3. Structural Systems Biology (Understanding Signal Transduction)

Understanding signal transduction pathways is capital for human health. Current efforts to do so involve knock out experiments and the use of small molecules or antibodies to interfere with selected protein-protein interactions along the pathway. However, signal transduction pathways in higher eukaryotes are characterized by the existence of multiple interactions for any of its particular components. Therefore, it is difficult to elucidate when knocking out a protein or blocking an activity with a drug what is the relationship between phenotype and the interaction affected. Moreover, there is ample evidence that the majority of the drugs in the market are not as specific as thought and they block more than one activity. Here we want to use a different tool that could allow the specific selection and activation, or inhibition, of specific routes in a pathway and also provide much higher specificity than small molecules. This tool is rational protein design. Structures of macromolecules and especially of molecular machines in combination with protein design could provide quantitative parameters, help to elucidate functional networks, or allow rational designed perturbation experiments for reverse engineering. Computer aided protein design has shown to be able to modify in a rational way protein-protein interactions, tuning specificity, affinity and interestingly kinetics of binding. Protein design can also be used to predict using structural information the partners of a particular domain, or protein.

As a scientific target we have selected the MAPK pathway and our final goal is to obtain a global quantitative understanding with the idea of designing better therapies for diseases involving its deregulation.

PUBLICATIONS

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"Association rate constants of ras-effector interactions are evolutionarily conserved".
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"Interaction of the human somatostatin receptor 3 with the multiple PDZ domain protein MUPP1 enables somatostatin to control permeability of epithelial tight junctions." FEBS Lett, Epub 2008 Dec 9.

Redondo P et al.
"Molecular basis of xeroderma pigmentosum group C DNA recognition by engineered meganucleases." Nature, 456(7218):107-111 (2008).

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"Crystal structure of I-DmoI in complex with its target DNA provides new insights into meganuclease engineering." Proc Natl Acad Sci USA, 105(44): 16888-16893 (2008).

Lenaerts T et al.
"Quantifying information transfer by protein domains: analysis of the Fyn SH2 domain structure." BMC Struct Biol, 8:43 (2008).

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"A molecular dynamics study of the interaction of D-peptide amyloid inhibitors with their target sequence reveals a potential inhibitory pharmacophore conformation."
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"DR4-selective tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) variants obtained by structure-based design". J Biol Chem, 283(29):20560-20568 (2008).

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"Effect of replacing glutamic residues upon the biological activity and stability of the circular enterocin AS-48". J Antimicrob Chemother, 61(6): 1256-1265 (2008). (*)

Esteras-Chopo A, Pastor MT, Serrano L and Lopez de la Paz M.
"New strategy for the generation of specific D-peptide amyloid inhibitors."
J Mol Biol, 377(5):1372-1381 (2008). (*)

Kiel C, Beltrao P and Serrano L.
"Analyzing protein interaction networks using structural information." Annu Rev Biochem, 77:415-441 (2008).

Campagna A, Serrano L and Kiel C.
"Shaping dots and lines: adding modularity into protein interaction networks using structural information." FEBS Lett, 582(8):1231-1236 (2008).

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"Computer design of obligate heterodimer meganucleases allows efficient cutting of custom DNA sequences."
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Di Ventura B, Funaya C, Antony C, Knop M and Serrano L.
"Reconstitution of Mdm2-dependent post-translational modifications of p53 in yeast."
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Pastor MT et al.
"Amyloid toxicity is independent of polypeptide sequence, length and chirality."
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Book chapters

Kiel C, Serrano L.
"Impact of structural proteomics on prediction of protein-protein interactions".
In: Structural Proteomics; JL Sussman; World Scientific Pub Co Inc; New Jersey/London/Singapore, pp. 29-46 (2008) (ISBN13: 978-9812772046)

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

PATENTS

Title: *Citoquina Modificada Para Su Estabilizacion*
Application number: ES2304957
Date: 1-11-2008
Inventors: Domingues Helena [De]; Oschkinat Hartmut [De]; Serrano Luis [De]; Peters Joerg [De].

Title: *Obligate Heterodimer Meganucleases And Uses Thereof*
Application number: WO2008093249
Date: 7-8-2008
Inventors: Fajardo Sanchez Emmanuel [Es]; Grizot Sylvestre [Fr]; Isalan Mark [Gb]; Serrano Pubul Luis [Es]; Stricher Francois [Es].

Title: *Cytokine Design*
Application number: US2008044376
Date: 21-2-2008
Inventors: Tur Vicente R [Es]; Van Der Sloot Albert Martinus [Es]; Mullally Margaret M [Nl]; Cool Robbert H [Nl]; Szegezdi Eva E [Ie]; Samali Afshin [Ie]; Fernandez-Ballester Gregorio [De]; Serrano Luis [Es]; Ouax Wilhelmus J [Nl].

SYSTEMS BIOLOGY

Group: Systems Analysis of Development
James Sharpe has a Senior ICREA Group Leader position.

Group structure:

Group Leader:	James Sharpe
Staff Scientist:	Jim Swoger
Postdoctoral Fellows:	Jean-Francois Colas, Henrik Westerberg, James Cotterell
Technician:	Laura Quintana
PhD Students:	Bernd Boehm, Luciano Marcon, Gaja Lesnicar-Pucko
Undergraduate:	Michael Rautschka

SUMMARY

Our lab has 2 primary goals:

(1) To further our understanding of developmental biology by bringing together a diverse range of techniques from biology, physics, imaging and computer science. The larger part of the lab focuses on a well-characterised standard model of development – the vertebrate limb (using both mouse and chick). For this project we constructing a collection of computer simulations which are based on high-quality quantitative empirical data generated by our own new 3D and 4D imaging technologies. A smaller but equally important project within this topic is a more abstract exploration of the patterning potential of gene network motifs.

(2) Building on the success of the 3D imaging technique developed within the lab called Optical Projection Tomography (OPT – Science 296:541, 2002), the other major goal of the lab is to continue developing and improving 3D and 4D imaging technology. Our most recent success in this direction is time-lapse OPT imaging of mouse limb development in-vitro (Nature Methods 5:609-12, 2008).

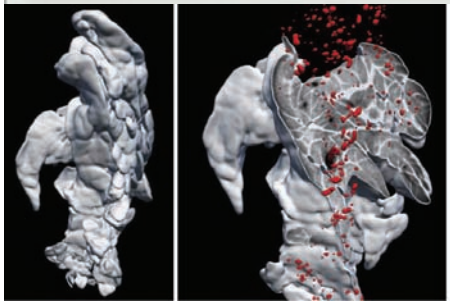
RESEARCH PROJECTS

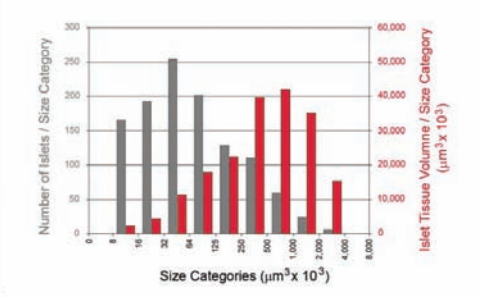
1. OPT imaging for quantitative assessment of mouse models of diabetes

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 (see figure above). 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, and at the beginning of 2007 we published a report (in collaboration with the lab of Dr. Ulf Ahlgren in Umea, Sweden) on this project in Nature Methods (4:31-33, 2007).

Top:
We have worked closely with the MRC to design a commercial version of the scanner.

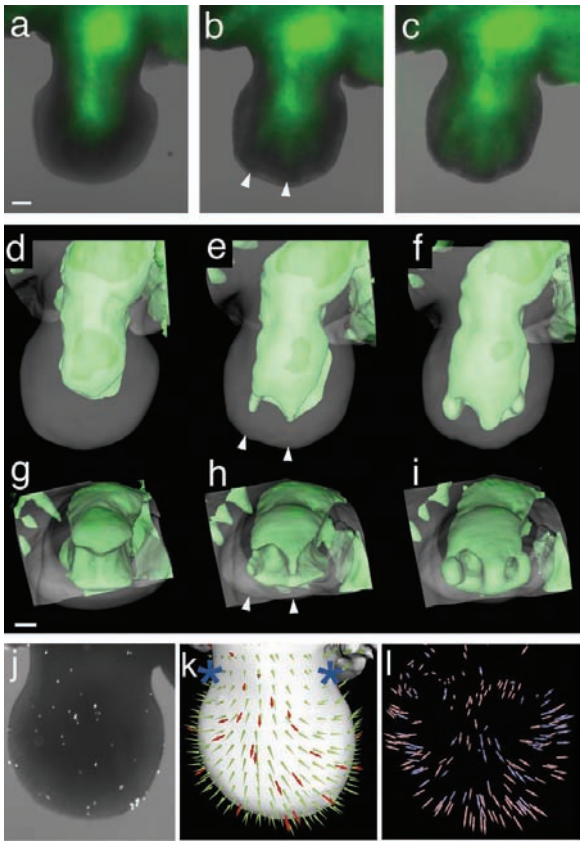
Bottom row: OPT scans of a whole adult mouse pancreas (left) which has been fluorescently-labelled with antibodies against insulin to highlight the 3D distribution of Islets of Langerhans (centre). This data can be morphometrically quantified to provide statistics on the volumetric size distribution (right) - Nature Methods (2007) 4:31-33.





2. 4D Time-lapse Optical Projection Tomography

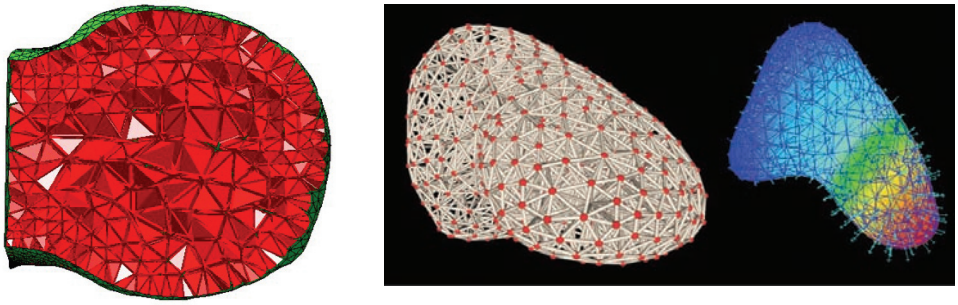
Optical projection tomography has proven to be a powerful tool for developmental biologists. But until recently OPT has been performed almost exclusively on fixed specimens which have been optically cleared to increase the quality of 3D images obtained. Building on the success of this new technology 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 data on tissue movements that is essential for subsequent computer modelling, and we have recently proven that it can also provide data on dynamic gene expression patterns, Nature Methods 5:609-12 (2008).



Time-lapse OPT. Panels (a-c) show a GFP transgenic mouse limb bud at 3 different stages of development in-vitro (Scx-GFP). Panels (d-i) show the same 3 stages as 3D reconstructions from two different orientations (d-f are a dorsal view, g-i are a distal view). The gradual emergence of the digital pattern of Scleraxis is highlighted by arrowheads. The bottom row illustrates the use of Live OPT for tracking global tissue movements. (j) Fluorescent microspheres are placed on the limb ectoderm, (k) these moving landmarks are then tracked over time (red arrows) and can be interpolated into a complete velocity vector field (green). Results from multiple experiments can be overlaid in 3D (l) to show the repeatability of the approach. All panels taken from Nature Methods 5:609-12 (2008).

3. 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 the dorso-ventral flattening of the limb bud, may be more complicated than previously thought. We have created a finite-element model (FEM) of limb development within which we are exploring different hypotheses. An essential aspect of this project is that we also perform lab-work 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.



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

4. 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. Using our new visualisation tools (above) are exploring the ways that these different principles might operate within the limb bud. We are now extending this analysis into a 3D simulation 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.

5. 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 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

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"Evidence that Fgf10 contributes to the skeletal and visceral defects of an apert syndrome mouse model."
Dev Dyn, [Epub ahead of print].

Alanentalo T, Lorén CE, Larefalk A, Sharpe J, Holmberg D, Ahlgren U.
"High-resolution three-dimensional imaging of islet-infiltrate interactions based on optical projection tomography assessments of the intact adult mouse pancreas".
J Biomed Opt, 13(5):054070 (2008).

McGinty J, Tahir KB, Laine R, Talbot CB, Dunsby C, Neil MAA, Quintana L, Swoger J, Sharpe J, French PMW.
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J Biophoton, 1(5):390-4 (2008).

Hajihosseini MK, De Langhe S, Lana-Elola E, Morrison H, Sparshott N, Kelly R, Sharpe J, Rice D, Bellusci S.
"Localization and fate of Fgf10-expressing cells in the adult mouse brain implicate Fgf10 in control of neurogenesis."
Mol Cell Neurosci, 37(4):857-68 (2008).

Boot MJ, Westerberg CH, Sanz-Ezquerro J, Cotterell J, Schweitzer R, Torres M, Sharpe J.
"In vitro whole-organ imaging: Quantitative 4D analysis of growth and dynamic gene expression in mouse limb buds".
Nature Methods, 5(7):609-12 (2008).

Kristen Summerhurst, Margaret Stark, James Sharpe, Duncan Davidson and Paula Murphy.
"3D representation of Wnt and Frizzled gene expression patterns in the mouse embryo at embryonic day 11.5 (Ts 19)."
Gene Expr Patterns, 8(5):331-48 (2008).

SYSTEMS BIOLOGY

Group: Gene Network Engineering

This group is part of the EMBL/CRG Research Unit in Systems Biology

Group structure:

Group Leader: Mark Isalan

Postdoctoral Fellows: Mireia Garriga, Emmanuel Fajardo, Frank Herrmann, Phil Sanders

Students: Andreia Carvalho, Marco Constante

Technician: Rebecca Baumstark

SUMMARY

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 and the other which employs these technologies, and related ones, to synthesise artificial gene networks.

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 1).

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.

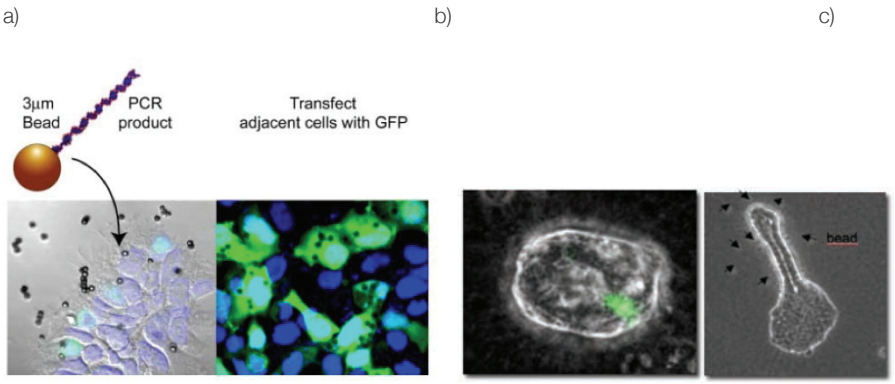


Figure 1:
Our system for magnetically-defined transfection. (a) A GFP-expressing PCR product is directed to cells using the paramagnetic bead as a scaffold. Note the multiple beads adjacent to transfected cells. This technique is being employed to engineer patterning gene networks in eukaryotic cells. The spatial control of transfection can be applied either to individual cells or to many cells in a culture. (b). Spatial transfection of a region of an MDCK cyst with GFP. (c). We have discovered that it is possible to induce single tubules from MDCK cysts using HGF-coated magnetic beads. This is the first step towards engineering a functional spatial pattern-forming gene network in this system.

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.

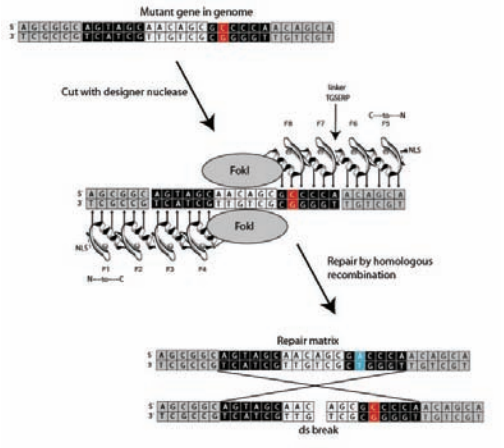
2. Synthesising zinc fingers for gene therapy and gene repair

Frank Herrmann, Rebecca Baumstark and Emmanuel Fajardo

As part of three EU-funded projects (Netsensor, EC Contract No. 012948 http://netsensor.embl.de/; Integra, EC Contract No. FP6 - 29025; FP7 ERC Zinc-Hubs) 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 2).

During 2008, we optimised a number of functional zinc fingers to recognise the p53 gene, resulting in two site-specific nucleases. Each nuclease recognises and cuts different 24 bp regions in the p53 gene sequence. The nucleases efficiently drive specific episomal gene repair in vivo, in a model cell assay. This will be a useful tool to study cancer because >50% of all human cancers have mutations in p53. The zinc finger technology will allow us to repair or mutate these regions in different cell lines at will.

a)



b)

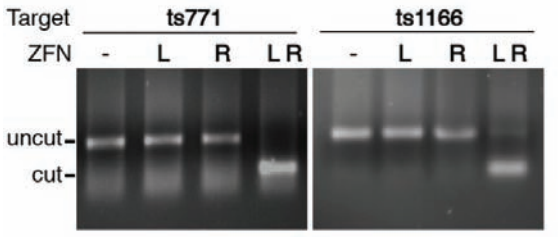


Fig. 2:
Mechanism of homologous gene repair by zinc finger nucleases. (a) In our laboratory, we are designing and synthesising a number of different zinc finger proteins to target unique disease-related genes. By linking the fingers to FokI nuclease, a cut is made in the genome. A gene repair matrix (blue) then recombines into the genome to repair the mutant gene (red). (b) In vitro DNA cutting by full zinc finger nucleases against the p53 tumour suppressor gene (mutated in over 50% of all cancers). p53 PCR target site (ts771, ts1166) is only cut by complete zinc finger nucleases when both the left (L) and right (R) halves are added together (LR).

PUBLICATIONS

Isalan M, Lemerle C, Michalodimitrakis K, Beltrao P, Horn C, Raineri E and Serrano L.
"Evolvability and hierarchy in rewired bacterial gene networks."
Nature 452, 840-845 (2008).

Michalodimitrakis K, Isalan M.
"Engineering prokaryotic gene circuits."
FEMS Microbiol Rev, 33(1):27-37 (2008).

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"Computer design of obligate heterodimer meganucleases allows efficient cutting of custom DNA sequences."
Nucleic Acids Res, 36(7):2163-73 (2008).

Book chapters

Herrmann F, Isalan M. "High-throughput 'On Chip' Protein and Nucleic Acid Transfection.
"In: Encyclopedia of Life Sciences"
(John Wiley & Sons, Ltd: Chichester) DOI: 10.1002/9780470015902.a0020899 (2008).

PATENT PENDING

Obligate Heterodimer Meganucleases And Uses Thereof. WO2008093152.

Group: Metazoan Systems
This group is part of the EMBL/CRG Research Unit in Systems Biology

Group structure:

Group Leader:	Ben Lehner, ICREA Researcher
Postdoctoral researchers (experimental):	Jennifer Semple, Olivia Casaneuva
Postdoctoral researchers (computational):	Tanya Vavouri, Rob Jelier, Benjamin Schuster-Böckler
PhD students (experimental):	Alejandro Burga Ramos, Angela Krüger
Technician (experimental):	Rosa Garcia-Verdugo
Visiting scientists (experimental):	Anna Marchetti (2008-2009), Alyson MacInnes (2008)
Visiting scientists (computational):	Mirko Francesconi (2008-2009), Alice Bossi (2007-2008)

SYSTEMS BIOLOGY

SUMMARY

We use computational and experimental analysis to address fundamental problems in genetics. For experimental work our primary model system is the simple animal C. elegans where we can perform both large-scale perturbation experiments and highly quantitative in vivo measurements at the resolution of individual cells. For computational work we use data from all sources that can be used to test the hypotheses that we are interested in.

RESEARCH PROJECTS

1. Towards predictive genetics

Individual genome sequencing is opening up a new era for genetics. To capitalise on this opportunity we are developing methods that can be used to relate how changes in DNA sequence correspond to changes in organism phenotype. One method is to use integrated gene networks to associate mutations in genes to phenotypic change (e.g. Lee et al., 2008). However most genetic variation changes gene expression rather than coding sequence, and we are therefore systematically dissecting the mechanisms by which changes in gene expression alter the phenotype of an organism (e.g. Semple et al. 2008).

2. Genetic redundancy and genetic interaction networks

A further important issue is that each of our genomes contains many different mutations, and we need to understand how mutations in multiple genes can combine to produce novel (synthetic) phenotypes (e.g. Tischler et al., 2008). Further we know that genomes encode redundant parts – that is multiple genes that can perform a common function. We have shown that this redundancy is evolutionarily stable (Vavouri et al. 2008), and we want to understand why this is.

3. Noise and robustness in genetic systems

Biological systems must function robustly to changes in the environment, to stochastic fluctuations in the levels of their components, and following genetic mutations. We aim to understand how cell states are determined robustly (without errors) in the context of stochastic and environmental variation, as well as the mechanisms that underlie existing stochastic responses to perturbations. We are also testing how individual variation may be useful to an organism. Finally we are interested in the implications of developmental robustness for evolution (e.g. Lehner, 2008), and the existence of global mechanisms that 'tune' the robustness of an organism.

4. Programming and re-programming cell fates

We are using systematic experimental and computational approaches to understand how the expression of genes and their interactions programs alternative cell fates, how these cell states are defined, how cell fates can be therapeutically re-programmed, and how the regulatory networks that define cell states evolve between species (e.g. Bossi and Lehner in press, Vavouri et al., 2007).

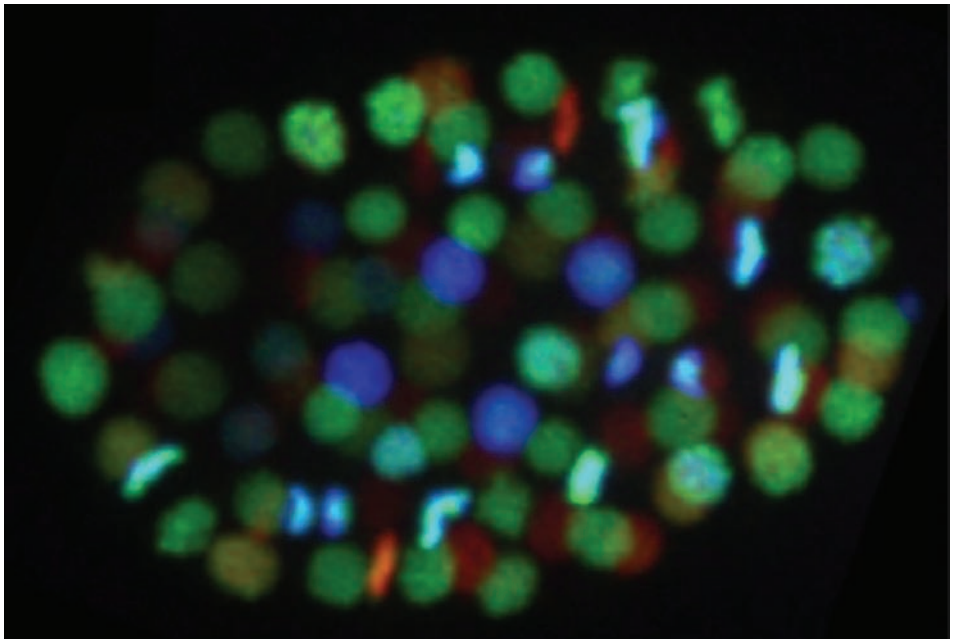

Figure
Dividing nuclei in a C. elegans embryo.

PUBLICATIONS

Bossi A, Lehner B.
"Tissue-specificity and the human protein interaction network".
Mol Syst Biol, In press.

Vavouri T, Semple J, Lehner B.
"Widespread conservation of genetic redundancy during a billion years of eukaryotic evolution".
Trends Genet, 24(10):485-8 (2008).

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"Selection to minimise noise in living systems and its implications for the evolution of gene expression." Mol Syst Biol, 4:170 (2008).

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"A simple principle concerning the robustness of protein complex activity to changes in gene expression".
BMC Syst Biol, 2:1 (2008).

Lee I, Lehner B, Crombie C, Wong W, Fraser AG, Marcotte E.
"A single network comprising the majority of genes accurately predicts the phenotypic effects of gene perturbation in an animal".
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Reviews

Lehner B, Lee I.
"Network-guided genetic screening: building, testing and using gene networks to predict gene function." Brief Funct Genomic Proteomic, 7(3):217-27 (2008).

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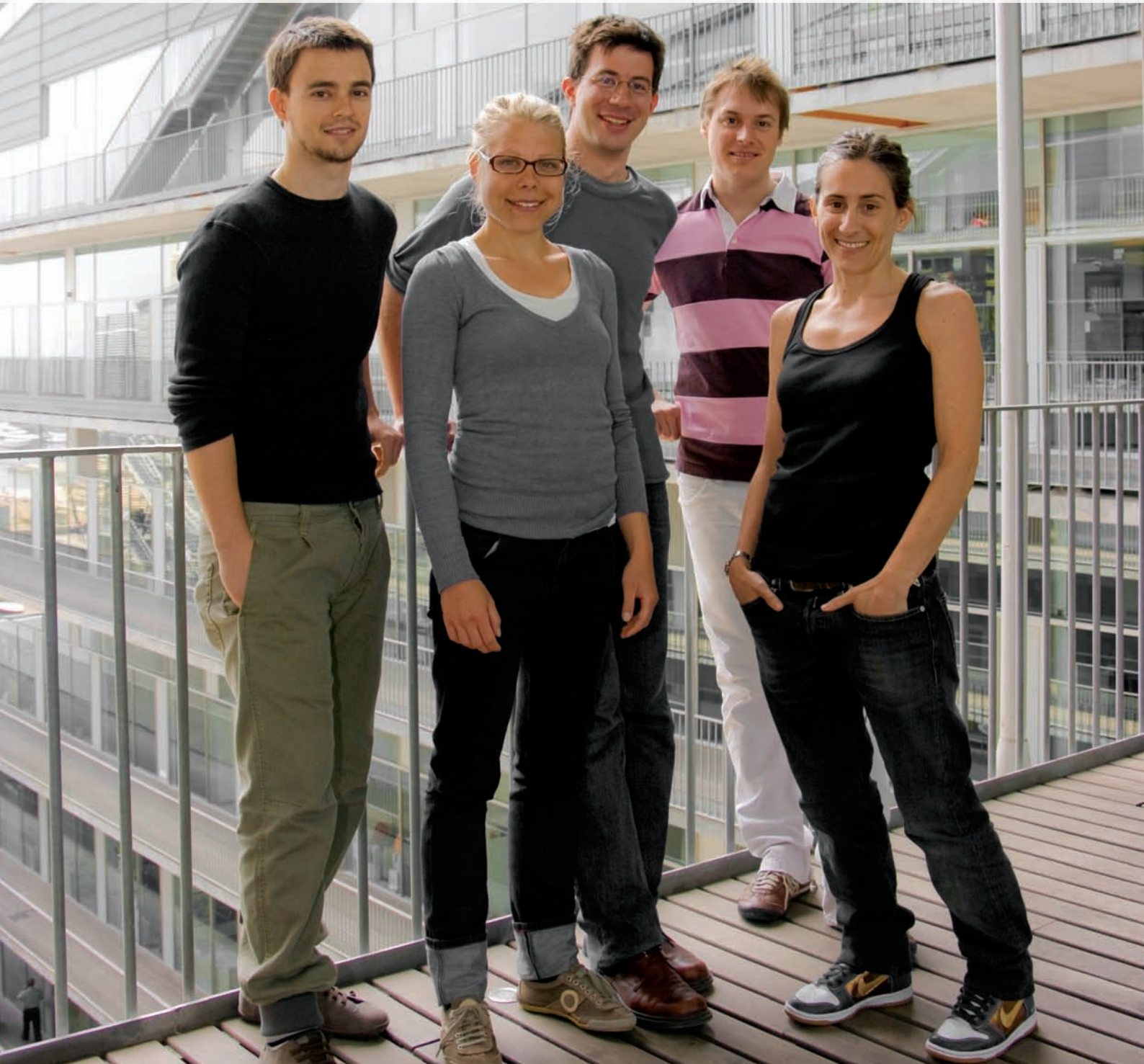
"Applications of RNAi in C. elegans research".
In: RNA interference Research Progress (Nova, New York) (2008).

SYSTEMS BIOLOGY

Group: Sensory Systems and Behaviour
This group is part of the EMBL/CRG Research Unit in Systems Biology

Group structure:

Group leader:	Matthieu Louis
Technician:	Mariana Lopez-Matas, Jordi Sanchez
Post-doctoral fellow:	Alex Gomez-Marin
Student:	Julia Riedl
Visiting student:	Magdalena Richter



SUMMARY

The main research interest of our group is to understand how sensory signals are encoded and processed by the brain and how they direct adaptive behaviour. We tackle this problem in the olfactory system of the fruit fly (Drosophila melanogaster) larva. By applying a combination of experimental and theoretical methods, we are studying the integrated function of the neural circuits encoding odour stimuli. We are also seeking to identify new centres involved in the processing of olfactory information and the making of behavioural decisions underpinning chemotaxis. Our goal is to improve our understanding of the structure-function relationships existing between neural circuits, sensory coding and behaviour.

RESEARCH PROJECTS

1. Functional analysis of the peripheral circuits representing olfactory signal

Mariana Lopez-Matas, Jordi Sanchez and Magdalena Richter

While the structure of the peripheral olfactory system of the larva is relatively well-characterized, the general principles underlying the coding of odours remains poorly understood. The larval 'nose' is composed of only 21 olfactory sensory neurons (OSNs), each expressing one (or occasionally two) type(s) of odorant receptors. How many functional OSNs are required to univocally code for the quality and intensity of an odour stimulus is still an open question. In previous works, we have shown that the information mediated by one type of odorant receptor is sufficient to monitor variations in odour intensity (Louis, Huber at al., 2008). We are now examining if a single type of odorant receptor is sufficient to code for the quality (nature) of an odour stimulus. Our goal is to explain the ability (or inability) of larvae with a single functional OSN to discriminate between two odours based on the patterns of neuronal activity (spike trains) elicited by these odours in individual OSNs. To establish precise correlations between sensory input (OSN activities) and behavioural output (orientation decisions), we have undertaken the development of new methods to carry out single unit recordings from identified neurons of the larval olfactory system (OSNs and projection neurons, Fig. 1).

Recently, we have shown that distinct larval OSNs have overlapping but not identical receptive fields (Asahina, Louis et al, 2009). Increasing odour concentrations of the same odour induce progressive activation of concentration-tuned OSNs and concomitant recruitment of inhibitory local interneurons in the antennal lobe (first relay centre of the fly olfactory system, Fig. 1). These findings suggest that the interplay of combinatorial OSN input and local interneuron activation allow larvae to detect odours across a large range of stimulus intensities. By combining electrophysiology and behavioural assays, we are now studying the combinatorial interactions between OSNs. Our long term goal is to integrate all our experimental data in computational models accounting for the integrated function of the larval antennal lobe.

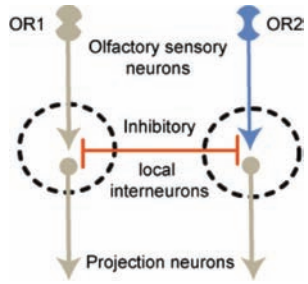


Figure 1: *Schematic of larval antennal lobe. Only 2 of the 21 olfactory sensory neurons expressing a different type of odorant receptors (ORs) are represented.*

2. Characterization of the neural circuits processing olfactory information

Julia Riedl and Alex Gomez-Marin

To perform scent-tracking, larvae compute odour gradients locally and follow the direction of the steepest concentration change (Louis, Huber et al., 2008). How this decision-making process is achieved is still unknown. A major goal of our research is to find the neural circuits implementing these decisions. Our preliminary observations suggest that larvae probe their environment by lateral head movements (sweeps). Our working hypothesis is that the concentrations detected at different positions are converted into a time series that is momentarily held in a short-term memory.

To identify the circuits involved in the processing of trains of odour stimuli and, in particular the spatial memory which potentially directs chemotaxis, we have started a behavioural screen. In adult flies, the mushroom bodies (MB) are known to be implicated in memory and learning. This centre represents a natural starting point for our research in larvae. Using Gal4-driver selectively expressed in the larval MB (Fig. 2), we are analyzing the effect of disabling sub-regions of the MB upon expression of the tetanus toxin C subunit TNT (synaptic transmission inhibitor).

Larvae with two functional sides chemotax more accurately than those with only one functional side (Louis, Huber et al., 2008). Since the left and right dorsal organs (larval 'noses') operate independently, we propose that the existence of two detectors significantly improves the signal-to-noise ratio in odour detection. To test this hypothesis, we have generated new transgenes to compare the behavioural performance of larvae with two functional OSNs expressing the same odorant receptor on one side with that of larvae expressing the same OR in one OSN on both sides. In the long run, our objective is to determine where and how the information coming from both sides is merged.

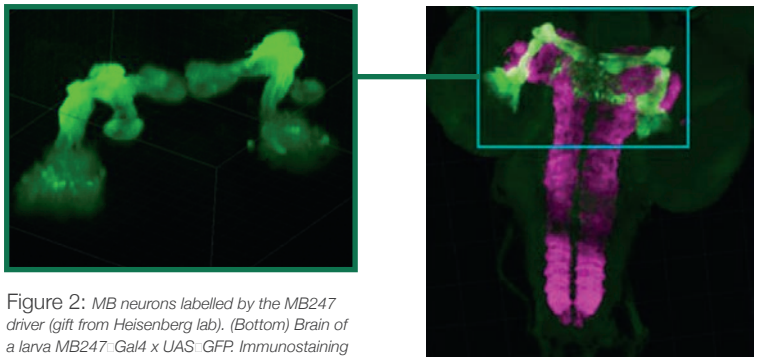


Figure 2: *MB neurons labelled by the MB247 driver (gift from Heisenberg lab). (Bottom) Brain of a larva MB247::Gal4 x UAS::GFP. Immunostaining against GFP (green) and nc82 (neuropil marker, cyan). (Top) Close-up view on the MBs.*

3. Generation of ultrasensitive responses by molecular titration

Matthieu Louis

Many regulatory proteins are sequestered into an inactive complex by repressors. The abundance of dominant-negatives to bZIP/bHLH transcription factors and stoichiometric inhibitors of kinases suggests that something special, and yet universal, underlies this molecular mechanism. Using mathematical and computational modelling, we have shown how and why protein-protein sequestration (called 'molecular titration') is capable of generating 'all-or-none' or ultrasensitive responses (Buchler and Louis, 2008). Our two main findings are: (i) molecular titration can generate threshold responses that are substantially larger than those achieved through molecular cooperativity; (ii) molecular titration can create large ultrasensitivity during time windows compatible with developmental processes, even in the absence of significant amplification at steady state. Our results provide a novel and testable framework which is likely to guide the work of experimentalists studying protein sequestration in regulatory networks.

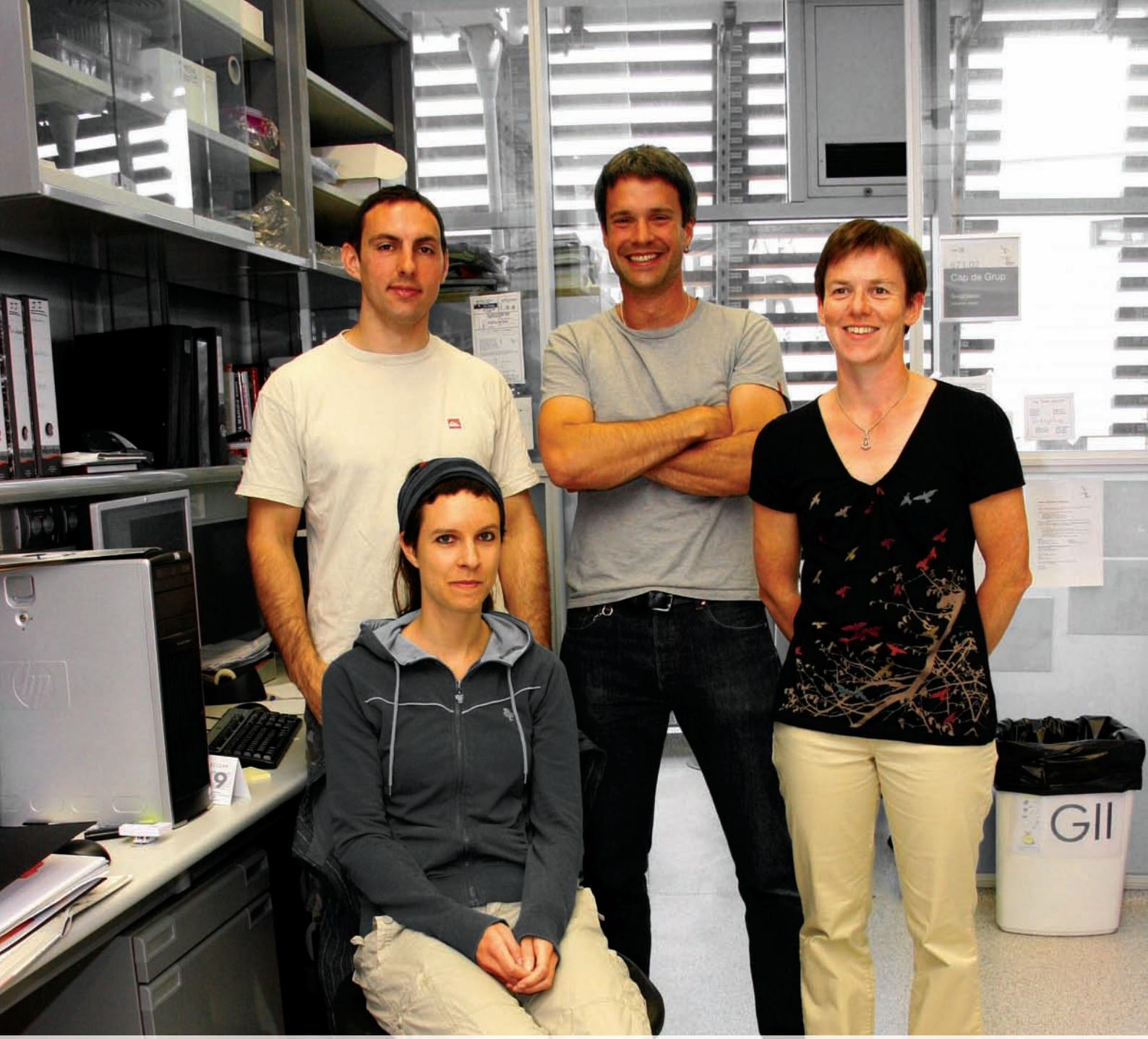
PUBLICATIONS

Louis M, Piccionotti S, Vosshall LB.
"High-resolution measurement of odor-driven behavior in Drosophila larvae."
J Vis Exp, (11). pii: 638. doi: 10.3791/638 (2008). (*)

Louis M, Huber T, Benton R, Sakmar T and Vosshall LB.
"Bilateral olfactory sensory input enhances chemotaxis behavior."
Nat Neurosci, 11:187-99 (2008). (*)

Buchler N and Louis M.
"Molecular titration and ultrasensitivity in regulatory networks."
J Mol Biol, 384:1106-1119 (2008).

(*) These articles are the result of the work of Dr. Matthieu Louis at the Rockefeller University, New York, USA.



SYSTEMS BIOLOGY

Group: Comparative Analysis of Developmental Systems (since October 2008)
This group is part of the EMBL/CRG Research Unit in Systems Biology

Group structure:

Group Leader: Johannes Jaeger

Postdoctoral Fellows: Karl Wotton

Technician: Hilde Janssens

SUMMARY

Our group is interested in how regulatory changes in gene networks lead to phenotypic change in an organism. We study gene network evolution by comparing the gap gene system—involved in segment determination in early development—between the fruit fly (Drosophila melanogaster) and other dipteran species (flies, midges and mosquitoes). To achieve this, we use an integrative approach, which combines quantification of gene expression with mathematical modeling.

We measure spatial patterns of gap gene expression in different dipteran species, such as the scuttle fly Megaselia abdita, the moth midge Clogmia albipunctata and the chironomid midge Chironomus reparus. We then use these data to infer the regulatory interactions necessary and sufficient to explain the observed expression patterns by fitting gene circuit models to data (Fig. 1). Gene circuits are computational tools to extract regulatory information from quantitative data, which have been successfully used to simulate and analyze the gap gene system in Drosophila melanogaster (Jaeger et al., 2004a,b; Gursky et al., 2004; Perkins et al., 2006; Jaeger et al., 2007; Ashyraliyev et al., 2008). Models from different species can then be compared to reveal which interactions are conserved and which have diverged during evolution. The models allow us to predict, which changes in regulatory interactions correspond to which differences in gene expression between species. We will test these predictions by using RNA interference in various species and reporter assays in Drosophila. To date, no such in silico reconstitution of the developmental and evolutionary dynamics of a real developmental system has been achieved in any other experimental system.

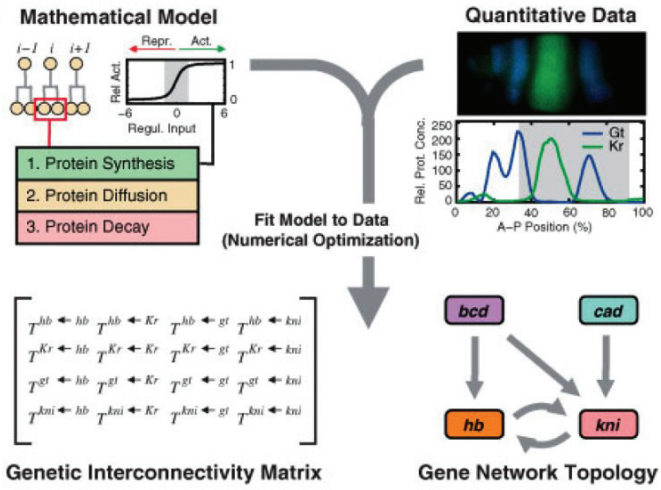


Fig. 1:
The Gene Circuit Method.

RESEARCH PROJECTS

1. A quantitative, comparative study of gap gene regulation in dipterans

Aim. We intend to quantify gap gene expression patterns by in situ hybridization and immunofluorescence in embryos of three species which represent distinct evolutionary branches in segment determination: Clogmia albipunctata, Chironomus reparus and Megaselia abdita (Fig. 2; see Janssens et al., 2005; Surkova et al., 2008 for data quantification methods). These data sets will then be analyzed to characterize spatial variability of gene expression domains and extents of dynamic shifts in the position of expression domains (Jaeger et al., 2004a; 2007). In addition, we will use these data to obtain gap gene circuit models for each species. The resulting network topologies will be used to identify conserved and divergent regulatory mechanisms.

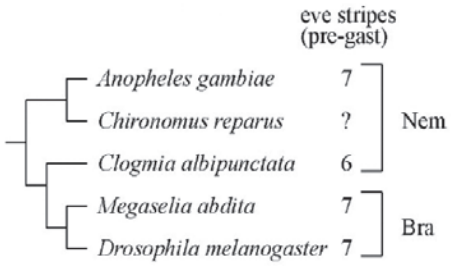


Fig. 2:
Dipteran Phylogeny.
Nem=Nematocerans, Bra=Brachycerans.

Results. In the first three months of this project, our main focus was the establishment of the Clogmia and Megaselia cultures. In addition, we have isolated genomic and cDNA, and are currently cloning gap genes from Megaselia in order to perform systematic in situ hybridization experiments and raise species-specific polyclonal antisera.

2. A quantitative study of gap gene mutants in Drosophila melanogaster

Aim. If we are to study evolutionary transitions using mathematical models of gene networks, we need a modeling formalism that captures the variational properties of the network. However, it remains unclear whether our current gene circuit models can correctly reproduce expression in mutant embryos. We will investigate these issues systematically by generating quantitative gene expression data for Drosophila gap gene mutants (with the group of Prof. J. Reinitz at Stony Brook University, NY, U.S.A.) and testing various modeling formalisms with regard to their ability to reproduce both wild-type and mutant patterns correctly.

Results. Preliminary quantitative data sets for mutants of Kr, kni (Reinitz) are already available, and we have an almost complete data set for mutants of the terminal gap gene tll. In the first three months of this project, we have established cultures of Drosophila melanogaster, and have started completing the tll mutant data set. As soon as it is finished, we will use these data to fit gene circuit models. These models will yield new insights into the dynamics of gap gene regulation. Furthermore, we will use all the available mutant data sets together to investigate why current gap gene models fail to reproduce gap gene null mutants correctly, and to obtain revised network models that are able to faithfully reproduce both wild-type and mutant expression. Such models can then be used for an in silico study of gap gene evolution.

3. Modeling the evolutionary dynamics of the gap gene network.

Aim. We want to explore the possibility that intermediate stages of evolution could be predicted (or reconstructed) using in silico evolution. Predictions from such an analysis can then be tested against the data sets obtained in Projects 1 and 2—or against qualitative expression data obtained from other suitable dipteran species.

Results. We are in the process of hiring a postdoctoral fellow with expertise in computer science and dynamical systems theory to carry out the in silico simulation studies required for this project.

PUBLICATIONS

Jaeger J, Irons D, Monk N.
"Regulative feedback in pattern formation: towards a general relativistic theory of positional information".
Development, 135(19):3175-83. Review. (2008) (*)

Ashyraliyev M, Jaeger J, Blom JG.
"Parameter estimation and determinability analysis applied to Drosophila gap gene circuits".
BMC Syst Biol, 2:83 (2008). (*)

Surkova S, Myasnikova E, Janssens H, Kozlov KN, Samsonova AA, Reinitz J, Samsonova M.
"Pipeline for acquisition of quantitative data on segmentation gene expression from confocal images."
Fly, 2:1–9 (2008). (*)

(*) These articles are the result of the work of Dr. Johannes Jaeger at the University of Cambridge, Cambridge, UK.

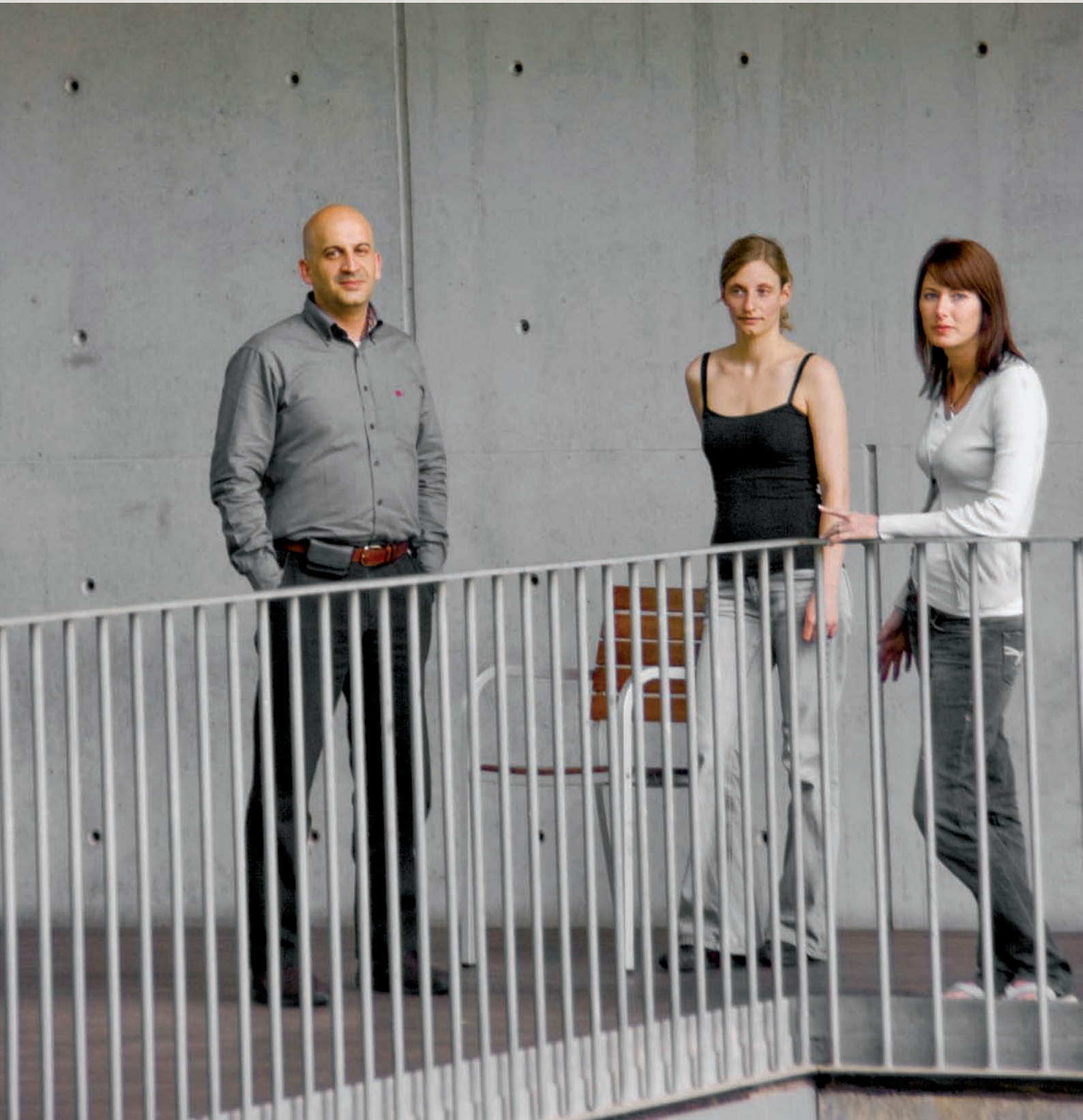
SYSTEMS BIOLOGY

Associated Core Facility: High Throughput Screening Unit

Unit structure:

Unit Responsible: Raúl Gómez

Technicians: Anja Leimpek, Katrina Broadbent, Christine Burn



SUMMARY

The High-Throughput Screening facility at CRG, is dedicated to provide exceptional services in advancing drug discovery research. Performing a variety of highly automated assay that covered biochemical, cell-based, siRNA as well as high content screening campaigns against a plethora of target classes.

The HTS facility has the capability to support cellular and biochemical assays using absorbance, fluorescent kinetics, time-resolved fluorescence , bioluminescence and cellular fluorescence imaging. Assay targets can include ion channels, receptors, enzymes, protein interactions, signalling pathways and cellular processes. The facility incorporates siRNA libraries, instrumentation, databases and personnel devote to establish assays according to the needs of each individual research project.

FACILITY OVERVIEW

Starting in March of 2008, a High-Throughput Screening platform was built featuring the following base set of equipment: a liquid handling system with washer and multi-drop dispenser (the Sciclone ALH 3000 workstation from Caliper), a multimode microplate reader (the Infinite 200M from Tecan), an automated incubator (Cytomat 2C from Thermo Scientific) and a robotic plate handler (KiNEDEx from Peak Robots, Inc.). In collaboration with Caliper, we augmented this suite with an epifluorescent microscope (ImageXpress Micro from Molecular Devices). This integration provides an exceptional combination of automated processes going from cell incubation, via transfection, to image acquisition and processing. Such a platform is unique in its kind in the South of Europe.

As part of a transversal project where antibodies needed validation, a high throughput ELISA system was required. It involved studying the response of serum against 3 different antigens with their respective controls (pre-immune serum). For that purpose, a program for scheduling all platform events was implemented allowing the ELISA assay to be run automatically with a maximum capacity of 16 assays per day.

The large amount of data generated using this system at high capacity led us to develop a data analysis software. “ELISA polyclonal Soft” was written in Matlab and compiled as a standalone application. It calculates the titer level and generates a web-based report for data publishing.

The complete high throughput ELISA system is now available as a service of the HTS facility and has been tested with 40 samples of serum from the lab of Isabelle Vernos.

For use with the newly purchased liquid handling system Zephyr (Caliper), a scripting program was implemented that automates plasmid purification with the corresponding Montage kit of Millipore. This was successfully tested with a yeast genome plasmid library provided by the group of Josep Vilardell. This implementation will be the object of a communication. It is now made available as a service of the HTS facility.

In November, an image processing workstation was set up with MetaXpress™ a software for high content imaging analysis. With it we provide to users with a range of tools to automate processing and analysis of cellular images. It cover the needs for study cell differentiation (Stem cell research), Neurodegenerative or neuroregenerative disease research, examining transfection efficiencies, intercellular signalling and receptor internalization.

By the end of 2009 the facility will expand its capacity on the implementation of new technologies for in-vivo assay.

Sequential waves of polyadenylation and deadenylation define a translation circuit that drives meiotic progression

All metazoans that reproduce sexually have the ability to form gametes. The gametes, the egg and the sperm, arise from germ cells after extensive differentiation, where they reduce by half their gene dosage through two sequential divisions (meiosis, Figure 1). In addition, the egg provides the zygote with the cytoplasm, which supports the development of the early embryo through precise expression patterns of maternally inherited messages. The two meiotic divisions and the early stages of embryonic development, where the basic body patterning is defined, take place in the absence of transcription and gene expression is controlled through the translational regulation of stored maternal mRNAs (Figure 1). Indeed, during the long period of oogenesis (This first meiotic arrest may last up to a few years in Xenopus or several decades in humans), prophase-I arrested oocytes store up to half of their genome as cytoplasmically silenced mRNAs. Then, the information stored in the dormant mRNAs has to be sequentially released at the right time and in the right place. This coordinated temporal and spatial regulation of the transcripts required to complete the two meiotic divisions, segregate correctly the chromosomes and establish the polarity in the egg, is orchestrated by RNA-binding proteins that recognize specific cis-acting elements in the 3'UTRs (for UnTranslated Regions following the coding region of the mRNAs) of groups of mRNAs implicated in similar functions. These RNA-binding proteins are organized in combinatorial networks stabilized by feedback loops that ensure unidirectional progression through meiosis to produce a functional egg. Another recurrent scenario during oocyte maturation and early development are the translational regulatory cascades or sequential waves of translational activation/repression. This hierarchical organization is used to control discrete temporal (i.e. meiotic-phase transitions) or spatial (i.e. local translation during axis determination) complex protein expression patterns. The general principle consists on a cascade where the translation of the mRNAs encoding translation-regulators is itself spatially or temporally controlled.

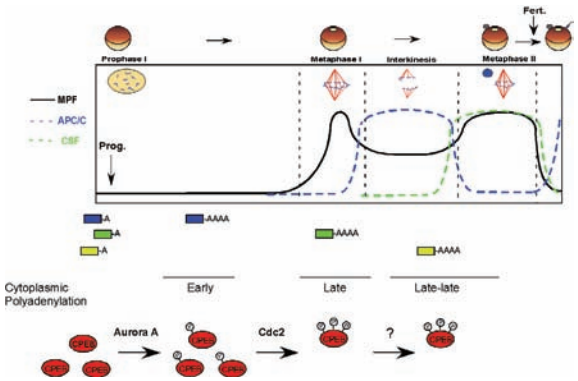


Figure 1. *Meiotic divisions. Vertebrate immature oocytes are arrested at prophase of meiosis I (PI). Meiotic maturation is comprised of two consecutive M-phases (MI and MII) without intervening S-phase. At MII the oocytes become arrested for a second time and await fertilization. Three key activities control meiotic progression. The Maturing Promoting Factor (MPF), an heterodimer of Cdc2 kinase and Cyclin B that catalyzes entry into M-phase of Meiosis I and II. The Anaphase-Promoting Complex (APC), which induces the ubiquitination and destruction of cyclins B. The Cytostatic Factor (CSF) that inhibits the APC stabilizing high MPF activity in MII. Meiotic progression is driven by the sequential translational activation of maternal mRNAs as the result of their cytoplasmic polyadenylation mediated by CPEB.*

The best-studied mechanism regulating the translation of maternally derived mRNAs in the oocyte cytoplasm is cytoplasmic polyadenylation. Not only is polyadenylation a nuclear processing event that fashions the 3'-end of almost all pre-mRNAs, but it also takes place in the cytoplasm during oocyte maturation and early embryo development. Nearly two decades ago, Xenopus oocytes were found to contain stored (silenced) maternal mRNAs harboring a small sequence in their 3' untranslated regions that control cytoplasmic polyadenylation and translational activation, which will drive the oocyte's re-entry into meiosis and early development. This cytoplasmic polyadenylation element (CPE) is the binding platform for CPE-binding protein (CPEB), which promotes polyadenylation-induced translation.

But Individual CPE-containing mRNAs display specific translational behavior during meiosis suggesting that individual features within their 3'UTRs determine their response to CPEB-mediated translational control. Thus, not every CPE-containing mRNA is masked and the activation of CPE-containing mRNAs does not occur en masse at any one time. Instead, the polyadenylation of specific mRNAs is temporally regulated. Despite the knowledge accumulated on the composition and regulation of the protein complexes that mediate translational repression and activation of CPE-containing mRNAs, the 3'UTR features that define whether an mRNA is a target for CPEB-mediated translational repression and how the time and extent of cytoplasmic polyadenylation-dependent translational activation is controlled were still unclear. In two recent works [1, 2] we have performed a systematic analysis of the combinations of cis-acting elements that define, qualitatively and quantitatively, the differential translational control of CPE-regulated mRNAs (Figure 2). The results of these works allow us to postulate a set of rules that can be used to predict the translational behavior of CPE-containing mRNAs during meiosis.

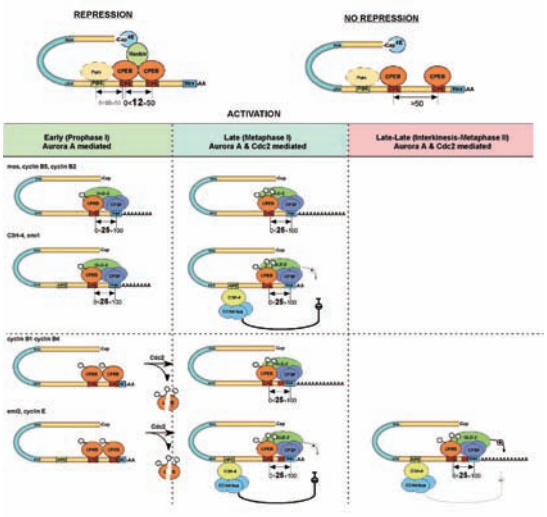


Figure 2. *Model for CPE/ARE-mediated translational control. Schematic representation of the cis-elements and trans-acting factors recruited, with their covalent modifications. The distances required for translational repression and activation as well as the time of activation are indicated. Optional factors/elements are displayed with dotted lines. AA indicates short Poly(A), AAAAAAA indicates long poly(A) and P indicates phosphorylation.*

But not only the mRNAs have to be activated at the right time, some of them have to be also specifically inactivated to allow meiotic progression into the next phase and this takes place thought a deadenylation negative feed-back loop mediated by another RNA-binding protein named C3H-4 [2]. Thus, the hierarchical translation of specific subpopulations of mRNAs at each meiotic phase is regulated through sequential waves of polyadenylation and deadenylation, and define a translational circuit that drives meiotic progression (Figure 3).

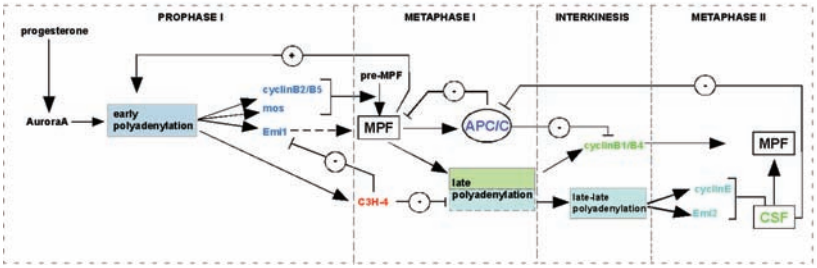


Figure 3. *The meiotic circuit Sequential translational regulation, through sequential waves of polyadenylation and deadenylation, of the maternal mRNAs ensures irreversible, self-sustaining, switch-like meiotic phase transitions by controlling discrete states of MPF, APC and CSF activities. Maturation-Promoting Factor (MPF), Anaphase-Promoting Complex (APC) and Cytostatic Factor (CSF) activities are indicated. The three waves of cytoplasmic polyadenylation (Early, Late and Late-late) are also depicted.*

Asymmetric localization of silenced mRNAs within the oocytes defines the basis for the embryonic axis formation and the establishment of germ cells, by coupling translational repression and localization. At the appropriate time and place, either in the oocyte itself or later in the embryo, the localized mRNAs will be reactivated by disassembling the repression complex and/or by cytoplasmic polyadenylation. Similarly to the temporal translational-cascades described above, spatial-cascades can be defining by generating gradients of translational regulators encoded by localized mRNAs. CPEB1 and C3H-4, localize preferentially to the animal pole of the oocyte, together with CPE-containing mRNAs. This localization seems to be microtubule directed and indeed, these mRNAs and their binding proteins later localize to the meiotic and mitotic spindles. The spindle-associated RNAs include structural components of the spindle, repressed centrosome-localized mRNAs for asymmetrical inheritance in embryonic divisions and CPE-regulated mRNAs encoding factors directly involved in chromosome segregation, spindle formation and meiotic progression. We have shown that the same combinatorial code of cis-acting elements contain the spatial information that dictates where these mRNAs have to be translationally activated. We have shown that, not only the time of translational activation has to be finely regulated, but also this local translation of CPE-regulated mRNAs at the meiotic spindles is required for meiotic progression and correct chromosome segregation [3] (Figure 4).

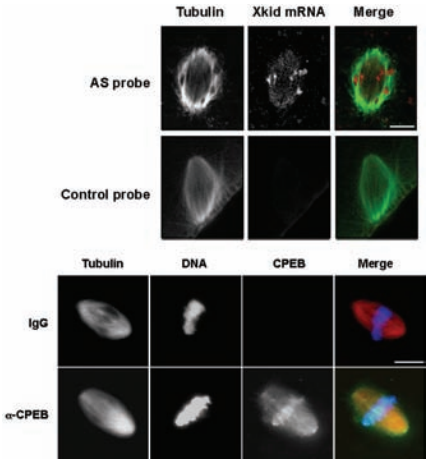


Figure 4. *Fluorescent in situ hybridization for CPE-regulated mRNA in MI-spindles. Antisense or Sense riboprobes for Xkid mRNA (red). Immunostaining with antibodies against Tubulin (green). Scale bar 10 µm. CPEB immunostaining. Immunofluorescence of spindles assembled in egg extracts with the anti-CPEB antibody (green). Microtubules are visualized in red and DNA in blue. Scale bar 10 µm*

Altogether, this combinatorial code defines 274 possible arrangements of cis-acting elements that result in 20 different translational-behaviors during meiosis and early embryonic development [4]. Experimental and computational analysis of these motif patterns identified hundreds of vertebrate mRNAs (up to 20% of X. Laevis, mouse or human genomes) potentially regulated by CPEs and AREs. The accuracy of these predictions has been validated both by a functional screening to identify CPE-regulated mRNAs activated in Prophase I or Metaphase I or by testing randomly selected candidates. The identified mRNAs potentially regulated by CPEB are enriched for genes functionally related to cell cycle and cell differentiation regulation, but also to other biological events such as chromosome segregation, synaptic stimulation, embryonic polarity or even implicated in angiogenesis and tumor development, etc. The conclusions of our work seem fully extrapolable to other systems beyond oocyte maturation, including differentiated tissues where the factors that exert this translational control are also present.

Piqué M, López JM, Foissac S, Guigó R and Méndez R.
"A combinatorial code for CPE-mediated translational control". Cell, 132(3):434-448 (2008).

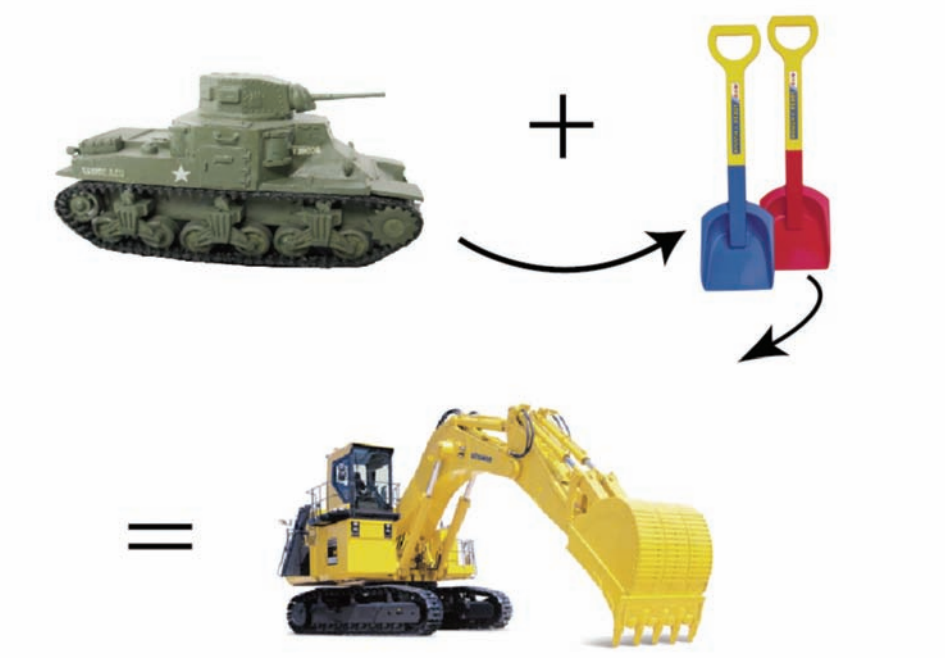
Belloc E and Méndez R.
"A deadenylation negative feedback mechanism governs meiotic metaphase arrest"
Nature, 452(7190):1017-21 (2008).

Elíscovich C, Peset I, Vernos I and Méndez R.
"Localized CPE-mediated translation controls meiotic chromosome segregation"
Nat Cell Biol, 10(7):858-865 (2008).

Belloc E, Piqué M and Méndez R.
"Sequential waves of polyadenylation and deadenylation define a translation circuit that drives meiotic progression". Biochem Soc Trans, 36(Pt 4):665-670, (2008).

SYSTEMS BIOLOGY PROGRAMME
Evolution shuffles its cards

Evolution continues to be one of the most fascinating subjects in Biology. How to explain the complexity of a human eye in terms of evolution is apparently impossible if we think that evolution proceeds by accumulating single point mutation after single point mutation with a layer of selection on top. When looking at the fossil record it looks like evolution does not proceed in a continuous way always but that once in a while it jumps and accelerates. We also know that genome organization is not static but rather dynamic. Comparison of bacteria separated in evolution by some million years looks like if someone throws to the air all the genes and when they fall to the floor join them by proximity. Similar things happen in eukaryotes, where chromosomes break and fuse, entire regions of the genome invert or split. As a result genes fall under the regulation of other regions of the DNA. This on one hand could result in disease like in the case of some types of leukaemia where a spontaneous chromosome rearrangement brings a Kinase (Abl) under the control of another chromosome region and is expressed when it should not. However, it also provide new raw material for evolution accelerating it by exploring new phenotypes in a brusque manner. The EMBL-CRG groups of Mark Isalan and Luis Serrano has explored how DNA rearrangement could produce new phenotypes. To do so they have used a working horse in Biology, the bacterium E. coli. The experimental setup was to select a number of transcription factors (proteins that regulate the expression of other genes) and to put them under the control of regulatory regions from other transcription factors (shuffling), while at the same time keeping the original arrangement. Then they analyzed the behavior of the bacteria with the shuffled genes under different environmental conditions. Surprisingly, they found that the bacteria survived more than 95% of reshuffled combinations, showing that shuffling per se is not necessarily a lethal barrier for evolution. They also found that a significant proportion of the bacteria with the shuffled transcription factors were more fit than the original wild-type bacteria under certain conditions. Moreover they found a superbug that could survive high temperatures and very adverse conditions in terms of nutrient depletion. These results indicate that gene shuffling is one of the major players in evolution, allowing new phenotypes to be explored in a quick manner without the standard procedure of accumulating spontaneous mutation and could be invoked to explain the acceleration steps found in evolution.



Appendix 1

VII ANNUAL SYMPOSIUM OF THE CENTRE FOR GENOMIC REGULATION
"MECHANISMS REGULATING CELL GROWTH AND DIVISION"

All mammalian cells, except the neurons, divide every 24 h. The life cycle of a cell can be divided into 4 distinct phases. These are G1, S, G2 and M. In G1 the cells generally grow. DNA is replicated in S-phase and in G2 the cells duplicate and separate their centrioles. The cells enter mitosis (M-phase) and the centrioles nucleate a mitotic spindle, which is used to separate the chromosomes. Once this has been achieved the cells undergo division (cytokinesis) to produce 2 daughter cells. These events are tightly linked and at each step of this cycle, the cells have evolved mechanisms to insure the completion of key events without any defects. If a defect is detected, the cells activate a checkpoint. The purpose of the checkpoint is to stall cells from progressing into the next stage. During this pause, special machinery is employed to mend appropriate defects. In case the defect cannot be corrected, the cells undergo a process called apoptosis or cell death. There are check points that are activated by defects in DNA replication, while others are activated when there is a defect in the formation of a normal spindle, organization/duplication of cellular compartments and separation of sister chromosomes.

The growth of cells and their subsequent division to produce two daughter cells are tightly regulated and connected by a series of biochemical reactions. A failure in detection and/or in the correction of these cellular defects underlies most of human pathologies. Aging is clearly linked to the in ability of cells to detect and correct the above-mentioned abnormalities. An understanding of the mechanisms regulating cell growth and their subsequent division is therefore of fundamental importance. Surprising as it may sound, the regulation of growth and division is highly conserved amongst species. Yeast, flies, worms, fish and the mammals all use a basic set of components to regulate cell growth and division. However, and not surprisingly, the complexity of this regulation increases with the increasing complexity of cellular interactions of the higher eukaryotes. This is a rapidly growing area of biology and there is tremendous interest for a basic understanding of the processes at molecular level. Biologists, chemists, physicists and mathematicians are using their individual talents to address these complicate and intricate regulatory pathways. Pharmaceutical companies are of course keen to develop compounds that affect cell growth and division, with the intent of generating valuable therapeutics.

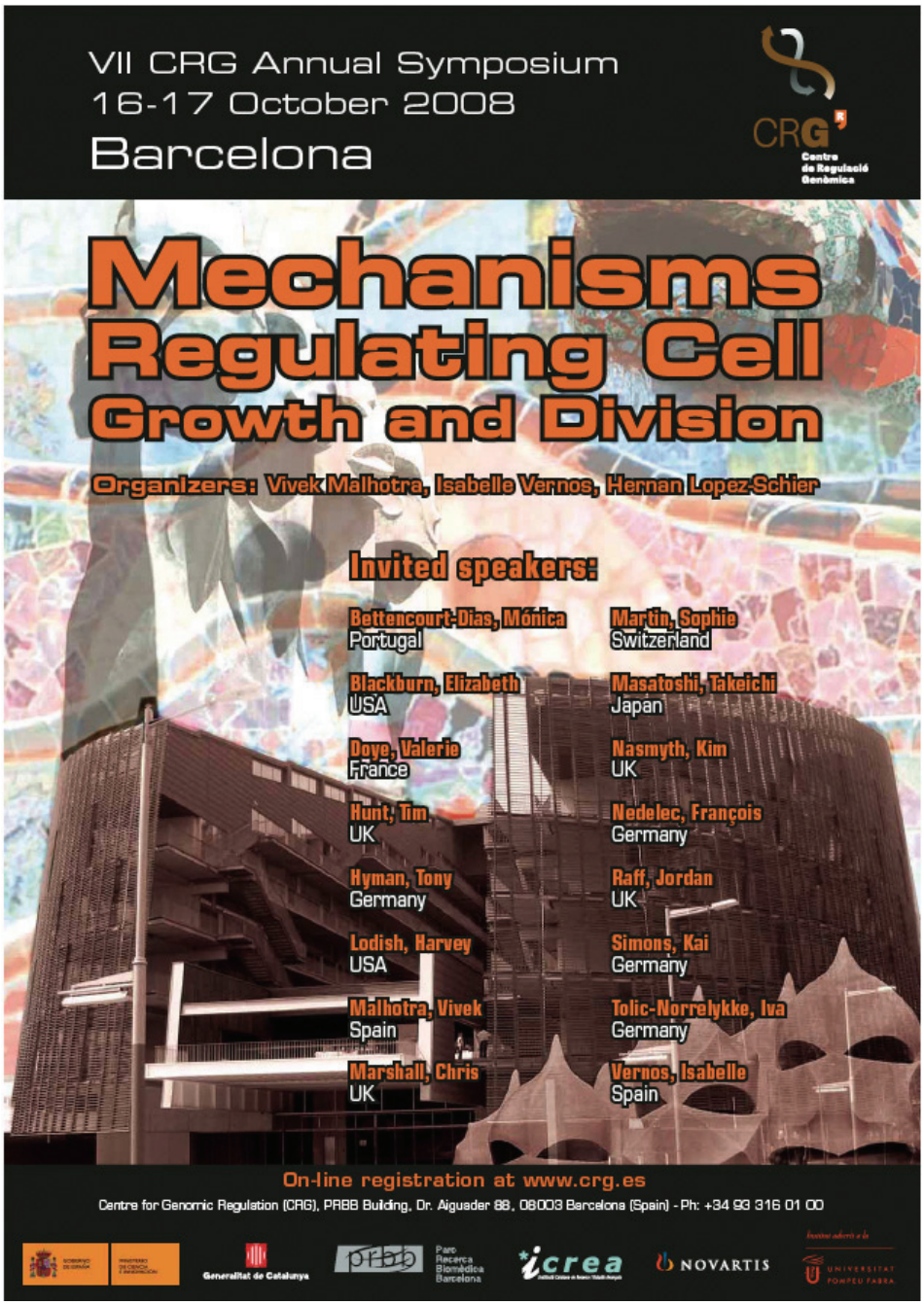
The aim of the research programme at the CRG dealing with these topics is to use the most advanced technologies, creativity and intellect to address key questions in the general area of cell growth and division. The current groups are involved in understanding the mechanism by which cells maintain their compartmentation during repeated cell divisions, replication of DNA, regulation of translation, the role of microRNA in cellular physiology, reconstitution and assembly of natural and artificial gene interactions, reprogramming of cells, the role of gene number and copies in cancer, and the molecular mechanism of Asthma, just to name a few.

In this scenario, the VII CRG Symposium titled "MECHANISMS REGULATING CELL GROWTH AND DIVISION" was held on 16th and 17th October 2008, at the PRBB Auditorium in Barcelona. The aim of this symposium was to use CRG as a forum to bring outstanding researchers from across the world to discuss the current understanding of mechanisms regulating cell growth and division, and to provide a picture or working hypothesis for future goals and directions. The symposium was open to everyone in the community and attracted a national and international audience and included 16 presentations of high level speakers divided into two blocks.

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.

The symposium contributed to increase the visibility of the CRG and Barcelona in this field and allowed offering a global panorama 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.



Appendix 2

PRBB-CRG SESSIONS 2008

05-12-08 John Reinitz
Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY, US
Canalization of transcription in the Drosophila blastoderm: from data to dynamical systems.

28-11-08 María Macías
Group Leader, Biomolecular NMR Spectroscopy, Structural & Computational Biology, IRB Barcelona, Spain
"FF domains, from structures to function"

21-11-08 Xose Bustelo
Centro de Investigación del Cáncer. University of Salamanca-CSIC. 37007 Salamanca, Spain
"Genetic analysis of the function of Vav oncoproteins in vivo".

17-11-08 Daniela Rhodes
MRC Laboratory of Molecular Biology; Cambridge, UK
Chromatin higher order structure and regulation of its compaction

14-11-08 Judy Lieberman
Immune Disease Institute, Harvard Medical School, Boston, USA
"Micromanipulating cell differentiation and proliferation"

31-10-08 Ivan Shatsky
Lomonosov Moscow State University, Russia
Molecular mechanism of translation initiation on the hepatitis C virus RNA

24-10-08 Barry J. Dickson
Institute of Molecular Pathology (IMP), Vienna, Austria
"Wired for sex: the genetics and neurobiology of Drosophila reproductive behaviour"

10-10-08 Isabel Fariñas
Unidad Mixta Universidad de Valencia - Centro de Investigaciones Príncipe Felipe, Spain.
"A vascular niche for stem cells in the adult brain"

04-10-08 Claudio Sette
Department of Public Health and Cell Biology, University of Rome "Tor Vergata", Rome, Italy
"Sam68: a multifunctional RNA-binding protein involved in human cancers"

26-09.-08 Dietmar Schmuker
Harvard Medical School, USA
"Ig-receptor diversity and specificity: Are thousands of membrane receptors controlling the precise assembly of neuronal circuits?"

19-09-08 Eric So
Haemato-Oncology Department. The Institute of Cancer Research, London, UK
"Transcriptional and epigenetic deregulation in AML stem cells"

15-09-08 Halldor Stefansson
EMBL, Heidelberg, Germany
"Minding the Science and Society gap: what's in for scientists?"

10-09.-08 Anna Di Rienzo
Professor, Dept. of Human Genetics, The University of Chicago, USA
"Human adaptations and the susceptibility to common diseases"

25-07-08 David A. Williams
Children's Hospital Boston, Boston, USA
"Rac and Rho: The Ying and Yang of Hematopoiesis"

24-07-08 Michael Egholm
Vicepresident R&D Roche-454 Lifesciences
"Towards Routine Human Sequencing"

18-07-08 M. Madan Babu
MRC-Laboratory of Molecular Biology, University of Cambridge, United Kingdom
"Tight regulation of intrinsically unstructured proteins: from transcript synthesis to protein degradation"

11-07-08 Scott Dougan
Dept. of Cellular Biology, The University of Georgia
"Pattern formation in zebrafish embryos"

07-07-08 Konrad Hochedlinger
Assistant Professor, Harvard Medical School, Harvard University, USA *"Pluripotency and Nuclear Reprogramming"*

04-07-08 Dannie Durand
Dept. of Biological Sciences, Carnegie Mellon University, Pittsburgh, USA
"Sequence similarity network reveals ancestry of multidomain proteins"

25-06-08 Antonio Reverter
Principal Research Scientist, Bioinformatics, CSIRO Livestock Industries, St. Lucia, Queensland, Australia
"Systems biology approaches to skeletal muscle development: A cow perspective"

05-06-08 Gonzalo de Polavieja
Neural Processing Lab, Theoretical Physics Dept., Faculty of Sciences, Universidad Autónoma de Madrid (UAM), Madrid, Spain
"Efficiency in brains: coding and wiring"

30-05-08 Alexander Schier
Dept. of Molecular & Cellular Biology, Broad Institute, Center for Brain Science, Harvard Stem Cell Institute, Harvard University, Cambridge, USA
"MicroRNAs in embryogenesis"

23-05.-08 Gilles Laurent
California Institute of Technology (Caltech), USA
"Coding and plasticity in an olfactory system"

9-05-08 David Sassoon
Director, UMR S 787 INSERM, "Groupe MYOLOGIE", Université Paris 6 Pierre et Marie Curie, Paris, France
"Identification of a novel resident population of postnatal skeletal muscle stem cells"

25-04-08 Jan Korbel
Postdoctoral Fellow, Bork Group, EMBL, Heidelberg, Germany
"Mapping Genome Structural Variation in Humans Using Novel Functional Genomics Approaches"

22-04-08 Didier Raoult
Faculté de Médecine, Unité des Rickettsies, WHO Collaborative Center for Rickettsial Reference & Research, CNRS UPRESA 6020, Marseille, France
"Lessons from Rickettsia genomics"

18-04-08 Michael Snyder
Department of Molecular, Cellular, and Developmental Biology, Yale University, USA
"Analysis of Human Variations and Regulatory Circuits"

11-04-08 Albert Sorribas
Grup Biomatematica i Bioestadistica, Dept. Ciencies Mediques Basiques, Institut Recerca Biomedica de Lleida, Universitat de Lleida, Spain
"Identifying selective pressures that shape the adaptive responses of yeast to stress: design and operational principles in metabolism"

28-03-08 Nick J. Proudfoot
Sir William Dunn School of Pathology, Oxford, UK
"Gene Punctuation in Eukaryotes".

17-03-08 Aravinda Chakravarti
Director, Center for Complex Disease Genomics, McKusick-Nathan Institute of Genetic Medicine, Johns Hopkins University School of Medicine, USA
"The many genetic faces of Hirschsprung disease"

14-03-08 Óscar Marín
Instituto de Neurociencias de Alicante, CSIC & Universidad Miguel Hernández
"Transcriptional control of neuronal migration"

05-03-08 Constantinos Sekeris
Emeritus Research Professor, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece
"Steroid and thyroid hormone receptors in mitochondria"

22-02-08 Keith J. Joung
Center for Cancer Research, Charlestown, USA
"Targeted Genome Manipulation Using Engineered Zinc Finger Nucleases"

07-02-08 Nick Baker
Dept. of Molecular Genetics, Albert Einstein College of Medicine, New York, USA
"The active role of corpse engulfment pathways during cell competition"

25-01-08 Des Higgins
Conway Institute, University College Dublin, Ireland
"Multiple Alignments and Multivariate Analysis"

PROGRAMME SEMINARS 2008
SYSTEMS BIOLOGY PROGRAMME

19-12-08 Gustavo PESCE
The Molecular Sciences Institute, Berkeley, California, USA
Genetic control of noise and cell to cell variation in the yeast pheromone response

16-12-08 Simon Sprecher
Dept. Biology, New York University, New York, USA
The Drosophila larval eye: development and function of a simple visual system

27-11-08 Ovidiu Radulescu
Institute for Mathematical Research of Rennes,
University of Rennes 1, Rennes, France
Mathematical framework for studying biological robustness

27-11-08 Jorrit Boekel
Dept. of Neuroscience, Karolinska Institutet, Stockholm, Sweden
Insights into renal responses to uropathogenic E. coli infection

21-11-08 Anton Crombach
Theoretical Biology / Bioinformatics Group, Universiteit Utrecht, Utrecht, The Netherlands
Evolution of evolvability in gene regulatoty networks

19-11-08 Alan Carleton
Neurosciences Fondamentales, Université de Genève, Geneva, Switzerland
From molecules to olfactory behvior:
analysis of the mechanisms underlying sensory processing.

11-11-08 Federico De Masi
Bulyk Lab, Brigham and Women's Hospital, Boston, USA
An Integrated C. elegans Basic Helix-Loop-Helix Dimerization and DNA binding Network

24-10-08 Denis Thieffry
Département Biologie, Université de la Méditerranée (Aix-Marseille), Marseille, France
Comparative analysis of logical models of the cell cycle in eucaryotes.

23-10-08 Nobuhiko Tokuriki
Weizmann Institute of Science, Rehovot, Israel
"The stability effect of mutations and enzyme evolution"

22-09-08 Phil Simmons
Sigma-Aldrich Corporation
Targeted Genome Editing in Mammalian Cells Using Engineered Zinc Finger Nucleases

19-09-08 M. Dierssen, P. Gorostiza, M. Sánchez-Vives, H. López-Schier, M. Louis
CRG, CREBEC, Fundació Clínic
Electrophysiology Connection

29-07-08 Álex Gómez-Marín
Departament d'Estructura i Consituents de la Matèria,
Facultat de Física, Universitat de Barcelona, Barcelona, Spain
"Brownian devices out of equilibrium: transport, energetics and fluctuations"

28-07-08 Kunihiko Kaneko
University of Tokyo, Komaba and ERATO Complex Systems Biology, JST, Japan
"Consistency Principe in Biological Dynamical System: Plastcity, Robustness, and Genotype-Phenotype Relationship"

17-07-08 Maria Serrano-Vega
MRC Laboratory of Molecular Biology, Cambridge, United Kindgdom
"Thermostabilization and structure determination of a b1 adrenergic receptor".

14-07-08 David Baker
Department of Biochemistry, University of Washington, Seattle, Washington, USA
"Novel enzymes, rapid structure determination, and an online computer game"

3-07-08 Albert Cardona
Molecular Cell and Developmental Biology Department, University of California, Los Angeles, USA
From micro to nano: mapping neuroanatomical data from confocal imaging to TEM serial sections and back

23-06-08 Lucas Pelkmans
ETH Zürich, Institute of Molecular Systems Biology, Switzerland
"Non-stochastic phenotypic variation in human cell populations"

10-06-08 Marianne Abildgaard Oerum
CLC bio - *CLC Combined Workbench Application Training*

02-06-08 Joan Teyra
Structural Bioinformatics Group, Biotechnologisches Zentrum, Dresden, Germany
"SCOWLP: Structural classification and analysis of protein interfaces"

30-05-08 Mónica Campillos
Biocomputing, Bork Group, EMBL Heidelberg, Germany
"Drug target identification using side-effect similarity"

23-05-08 Attila Becksei
Institute of Molecular Biology / UZH, Zürich, Switzerland
Readout of weak protein-DNA binding by transcriptional circuits

09-05-08 Stefano Cardinale
Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, United Kingdom
Inactivation of a synthetic human kinetochore by specific targeting of chromatin modifiers

14-03-08 Alyson MacInnes
Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, USA
"Ribosomal Protein Gene Mutations and the Loss of p53"

17-01-08 Phil Kim
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, USA
SB Group Leader Selection - "Jumping scales: How 3D structures and molecular genetics meet in protein networks"

17-01-08 Tom Shimizu
Department of Molecular and Cellular Biology, Harvard University, Cambridge, USA
SB Group Leader Selection - "In vivo physiology of microbes: models, measurements and beyond"

PROGRAMME SEMINARS 2008
GENE REGULATION PROGRAMME

11-12-2008 Susana Rodríguez-Navarro
Centro de Investigación Príncipe Felipe, Valencia. Spain
The transcription/export factor Sus1 is a small protein with many faces

28-11-08 Gordon Hager
Laboratory of Receptor Biology and Gene Expression, Hormone Action and Oncogenesis Section. NCI, NIH, USA
"Interaction of Nuclear Receptors with Regulatory Elements"

13-11-08 Tomás Aragón
Department of Biochemistry and Biophysics, University of California at San Francisco, USA
mRNA targeting to ER stress signaling centers

11-11-08 Clélia Laitem
Institute of Biologie of Lille, France
Ets-1 p27: a novel Ets-1 isoform with a dual-acting dominant negative function which represses the tumorigenicity of mammary carcinoma cells

30-10-08 Antje Ostareck-Lederer
Martin-Luther-Universität Halle Wittenberg, Germany
Post-transcriptional control of gene expression during erythroid cell maturation

22-10-08 Eleonora Leucci
Copenhagen Biocenter BRIC, Copenhagen, Denmark
Role of microRNA in B cell differentiation and cancer

19-09-08 Doris Meder
Max Planck Institute of Molecular Cell - Biology and Genetics
"Fostering Scientific Excellence with Professionally Managed Core Facilities"

19-09-08 Jiri Zavadil
Assistant Director, NYU Cancer Institute - Genomics Facility
"Core Facilities in he Service of Competitive Biomedical Research"

16-09-08 Hans Reinke
Département de Biologie Moléculaire, Université de Genève, Switzerlan
Peripheral circadian oscillators: time, food and temperature

16-09-08 Hernán Diego Folco
Wellcome Trust Centre for Cell Biology, Institute of Cell and Molecular Biology.University of Edinburgh, United Kingdom
Heterochromatin and centromeric function in fission yeast

16-09-08 Joaquín Espinosa
Department of Molecular, Cellular, and Developmental Biology. University of Colorado at Boulder, Colorado, USA
Novel insights on mechanisms of gene expression control and cell fate choice from the p53 field

23-07-08 Ernest Martínez
Associate Professor. Department of Biochemistry. University of California, USA
Multiprotein Complexes in Regulation of Chromatin, Transcription & MYC Oncoprotein Function

17-07-08 Jesús Alfredo Cortés Hermosillo
Unidad Académica de Biología Experimental, Universidad Autónoma de Zacatecas, México
FROM THE AUTOIMUNITY TO THE PLANTS

12-06-08 Charles Query
Albert Einstein College of Medicine, New York, USA
Alteration of branch region–U2snRNA dynamics in spliceosome assembly impacts fidelity and flexibility of gene expression.

10-06-08 Giovanni Perini
Associate professor of Genetics. Department of Biology, University of Bologna, Italy
Role and mechanisms of Myc factors in controlling transcription regulation of ATP-binding cassette drug transporters in cancer

13-05-08 Joaquín Espinosa
University of Colorado at Boulder, USA
'Understanding how gene networks control cell behavior: the p53 paradigm'

22-04-08 Andrea Barta
Max F. Perutz Laboratories, Vienna Biocenter, Vienna, Austria
SR proteins and alternative splicing in plants

11-04-08 Elias Bechara
Université Nice-Sophia Antipolis, Instabilité génétique, Maladies rares et cancers, France
"Dissecting the RNA binding properties of the Fragile X Mental Retardation Protein".

PROGRAMME SEMINARS 2008
DIFFERENTIATION & CANCER PROGRAMME

09-12-08 Timm Schroeder
Institute of Stem Cell Research, Helmholtz Center München, Neuherberg / Munich, Germany
Tracking of stem cell behavior at the single cell level: New tools for old questions?

25-11-08 Anton Wutz
IMP Vienna, Austria
"Silencing chromosomes with Xist in embryos and tumors".

12-11-08 Andre Nussenzweig
National Institutes of Health, NCI, Bethesda, USA
"pathways that trigger and prevent cancer-causing chromosomal translocations."

30-10-08 Matthias Stadtfeld
MGH Cancer Center and Harvard Stem Cell Institute, Boston , USA
"Induction of pluripotency in differentiated cells"

22-10-08 Joern Toedling
EMBL - European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom
"Analyzing ChIP-chip data using Bioconductor"

21-10-08 Roger Revilla
Research Institute of Molecular Pathology, Vienna, Austria
"Elucidating the Gene Regulatory Network that controls the specification of the skeletogenic lineage in the sea urchin embryo".

18-09-08 Yoshikuni Nagamine
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
The DEAH helicase RHAU in hematopoiesis and ras transformation

18-07-08 William Keyes
Cold Spring Harbor Laboratory, Cold Spring Harbor, USA
"Senescence and Stem Cells: How p63 Mediates Cancer and Aging"

18-07-08 Taku Naito
Cutaneous Biology Research Center, Massachusetts General Hospital, Charlestown, USA
"Epigenetic regulation of T cell differentiation by Ikaros/Mi2b complex".

18-07-08 Elsa Quintana
Center for Stem Cell Biology, HHMI/Department of Internal Medicine, Life Sciences Institute, University of Michigan, USA
"Improved xenotransplantation assay reveals that many human melanoma cells are tumorigenic"

09-07-08 Claudio Basilico
Professor of Molecular Pathogenesis, Chairman, Dept. of Microbiology, NYU School of Medicine, USA
Regulation of skeletal development by FGF signaling

26-03-08 Paul Frenette
Mount Sinai School of Medicine, Department of Medicine and Department of Gene and Cell Medicine, New York, USA
"Circadian release of hematopoietic stem cells".

19-02-08 Enrico Avvedimento
Dipartimento di Biologia e Patologia Molecolare e Cellulare, "Federico II" University of Naples, Italy
"Oxidation driven transcription: demethylation of histone H3 drives estrogen induced transcription"

15-02-08 Suzanne van de Nobelen
Frank Grosveld' lab, Erasmus University, Rotterdam, The Netherlands
"Identification of CTCF protein partners"

08-01-08 Carme Caelles
Cell Signalling Research Group. Institute for Research in Biomedicine, IRB Barcelona, Parc Científic de Barcelona, Spain
"Pharmacological actions mediated by the nuclear receptor - JNK pathway negative crosstalk".

PROGRAMME SEMINARS 2008
BIOINFORMATICS & GENOMICS PROGRAMME

21-11-2008 Philipp Bucher
Swiss Institute of Bioinformatics and Swiss Institute for Experimental Cancer Research Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland
"Chromatin structure-driven computational analysis of regulatory genomic sequences"

30-10-2008 Albert Vilella
European Bioinformatics Institute, United Kingdom
"Comparative genomic resources in Ensembl: gene trees and whole-genome multiple alignments"

23-10-2008 Audrey Kauffmann
European Bioinformatics Institute, United Kigdom
"Microarray quality assessment with arrayQualityMetrics"

21-10-2008 Gad M. Landau
University of Haifa, Israel
"Haplotype Inference Constrained by Plausible Haplotype Data"

21-10-2008 Ron Y. Pinter
Technion, Haifa, Israel
"Pathway-based Phylogeny Reconstruction"

25-09-2008 Sonja Hänzelmann
Nijmegen Centre for Molecular Life Sciences, The Netherlands
"Quantitative Label Free Proteomics - Correction of XIC Peak Areas based on Amino Acid Composition"

17-09-2008 Wyeth Wasserman
University of British Columbia. Vancouver, Canada
"Bioinformatics and gene regulation: designing cell-selective promoters for brain expression"

09-09-2008 Karen B. Avraham
Chair, Department of Human Molecular Genetics and Biochemistry Sackler, School of Medicine, Tel Aviv University, Israel
"Identification of microRNA target pairs in the inner ear by microarray, proteomics and bioinformatics analysis"

09-09-2008 David González
Trinity College, Dublin, Ireland
"Recent de novo origin of human protein-coding genes"

03-09-2008 Jean-François Taly
Unité Mathématique Informatique et Génome (MIG), Institut National de la Recherche Agronomique (INRA), France
"Can molecular dynamics simulations help in discriminating correct from erroneous protein 3D models?"

29-07-2008 Attila Gyenesei
Abo Academy / University of Turku, Finland
Identification of co-regulates gene networks in global transcriptomics using advanced data mining

29-07-2008 Robert Hermann
Abo Academy / University of Turku, Finland
Genetics of complex disease: Will whole genome scans help us?

11-07-2008 Christian Blum
Dept. Llenguatges i Sistemes Informàtics, Universitat Politècnica de Catalunya (UPC), Spain
"Ant Colony Optimization"

10-07-2008 Thomas Manke
Max Planck Institute for Molecular Genetics, Germany
"Quantitative Models of Regulatory Interactions and Molecular Networks"

16-07-2008 Jose M. Muiño
Institute of Plant Genetics, Polish Academy of Science, Poland
"Genome-wide analysis of direct target genes of SEP3 and AP1 transcription factors in Arabidopsis flower development"

12-06-2008 Mariano Allo
Universidad de Buenos Aires, Argentina
"siRNA Mediated Regulation of Alternative Splicing"

09-06-2008 Feng-Chi Chen
Bioinformatics group of Genomics Research Center, Academia Sinica, Taiwan
"Human-specific insertions and deletions inferred from mammalian genome sequences"

09-06-2008 So Nakagawa
Center for Information Biology and DNA Data Bank of Japan (DDBJ), National Institute of Genetics, Japan
"Diversity of preferred nucleotide sequences around the translation initiation codon in eukaryote genomes"

09-06-2008 Trees-Juen Chuang
Genomics Research Center of Academia Sinica, Taiwan
"Identification and analysis of ancestral hominoid transcriptome inferred from cross-species transcript and processed pseudogene comparisons"

29-05-2008 Ross Hardison
T. Ming Chu Professor of Biochemistry and Molecular Biology, The Pennsylvania State University, USA
"Biological Functions of DNA Occupied by the Erythroid Transcription Factor GATA-1: Evolutionary History and Current Events"

16-04-2008 Jens Kleinjung
Division of Mathematical Biology. National Institute for Medical Research, London, United Kingdom
"Alignment algorithms and fragment alphabets for structure modelling and design"

03-04-2008 Anne Bergeron
Comparative Genomics Laboratory, Universite du Quebec a Montreal, Canada
"Genome rearrangements with applications to breakpoint reuse"

27-03-2008 Dmitri Pervouchine
Institute for Information Transmission Problems, Moscow, Russia
"Large-scale analysis of RNA secondary structures involved in regulation of splicing"

17-03-2008 Gabriele Sales
University of Turin, Italy
"Identification of functional sequences though whole genome alignment"

11-03-2008 Doris Bachtrog
Division of Biological Sciences, University of California, San Diego, USA
"Evolutionary genomics of sex chromosomes"

11-03-2008 Raul Rabadan
School of Natural Sciences, Institute for Advanced Study, Princeton, USA
"The Evolution of Influenza A"

11-03-2008 Ravi Sachidanandam
Cold Spring Harbor Laboratory, New York, USA
"Splice Site Variations in Human Disorders"

10-03-2008 Boris Adryan
MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
"Exploring the regulatory landscape of the Drosophila genome"

10-03-2008 Daniela Delneri
Faculty of Life Science, University of Manchester, United Kingdom
"Genomic approaches to fitness and speciation in yeasts"

10-03-2008 Fyodor Kondrashov
Section on Ecology, Behaviour and Evolution. Division of Biological Sciences, UCSD-San Diego, USA
"The molecular basis for compensating disease mutations"

10-03-2008 Tomas Vinar
Biological Statistics and Computational Biology Cornell University, New York, USA
"Computational methods to study genes and their evolution"

10-03-2008 Toni Gabaldon
Centro de Investigaciones Príncipe Felipe, Valencia, Spain
"Using evolutionary analyses to understand cellular evolution and unravel new disease mechanisms"

20-02-2008 Jens Stoye
Genome Informatics, Bielefeld University, Germany
"Computational Characterization of Short Environmental DNA Fragments"

24-01-2008 Juan A. Subirana
Chemical Engineering, UPC, Barcelona, Spain
"Structural families and comparative study of microsatellites in diverse genomes"

10-01-2008 Steven Skiena
Department of Computer Science, State University of New York at Stony Brook, USA
"Assembly for Double-Ended Short-Read Sequencing Technologies"

PROGRAMME SEMINARS 2008
CELL & DEVELOPMENTAL PROGRAMME

02-10-2008 Eugenio Marco
Harvard Medical School, USA
"Quantitative Biology of the Cytoskeleton in Cell Division"

02-10-2008 Jerôme Solon
European Molecular Biology Laboratory, Heidelberg, Germany
"Force generation and collective cell behavior during morphogenesis"

02-10-2008 Thomas Vaccari
University of California, USA
"The Endocytic Control of Cell-Cell Signaling, Growth and Tumor Suppression in Drosophila Melanogaster"

13-06-2008 Victoria Sanz Moreno
Institute of Cancer Research, United Kingdom
"Rac activation and inactivation control plasticity of tumour cell movement"

13-05-2008 Alioune Ndoye
Molecular Devices
"Introduction to the ImageXpress Micro Screening microscope"

06-05-2008 Monika Marx
Carl Zeiss MicroImaging GmbH, Germany
LSM 710 A New Dimension in Confocal Microscopy

21-04-2008 Juan Luis Monteagudo
Leica Microsistemas
"Leica TCS LSI: from cell to embryo"

15-04-2008 Stephanie Urschel
Field Application Scientist. ThermoScientific - Dharmacon
"AccelIT siRNA: Access a New World of RNAi Discovery"

31-03-2008 Rolf Borlinghaus
Leica Microsystems Manheim, Germany
Nuevas Tecnologías en Microscopía Confocal: STED

04-02-2008 Izabela Sumara
Swiss Federal Institute of Technology, Switzerland
"A novel, Cul3-based E3-ligase is required for faithful mitosis in human cells"

04-02-2008 Romeo Ricci
Institute of Cell Biology, Zurich
"Novel functions of non-canonical p38 MAPKs in metabolism and inflammation"

30-01-2008 SPEAKER
Bitplane AG
"Presentation of Imaris and Surpass Software for the rendering and analysis of microscopy data"

PROGRAMME SEMINARS 2008
GENES & DISEASE PROGRAMME

25-07-2008 Claudio Lottaz
Computational Diagnostics, Institute for Functional Genomics, University of Regensburg, USA
"Meta-Analysis of gene expression studies based on gene rankings"

30-06-2008 Frédéric J. de Sauvage
Genentech Inc., USA
"Targeting the Hedgehog Pathway in Cancer"

04-06-2008 Aaron Del Duca / Michael Wodkowski
DNA Genotek, Ottawa, Canada
"Nucleic Acid Specimen collection for population-based genetic research and diagnostics - Oragene (DNA from saliva) as the new 'gold standard'"

15-01-2008 Heinz Himmelbauer
Max-Planck-Institut fur Molekulare Genetik, Germany
"Genome analysis utilising short and ultrashort reads"

15-01-2008 Olga Durany
International Biotech Company
"Ultrasequencing Facilities (Illumina/Solexa 1g; Roche 454 GS-FLX). Plan Proposal for Application on Funtional Genomics Research"

Appendix 3

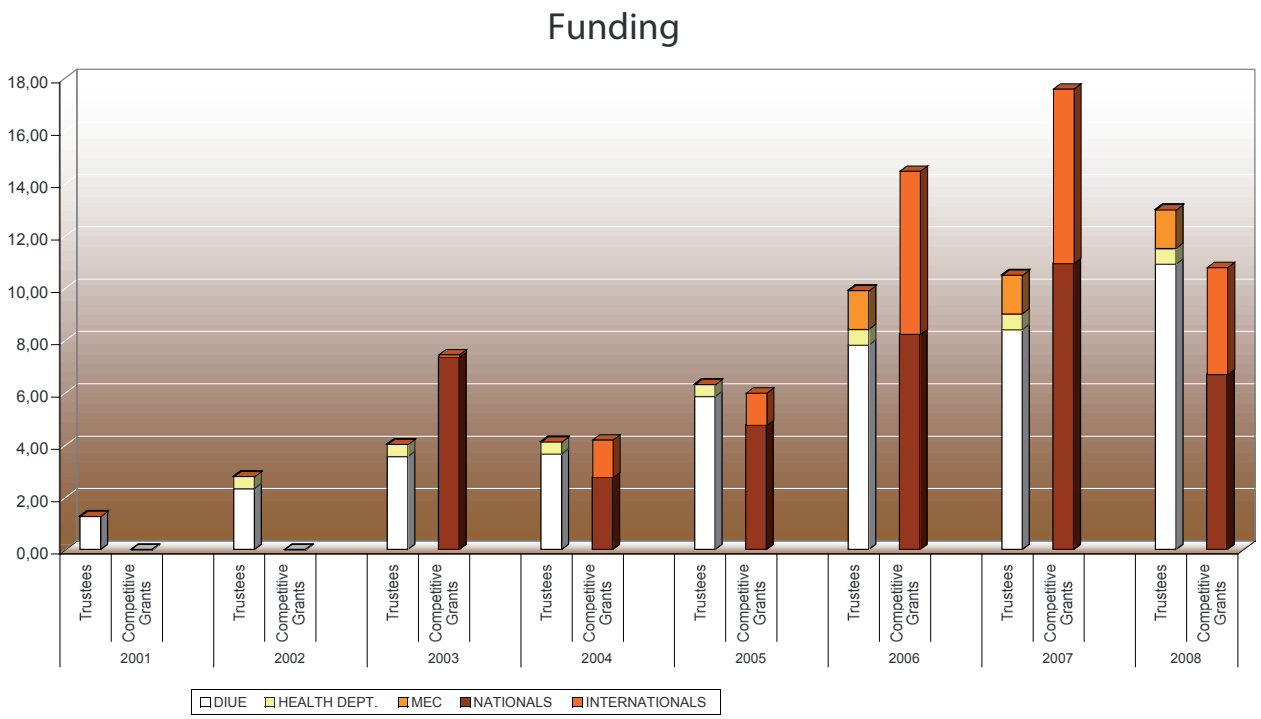
GRANTS

The grants that the CRG obtained from 1st January to 31st December 2008 are the following:

ORGANISM	AMOUNT (€)
MINISTERIO DE EDUCACION Y CIENCIA	5.342.515,81
EUROPEAN COMMISSION	3.691.888,67
FUNDACION DESARROLLO INVESTIGACION GENOMICA Y PROT	766.824,88
FUNDACION MARCELINO BOTIN	440.000,00
NATIONAL INSTITUTE OF HEALTH NIH	286.833,90
AGAUR - AGENCIA GESTIO D'AJUTS UNIVERSITARIS	249.318,75
FUNDACIO LA CAIXA	232.687,00
FUNDACIO MARATO TV3	223.929,55
MINISTERIO DE SANIDAD Y CONSUMO-FIS	214.129,06
GRANTS MISCELANIOUS	127.140,19
ASSOCIATION FOR INTERNATIONAL CANCER RESEARCH	98.785,11
FONDATION JEROME LEJEUNE	91.000,00
MINISTERIO DE CIENCIA E INNOVACION	74.251,94
MUSCULAR DYSTROPHY ASSOCIATION	71.824,12
NOVARTIS FARMACEUTICA S.A.	54.000,00
FUNDACIÓN ALICIA KOPLOWITZ	50.000,00
FUNDACION ESPAÑOLA PARA LA CIENCIA Y LA TECNOLOGIA	42.075,00
MINISTÉRIO DA CIÉNCIA E DO ENSINO SUPERIOR	36.901,25
EUROPEAN MOLECULAR BIOLOGY ORGANIZATION	30.000,00
FUNDACIÓN RAMON ARECES	29.862,55
FUNDACION INVESTIGACION Y PREVENCIÓN DEL SIDA	25.305,00
FUNDAÇAO PARA A CIÊNCIA E A TECNOLOGIA	25.000,00
F-STAR BIOTECHNOLOGISCHES FORSCHUNGS-UND ENTWICKLU	18.750,00
FUNDACIÓN DE INVESTIGACIÓN MÉDICA MUTUA MADRILEÑA	15.950,00
ASSOCIATION FRANÇAISE CONTRE LES MYOPATHIES AFM	11.000,00
INSERM TRANSFERT SA	8.000,00
CELL CENTRIC LIMITED	5.000,00
INSTITUTO DE CULTURA. AJUNTAMENT DE BARCELONA.	3.000,00
FENS - FEDERATION OF EUROPEAN NEUROSCIENCE SOCIETI	1.000,00
TOTAL AMOUNT	**12.266.972,78**

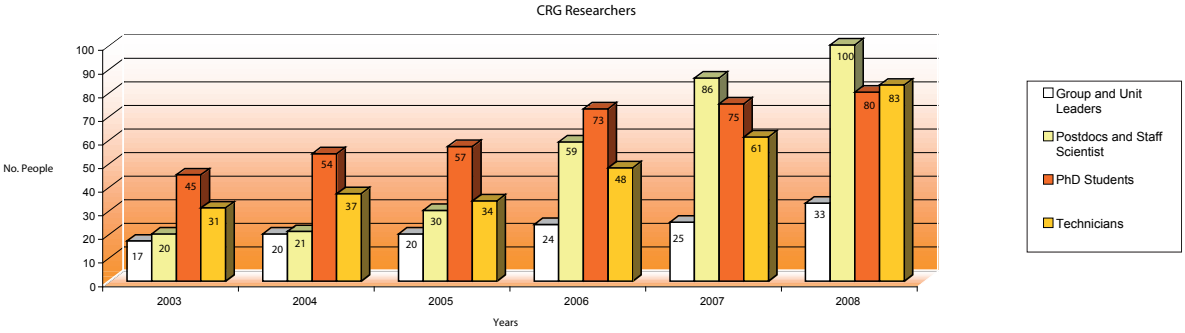
Appendix 4

FINANCE & PERSONNEL EVOLUTION AT THE CRG



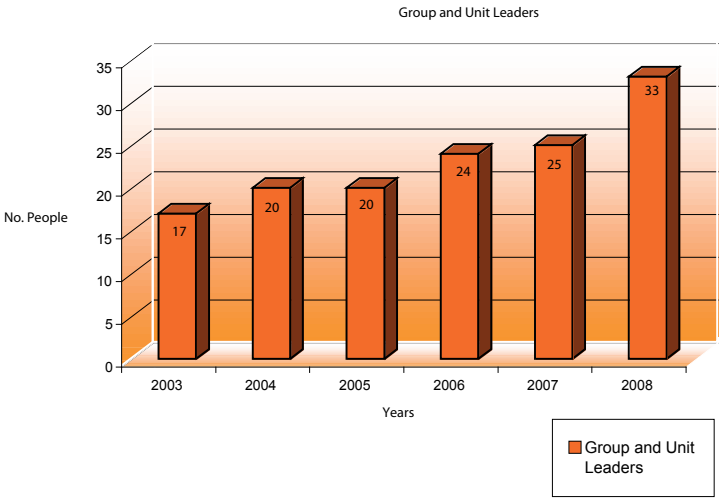
CRG
Researchers

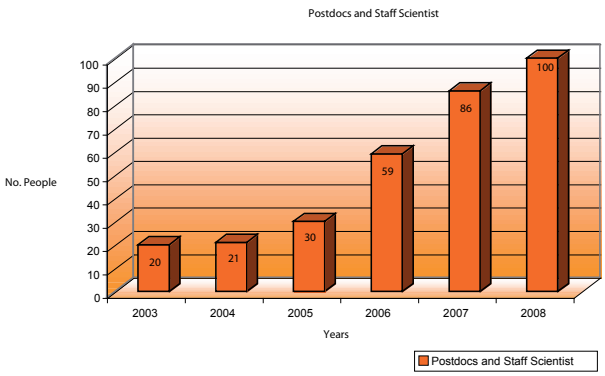
	Group and Unit Leaders	Postdocs and Staff Scientist	PhD Students	Technicians
2003	17	20	45	31
2004	20	21	54	37
2005	20	30	57	34
2006	24	59	73	48
2007	25	86	75	61
2008	33	100	80	83



CRG
Personnel: 31-12-2008

	Group and Unit Leaders
2003	17
2004	20
2005	20
2006	24
2007	25
2008	33





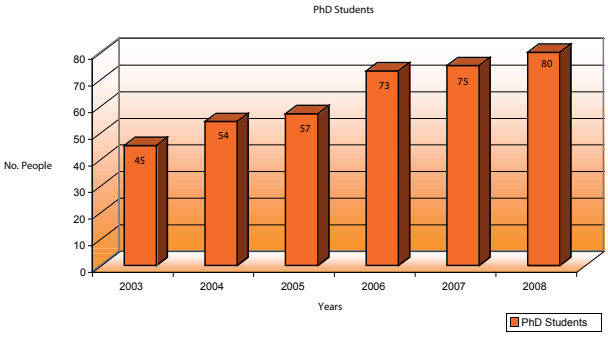
CRG
Personnel: 31-12-2008

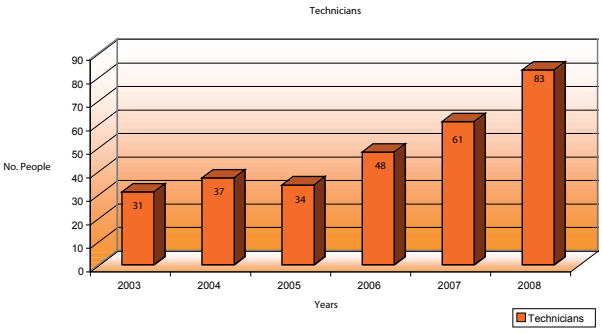
	Postdocs and Staff Scientist
2003	20
2004	21
2005	30
2006	59
2007	86
2008	100

CRG
Personnel: 31-12-2008

PhD Students

2003	45
2004	54
2005	57
2006	73
2007	75
2008	80





CRG
Personnel: 31-12-2008

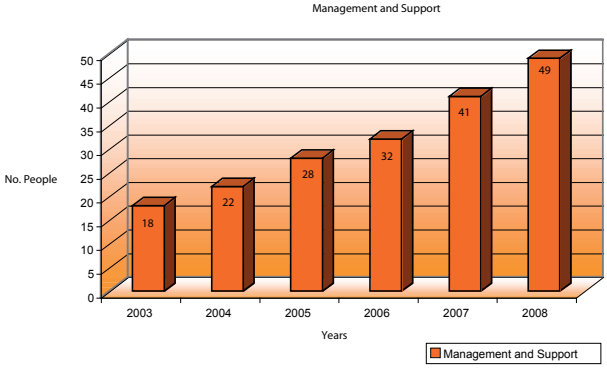
Technicians

2003	31
2004	37
2005	34
2006	48
2007	61
2008	83

CRG
Personnel: 31-12-2008

Management and Support

2003	18
2004	22
2005	28
2006	32
2007	41
2008	49



Appendix 5

PRESS CLIPPING

ABC

La Obra Social «la Caixa» dota con 340 becas su programa universitario

M. A.
MADRID. La Obra Social «la Caixa» acaba de abrir su nueva convocatoria de becas para universitarios españoles. Para este año, la entidad ofrece 340 becas, 90 más que el año pasado.

bién este año, 160 becas para cursar un doctorado internacional en Biomedicina que se realizarán en los cuatro centros de excelencia en investigación biomédica en España: Centro Nacional de Investigaciones Oncológicas (CNIO), Centro Nacional de Biotecnología (CNB-CSIC), Centro de Regulación Genómica (CRG) e Instituto de Investigación Biomédica (IRB Barcelona).

Doctorado en Biomedicina

Al programa de formación universitaria se añaden, también este año, 160 becas para cursar un doctorado internacional en Biomedicina que se realizarán en los cuatro centros de excelencia en investigación biomédica en España: Centro Nacional de Investigaciones Oncológicas (CNIO), Centro Nacional de Biotecnología (CNB-CSIC), Centro de Regulación Genómica (CRG) e Instituto de Investigación Biomédica (IRB Barcelona).

Al impulso a la formación universitaria y de investigación hay que añadir también el programa de becas de «la Caixa» para la capacitación profesional de recién nacidos, nacido en 2005 en Cataluña y que en 2006 se extendió al resto de España. Sumando sus diferentes modalidades, en 2008 «la Caixa» concederá más de 1.500 becas, con una inversión superior a los 18,4 millones de euros, un 47% más que en 2007.

MIGUEL BEATO, DIRECTOR DEL CENTRE DE REGULACIÓ GENÒMICA, ORTÉ EL XIX PREMI FCRI

"A Barcelona estic tenint un renaixement científic"

Miguel Beato va començar el Centre de Regulació Genòmica
de passar gran part de la seva carrera científica a Alemanya
"Hi ha un tipus d'entusiasme que a Alemanya, després de la reunifi
"Em trobava en l'última fase de la meva carrera i la perspectiva
lona ha estat la millor decisió que he pres, perquè hi estic tenint
científic", afirma. I el motiu d'aquest renaixement ha estat el C
amb criteris d'excel·lència científica i d'internacionalització, i
Fundació Catalana per a la Recerca i la Innovació (FCRI) ha decidit
guel Beato. Amb aquest premi, "que estimula a seguir treballant fort", es reconeixen
les seves contribucions d'abast internacional en l'avenç de la biologia molecular.

Enguany ha estat distingit amb el Premi FCRI, per "les seves contribucions d'abast internacional en l'avenç de la biologia molecular, en concret al camp del control de l'expressió gènica per hormones esteroides i dels seus efectes en la remodelització de la cromatina i en la proliferació cel·lular". Què el va conduir cap a aquest camp de recerca?

Jo soc metge, tinc el títol de ginecòleg i tocòleg, i vaig fer una mica de pràctica mèdica, però les condicions eren força precàries. Era la seguretat social reduïda a un esquelet mínim i era molt frustrant.

Al cap d'un cert temps vaig comprendre que no era el que volia fer durant tota la meva vida. Durant la pràctica mèdica m'havia dedicat a la ginecologia, on les hormones esteroides juguen un paper molt important. De fet, estava en una consulta d'esterilitat i reproducció i el nivell hormonal era clau per a la fisiologia dels pacients. D'aleshores

Vaig fer la t
te de les hormon
sobre la prolifera
dometri, sobre e

4 | teraflop

El CRG es regeix pels mateixos criteris de mobilitat i internacionalitat que l'EMBL

20 minutos

adn » tecnología

El amor desactiva la cap querida

Los últimos estudios científicos apunta relevancia a la hora de establecer relaci

EFE . Barcelona | 24/03/2008 - hace 3 días | comentarios

Las últimas investigaciones sobre el funcionamien pierden la cap de criticar a sus parejas, es a confirmar r refrán que asegura que

Al menc sucede en los casos de s ante r imientos, se activan las mi Mad ligadora del Centro de Regula

del caso, sin embargo, es qu rales, en ambos

de las

adn.es

Cere

El Centro

La Voz de Galicia

SOCIEDAD

INVESTIGACIÓN

Un estudio muestra cómo las células madre contribuyen a formar músculos más grandes

El estudio, publicado en la revista Cell Metabolism, podría irse en tratamientos para invertir o mejorar la pérdida sa muscular.

/1/2008 | 10:09 h

gadores del Centro de Regulación Genómica (CRG) de ona **han demostrado** que las células madre conocidas élulas satélite contribuyen a formar músculos más s en respuesta a un esfuerzo.

adn » tecnología

Identifican un mecanismo que regula el 10% del genoma

El descubrimiento podría implicar "importantes avances" en el diagnóstico de enfermedades tumorales, así como en el conocimiento de males relacionados con los cromosomas, como el Síndrome de Down

EFE . Madrid | 06/06/2008 - hace 16 horas | comentarios | +0 -0 (0 votos)

Científicos del Centro de Regulación Genómica (CRG) han identificado un nuevo mecanismo relacionado con la regulación del 10% del genoma humano, un descubrimiento "pionero", según los expertos, que puede permitir "importantes avances" en el diagnóstico de enfermedades tumorales.

«En aquest país no hi ha tradició de divulgació científica

Aquesta neurobiòloga nascuda a Cantàbria, d'origens alemanys i resident a Barcelona, ha estat gu
Pensament i Cultura Científica per projecte d'investigació genètica sobre la sín

Un 'camarero' al servicio de

Desarrollo de un biochip que amplía el diagnóstico prenatal

CIENCIA ▶ El Centro de Regulación Genómica, la Universitat Pompeu Fabra y los c ficos Xavier Estivill, Llui Armengol y Luis Pérez J do han creado una empr (Quantitative Genomic M cine Laboratories) que cc cializará un chip de AD ideado por estos científi y que detecta más anom genéticas en el feto que test actuales y de forma precoz. / Redacción

¿QUÉ LE DISTRAE?

Xavier Estivill pasa la mayor parte del día en el Centro de Regulación Genómica y cuando está con su familia suele leer artículos científicos. No obstante, hay dos actividades que logran distraerle un poco: la poda de viñas cuando es el tiempo y la construcción de maquetas de monumentos históricos, en vacaciones.

–Vostè ha estat premiada, entre altres motius, pel programa de *Gens i malalties*, del Centre de Regulació Genòmica de Barcelona. Me'l pot explicar?

–«Treballem en dues línies [illegible] igació per investigar les bases [illegible] les malalties [illegible] n d'identifi- [illegible] alteracions o quins gens poden contribuir a fer desenvolupar malalties mentals. Una d'aquestes línies estudia el retard mental. No només la síndrome de down, sinó també l'autisme o el trastorn de Rett.»

EL PUNT BARCELONA

Los genes no discriminarán

EE.UU. aprueba una ley para evitar el abusivo de los datos genéticos

EUSEBIO VAL
Washington. Corresponsal

Estados Unidos, líder mundial en investigación genética, ha dado un paso decisivo para evitar que sus ciudadanos sean discriminados por los resultados de los análisis genéticos que se realizan en los centros de investigación.

Los análisis genéticos permiten conocer la predisposición a sufrir algunas enfermedades

1 Se obtiene una muestra de células de la saliva o de la sangre

España ya ficha a cracks de la ciencia

Los nuevos centros de investigación revierten la fuga de cerebros

Los cerebros ya no se fugan

Investigadores destacados eligen España para desarrollar sus proyectos

España, en quinto lugar europeo en proyectos biomédicos de excelencia

"Si algun científico no vuelve a España es porque no quiere", afirma Manuel Serrano

El éxito del programa Ieea. Cuatro de los científicos que reciben financiación del Consejo Europeo de Investigación y que han llegado a Catalunya gracias a programa Ieea. Juan Carlos Garavaglia, Luis Serrano, Joachim Vilth y Macej Lewenstein

BEN LEHRER
Centro de Regulac

● Llegó al CRG, el Parc de Rece junto al hospita procedente de la Universidad de Cambridge. Lidera uno de los grupos de investigación sobre sistemas biológicos más potentes de Europa.

Europa reconoce a 15 investigadores que trabajan en centros catalanes

BARCELONA Redacción

La investigación que se realiza en centros y universidades catalanas acaba de recibir un espaldarazo. Un total de 15 investigadores que trabajan en Cataluña han colocado sus proyectos entre los 300 que apoyará y financiará el Consejo Europeo de Investigación.

Este Consejo fue creado para promover la investigación de excelencia con fondos europeos. Una de las actividades que puso en marcha fue la convocatoria *Becas de Salida (Starting Grants)*. Se presentaron 9.000 propuestas, de las que se han seleccionado 300, atendiendo a criterios de calidad científica. De los seleccionados, 24 trabajan en instituciones de investigación españolas y de estos, 15 lo hacen en centros y universidades catalanas (un 5,3%, del total).

De los investigadores que trabajan en Catalunya, siete lo hacen en centros no universitarios: tres en el Centro de Regulació Genòmica, dos en el Institut Català de Nanotecnologia, uno en el de Recerca Biomèdica y otro en el de Bioinginyeria. También el centro mixto CSIC-IRTA y el Centro Nacional de Microelectrònica del CSIC cuentan con seleccionados.

En cuanto a las universidades, seis son los investigadores reconocidos: dos son de la Pompeu Fabra (UPF), mientras que [illegible] Rovira i Virgili (URV), la Autònoma de Barcelona (UAB), tienen un investigador cada una y el Institut Català de Recerca i Estudis Avançats (ICREA) cuenta con cinco investigadores entre los reconocidos.

En el resto de España, la institución más destacada es el Centro Nacional de Investigaciones Oncológicas (CNIO), dirigido por Mariano Barbacid, que cuenta con tres seleccionados entre los cien primeros.

Los 300 investigadores seleccionados son doctores recientes, han defendido su tesis doctoral hace entre dos y nueve años, con una media de edad es de 35 años y el 26% son mujeres. La dotación presupuestaria para financiar los proyectos puede llegar a los dos millones de euros, y tienen de entre 3 y 5 años para llevarlo a cabo.●

El Consejo Europeo de Investigación prevé financiar 300 proyectos, de los que 24 se desarrollan en España

Del DNI a l'ADN

Spain is revitalizing its science base, with Barcelona surging ahead as a Mediterranean science hub, reports **Quirin Schiermeier**.

"In the past five years, Barcelona has turned into a premier location for biomedical research."
— Luis Serrano

Barcelona in the lead

The Barcelona Biomedical Research Park attests to the city's ambitions in science.

The Barcelona Biomedical Research Park attests to the city's ambitions in science.

CATALONIAN POWERHOUSE

EL PAIS

bros importados

de Regulación Genómica atrae a los investigadores en biomedicina

+Ciència

Especialistes en genes. Un científico del Centre de Regulació Genòmica de Barcelona, que desarrolla investigaciones de biología sintética, ayer en el laboratorio

podrem conèixer els elements bàsics que ens fan vulnerables a malalties com el càncer. Científics de tot el món treballen per fer realitat aquest

La VANGUARDIA

FUNDADA EN 1881 POR DON CARLOS Y DON BARTOLOMÉ GODÓ

AVISO A ASEGURADORAS

La ley prohíbe que las aseguradoras ordenen a sus clientes hacerse pruebas de ADN

HITO EN LOS DERECHOS CIVILES

Se trata del primer gran avance en derechos civiles del siglo XXI

AVUI+

El pla, dotat amb 19,5 milions d'euros, finançarà 160 beques de 4 anys entre el 2008 i el 2011 per cursar un doctorat internacional en biomedicina al Centre de Regulació Genòmica, a

es beques de La Cai ran joves talents en nbit de la biomedicina

La xifra

EL DATO

Premio al científico Miguel Beato

Miguel Beato, director del Centre de Regulació Genòmica, fue galardonado ayer con el premio de la Fundació Catalana per a la Recerca i la Innovació por sus descubrimientos que han contribuido a comprender mejor cómo se regula la actividad de los genes.

TERESA PÉREZ

'NEUROBIOLOGIA DE L'ENAMORAMENT'.
CCCB.
Montalegre, 5.
Entrada libre.

Mara Dierssen (Santander, 1961), investigadora del Centre de Regulació Genòmica (CRG) de Barcelona, participa hoy junto con el catedrático Adolf Tobeña y la psicóloga Elena Crespi en el debate *Neurobiologia de l'enamorament*. Es un café científico que el centro organiza para abrir la ciencia al gran público. El debate comienza con la pregunta *Què passa al nostre cervell quan ens enamorem?*. Mara Dierssen nos da un adelanto. Lean con cariño.

el Periódico

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