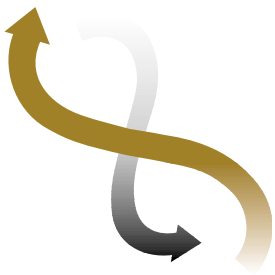

CRG

Centre
de Regulació
Genòmica

CENTER FOR GENOMIC REGULATION **ANNUAL REPORT 2004**

CENTER FOR GENOMIC REGULATION
Annual Report 2004

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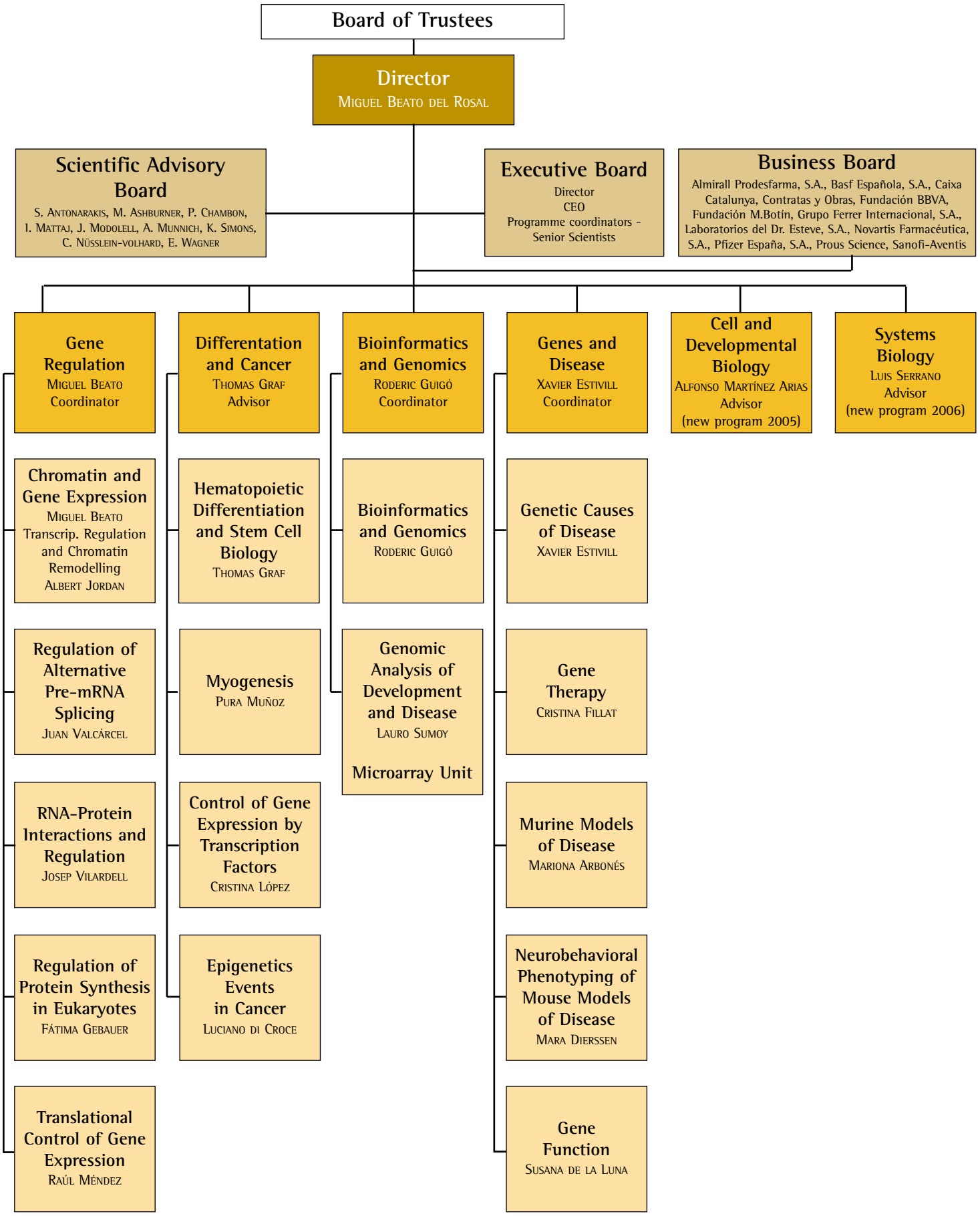
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Susana de la Luna

Management Structure

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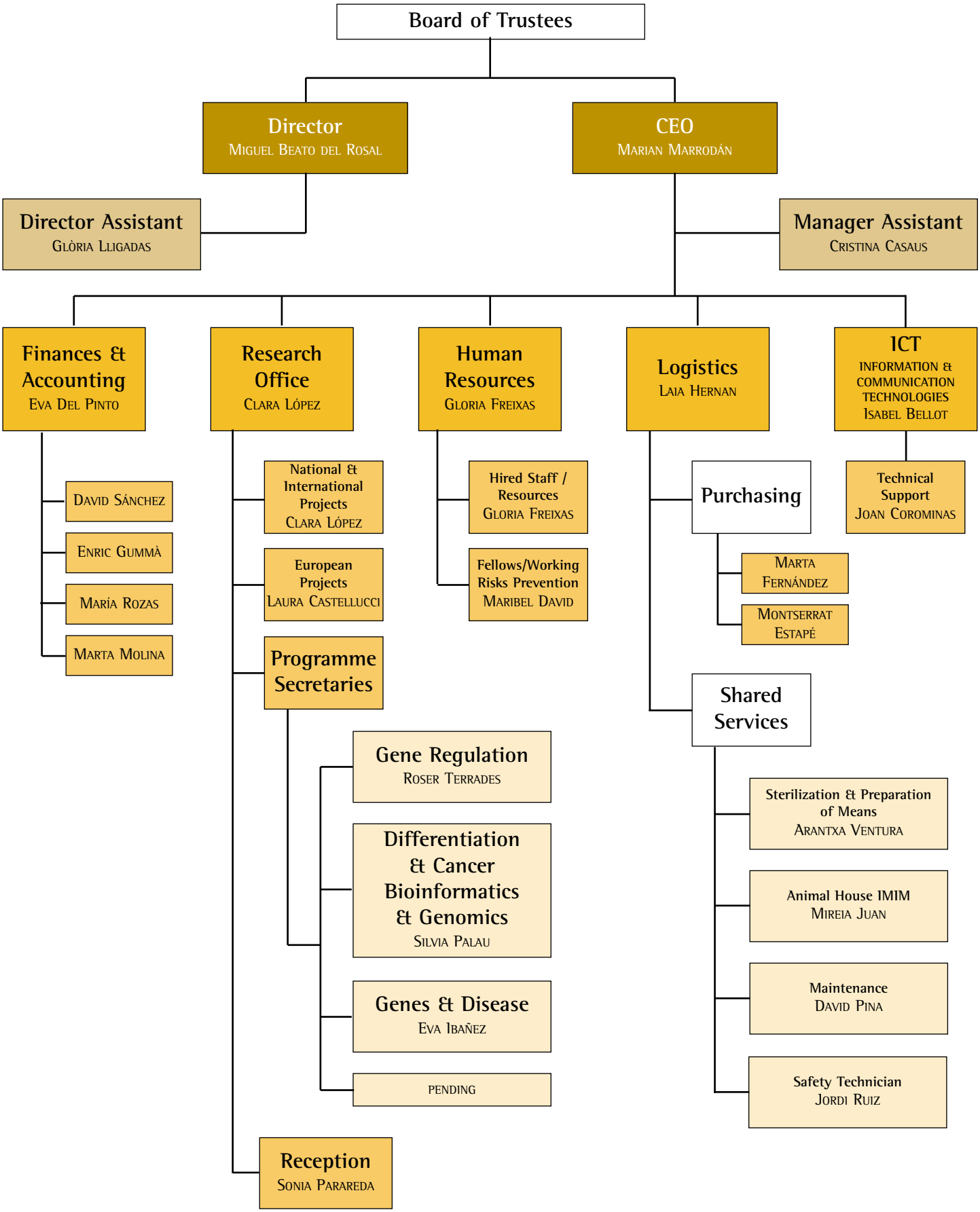
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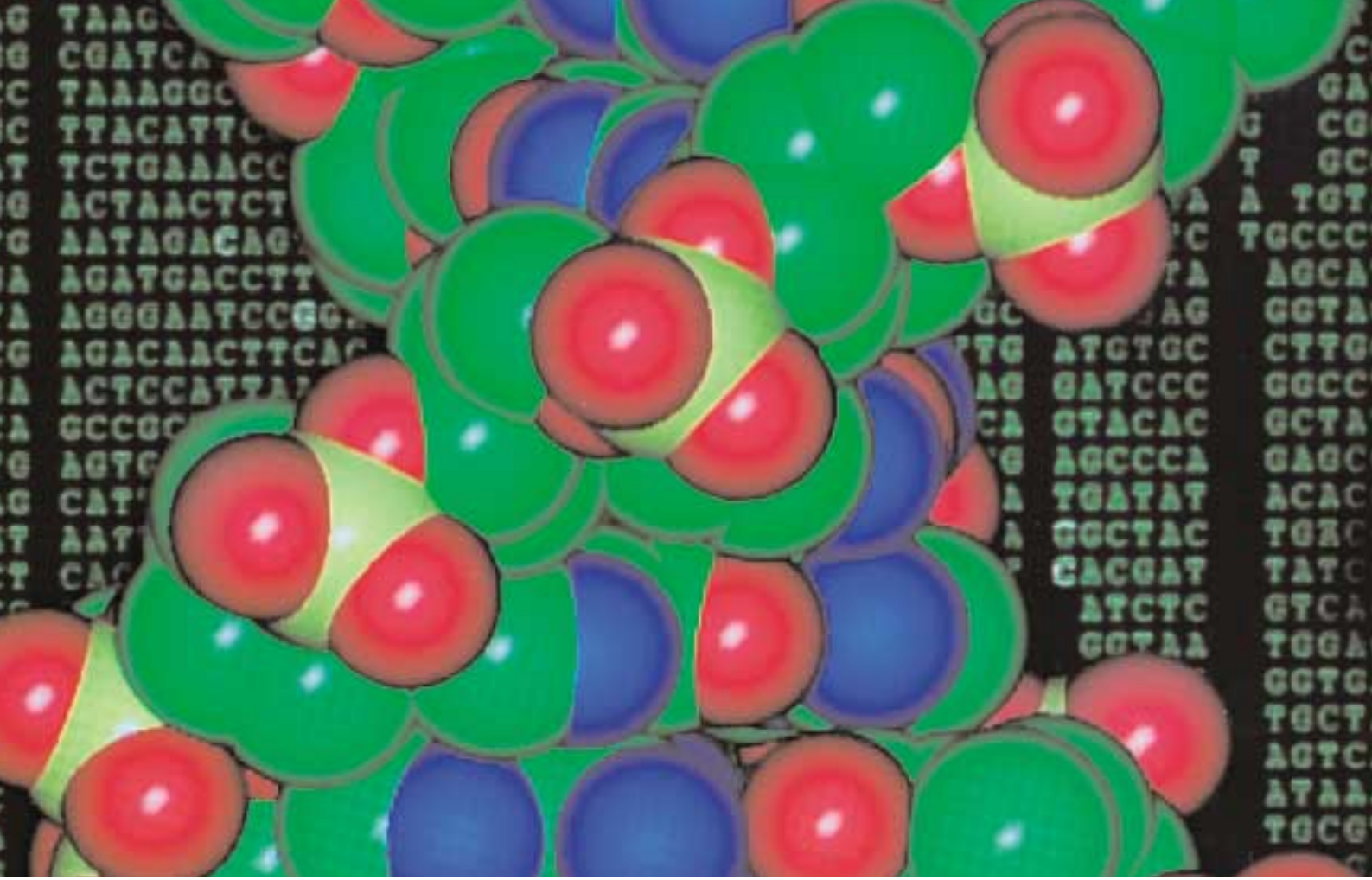
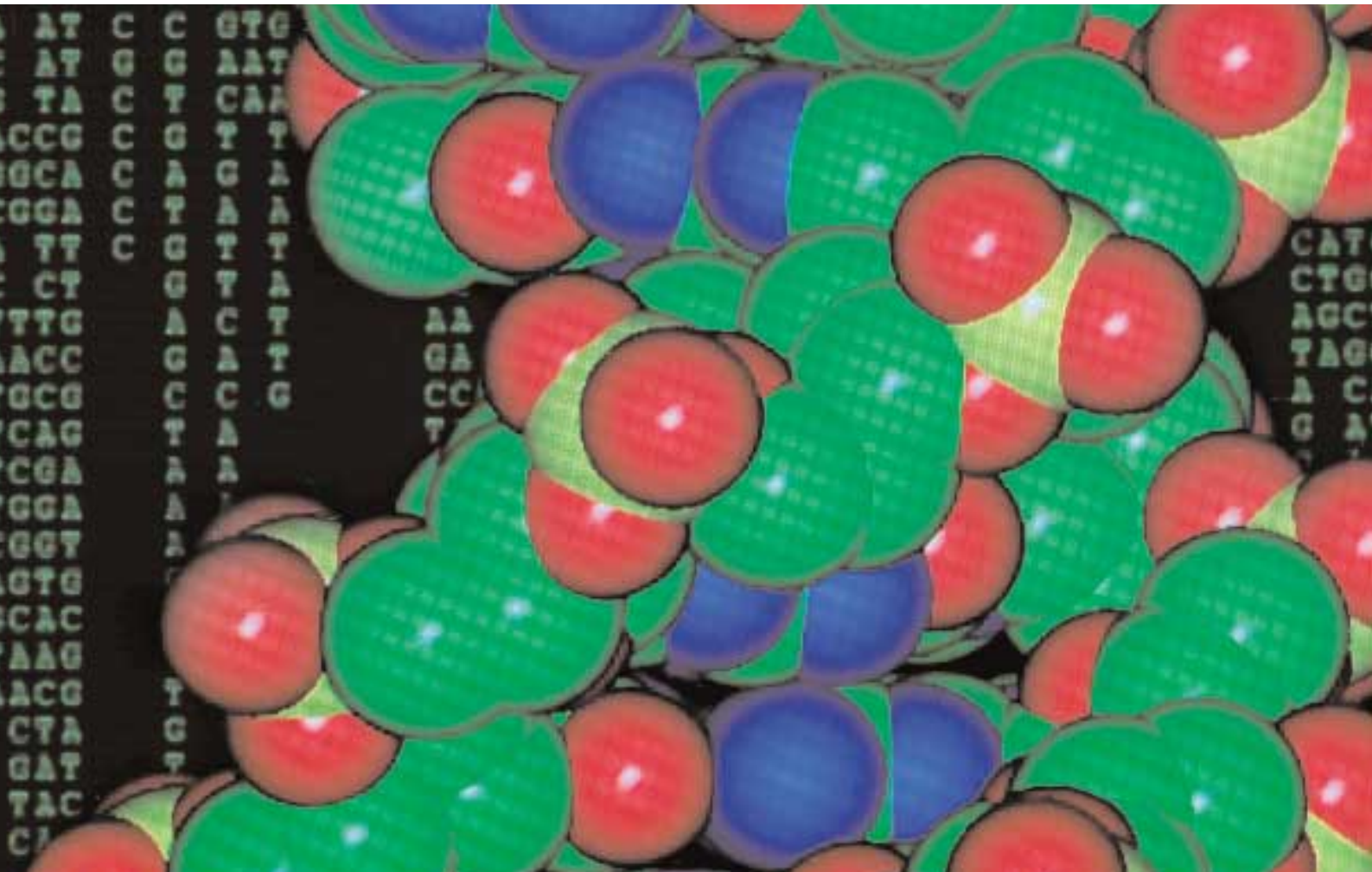


Introduction

Director of the CRG: *Miguel Beato del Rosal*



During the past year the CRG has finished its definition of the scientific career, has continued with the consolidation of the four initial programmes and the core facilities and has progressed in creating the conditions for the establishment of two new programmes on *Cell and Developmental Biology* and on *Systems Biology*. Moreover we have created the *CRG Business Board*, which has already taken important decisions for fostering our connections with pharmaceutical companies. All this has happened in the context of important changes in the Spanish and Catalan Governments, initiated in the spring of 2004, as a consequence of which our 2004 budget was kept at the level of 2003. On the other hand, the envisaged date for moving to the new building has been delayed by more than one year and is now planed for the end of 2005 or the beginning of 2006. The adaptation to these political and structural changes has been complex, but we can say that by the end of 2004 the CRG was back to its agenda, reinforced by an even stronger support from the administration.

The four established programmes have continued their activity without major changes, due mainly to space limitations. The year has seen a reinforcement of the collaboration of groups within programmes and across programmes, which is contributing to a fertile scientific atmosphere in the centre. As for the new programs, we have confirmed the commitment of the external advisors and have started recruiting new groups. Alfonso Martínez-Arias, from the University of Cambridge, UK, has headed a searching committee that among the almost one hundred candidates who applied for the group leader positions of the *Cell and Developmental Biology* programme selected six for interviews. After a first round, offers were made to two candidates, Isabelle Vernos from EMBL and Hernán López-Schier from Rockefeller University. In the meantime both scientists have accepted and Isabelle has obtained an ICREA position from the Catalan government. She will move with her group to the provisional facilities of the CRG in July 2005.

The *Systems Biology* programme continues to count on the advice of Luis Serrano and has also initiated the recruitment of group leaders. An announcement was placed in *Nature* and over 60 scientists, 40 of them foreigners, applied. A small group of 6 candidates was selected and has been invited to attend a mini-symposium early next year in Heidelberg, where a searching committee headed by Luis Serrano and including two other scientists from the CRG, Roderic Guigó and Alfonso Martínez-Arias, and two scientists from EMBL, Ian Mattaj and Bob Russell will interview them. In the same symposium 5 candidates for a new group leader position in the *Bioinformatics and Genomics* programme will also be interviewed by the searching committee.

We have continued to develop our core facilities. The *Genotyping Unit* established in the context of the Barcelona Genotyping Node of the CeGen is now set up for high throughput genotyping and many projects include now SNPs studies, which will be performed in the facility. The *Microarray Unit* continues to expand and provides now bioinformatics advice and technical support for many projects from the PRBB and for external

scientists. We have also established a microinjection service in the context of the *Transgenesis Unit*, which provides technical help to scientists in the PRBB. Finally we have installed a new FACS to complement the *FACS facility* run by the CEXS/UPF.

During 2004 the CRG has continued with the optimization of the ***communication and informatics infrastructure***, in particular we have initiated the implantation of the Integrated Management System from Oracle, which will allow direct access of each scientist to the financial state of his/her projects. We are also advancing in the construction of a scientific portal and an effective intranet system, and have gained direct access to the Internet via the scientific ring of the CESCA (Centre for Supercomputation of Catalonia) with a present capacity of 10 Mbps that could be expanded in the future.

In January 21/22 the CRG group leaders met for the second time in the context of two-days ***retreat*** in a Hotel at the Montseny near Barcelona. The meeting was also attended by Alfonso Martínez-Arias as advisor for the new programme on Cell Biology and Development. There was a brief review of the different project followed by a very intense brainstorming about future lines of research and collaborations. We want to repeat this retreat next year focusing on identifying the relevant questions in each field.

In the summer of 2004 the Department of Universities, Research and Information Society (DURSI), after evaluating our performance indicators during 2003, has approved a ***three-year financial plan*** (2004-2006) for the CRG that foresees the establishment of the two new programmes on Cell and Developmental Biology and on Systems Biology and the expansion of the other four programmes to a final size of six groups each. The full development of the programmes will only be possible after completion of the new building of the Parc de Recerca Biomedica de Barcelona (PRBB), expected for the end of 2005 or the beginning of 2006.

After a long period of preparation, on May 24, the ***Business Board*** of the CRG was appointed by the Conseller Carles Solá, and presented in a public act with a high concentration of press and television. It is composed of 12 leading representatives of local and multinational pharmaceutical companies, as well as other relevant companies and foundations. Shortly thereafter, on July 14, the first working meeting of the Board took place, the executive team was chosen, and several initiatives were suggested. The first was to organize a meeting to present the science made in the CRG to the specialists of the different companies. This meeting, called "Open CRG", was held on November 16 and resulted in the proposal to appoint a project manager for catalyzing the exchange of ideas and projects between the scientists at the CRG and their colleagues in the companies represented in the business Board. Moreover, Novartis announced the granting of a two-year postdoctoral fellowship to support the research projects of the CRG.

The delay in the construction of the ***PRBB building*** was partly due to the incorporation into the PRBB project of a newly approved Centre for Regenerative Medicine of Barcelona and to the resulting need to expand the size of the animal facility. In the meantime the new plans are accepted and the construction progress well. The plan for the equipment of the building has been approved and the work on the installations is expected to begin next spring. The plans are now that the building will be finished and ready for moving in early 2006.

Collaborations with other colleagues from the CEXS/UPF and the IMIM are supported by their common participation in various networks funded by the Spanish government (National Network of Cancer Centres, National Network of Genotyping, National Network of Genomic and Proteomic) and by the government of Catalonia (Network of Hormone-Dependent Cancers, Thematic Network of Gene Therapy, Animal Models of Disease related to the Central Nervous System). During the year 2004 we have elaborated the text of an agreement with the UPF to become a University Associated Institute, which will be official soon. This

agreement gives the CRG group leaders access to some of the privileges of university professor, such as directing PhD thesis and applying for PhD fellowships, as well as access to the university library. In exchange, CRG scientists participate in advanced teaching of Biology students at the UPF, in particular in the fifth year of the Basic Research itinerary and in the International PhD programme on Basic Biomedical Research, and the CRG contributes to financing the Journal subscriptions of the UPF/CEXS.

Integration with the other Institutions in the PRBB has been favoured by the 29 ***Friday Seminars*** with foreign speakers organized by the CRG, which are regularly attended by scientists and students from the CEXS/UPF and the IMIM. In addition the various programmes of CRG organized 25 Seminars with external speakers, which were also very appreciated by the other PRBB colleagues.

The ***third CRG Symposium*** was held on October 15-16, 2004. It was organized by Alfonso Martínez-Arias, and attracted more than one hundred colleagues, who came to listen to 15 experts in "*The Cell in Development*". The symposium opened with a plenary lecture by Wolpert, Cambridge UK, and was characterized by excellent talks full of new information and by lively scientific discussions.

The expanded ***Scientific Advisory Board (SAB)*** met for two days in November and listened to presentations of the scientific projects of all group leaders. They also visited the laboratories, approved the text of the scientific career at the CRG, and made important recommendations to the director. There was general agreement that in view of the new move to the PRBB building the evaluation of the first two programmes, Gene Regulation and Genes and Disease should be postponed until 2007.

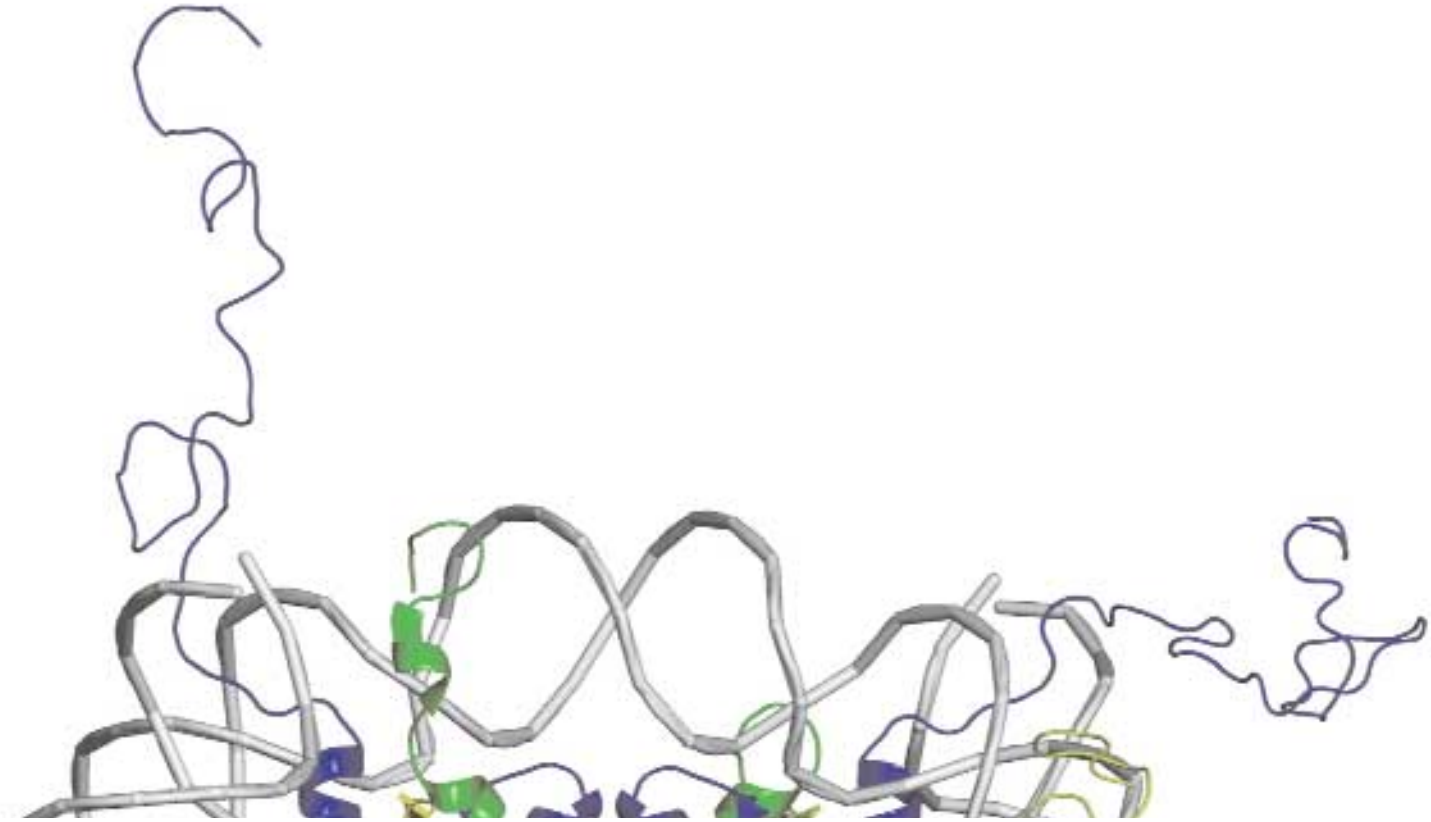
Two days after the meeting of the SAB, on November 15, the CRG hosted a ***Symposium on "Cancer, Transcription and Development"*** in the honour of Thomas Graf and organized by his former collaborators and students. It included 10 renamed international expert presentations and was attended by over 100 colleagues, who participated in a lively discussion.

Numbers. By the end of 2004 the CRG had 16 research groups and a total of 154 people, including 41 senior and postdoctoral scientists, 54 graduate students, 37 technicians and 22 administrative and support. Relative to 2003, this represents a 17.5 % increment in the number of people working at the CRG.

As detailed in the reports of the individual groups and in the Appendix 3, scientists at the CRG continue to be successful in obtaining ***external grants*** and financial support for their scientific projects. The total amount of competitive resources granted to CRG scientists in 2004 exceeded 4,2 million.

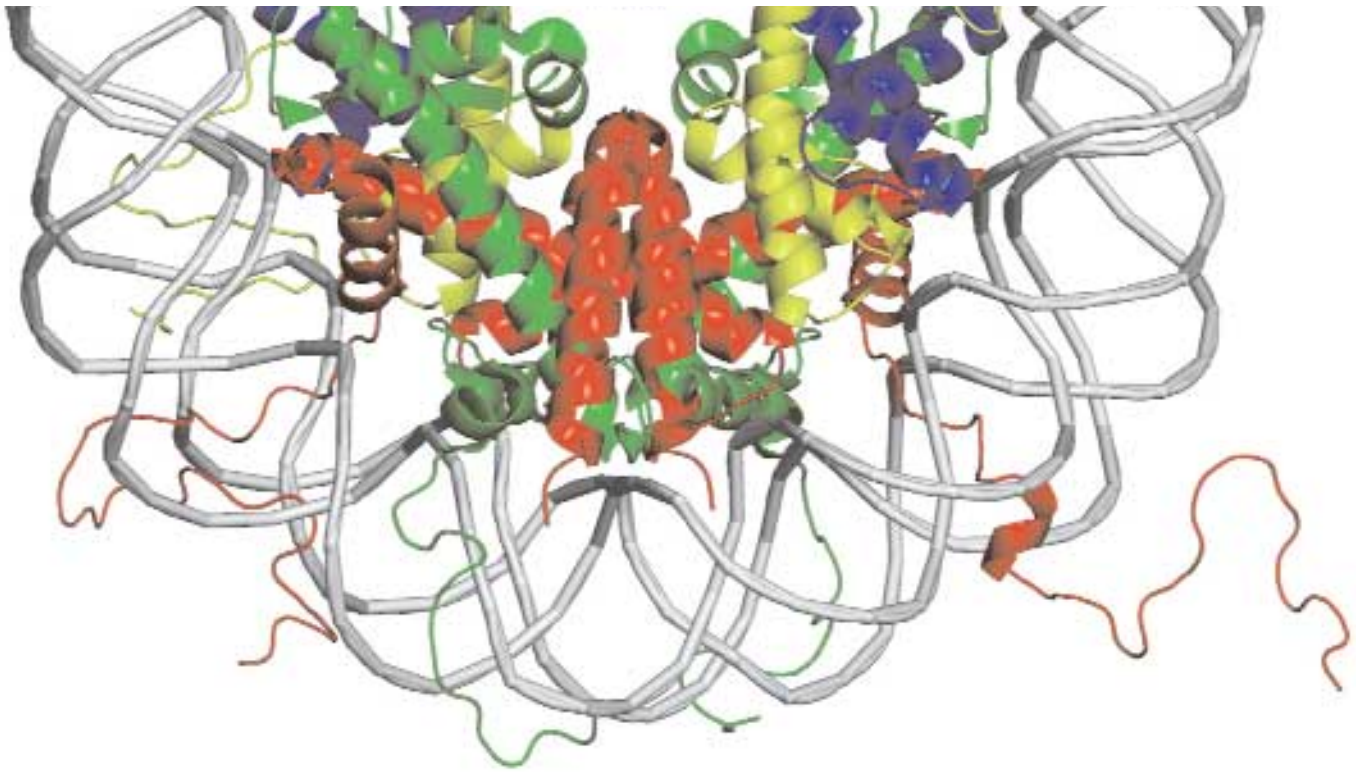
The first ***publications*** of results obtained in the CRG are starting to appear. During the last year there were 78 papers published or in press with authors affiliated at the CRG, and the average impact factor per paper was 9,14. These numbers reflect the difficulties associated with starting new ambitious groups in an institution installed in provisional space and still lacking experienced general services and core facilities.

Outlook. By the end of 2004 the provisional labs of the CRG as well as the offices of the administrative area are very crowded and one can feel a certain tension. The CRG continues to exhibit a strong tendency to grow and we are all looking forward to the time when we will be able to expand in the new building. This is essential for implementing the two new programs, for developing the Bioinformatics and Genomic program and for implanting strong and state of the art core facilities, specially the animal facility. The rhythm at which the CRG project has developed during the initial phase cannot be constrained much longer without damaging the institution. We firmly hope and are contributing with all our energy to ensure that the date for completion of the PRBB building will not be postponed again.



Gene Regulation

Coordinator: *Miguel Beato del Rosal*



During the last year the five groups of the programme (Fátima Gebauer, Raúl Méndez, Juan Valcárcel, Josep Vilardell, Miguel Beato,) have continued their consolidation with the incorporation of new students, the development of the established projects, and the securing of new project grants. In general, there was no possibility of expansion because of the space limitations. Thus the size of the programme has remained stable, compared to 2003.

Collaborations among groups in the programme are starting to develop partly as a result of discussions during the well-attended Monday data seminars, in which each scientist of the programme presents the progress of his/her projects to the other scientists, including colleagues from other programmes and from other institutions of the near environment. As one indicator of these starting collaborations, the groups of Juan Valcárcel and Miguel Beato organized an international workshop in Baeza (Jaén) on the *"Coupling between transcription and RNA processing"* that was a great success (see the Meeting Review by M. Ares and N.J. Proudfoot in *Cell* 120, 163-166, 2005). The small groups have common lab meetings and journal clubs in addition to the general Journal Club of the programme each Wednesday. In addition a large fraction of the CRG Friday seminars are on topics related to Gene Regulation and with speakers invited by scientists of the programme, and additional programme seminars by external speakers have been held on other weekdays.

Collaborations are also ongoing with scientists from other CRG Programmes as well as with scientists from the other institutions grouped in the PRBB. Strong collaborations are already established with the Bioinformatics and Genomics programme. The group of Juan Valcárcel collaborates with the group of Roderic Guigó to combine computational and molecular tools to identify sequences involved in alternative splicing regulation. Miguel Beato's group collaborates with the microarray unit and the group of Lauro Sumoy in projects aiming at the definition of pathways and gene networks regulated by ovarian hormones in breast and endometrial cancer. Along with the Department of Pathology of the Hospital del Mar they have developed a small cDNA microarray with which the expression of 800 genes relevant for breast cancer can be studied in primary tumour material. Collaborations are also ongoing with the Cell Differentiation and Cancer programme for studying the epigenetic regulation of the $p21^{Cip1/Waf1}$ gene promoter and with the Gene and Disease programme for genotyping breast cancer patients.

External funding continues to be very successful but in terms of publications 2004 was a difficult year. Most projects carried over from the laboratories of origin of the group leaders have already been concluded and the new projects started at the CRG are in general not advanced enough to yield publications. Nevertheless, the first papers are beginning to appear with results obtained exclusively or largely in the CRG.

GENE REGULATION

Chromatin and Gene Expression

Group structure:

Group Leader
Miguel Beato del Rosal

Postdoctoral Fellows
Cecilia Ballaré
Mike Edel (since November 2004)
María Jesús Meliá
Guillermo Vicent

PhD Students
Thomas Bechtold
Verónica Calvo
Jaume Clausell
Vladimir Maximov
Roser Zaurin

Technician/s
Jofre Font
Silvina Nacht
Nora Spinedi

Visitors
Luciana Rocha-Viegas
Griselda Vallejo
Patricia Saragüeta

Subgroup

Transcriptional Regulation and Chromatin Remodelling

Staff Scientist
Albert Jordan (subgroup leader)

PhD Students
Ignacio Quiles
Mónica Sancho
Alicia Subtil

Technician/s
Nora Spinedi

The group is interested in understanding the mechanism of signal transduction and gene regulation in eukaryotes and uses as main experimental model gene induction by steroid hormones. The main lines of research have been the regulation of transcription by glucocorticoids and progestins and the crosstalk between estrogen and progesterone receptors and other signalling pathways. The role of these hormones in breast and endometrial cancer cell proliferation and apoptosis is a more applied line of research of the group.

RESEARCH PROJECTS

1. Regulation of MMTV transcription

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

After clarifying the complex role of histone H1 in the induction process, the main focus of this project has been the elucidation of the structural changes accompanying activation of MMTV promoter chromatin and how they are catalyzed. We have initially focused on the modifications of the core histones. We found that upon progesterone treatment of breast cancer cells carrying an integrated copy of the MMTV promoter, a Brg-1 containing complex is recruited to the promoter along with PR, co-activators and the RNA polymerase II (Vicent *et al.*, 2004). Simultaneously we detected the displacement of histones H2A and H2B from the promoter nucleosome containing the HREs but not from the adjacent nucleosomes (Figure 1). On mononucleosomes assembled with recombinant histones, SWI/SNF catalyzes displacement of H2A/H2B dimmers from MMTV promoter but not from positioned nucleosomes containing ribosomal promoter DNA (Vicent *et al.*, 2004). A similar result was obtained with recombinant nucleosomes arrays containing several nucleosomes from the MMTV LTR: only the nucleosome containing the HREs looses H2A and H2B upon incubation with purified SWI/SNF and ATP. We conclude that the nucleotide sequence of DNA determines the outcome of nucleosome

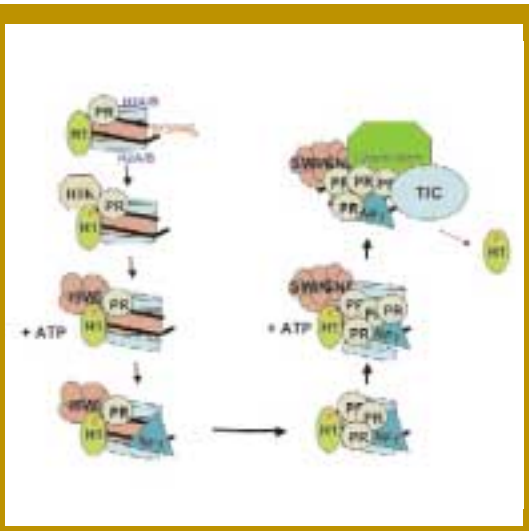


Figure 1. Steps in the activation of the MMTV promoter. PR binds preferentially to the exposed HRE1 on the MMTV nucleosome. It first recruits a histone H1 kinase (HIK) that phosphorylates H1, as a prerequisite for the ATP-dependent remodelling by the recruited ISWI-containing complex. This remodelling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

remodelling by a purified remodelling complex. We are now planning to extend these studies to other nucleosomes in order to understand the properties of the DNA sequence responsible for the differential responses to SWI/SNF action.

As an additional tool for the study of the role of chromatin in the regulation of gene expression we have established a simple method for the preparation of whole cell extract from *Saccharomyces cerevisiae* able to assembled exogenously added plasmid DNA and core histones into regular nucleosomes (Rodríguez-Campos *et al.*, 2004). This *in vitro* assembled chromatin can be used as template for cell-free transcription in a nuclear HeLa cell extract and recapitulates the synergism between hormone receptor and NF1 characteristic of MMTV promoter activation.

2. Crosstalk between hormone receptors and other signalling pathways

A. Jordan, C. Ballaré, T. Bechtold, A. Subtil, N. Spinedi

In previous work we found that progesterone can activate transiently the Src/Ras/Erk pathways via an interaction of PR with the estrogen receptor alpha (ERα), and that the kinase activation is essential for the proliferative response of breast cancer cell lines (Figure 2). We found that the ligand binding domain of ERα interacts with two different regions in the N-terminal half of PR, ERID-I and ERID-II, which are both required for progestin activation of the Src/Ras/Erk cascade. We are now identifying the amino acids implicated in the interaction between PR and ERα. In the meantime we have generated PR mutants with defects in ERID-I and/or ERID-II that interfere with binding to ERα, in order to study the role of the progestin activation of the Src/Ras/Erk cascade in cell culture and eventually in transgenic mice. A considerable effort was devoted to establishing stable cell lines expressing inducible versions of these mutated PRs, but the phenotype of these cell lines was not maintained in culture. We are now using new cell lines constitutively expressing PR mutants (see 4, bellow) to study their influence on chromatin dynamics during progesterone induction of gene expression.

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

M.J. Meliá, V. Calvo, M. Edel, L. Rocha-Viegas, G. Vallejo, P. Saragüeta

In the context of a collaboration with the Department of Pathology of the Hospital del Mar, Barcelona, we are studying the gene networks regulated by estrogens and progestins in breast cancer cells and in primary tumour material and how this parameter is influenced by the products of the BRCA genes. For these studies we are collaborating closely with Belen Miñana y Claudio Sumoy of the Microarray Unit of the CRG. We are combining the array information with conventional molecular biology techniques for analyzing how the

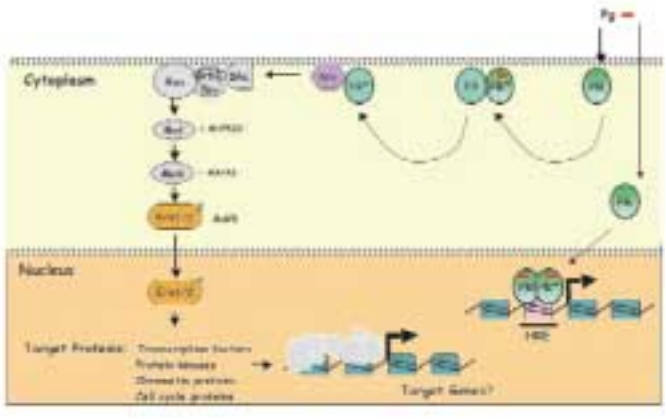
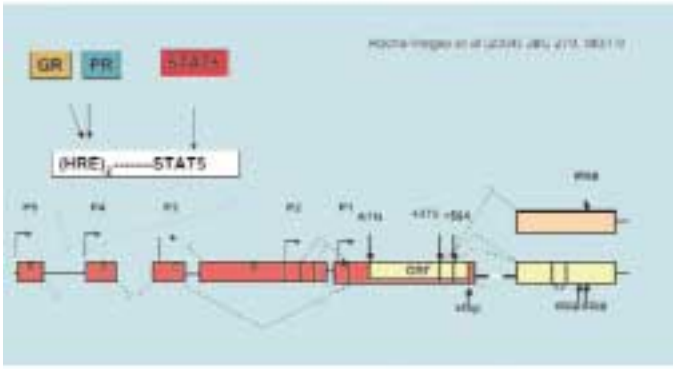


Figure 2.
Crosstalk between PR and ER in the inner side of the cell membrane. In addition to the direct activation of PR followed by homodimerization and binding to HREs in chromatin (right side), progesterone can induce the activation of a preformed ER-PR complex, leading to interaction of ER with c-Src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

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



interactions between hormone receptors and BRCA proteins modulate gene expression and chromatin remodelling.

In a collaboration with the group of Adalí Pecci, University of Buenos Aires, we are studying the role of glucocorticoids and progestins in the control of apoptosis in transformed endometrial cells, which is mediated in part via a regulation of *bcl-X* transcription and splicing. We have identified the HREs responsible for progesterone induction of the bcl-X gene in mammary epithelial cells (Rocha-Viegas *et al.*, 2004). We are now analyzing the molecular mechanism involved in the tissue-specificity of the apoptotic response, focusing on the differential effect of glucocorticoids in thymocytes, where they induced apoptosis, and in mammary epithelial cells, where they prevent apoptosis. We find that this difference is mediated by selective functional interactions with members of the STAT5 family of transcription factors (Figure 3).

In collaboration with the group of Patricia Saragüeta, CONYCET, Buenos Aires, we are studying the response of endometrial stromal cells to estrogens and progesterone using cell biological techniques and cDNA microarrays, with a focus on understanding the decidual reaction and the mechanism of blastocyst implantation (Figure 4). We found that very low concentrations of progestins induce cell proliferation via activation of the crosstalk of PR with ERß and the mitogenic kinase cascades (G. Vallejo, C. Ballaré, L. Baranao, M. Beato & P. Saragüeta, submitted).

In a new project we are studying the role of $p21^{Cip1/Waf1}$ in the progesterone control of cell proliferation and cell differentiation in breast cancer cells.

PUBLICATIONS

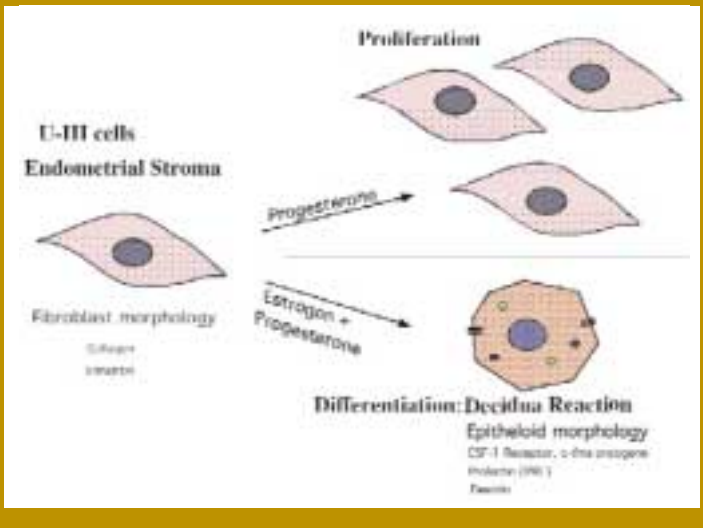
- Rocha Viegas L, Vicent GP, Baranao JL, Beato M, Pecci A "Steroid hormones induce bcl-X gene expression through direct activation of distal promoter P4." J Biol Chem, 279, 9831-9839 (2004)
- Rodríguez-Campos A, Koop R, Faraudo S, Beato M "Transcriptionally competent chromatin assembled with exogenous histones in a yeast whole cell extract" Nucleic Acids Res, 32, E111 (1-6) (2004)
- Vicent, GP, Nacht AS, Smith CL, Peterson CL, Dimitrov S, Beato M "DNA instructed displacement of H2A and H2B at an inducible promoter" Mol Cell, 16, 439-452 (2004)

Research Subgroup
Transcriptional Regulation and Chromatin Remodelling

A. Jordan, I. Quiles, M. Sancho, A. Subtil, N. Spinedi

We are interested on distinguishing between direct effects of nuclear hormone receptors and those

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



mediated by signal transduction pathways on transcription of target genes (Figure 2). For this, we are currently constructing breast cancer-derived cell lines that express tagged forms of PR mutated at residues involved either in the nuclear action of the receptor or in its ability to interact with components of signal transduction pathways. The MMTV promoter is used as a reporter to study the transcriptional effect of receptor variants. In addition, expression of tagged receptor is being used to perform proteomic studies of nuclear, cytoplasmic and membrane-associated purified complexes containing PR in the absence or presence of hormone.

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

During the process of MMTV promoter activation by PR in *Drosophila* extracts, histone H1 is phosphorylated and leaves the promoter. At least six H1 variants exist in mammalian somatic cells that bind to the nucleosome core particles and linker DNA. We are now developing RNA interference to create stable breast cancer cell lines lacking expression of each of the H1 variants specifically. With these cells we plan to investigate the role of each variant on MMTV promoter repression, activation and chromatin remodelling, as well as on global gene expression by using microarrays. In parallel, we are developing specific antibodies for H1 isoforms 1 to 5 that will be used on ChIP experiments devoted to determine the presence of each form in several target promoters, as well as at a genome level using promoter microarray (chip-on-ChIP).

Finally, to gain insight into the process of MMTV activation *in vivo*, we have started an approach to freeze the ordered recruitment of transcription factors and chromatin modifications induced by hormone addition. This is achieved by the integration in a breast cancer cell of MMTV promoters mutated at different *cis* regulatory elements (TATA, NF1 binding, HREs). To avoid variable effects of flanking chromatin, we are developing a method to integrate repeatedly different MMTV constructs into the same *locus* of the genome, based on the Cre/loxP technology.

PUBLICATIONS

- Larsson KM, Jordan A, Eliasson R, Reichard P, Logan DT, Nordlund O
  "Structural mechanisms of allosteric substrate specificity regulation in a ribonucleotide reductase"
  Nature Struct Mol Biol, 11, 1142-1149 (2004)

GENE REGULATION

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

Group structure:

Group Leader
Juan Valcárcel

Postdoctoral Fellows
Brendan Bell (until July 2004)
Claudia Ben-Dov
Daniel Bilbao (until October 2004)
Sophie Bonnal
Veronica Raker

Students
Mafalda Araujo
Nuria Majos
Luis Mendes

Technicians
Alicia Ezquerra (since April 2004)
Conchi Martínez (until September 2004)

Our group is interested in understanding how cells choose between alternative splice sites to generate multiple mRNAs and proteins from a single primary transcript. We study both detailed mechanisms of splicing repression and activation in specific genes, as well as global changes in regulation using microarrays. During last year we have made progress in understanding how various splicing factors modulate exon definition to regulate splicing of the apoptosis-controlling gene Fas. We also identified a novel mechanism for splice site proofreading involving the protein product of the proto-oncogene DEK. Finally, results using splicing-sensitive microarrays revealed ectopic expression of neuron-specific splicing regulators in high grade Hodgkin lymphomas.

RESEARCH PROJECTS

1. Alternative splicing of the Fas receptor: mechanisms of exon definition

Fas pre-mRNA exon 6 is alternatively spliced to generate mRNAs encoding either the pro-apoptotic, membrane-bound form of the receptor, or a soluble isoform that prevents programmed cell death. We have studied how four proteins (TIA-1, PTB and the products of the tumour-related genes EWS and RBP5) modulate Fas exon 6 splicing. The picture emerging from these studies is that regulation targets molecules that stabilize interactions between splicing factors bound to the splice sites that border the exon. The nature of these bridging molecules and their interactions has been elusive, despite the importance of this process - known as exon definition- for proper splicing and regulation of internal exons. We hope that our work will provide clues about the molecular mechanisms of exon definition.

2. Proofreading 3'splice site recognition

A paradox of the splicing process is that the high accuracy required to precisely excise introns is apparently based on recognition of splicing signals that are rather short and degenerate. One way to enforce accuracy is the existence of proofreading mechanisms, but little is known about how such mechanisms operate in the spliceosome. The 3' end of introns is almost invariably an AG dinucleotide,

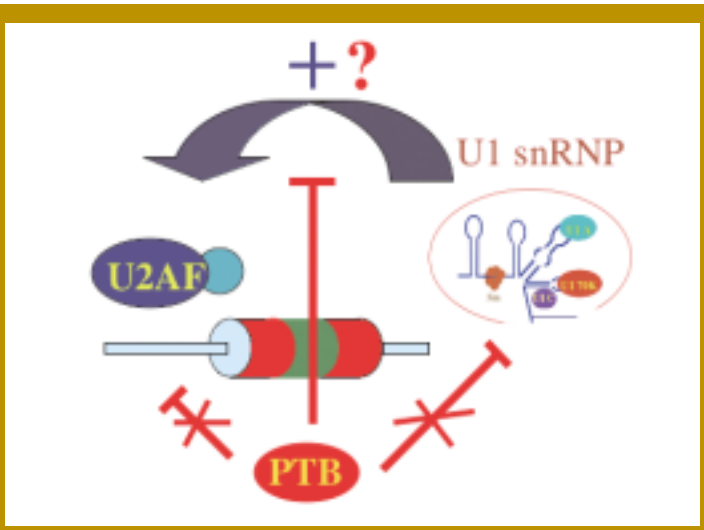
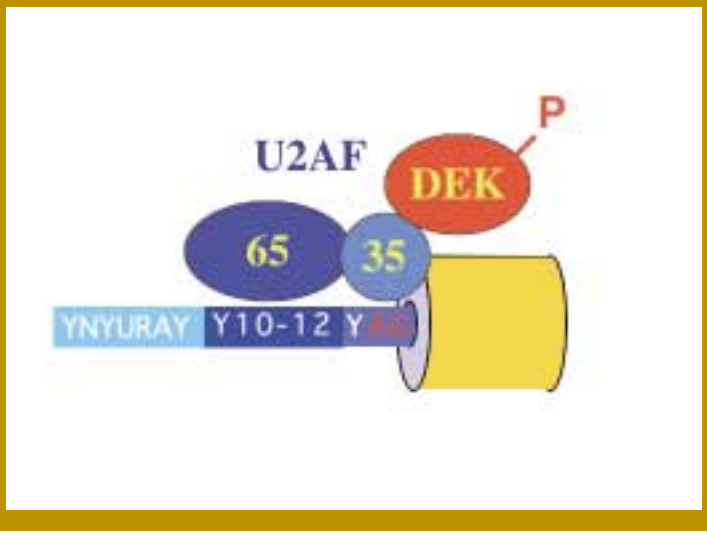


Figure 1.
Mechanism of PTB-induced exon skipping. Binding of PTB to an exonic splicing silencer in Fas exon 6 causes exon skipping. Inclusion of this exon is heavily dependent on early stabilizing interactions involving factors bound to the splice sites flanking the exon (U1 snRNP and U2AF). PTB does not directly interfere with the binding of these factors, but rather blocks "across the exon" interactions leading to exon definition.

Figure 2.
Proofreading of 3´ splice site recognition by DEK. 3´splice sites are recognized by the two subunits of the splicing factor U2AF. Its 35 KDa binds to the invariant 3´splice site AG dinucleotide. We have found that specificity of U2AF35 for AG is reinforced by the protein product of the proto-oncogene DEK. DEK phosphorylation at two serine residues in its amino-terminus is important both for interaction with U2AF35 and for enforcing 3´splice site specificity.



which is recognized by the splicing factor U2AF35. We have observed that the specificity of U2AF35 for AG is enforced by DEK, a protein previously implicated in transcription regulation. Our results also indicate that both this activity of DEK and its ability to interact with U2AF35 depend on a phosphorylation switch. We are now investigating whether DEK phosphorylation can act as a mechanism for regulating splice site selection.

3. Changes in splicing regulation during tumour progression

We have designed oligonucleotide microarrays able to discriminate between alternatively spliced mRNA isoforms, and used them to analyze changes in alternative splicing of genes involved in cell signalling, proliferation and adhesion in cell lines corresponding to different stages of progression of Hodgkin lymphoma. The results suggest that alternative splicing can be used as an indicator of tumour grade. The microarrays also revealed changes in the expression of splicing regulators. For example, expression of neuron-specific regulators of the NOVA family in some cell lines results in increased expression of neuron-enriched isoforms of JNK2 kinase that can influence cell proliferation. We are now extending these studies to other genes and tumours. The hope if that these global studies will provide a better understanding of the impact of alternative splicing in various biological processes, and that will also help to reveal the molecular logic behind regulatory networks established by differential expression of splicing factors.

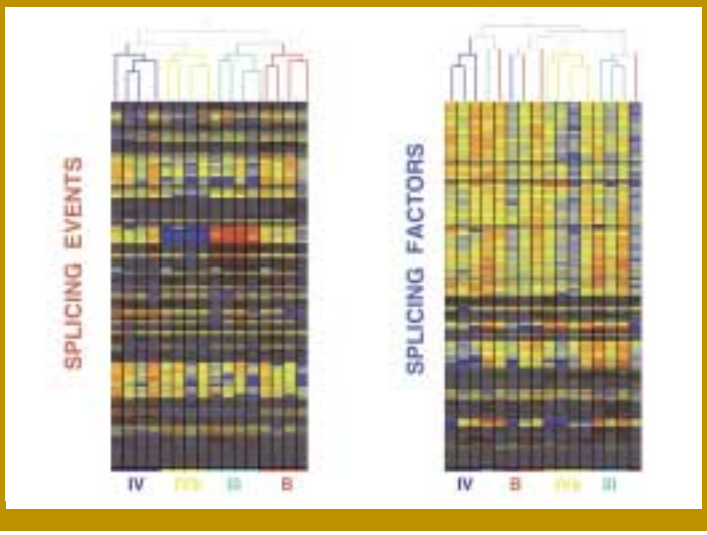
Additional future plans

The following additional projects have been started recently or will be started shortly:

- In collaboration with the computational group of Roderic Guigó, we are identifying sequences that influence splice site recognition and the factors that recognize them.
- In collaboration with the group of Francesc Real (IMIM/UPF), we are studying alternative splicing of the transcription factor Pax 6, and its alterations in pancreatic cancer and aniridia.
- In collaboration with Maria-Carmo Fonseca (University of Lisbon), we are using our microarray approach to study changes in alternative splicing in cellular models of muscular dystrophies.
- In collaboration with Reinhard Lührmann (MPI, Göttingen), we plan a large screen for functional characterization of splicing factor isoforms using RNAi and microarrays.
- We have started projects aiming to understand the impact of activation of signal transduction pathways on alternative splicing, as well as the regulatory mechanisms underlying these changes.

Figure 3.

Analysis of splicing regulation using microarrays. Splicing-sensitive microarrays have been used to analyze changes in alternative splicing of genes involved in cell proliferation and metastasis, in parallel with changes in expression of splicing factors and regulators, in cell lines derived from Hodgkin lymphomas at different stages of tumour progression. While a correlation can be found between changes in alternative splicing and tumor grade, such overall correlation is not apparent for expression of splicing regulatory factors.



PUBLICATIONS

- Banerjee H, Rahn A, Gawande B, Guth S, Valcárcel J, and Singh R
  "The conserved RNA Recognition Motif 3 of U2 snRNA Auxiliary Factor (U2AF65) is essential in vivo but dispensable for activity in vitro."
  RNA, 10, 240-253 (2004)
- Pacheco TR, Gomes A, Benes V, Ansorge W, Wollerton M, Smith CWJ, Valcárcel J and Carmo-Fonseca M
  "Diversity of vertebrate splicing factor U2AF35: identification of alternatively spliced U2AF1 mRNAs."
  J Biol Chem, 279, 27039-49 (2004)
- Harrington ED, Boue S, Valcárcel J, Reich JG and Bork P
  "Estimating rates of alternative splicing in mammals and invertebrates."
  Nat Genet, 36, 916-7 (2004)
- Relogio A, Ben-Dov C, Baum M, Ruggiu M, Benes V, Darnell RL and Valcárcel J
  "Splicing microarrays reveal functional expression of neuron-specific splicing regulators in Hodgkin lymphoma cells".
  J Biol Chem (in press)
- Singh R and Valcárcel J
  "Building specificity with non-specific RNA binding proteins".
  Nature Structural and Molecular Biology (in press)

GENE REGULATION

Group: RNA-Protein Interactions and Regulation

Group structure

Group Leader
Josep Vilardell

Postdoctoral Researcher
Estefanía Muñoz (since October 2004)

PhD Students
Mireia Bragulat
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Technician
Judit Peix

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* and the gene *RPL30*, which encodes the ribosomal protein L30. Thru binding to a structure present in its own transcript L30 can regulate RNA processing at several steps. Our main interest is on control of splicing (see figure), 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.

RESEARCH PROJECTS

1. Genetic screen to select mutants in regulation of splicing

M. Bragulat

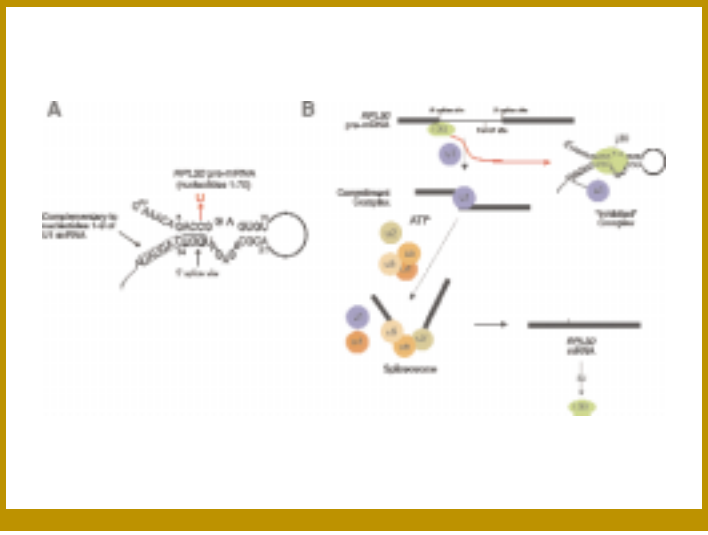
In order to understand how L30 inhibits splicing a genetic approach is being set up, with the use of two reporter genes (*CUP1*, *GFP*), placed under the post-transcriptional control of L30, to better aim our screens. Because this regulation is subtle, the system must be tuned precisely to obtain good discrimination between the mutants and the wt. In addition, and based on our results from the biochemical approach, a further screening asking for regulation of a mutant (C9T, see figure) pre-mRNA de-repressed, is being pursued. In the first approach a lack of regulation will be needed for viability; while the opposite is required for the second. We pursue the identification of activities involved in regulation of the splicing machinery. The required controls to aim our focus on *trans* mutants, other than L30, are in place. We are analyzing the products of the first screenings. This project has ran into multiple difficulties, mostly in the generation of strains and constructs, and an unexpected number of mutants in *RPL30*, which are not being sought at the moment. Should the new screens fail we will perhaps a complete change in strategy will be necessary.

2. Characterization of the "inhibited" complex

S. Macias

As shown in the figure, during L30 regulation of splicing a new complex is formed. We are interested on the nature of this complex, its components and their interactions. Cross-linking and western approaches together with biochemical purification procedures are being followed. We have generated interesting data showing that U1 RNA can be crosslinked to the 5' SS and by co-IPs with extracts with tagged splicing factors a picture of the regulation mechanism is becoming clear, suggesting that L30 is likely to disrupt the crosstalk between splicing signals. For the biochemical purification we are following a TAP based approach, using the L30 orthologue from the

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



archaebacterium *Sulfolobus acidocaldarius*, which can not be incorporated into yeast ribosomes but can regulate *RPL30* splicing in vivo. The first purifications are being performed.

3. Genetic interactions in positions 3 and 4 of the intron

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

The consensus sequence of the yeast 5' ss is GUAUGU, while that of L30 is GUCAGU, with A3C+U4A changes, evolutionarily conserved. However, it is known that A3C can be deleterious for splicing, while U4A has practically no effect. Could U4A suppress A3C? Are U6 or the 3' ss involved? We have tested all possible genetic interactions between positions 3 and 4 of the 5'ss and –3 of the 3'ss (xAG). The results show that in fact position 4 is able to suppress many second step mutations besides A3C, and support a recently proposed new model for the spliceosome mechanism. This is probably the most advanced project in the lab, and a manuscript is in preparation.

4. Other *S. cerevisiae* genes with regulated splicing

E. Muñoz

The *RPL30* gene is providing information on possible ways to regulate splicing, validating these type of studies. Can we find other cases of regulation? Using bioinformatics, all *Saccharomyces* 5' splice sites (5'SS) have been folded and those in which the 5'SS is likely to be in a stem have been selected for further study. Some of them (i.e. *SAR1*) had been already described as being postranscriptionally regulated, albeit by unknown mechanims. Using our experience we intend to uncover new instances of regulated splicing. Estefanía Muñoz has been awarded a fellowship from the Andalusian Government to follow this project.

PUBLICATIONS

No publications since November 2003.

GENE REGULATION

Regulation of Protein Synthesis in Eukaryotes

Group Structure

Group Leader
Fátima Gebauer

Postdoctoral Researcher
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Students
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Solenn Patalano
Aida Martínez

Technician
Olga Coll

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

RESEARCH PROJECTS

1. Translational control of dosage compensation in Drosophila

Dosage compensation in *Drosophila* is achieved by hypertranscription of the male X chromosome via the action of a ribonucleoprotein complex known as the MSL (for male specific lethal). This process is inhibited in female flies primarily because the expression of one of the MSL components, the protein MSL-2, is repressed. Sex-lethal (SXL), a female-specific RNA-binding protein, binds to stretches of uridines present in the 5' and 3' UTRs of *msl-2* pre-mRNA, which ultimately results in inhibition of *msl-2* mRNA translation. Translational repression requires additional factors that are nucleated by SXL in the 3´UTR of msl-2. We have isolated and identified a co-repressor. Current research focuses on the study of the interactions between this co-repressor, SXL and the mRNA, as well as on the *in vivo* significance of these interactions.

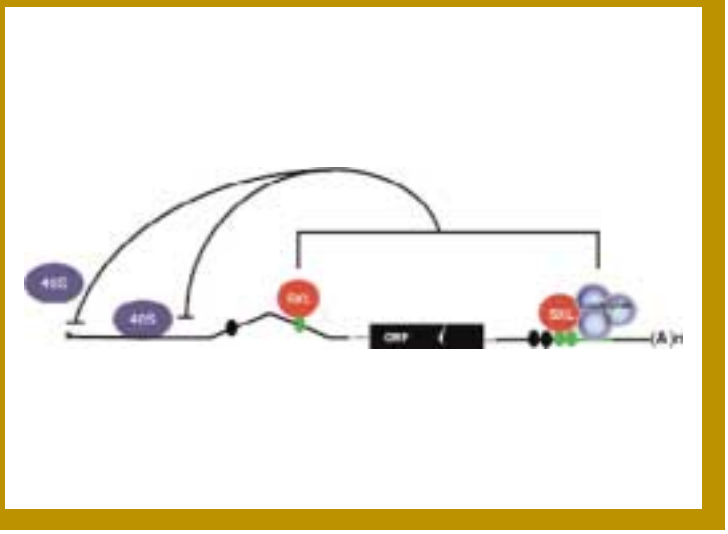
2. Translational regulation of early embryonic patterning in Drosophila

A number of transcripts encoding factors important for antero-posterior and dorso-ventral axis formation in the *Drosophila* embryo are translationally activated by cytoplasmic polyadenylation. The cis-acting sequences and the factors regulating cytoplasmic poly(A) tail elongation in *Drosophila* are largely unknown. We have previously described a poly(A)-dependent cell-free translation system derived from *Drosophila* embryos. We have now optimized and characterized this system for cytoplasmic polyadenylation, We are using the cytoplasmic polyadenylation/ translation system to study the translational regulation of bicoid and toll mRNAs and have identified a novel cytoplasmic polyadenylation element.

3. Regulation of $p27^{kip}$ mRNA translation in mammals

$p27^{kip}$ is a cyclin-dependent kinase (cdk) inhibitor that blocks the mammalian cell cycle in G1. Proper

Figure 1.
Translational repression by SXL. SXL binds to specific sites in both the 5´and 3´UTRs of msl-2 mRNA and recruits co-repressors to the 3´UTR in order to inhibit the stable association of the small ribosomal subunit with the mRNA.



modulation of $p27^{kip}$ levels is essential for cell proliferation. One of the mechanisms that modulate the level of $p27^{kip}$ is the translational regulation of its mRNA. Our goal is to identify factors that specifically regulate $p27^{kip}$ mRNA translation. As a first step, we are trying to identify regulatory sequences for translation in $p27^{kip}$ mRNA by using cell transfection approaches as well as in vitro translation in extracts generated from synchronized cells. Our results suggest that $p27^{kip}$ mRNA translation is complex and involves both cap-dependent and cap-independent mechanisms.

PUBLICATIONS

- Gebauer F and Hentze MW
  "Molecular mechanisms of translational control."
  Nat Rev Mol Cell Biol, 5, 827- 835 (2004)
- Xi Q, Cuesta R, Schneider RJ
  "Tethering of el F4G to adenoviral mRNAs by viral 100k protein drives ribosome shunting."
  Genes Dev, 18, 1997-2009 (2004)
- Cuesta R, Xi Q, Schneider RJ
  "Structural basis for competitive inhibition of eIF4G.Mnk1 interaction by the adenovirus 100-kilodalton protein."
  J Virol, 78, 7707-7716 (2004)

GENE REGULATION

Translational Control of Gene Expression

Group structure

Group Leader
Raul Méndez

Postdoctoral Researchers
Isabel Novoa (Ramón y Cajal-awarded)
Maria Pique (Technician)

Students
Carolina Eliscovich (Graduate Student)
Eulalia Belloc (Graduate Student)
Ana Igea (Graduate Student)

Technician
Javier Gallego

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

RESEARCH PROJECTS

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

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

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

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

Once polyadenylation takes place during oocyte maturation, most of the CPEB (~90%) is destroyed; virtually all that remains stable is confined to animal pole blastomeres where it is strongly associated with spindles and centrosomes. When injected into embryos, reagents that are known to disrupt polyadenylation-induced translation (e.g., CPEB antibody or a CPEB dominant negative mutant) inhibit cell division and produce abnormal mitotic structures. These results suggest that cell division requires polyadenylation-induced translation, but they do not indicate which mRNA(s) might be involved.

Based on the combination of cis-acting elements defined above. We have identified two maternal mRNAs, encoding for proteins that regulate spindle formation, which are polyadenylated in response to progesterone and targets for CPEB-regulated translation. We are currently investigating whether the 3'-UTRs of these mRNAs mediate spindle localized translation. For that purpose we have established a collaboration with Isabelle Vernos at the EMBL.

3. Functional screening to identify new cytoplasmically polyadenylated mRNAs that regulate cell cycle progression

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

The results from the screening have allowed us to validate the model proposed above and to identify many new mRNAs that are potentially regulated by CPEB. The next step we are undertaking is to determine which of the identified mRNAs have to be translated during meiotic progression. For that purpose we are removing the 3'-UTRs of the target mRNAs by microinjecting antisense-oligonucleotides and analyzing the effect of this treatment on the PI-MI and MI-MII transitions. With this approach we have already identified new factors regulated by CPEB and required for the exit from MI.

4. Translational control of mitotic cell cycle

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

In addition, we are adapting the above-mentioned functional screening to identify mRNAs that display changes in poly(A) tail length during cell cycle.

Once identified potential targets we will analyze their role in M-phase and S-phase entry by using cell transfection approaches.

PUBLICATIONS

- Sarkissian M, Mendez R, Richter JD
  "Progesterone and Insulin Stimulation of CPEB-Dependent Polyadenylation is regulated by Aurora A and Glycogen Synthase Kinase-3."
  Gene Dev, 18, 48-61 (2004)

Book chapter

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

Differentiation and Cancer

Acting coordinator: *Thomas Graf,*
Albert Einstein College of Medicine, New York

Since its inception early in 2002, the Programme hosts four groups headed by Pura Muñoz, Cristina Lopez Rodriguez, Thomas Graf, and Luciano di Croce. It is intended to hire two additional groups in 2006, when the CRG moves to the PRBB building under construction.

Research within the Programme covers the areas of:

1. Transcription factors in the development and function of specialized cells, in particular, blood and muscle cells

2. Adult stem cells, plasticity and tissue regeneration

3. Epigenetic events in leukemia and the reprogramming of hematopoietic cells

All four groups work with mammalian cell lines and with mice, sharing their expertise in various technologies, such as FACS analyses, fluorescence microscopy and mouse genetics. They also have links and collaborations with members of the other Programmes within the CRG. Group leaders, postdocs and students actively participate in Work in Progress and Journal Club Seminars of the Programme. They also attend the internal seminars organized by the other Programmes, in particular, the Gene Regulation Programme.

DIFFERENTIATION AND CANCER

Hematopoietic Differentiation and Stem Cell Biology

This group is split between the Albert Einstein College of Medicine (New York, USA) and CRG (Barcelona, Spain)

Group structure:

Group Leader
Thomas Graf

At Albert Einstein:

Postdoctoral Fellows
Min Ye
Weimin Ci

PhD Students
Huafeng Xie
Matthias Stadfeld
Cathy Laiosa
Ines Petersen

Technicians
Jinghang Zhang

At the CRG:

Postdoctoral Fellows
Florencio Varas
Alexis Schubert

Technicians
Luisa Irene de Andrés

The lab is interested in the pathways of blood cell differentiation, in the role of transcription factors during this process and in their ability to reprogram differentiated cells. We are also attempting to model fusion oncoprotein-induced leukemias in mice.

RESEARCH PROJECTS

1. Re-programming lymphoid cells into macrophages by enforced transcription factor expression

During blood cell formation, the earliest multipotent progenitors branch into a common lymphoid and a common myeloid precursor. However, it is largely unknown which transcription factors determine the branching between the two compartments. In attempts to answer this question, instead of studying multipotent precursors we have chosen the approach used in our earlier work with chicken cell lines which consists in the reprogramming of already differentiated ('committed') blood cells by enforced transcription factor expression. We have concentrated on the dichotomy between B cells and macrophages on the one hand and T cells and macrophages on the other since both types of bipotent precursors for these cell types exist in vivo.

We found that the bZip type transcription factor C/EBP alpha, which is expressed in macrophages but not B cells, effectively induces a switch of B cell precursors towards functional macrophages. The activation of myeloid genes requires a collaboration between C/EBP and the transcription factor PU.1, which is expressed in B cell precursors and B cells. Interestingly, the extinction by C/EBP of the late B cell marker CD19 is PU.1 independent, and is caused by an inhibition of the CD19 regulator, Pax5. We have now extended these studies to T cells at different stages of differentiation. The earliest stages, double negative T cell precursors, can be reprogrammed in a similar way by C/EBP as the B cells, again requiring PU.1 for myeloid gene activation. However, the CD4, CD8 double positive cells can only be partially be reprogrammed by C/EBP, in that the expression of the two antigens becomes extinguished, without myeloid gene activation. Finally, CD4 only positive T cells are completely refractory to reprogramming. These experiments show that differentiation plasticity is gradually lost in a defined blood cell lineage and that cross antagonisms and synergisms between transcription factors determine the bifurcation of lymphoid and myelomonocytic cells. Current efforts

concentrate to elucidate the mechanisms by which enforced expression of C/EBP leads to the extinction of multiple lymphoid markers, a crucial aspect in the programming and re-programming of blood cells.

2. Is the reported plasticity of hematopoietic cells a normal developmental process?

Work by numerous labs have shown that, following transplantation of marked hematopoietic cells, non-hematopoietic cells of donor origin (such as hepatocytes, endothelial and muscle cells) can be found in the recipient mice. To study whether 'lineage switches' actually occur in normal cells during embryonic development or as an ongoing process in adults we 'translate' expression of a hematopoietic restricted gene into an irreversible change in the DNA, again using a Cre recombinase approach. For this purpose we generated a transgenic mouse line that expresses Cre under the control of the pan-hematopoietic gene Vav. Analysis of a cross of this mouse with ROSA26 reporter mice generated mice ('vav ancestry mice') in which essentially all hematopoietic stem cells express EYFP. This made it possible to ask whether any non-hematopoietic cell types are also EYFP labeled, indicating that they have a hematopoietic origin. Sections through the fetal liver - an organ that starts out as a hematopoietic tissue and becomes fully hepatic only around birth - of a control mouse, a chimeric vav ancestry mice in which approx. 40% of the cells from ALL tissues are labeled, showed that the technique was reliable in identifying different types of EYFP labeled cells (Fig. 1). Analysis of the fetal liver of the experimental mice revealed that a very small proportion of the hepatocytes have a hematopoietic origin, and that these cells are derived from the fusion of macrophages. Injury of the liver causes a 3 to 4 fold expansion of the labeled hepatocytes, mostly because of the division of pre-existing labeled hepatocytes. We have also extensively analyzed the endothelial cells in the liver of these mice, and found that they do not contain any cells of hematopoietic origin. Together, these results support the notion of stability rather than plasticity of tissues, once they are specified. The failure to detect labeled endothelial cells in our system also questions reports claiming that hematopoietic stem cells can convert into endothelial cells.

3. A CD41 EYFP knock-in mouse

CD41/GpIIb is one of the earliest markers detectable on definitive hematopoietic stem cells and is also a lineage specific marker of megakaryocytes/platelets. To study the development of this lineage from stem cells we have introduced the EYFP gene into the CD41 gene by homologous recombination. In this mouse, megakaryocytes and platelets are EYFP labeled, while no cells from other lineages express the reporter gene. However, only about 10-20% of the megakaryocytes and platelets in this mouse are labeled. We now found that yolk sac derived

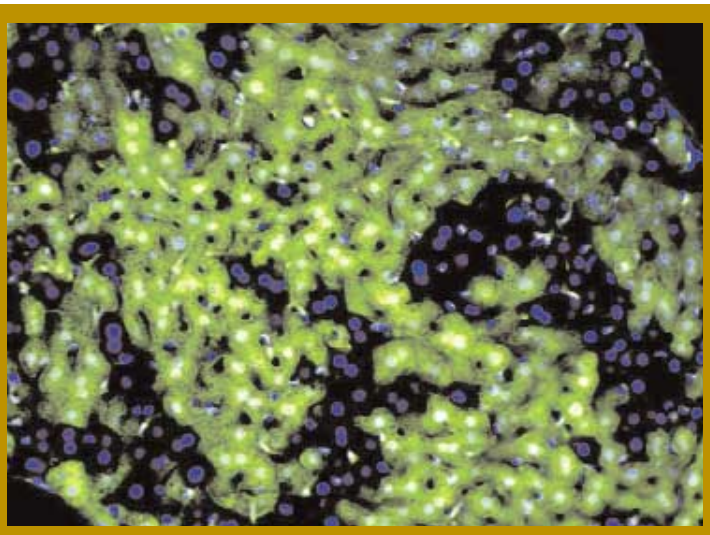


Figure 1.
The figure shows a section through the liver of a vav ancestry mouse, with YFP positive cells in yellow and nuclei in blue. All macrophages (Kupffer cells) are YFP labelled (small cells throughout the section) and about 60% of the hepatocytes (islands of large cells).
From: Stadtfeld and Graf, Development, Jan. 2005

megakaryocytes colonies are completely EYFP negative, while fetal liver derived colonies are a mixture of negative and positive colonies. These observations, together with additional results, raise the possibility that the knock-in traces a sublineage of megakaryocyte/platelets in adult mice. This hypothesis is being investigated.

4. Mechanisms of leukemogenesis

Retroviruses are known to integrate within the host genome and can thus activate ectopically endogenous genes by 'promoter insertion'. An example of this mechanism was uncovered when two children that were part of a gene therapy trial with the cytokine receptor gamma chain developed a T cell leukemia in which the LMO2 gene (a LIM domain transcription factor) was found to be activated. We are now investigating whether the co-expression of LMO2 and the gamma receptor in human T cell progenitors is sufficient to cause leukemia. For this purpose we use a mouse model for human hematopoietic cells (in collaboration with Harris Goldstein, Albert Einstein College of Medicine). This consists in the infection of human fetal liver stem cells with retroviruses encoding the two genes plus either GFP or YFP. These cells are then transplanted into a human thymus graft prepared two months earlier in SCID mice. The implanted grafts can then be removed at different times thereafter and cells infected by each of the two viruses traced by the indicator genes. Preliminary results suggest that the infected cells persist for months after transplantation and in some cases expand.

5. Retrovirus insertions as a tool to discover genes that accelerate stem cell expansion

Recent reports support the notion that the retroviral insertion sites into infected host cell DNA is less random than was previously assumed. Transcriptional active cellular genes are preferentially targeted and even cell type dependent hot spots for retroviral insertion have been described on specific chromosomes. In addition, it appears that in retrovirus infected stem cells transplanted into irradiated recipients a few clones become dominant. This may be due to the activation of a gene favoring the expansion of stem cells and may also constitute one of the contributing factors to the development of leukemias. To study whether specific retroviral insertions enhance the repopulation potential of hematopoietic stem cells a technique was developed to map to efficiently retroviral integration sites in the progeny of infected and transplanted stem cells.

PUBLICATIONS

- Xie H, Ye M, Feng R and Graf T
  "Stepwise reprogramming of B cells into macrophages."
  Cell, 117, 663-676 (2004) (See also commentary by C. Murre in the same issue of Cell)
- Stadtfeld M, Varas F and Graf T
  "Fluorescent protein-cell labeling and its application in time-lapse analysis of hematopoietic differentiation."
  In: Developmental hematopoiesis, Humana Press (M. Baron, ed.) (in press)
- Markart P, Faust N, Graf T, Na CL, Weaver TE, Akinbi HT
  "Comparison of the microbicidal and muramidase activities of mouse lysozyme M and P"
  Biochem J, 380, 385-92 (2004)
- Puri KD, Doggett TA, Douangpanya J, Hou Y, Tino WT, Wilson T, Graf T, Clayton E, Turner M, Hayflick JS, Diacovo TG
  "Mechanisms and implications of phosphoinositide 3-kinase delta in promoting neutrophil trafficking into inflamed tissue."
  Blood, 103, 3448-3456 (2004)
- Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J, Condeelis J
  "A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors"
  Cancer Res, 64, 7022- 7029 (2004)

DIFFERENTIATION AND CANCER

Control of Gene Expression by Transcription Factors

Regulation of gene expression by NFAT5 during the immune response

Supported by a Leukemia & Lymphoma Society career development program

This group began its research activities at CRG in May 2003.

Group structure:

Group Leader
Cristina López-Rodríguez

Students
Vanessa dos Reis Ferreira

Undergraduate students
Anaïs Estrada Gelonch

Technicians
Ana Marina Mosquera

Our major interest is to elucidate the mechanisms by which transcription factors regulate gene expression. We use primarily immune cells as a model system to analyze the transcriptional control of cellular growth, proliferation and differentiation and how disturbance of these basic cellular processes causes autoimmunity, senescence and cancer.

Our research focuses on NFAT5, a recently discovered transcription factor that shares structural and functional characteristics with members of the NF-*kappaB* and NFAT families of proteins. NFAT5 is expressed and activated when cells are exposed to different stimuli like hypertonicity, antigen receptor-induced activation of T lymphocytes and *alpha6/beta4* integrin signaling. Despite their differences, these stimuli affect cellular growth/size and have a major impact on cell communication and proliferation, influencing human diseases such as hypertension, diabetes, inflammation, autoimmunity and cancer.

Our goal is to understand how NFAT5 regulates specific gene transcription in vivo by dissecting how the presence of NFAT5 influences either the accessibility or repression of local chromatin regions. The mechanism of activation of NFAT5 indicates that the analysis of its regulation and function could provide clues for manipulating immune responses and treating multiple diseases.

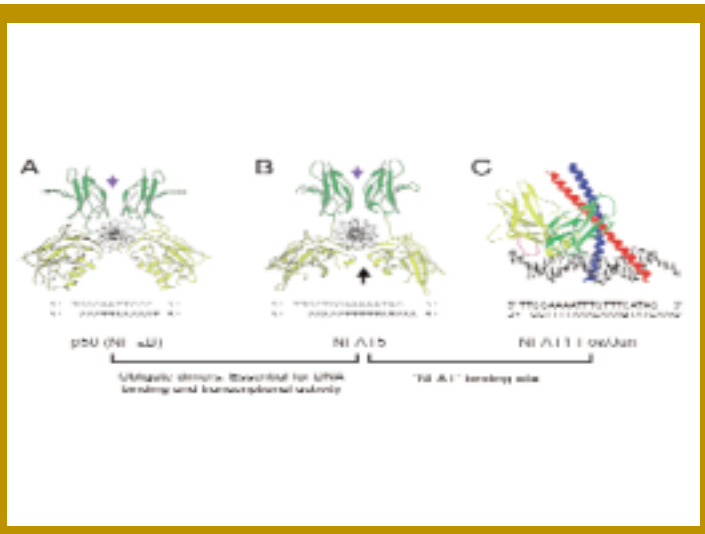


Figure 1.
NFAT5 (B) is a structural hybrid between NF-kB (A) and NFATCc (C) proteins. Three different Rel-domain-containing families of proteins. NFAT5 binds DNA sequences identical to the ones recognized by NFATc proteins. However, NFAT5 is a constitutive dimeric protein that selectively conserves the dimerization suface (black arrow) that, together with the one conserved from NF-kB, contributes to encircle its DNA-target sites.

1. Function of NFAT5

Our approach to understand the role of NFAT5 in vivo is to analyze mice genetically modified to lack this protein. We have targeted the NFAT5 locus in mouse embryonic stem cells to generate an NFAT5-null model that does not express NFAT5 protein. We will characterize the function of NFAT5 both ex vivo and in vivo by using primarily immune cells from the animal model that does not express NFAT5. Due to the fact that all Rel proteins (NFAT and NF-*kappa*B) regulate specific gene expression in immune cells, we are interested in studying how NFAT5 regulates the development of the different immune-cell populations as well as their response to antigen. Beyond the analysis of NFAT5 in the immune system, we are also interested in characterizing the contribution of NFAT5 to major cellular processes.

2. Gene expression pattern regulated by NFAT5

We are interested in studying what specific pattern of gene expression is under the control of NFAT5 and not Rel proteins (NFAT and NF-*kappa*B). We will use NFAT5 deficient cells to analyze the expression levels of multiple genes -by means of cDNA arrays- in order to discover what genes are selectively transcribed or silenced due to NFAT5 activity in vivo. We are also interested in studying the molecular mechanism by which NFAT5 regulates transcription. We will study what exact gene-regulatory regions confer NFAT5-mediated gene expression. In addition to that, we are interested in characterizing what domains of NFAT5 are the ones that direct its activity by binding other proteins or undergoing post-translational modifications.

PUBLICATIONS

- López-Rodríguez C, Antos CL, Shelton J, Richardson JA, Fangming L, Novobrantseva TI, Bronson RT, Igarashi P, Rao A, and Olson EN
  "Loss of NFAT5 results in renal atrophy and lack of tonicity-responsive gene expression."
  Proc Natl Acad Sci, 101, 2392-2397 (2004)

DIFFERENTIATION AND CANCER

Myogenesis

Group structure

Group Leader
Pura Muñoz Cánoves

Postdoctoral Fellows
Mònica Suelves

PhD Students
Frederic Lluís
Bernat Baeza-Raja
Berta Vidal
Vanessa Ruíz

Technicians
Mercè Jardí
Isabel-Cuartas

Ramón y Cajal Investigator
Antonio Serrano

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

I Mechanisms controlling myogenesis in vitro: role of p38 MAPK.
II Role of the plasminogen system in skeletal muscle regeneration in vivo.
III Molecular mechanisms regulating the muscle phenotype in vivo.

RESEARCH PROJECTS

1. Mechanisms controlling myogenesis in vitro: role of p38 MAPK

Myogenesis is largely controlled by the basic helix-loop-helix (bHLH) family of muscle regulatory factors (MRFs), including MyoD, Myf5, myogenin and MRF4. The MRFs exert their function by promoting muscle-specific gene transcription through a specific DNA sequence, the E-box. Selective and productive recognition of chromatin targets requires heterodimerization of MRFs with the ubiquitous E proteins, E12 and E47. Thus, formation of the functional MRF/E47 heterodimer is pivotal in controling muscle gene expression.

1. p38 regulates formation of functional MRF-E47 dimers in myogenesis.
Our recent results provide a novel mechanism demonstrating that p38 MAPK activity regulates MyoD/E47 association *in vitro* and *in vivo*. (*Lluis F, Ballestar E, Suelves M, Esteller M, Muñoz-Cánoves P; EMBO J; in press*).

2. *Differential role of p38 MAPK in early and late myogenesis: role of p38 on MRF4 activity.*
Our results show that p38 MAPK represses the transcriptional activity of MRF4 (involved in late stages of myogenesis), but not of MyoD, resulting in downregulation of specific muscle genes. *(Suelves M., Lluis F, Ruiz V, Nebreda AR, Muñoz-Canoves P. EMBO J. 33: 365-375, 2004).*

3. Crosstalk between p38 and NF-kB signaling pathways in myogenesis.
Our results show that NF-kB activation is dependent on p38 activity during differentiation, being NF-kB an effector of p38, thus providing a novel mechanism for the promyogenic effect of p38 *(Baeza-Raja B, Muñoz-Cánoves P. Mol. Biol. Cell. 15: 2013-2026, 2004).*

2. Role of the plasminogen system in skeletal muscle regeneration *in vivo.*

We had previously observed a muscle regeneration defect in uPA (urokinase plasminogen activator)-

Figure 1.
Possible phosphorylation targets of p38 MAPK that may account for the key role of this MAPK in myogenic differentiation.
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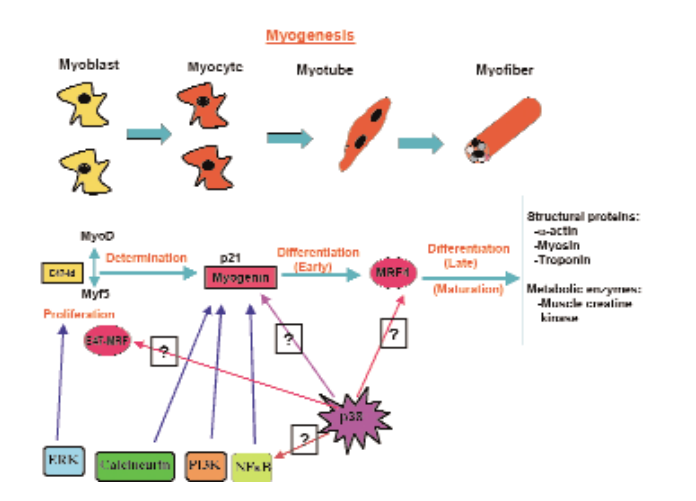


deficient mice after injury, which correlated with fibrin deposition and a decreased recruitment of blood-derived monocytes and lymphocytes to the damaged muscle.

1. Role of uPA-dependent fibrinolysis in muscle regeneration and in mdx dystropninopathy.

- We have shown that uPA ameliorates dystrophy in *mdx* mice, an animal model of Duchenne Muscular Dystrphy (DMD), and injury-induced muscle regeneration via two mechanisms: fibrin degradation and mobilization of bone marrow cells during muscle repair. *(Suelves M, Roma J, Luttun A, Baeza-Raja B, Lluís F, Jardí M, Dewerchin M, Roig M, Carmeliet P, Muñoz-Cánoves P. In revision).*

- We have demonstrated a role for uPA/plasmin-mediated pericellular fibrinolysis in myogenesis *in vitro* (*Lopez-Alemany R, Suelves M, Muñoz-Canoves P. Thromb Haemost. 90: 724-733, 2003; Lopez-Alemany R, Suelves M, Vidal B, Muñoz-Canoves P. Frontiers in Bioscience, in press).*

2. Role of PAI-1 as a regulator of muscle growth.
Preliminary results show that muscle size of PAI-1-deficient mice is larger than that of wild type mice, suggesting that PAI-1 may be negatively regulating muscle growth. The mechanisms underlying PAI-1 function in muscle regeneration are being analyzed at present. We hypothesize that PAI-1 may be regulating negatively IGF-1 signaling in muscle cells, counteracting the IGF-1-mediated growth promoting effect in muscle.

3. Molecular and cellular mechanisms involved in the regulation of muscle phenotype: therapeutical implications

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

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

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

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

PUBLICATIONS

- Suelves M., Lluis F, Ruiz V, Nebreda AR, Muñoz-Canoves P
  "Phosphorylation of the transactivation domain of MRF4 by p38 MAPK mediates repression of specific myogenic genes."
  EMBO J, 33, 365-375 (2004)
- Baeza-Raja B, Munoz-Cánoves P
  "p38 MAPK-induced NF-kB activity is required for skeletal muscle differentiation."
  Mol Biol Cell, 15, 2013-2026 (2004)
- Lopez-Alemay R, Suelves M, Diaz-Ramos A, Vidal B, Muñoz-Canoves P
  "Alpha-enolase plasminogen receptor in myogenesis."
  Frontiers in Bioscience (in press)
- Lluís F, Ballestar E, Suelves M, Esteller M, Sartorelli V, Muñoz-Cánoves P
  "E47 phosphorylation by p38 MAP kinase promotes MyoD/E47 association and muscle-specific gene transcription"
  EMBO J (in press)
- Vidal B, Parra M, Jardí M, Saito S, Paella E, Muñoz-Cánoves P
  "The alkylating carcinogen MNNG activates the plasminogen activator inhibitor-1 (PAI-1) gene through sequential phosphorylation of p53 by ATM and ATR kinases"
  Thromb Haemostasis (in press)
- Nagamine Y, Medcalf R, Muñoz-Cánoves P
  "Transcriptional and posttranscriptional regulation of the plasminogen activator system"
  Thromb Haemostasis (in press)
- McCullagh KJ, Calabria E, Pallafacchina G, Ciciliot S, Serrano AL, Argentini C, Kalhovde JM, Lomo T, Schiaffino S
  "NFAT is a nerve activity sensor in skeletal muscle and controls activity-dependent myosin switching".
  Proc Natl Acad Sci, 101, 10590-5 (2004)

Reviews

- Serrano AL, Andres V.
  "Telomeres and cardiovascular disease: does size matter?"
  Circ Res, 94, 575-84 (2004)

Patents

- Spain Patent Application No. P200402804 (filled 16/11/2004). "Agent for the treatment of Duchenne Muscular Dystrophy (DMD)". Muñoz-Cánoves P, Suelves M, Jardí M (Centre de Regulació Genòmica)

DIFFERENTIATION AND CANCER

Epigenetics Events in Cancer

The group was created in June 2003. Luciano Di Croce has an ICREA Permanent Group Leader position.

Group structure

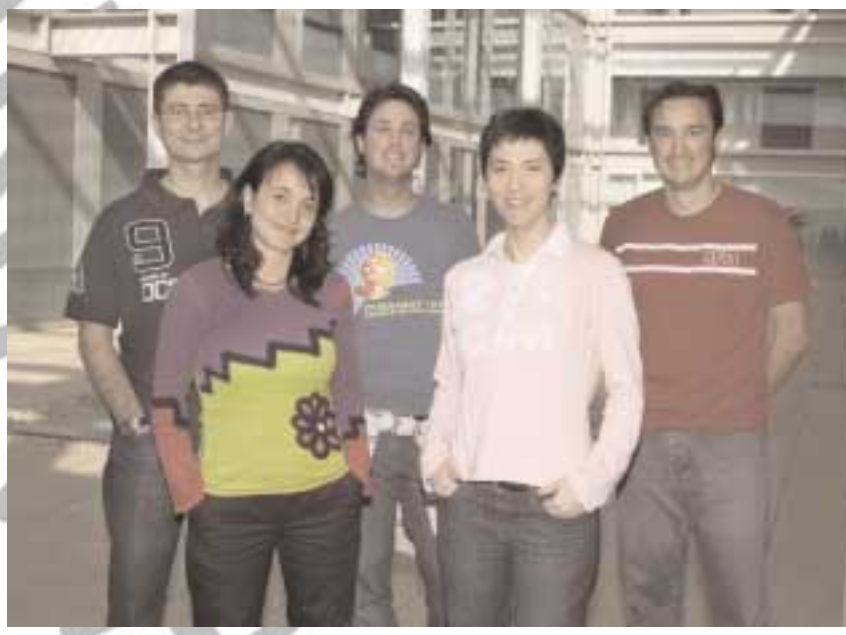
Group Leader
Luciano Di Croce

Postdoctoral
Marcus Buschbeck

PhD Students
Lluis Morey
Raffaella Villa

Undergraduate Student
Iris Joval Granollers

Technicians
Arantxa Gutierrez



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

RESEARCH PROJECTS

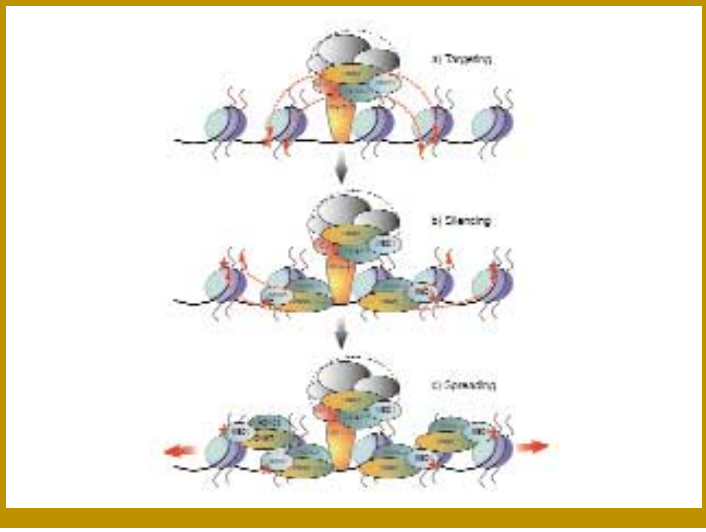
1.Biochemical link(s) between DNA methylation and transcriptional silencing

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

2. Role of epigenetic modification in cancer

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

Figure 1.
Model of stepwise mechanism of epigenetic silencing in leukemia



3. Histone tail modification and heterochromatin

Heterochromatin DNA is characterized by the presence of both a "closed" chromatin conformation and the presence of the Polycomb group (PcG) of proteins. The recruitment of the PcG protein complexes, as well as their contribution to cancer progression, is also investigated, in collaboration with PierGiuseppe Pelicci and Saverio Minucci (IEO, Milan). Preliminary results suggest that members of the PcG interact with PML-RAR and are recruited to its target genes. Indeed changes in the "histone code" are also observed in proximity of PML-RAR binding sites. The heterochromatin formation could be thus responsible for the observed stable gene silencing.

PUBLICATIONS

- Buschbeck M, Hofbauer S, Di Croce L, Keri G and Ullrich A
  "Abl kinase-sensitive levels of ERK5 and its intrinsic basal activity contribute to leukemia cell survival."
  EMBO Reports (in press)
- Brenner C, Deplus R, Didelot C, Danovi D, Amati B, Kouzarides T, de Launoit Y, Di Croce L and Fuks F
  "Myc represses transcription through recruitment of DNA methyltransferase corepressor."
  EMBO J (in press)
- Di Croce L, Buschbeck M, Gutierrez A, Joval I, Morey L, Villa R and Minucci S
  "Altered epigenetic signals in human disease."
  Cancer Biol Ther (in press)
- Villa R, De Santis F, Gutierrez A, Minucci S, Pelicci PG and Di Croce L
  "Epigenetic gene silencing in acute promyelocytic leukemia."
  Biochem Pharmacol, 68, 1247-1254 (2004)

Genes and Disease

Coordinator: *Xavier Estivill*

Our understanding of the molecular basis of common human disorders is still very limited. With most of the human genome sequenced and with a large amount of information on the variability of the human genome in hand, we now have the opportunity to identify molecular mechanisms that are responsible for human disease, to define major pathways that are altered, and to develop strategies that could correct the biological defects that lead to disease. The Genes and Disease (G&D) Programme of the CRG has the ambition to characterize sequence and genomic variability related to disease, to study the function of genes with a potential role in human disorders, to develop and characterize models of human disease, and to design therapeutic approaches to cure diseases.

Research Groups:

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

Several human disorders, including mental retardation, psychiatric disorders and hearing impairment, are studied by the five groups from different points of view. Efforts in the G&D Programme are engaged in the analysis of sequence and genomic variants of the human genome that could participate in the predisposition to human disorders, with particular emphasis in psychiatric diseases. These studies are complemented with the neuropathological and behavioural characterization of mouse models of candidate genes. Specific collective work within the Programme is focused on understanding the function of genes with potential implications in Down syndrome phenotypic traits. It is expected that dosage-sensitive genes will have critical consequences that can be explored by modifying the levels of their expression at the cellular level in vitro or in murine models in vivo. Both approaches are followed by different groups in the Programme. Murine models are being also used for the development of therapeutic approaches that could correct features involved in mental retardation, anxiety disorders and other abnormalities, including models of cancer. Related to this, research in the gene therapy group is directed to the development of efficient gene delivery approaches for local or systemic production of therapeutic proteins. The Programme has made a great effort to set up facilities for high-throughput genotyping, such as the Barcelona Genotyping Node (CeGen), and to establish technological facilities and scientific skills to perform neurobehavioral characterization of murine models generated in the Programme or by other investigators.

The G&D Programme participates in several networks supported by the "Instituto de Salud Carlos III – Fondo de Investigación Sanitaria" (ISCIII-FIS), including Clinical Genetics, Neuroscience, Hearing impairment, Psychiatric Genetics, and Cancer, and by other local funding bodies (Gene Therapy Network and Murine Models Network). The Programme participates in teaching

activities in Human Genetics and Human Pathology at UPF. Members of the Programme have strengthened links with several investigators of the PRBB: Luis Pérez-Jurado, Paco Real, Núria Malats and Arcadi Navarro (Estivill's Group); Miguel Angel Valverde (Eulàlia Martí); Margarita Puig and Rafa Maldonado (Dierssen's Group); and Jordi Segura, David Andreu and Emili Martínez Miralles (Fillat's Group), among others.

During this year, the groups in the programme have continued their consolidation with the incorporation of new PhD students and post-doctoral fellows. Data Clubs and Discussion Groups have become an important part of the scientific life of all the members of the Programme. The tendency for growth is reflected also in the fact that the programme has more than 20 ongoing research projects and 30 research fellows supported by national or international funding bodies. All together, external funds obtained by the G&D Programme represent over 2 million euros during 2004.

Transgenics Unit

Technician
Luís Sánchez Palazón (CRG)

During 2004 the microinjection lab has been set up and has produced transgenic mice by pronuclear injection for CRG and UPF labs. It is expected that our move to the new PRBB building will bring us the opportunity to offer additional services including transgenesis via stem cells.

GENES AND DISEASE

Genetic Causes of Disease

Group structure

Group Leader
Xavier Estivill

Staff Scientist
Eulàlia Martí (since November 2004)

Scientific Officer
Àurea Rodríguez (since February 2004)

Postdoctoral Fellows
Mònica Bayès (Ramón y Cajal)
Yolanda Espinosa (Ramón y Cajal)
Mònica Gratacòs
Heidi Howard (since February 2004)
Miroslava Ogorelkova

PhD Students
Lluís Armengol
Ester Ballana
Nina Bosch
Celia Cerrato
Marc Gómez (since October 2004)
Monica Guidi
Josep Maria Mercader
Margarita Muiños (since July 2004)
Marta Ribasés
Selma Serra (since July 2004)
Marina Ventayol

Technicians
Carles Arribas
Anna Carreras
Rafael De Cid
Cecilia Garcia
Manel García
Marta Morell
Imma Ponsa
Anna Puig

The group focuses on the analysis of the variability of the human genome at the nucleotide and genomic levels and their relation with disease predisposition. The group has contributed to the detection of genetic variants involved in hearing impairment, anxiety and eating disorders. This research has led to the identification of SNPs in BDNF and NTRK2 that predispose to eating disorders and several gene variants involved in hearing loss. The group is interested in studying the contribution of non-coding RNAs and segmental duplications to human disease. The group has set up the analytical basis for the study of human genome diversity throughout genotyping nucleotide variants. Thus, the group has set up the Barcelona genotyping facility, which is a join centre (CeGen) with the Pompeu Fabra University, and is supported by Genome Spain. Through the CeGen facility, the group is searching for genes involved in the modification of non-syndromic hearing loss, the analysis of clinical variability in the response to methadone and to nicotine treatments, the study of several psychiatric disorders, and the analysis of non-coding and small RNAs in the susceptibility to complex disorders.

RESEARCH PROJECTS

1. Segmental duplications, variability and human disease

Ll. Armengol, N. Bosch, C. Cerrato, Y. Espinosa, M. Gómez, Manel García, M. Guidi, I. Ponsa, M. Morell, M. Muiños and M. Ogorelkova

Large-scale segmental duplications have played an important role in hominoid evolution and can be 'hot spots' for non-allelic homologous recombination leading to deletion, duplication, inversion or translocation. We have initiated a systematic analysis of the role of segmental duplications in disease. We have precisely localized all human genome segmental duplications with lengths larger than 5 kb and a level of identity >90%. Over 3,000 segmental duplications have been detected, of which about 50% are intrachromosomal. The group has characterized two genomic regions that contain a complex organization of segmental duplications, one on chromosome 15q11-q13, and the other on chromosome 8p23.1. The 15q11-q13 region is involved in several genomic disorders, including Angelman syndrome and Prader-Willi syndrome. Three clusters of small nucleolar RNAs (snoRNAs), expressed in the central nervous system, are being studied for their potential implication in psychiatric disorders. We have identified a high rate of gene conversion events at some of these snoRNAs. Another interesting region is a five-Mb segment of human chromosome 8p23.1, which is inverted in a significant proportion (about 25%) of individuals of the general population. Genes located at the segmental duplication that flank the inversion, vary in copy number in different individuals. A complete BAC collection (32K) covering the draft sequence of the human genome has been selected for the development of BAC microarrays for comparative genome hybridisation (CGH) studies. We have developed arrays containing regions that within and between segmental duplications, subtelomeric regions and evenly spaced loci for each human chromosome. Moreover, we have compared the human, mouse and rat genome sequences and have demonstrated that recent segmental duplications correlate with breaks of synteny

between these three species. Our preliminary data suggest that segmental duplications have participated in the recent evolution of these genomes.

2. Genetic factors involved in psychiatric disorders

M. Gratacòs, H. Howard, J.M. Mercader and M. Ribasés

We are studying the genetic contribution to the susceptibility to develop psychiatric diseases, including eating disorders, anxiety disorders, and variability in the response to methadone and to nicotine treatments. We have identified that SNPs located in the brain-derived neurotrophic factor (BDNF), –270C/T and Val66Met, are involved in the predisposition to anorexia and bulimia. A combined population and family-based study in patients from seven European countries revealed that the met66 variant is strongly associated to all eating disorders subtypes (anorexia nervosa, restricting, binge-eating/purging and bulimia nervosa). Moreover, the –270C BDNF variant has an effect in anorexia nervosa, bulimia nervosa and late age of onset of weight loss. The group is now setting up functional studies to evaluate the contribution of these variants to anorexia and weight loss in mice. The group has coordinated a National Genotyping Network for Psychiatric Disorders, supported by the FIS (http://davinci.crg.es/rgpg/). Several genotyping projects have been initiated in collaboration with several clinical groups. These projects include postpartum depression, eating disorders, autism, attention deficit and hyperactivity disorder, among others.

3. Genetic factors that predispose to hearing impairment

E. Ballana and M. Ventayol

Three genes (GJB2, GJB3 and GJB6) encoding for connexin proteins are involved in hearing impairment. The group has shown that most cases of congenital deafness are due to mutations in GJB2 and has demonstrated that GJB3 causes both sensorineural deafness and peripheral neuropathy. The group has found that a mutation in the mitochondrial genome (A1555G) is the commonest genetic cause of familial progressive hearing loss and is currently searching for genes and environmental factors that modify hearing impairment in subjects that carry mutation A1555G. The group has made progress in the identification of mutations in the GJB1/GJB3 genes and in the characterization of genomic regions that are likely involved in the variability of the deafness phenotype due to the mitochondrial mutation A1555G. A five-Mb region of human chromosome 8p23.1 region is inverted in a significant proportion (about 25%) of individuals of the general population. The positive linkage region associated with the A1555G deafness phenotype was described around markers D8S277-D8S1819, located telomeric with respect to the inverted region. Several clusters of genes encoding defensins are located within these segmental duplications. The number of repeat units varies between control individuals, and the potential clinical implications have to be defined. In order to analyze the role of 8p23 genomic architecture as a modifying target for the A1555G phenotype, microsatellite markers located in the region have been screened in families carrying the A1555G variant. It is expected that more than one modifying factor is involved in the hearing impairment phenotype due to the A1555G mutation. Therefore, several other genetic factors are being studied, including the presence of heteroplasmia for mutation A1555G in samples of subjects that do not have the hearing loss phenotype.

4. Role of DSCR1 as a protective factor to neuronal degeneration

E. Martí and S. Serra

Oxidative stress (OS) is a key pathogenic factor common to many neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease, Down's syndrome (DS) and amyothrophic lateral sclerosis. Whether OS is a primary cause or an amplifying downstream consequence of the neurodegenerative process is still an open question. Several data suggest an involvement of DSCR1/Calcipressin1 (Calp1), an endogenous inhibitor of calcineurin (CaN) activity, in the

neuropathology associated to DS and AD. On the other hand, Calp1 expression has been associated to a differential susceptibility to stress in mammalian cells. Our most recent data indicate that suppression of the Dscr1 gene in a murine model increases neuronal resistance to OS insults. OS induces cellular Ca2+ overload and subsequent entry into the mitochondria, where disrupts normal metabolism leading to cell death. Our preliminary data points to the regulation of this Ca2+ pathway as a direct or indirect (via modulation of CaN activity) target of Calp1. In this context, our main interest is to understand the role of Dscr1/Calp1 in the neuronal response to oxidative stress, with special interest in the molecular mechanisms involved in the Calp1 regulation of Ca2+ influx, mitochondrial function, cellular antioxidant defenses and ATP release. This project is a first step to establish Calp1 as a new target molecule to consider in oxidative stress and neurodegeneration-related therapeutic strategies.

5. National Network of Genotyping (CeGen)

M. Bayès, A. Puig, C. García, C. Arribas, A. Carreras, R. De Cid

We have set up a hightroughput genotyping facility for the study of complex disorders and for phramacogenomic research. With the current equipment we are able to produce up to 280.000 genotypes per week. During 2004, the unit has performed a trial project focused on several psychiatric diseases and on evolution biology. This genotyping facility is the Barcelona Network of the National Genotyping Centre (CeGen), supported by Genome Spain and is a joint effort between the CRG and the Pompeu Fabra University (UPF). CeGen Barcelona will be operative for external genotyping projects starting on 2005. Investigators should obtain their own funds for genotyping, while CeGen should cover the costs of equipment and personnel, as well as provides support for project design and analysis of data.

PUBLICATIONS

- Gabrovsek M, Brecelj-Anderluh M, Bellodi L, Cellini E, Di Bella D, Estivill X, Fernandez-Aranda F, Freeman B, Geller F, Gratacòs M, Haigh R, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Nacmias B, Ribases M, Remschmidt H, Komel R, Sorbi S, Tomori M, Treasure J, Wagner G, Zhao J, Collier DA
  "Combined family trio and case-control analysis of the COMT Val158Met polymorphism in European patients with anorexia nervosa."
  Am J Med Genet, 1;124B(1), 68-72 (2004)
- Groman JD, Hefferon TW, Casals T, Bassas L, Estivill X, et al
  "Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign."
  Am J hum Genet, 74, 176-179 (2004)
- Liu X, Li X, Li M, Acimovic YJ, Li X, Scherer SW, Estivill X, Tsui LC
  "Characterization of the segmental duplication LCR7-20 in the human genome." Genomics, 83, 262-269 (2004)
- Martínez de Lagrán M, Altafaj X, Gallego X, Martí E, Estivill X, Sahún I, Fillat C, Dierssen M.
  "Motor phenotypic alterations in TgDyrk1a transgenic mice implicate DYRK1A in Down syndrome motor dysfunction."
  Neurobiol Dis, 15, 132-142 (2004)
- Donaudy F, Snoeckx R, Pfister M, Zenner HP, Blin N, Di Stazio M, Ferrara A, Lanzara C, Ficarella R, Declau F, Pusch CM, Nurnberg P, Melchionda S, Zelante L, Ballana E, Estivill X, Van Camp G, Gasparini P, Savoia A
  "Nonmuscle Myosin Heavy-Chain Gene MYH14 Is Expressed in Cochlea and Mutated in Patients Affected by Autosomal Dominant Hearing Impairment (DFNA4)."
  Am J Hum Genet, 74(4), 770-6 (2004)
- Casals T, Aparisi L, Martinez-Costa C, Gimenez J, Ramos MD, Mora J, Diaz J, Boadas J, Estivill X, Farre A
  "Different CFTR mutational spectrum in alcoholic and idiopathic chronic pancreatitis?" Pancreas, 28(4), 374-9 (2004)
- Shaw CJ, Stankiewicz P, Bien-Willner G, Bello SC, Shaw CA, Carrera M, Perez Jurado L, Estivill X, Lupski JR
  "Small marker chromosomes in patients with segmental aneusomy for proximal 17p." Hum Genet. 115(1), 1-7 (2004)
- Bykhovskaya Y, Mengesha E, Wang D, Yang H, Estivill X, Shohat M, Fischel-Ghodsian N
  "Human mitochondrial transcription factor B1 as a modifier gene for hearing loss associated with the mitochondrial A1555G mutation."
  Mol Genet Metab, 82(1), 27-32 (2004)

- Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M, Cavallini MC, Cellini E, Di Bella D, Erzegovesi S, Foulon C, Gabrovsek M, Gorwood P, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Kipman A, Komel R, Nacmias B, Remschmidt H, Ricca V, Sorbi S, Wagner G, Treasure J, Collier DA, Estivill X
  "Association of BDNF with anorexia, bulimia and age of onset of weight loss in six European populations."
  Hum Mol Genet, 13(12), 1205-12 (2004)
- Casals T, De-Gracia J, Gallego M, Dorca J, Rodriguez-Sanchon B, Ramos M, Gimenez J, Cistero-Bahima A, Olveira C, Estivill X
  "Bronchiectasis in adult patients: an expression of heterozygosity for CFTR gene mutations?"
  Clin Genet, 65(6), 490-495 (2004)
- Fotaki V, Martinez De Lagran M, Estivill X, Arbones M, Dierssen M
  "Haploinsufficiency of Dyrk1A in mice leads to specific alterations in the development and regulation of motor activity."
  Behav Neurosci, 118(4), 815-21 (2004)
- Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M, Cristina Cavallini M, Cellini E, Di Bella D, Erzegovesi S, Foulon C, Gabrovsek M, Gorwood P, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Kipman A, Komel R, Nacmias B, Remschmidt H, Ricca V, Sorbi S, Tomori M, Wagner G, Treasure J, Collier DA, Estivill X
  "Association of BDNF with restricting anorexia nervosa and minimum body mass index: a family-based association study of eight European populations".
  Eur J Hum Genet, Dec 22 (2004)
- Bykhovskaya Y, Mengesha E, Wang D, Yang H, Estivill X, Shohat M, Fischel-Ghodsian N
  "Phenotype of non-syndromic deafness associated with the mitochondrial A1555G mutation is modulated by mitochondrial RNA modifying enzymes MTO1 and GTPBP3".
  Mol Genet Metab, 83(3), 199-206 (2004)
- Benavides-Piccione R, Ballesteros-Yanez I, de Lagran MM, Elston G, Estivill X, Fillat C, Defelipe J, Dierssen M
  "On dendrites in Down syndrome and DS murine models: a spiny way to learn."Prog
  Neurobiol, 74(2). 111-26 (2004)
- Ribases M, Gratacos M, Badia A, Jimenez L, Solano R, Vallejo J, Fernandez-Aranda F, Estivill X
  "Contribution of NTRK2 to the genetic susceptibility to anorexia nervosa, harm avoidance and minimum body mass index."
  Molecular Psychiatry (in press)
- Benavides-Piccione R, Elston GN, Arbones ML, Fotaki V, Estivill X, DeFelipe J, Dierssen M
  "Alterations in the phenotype of neocortical pyramidal cells in the DYRK1A+/- mouse." Neurobiol Disease (in press)

Book chapters

- Estivill X
  "Genética médica. Bases Moleculares de la herencia. Genes y Enfermedad. Principios Generales del análisis genético y mapa del genoma humano. Enfermedades hereditarias complejas."
  XV Edición Farreras Rozman. Tratado de Medicina Interna (in press)
- Dierssen M, Benavides-Piccione R, Martínez-Cué C, Estivill X, Baamonde C, Fillat C, Martínez de Lagrán M, Altafaj X, Flórez J, Elston GN , de Felipe J
  "Genotype-phenotype neural correlations in trisomy 21."
  In: The Adult with Down syndrome. A. Rondal, Alberto Rasore-Quartino and Salvatore Soresi Eds. Whurr Publishers, Volume 2, 15-30 (in press)

GENES AND DISEASE

Gene Therapy

Group structure

Group Leader
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PhD Students
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Technicians
Núria Andreu

Gene therapy is an emerging field that holds the promise of treating a wide variety of diseases. However, before this can be achieved, successful vector systems must be developed to deliver therapeutic genes and successful preclinical studies in 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 molecular aspects of Pancreatic cancer, Down syndrome and Wiskott-Aldrich syndrome disorders and more importantly is interested in the development of optimal gene therapy approaches.

The group is also working in the development of efficient gene delivery strategies for the local or systemic production of therapeutic proteins. These approaches allow us to study the role of hHGF as a renoprotective factor and to model the misuse of gene therapy for doping control.

RESEARCH PROJECTS

1. Pancreatic cancer

One of the most devastating diseases that our society is presently facing is cancer. Particularly pancreatic cancer is the fifth cause of cancer deaths in industrialized countries. This neoplasia has a very bad prognosis mainly due to the late diagnosis together with the fact that current therapies are very inefficient. Gene therapy emerges as a candidate approach for their treatment. The group has been involved in the past few years in exploring the feasibility of suicde gene therapy in pancreatic cancer, showing some but limited effects. Based on those observations we are presently interested in the development of more potent and selective agents. To be able to increase their potency we are working with different systems that may facilitate the spreading of the cytotoxic compound into the tumor mass. To increase selectivity we are developing vectors that can target specific cellular receptors with the therapeutic gene modified to be transcriptionally active only in tumor cells.

2. Down syndrome (DS)

Down syndrome is the most common autosomal trisomy; the trisomy 21. It is a multi-system disorder with a wide range of physical features, health and development problems. As we begin to understand the role of specific genes and we can identify the contribution of individuals genes to the overall phenotype thinking on partial gene therapy for specific defects might be a good approach for the treatment of certain diabilities. From the results of our group and others we have strong evidences to believe that Dyrk1A can be one of the genes that will highly contribute to the Down syndrome phenotype. In fact, transgenic mce that overexpres Dyrk1A, present neurodevelopmental delay, motor alterations and some conginitve deficits, similar to those described in Down syndrome patients. We are currently testing the feasibility of a gene transfer approach, based on reducing DYRK1A overexpression in brain by RNA interference technology to rescue defined phenotypes in

TgDyrk1A and in the most complete DS model described, theTs65Dn mouse.

3. Wiskott-Aldrich syndrome (WAS)

Wiskott-Aldrich syndrome is an X-linked recessive disorder. We and others have identified and characterized mutations in the WASP gene that can be responsible for the disease. In a Spanish population study we have identified a broad spectrum of the mutations with an uneven distribution throughout the gene. Being missense mutations preferentially located in the amino-terminal part of the protein, and mainly stop and frameshift mutations in the carboxy-terminal region. Although some genotype-phenotype analysis could be established, in particular cases we observed high intrafamilial clinical variability. Interestingly we have also reported a female case of WAS, showing that a skewed X-inactivation, favoring the expression of the WASP-mutated allele was occurring. We are currently studying new mutations in WAS patients and understanding their molecular mechanism. The only current treatment available for WAS is allogeneic stem cell transplantation, however many patients lack a suitable matched sibling donor. Thus, autologous transplantation of patient's WASP-corrected cells will be a candidate approach for WAS treatment. We are working on a gene therapy approach for WAS and we are presently evaluating the phenotypical correction of WASP-transduced lymphocytes from WAS patients.

PUBLICATIONS

- Cascalló M, Calbó J, Capellà G, Fillat C, Pastor-Anglada M, Mazo A
  "Adenovirus-mediated p53 transfer enhances gemcitabine activity by inducing apoptosis in human pancreatic tumors."
  Oncology (in press)
- Cruzado JM, Lloberas N, Torras J, Riera M, Fillat C, Herrero-Fresneda I, Aran JM, Alperovich G, Vidal A, Grinyó JM
  "Regression of advanced diabetic nephropathy by hepatocyte growth factor gene therapy in rats."
  Diabetes, 53, 1119-1127 (2004)
- Benavides-Piccione R, Ballesteros I, Martínez de Lagrán M, Lestón G, Estivill X, Fillat C, DeFelipe J, Dierssen M
  "On dendrites in Down syndrome and DS murine models: a spiny way to learn."
  Prog Neurobiol, 74, 111-126 (2004)
- Roig JM, Molina MA, Cascante A, Calbó J, Carbó N, Wirtz U, Sreedharan S, Fillat C, Mazo A
  "Adenovirus-mediated Retinoblastoma 94 gene transfer induces human pancreatic tumor regression in a mouse xenograft model."
  Clin Cancer Res,10, 1454-1462 (2004)
- Martínez de Lagrán M, Altafaj x, Gallego X, Martí E, Estivill X, Fillat C, Dierssen M "Motor phenotypic alterations in TgDyrk1A transgenic mice implicate DYRK1A in Down syndrome motor dysfunction."
  Neurobiol of Disease, 15, 32-142 (2004)
- Riera M, Chillon M, Aran JM,. Cruzado JM, Torras J, Grinyó JM and Fillat C.
  "Intramuscular SP1017-formulated DNA electrotransfer enhances transgene expression and distributes hHGF to different rat tissues."
  J Gene Med, 6, 111-118 (2004)

Book Chapters

- Fillat C, Arán JM, Gómez-Foix AM
  "Genética y tratamiento."
  XV Edición Farreras Rozman. In: Tratado de Medicina Interna, 161, 1289-1293 (2004)
- Dierssen M, Benavides-Piccione R, Martínez-Cué C, Estivill X, Baamonde C, Fillat C, Martínez de Lagrán M, Altafaj X, Flórez J, Elston GN, de Felipe J
  "Genotype-phenotype neural correlations in trisomy 21."
  In: The Adult with Down syndrome. A. Rondal, Alberto Rasore-Quartino and Salvatore Soresi Eds. Whurr Publishers, Volume 2, 15-30 (2004)

GENES AND DISEASE

Murine Models of Disease

Group structure

Group Leader
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Our interest is to study the in vivo function of particular genes that are relevant for specific physiological and pathological conditions. Our current research focuses on two chromosome 21 genes *DYRK1A* and *DSCR1*, which are highly expressed in central nervous system and are considered candidate genes for several phenotypic traits in Down syndrome. As experimental approach we use genetically modified mice.

RESEARCH PROJECTS

1. DYRK1A

Comprehension of DYRK1A function in the mammalian nervous system has raised an increased interest in the scientific community because transgenic mouse models overexpressing this gene recapitulate some of the neurological alterations in Down syndrome. We have previously shown that mice carrying a *Dyrk1A* null allele display a phenotype, characterized by intrauterine growth retardation and microcephaly, which is similar to that shown by patients with a segmental deletion of a small chromosomal region that includes *DYRK1A*. This supports the notion that changes in *DYRK1A* dosage have remarkable consequences in mammalian central nervous system (CNS) development leading to detrimental alterations in brain morphology and function. More recently, we showed that *Dyrk1A*+/- mice present a decreased number of particular neurone subtypes and alterations in the morphology of the neuronal processes indicating the implication of this protein kinase in mechanisms that control the number of progenitor cells and/or cell-fate decision and differentiation. Our present interest is to define the physiological role of DYRK1A in neurogenesis. With this aim, we are using transgenic mice with different number of functional copies of *Dyrk1A* to analyze, *in vivo* and *in vitro*, the proliferation and cell diversification capacities of particular neuronal progenitor cell populations.

Figure 1.
Sections from two central nervous system structures showing a reduction in the number of particular neuron subtypes in *Dyrk1A*+/- mice
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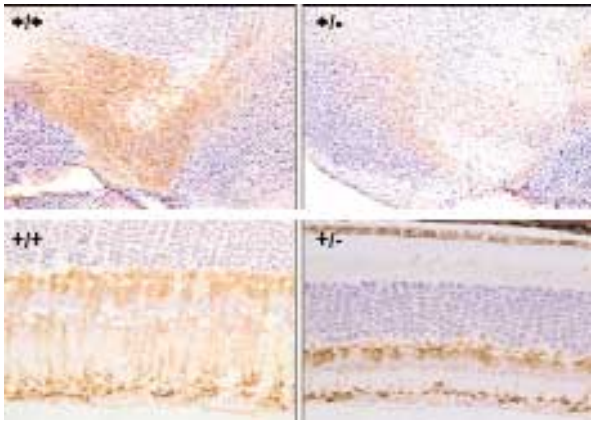

Figure 2.
CALP1 is an inhibitor of calcineurin-mediated signalling pathways
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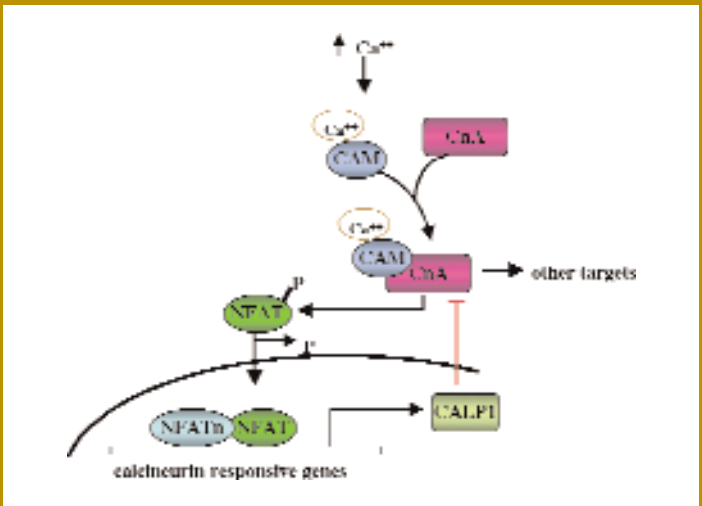

2. DSCR1

Calcipressin1 (CALP1), encoded by the Down Syndrome Candidate Region 1 (*DSCR1*) gene is a functional inhibitor of calcineurin (CaN), a calcium and calmodulin dependent phosphatase. CaN is a ubiquitous and multifunctional calcium-activated protein phosphatase involved in immune response, synaptic plasticity, neurite outgrowth, skeletal muscle growth and differentiation, heart valve formation and cardiac hypertrophy. The only calcineurin inhibitor which expression is regulated by calcium-calcineurin signalling is CALP1, indicating that this protein can function in a feedback inhibition loop to suppress sustained calcineurin activity. In situations of physiological intracellular calcium concentrations, *DSCR1* is expressed in different adult mammalian tissues including brain, heart and skeletal muscle. During development, the gene is widely express in the central nervous system and in the primitive ventricle of the heart. These data indicates that CALP1 acts as a calcineurin regulator in a variety of tissues. Our present interest is to elucidate the role of this regulator in central nervous system development and its possible implication in processes related to learning and memory. To address this question we generated a Dscr1 knockout mouse model and we are characterizing it at the molecular and histological levels. Specific behavioural tests have been performed to reveal any possible neurological and cognitive alterations in these mice.

PUBLICATIONS

- Fotaki V, Martinez de Lagran M, Estivill X, Arbones ML, Dierssen M
  "Haploinsufficiency of Dyrk1A in mice leads to specific alterations in the development and regulation of motor activity."
  Behav Neurosci, 118, 815-821 (2004)
- Benavides-Piccione R, Elston GN, Arbonés ML, Fotaki V, Estivill X, DeFelipe J, Dierssen M
  "Alterations in the phenotype of neocortical pyramidal cells in the DYRK1A+/- mouse."
  Neurobiol of Disease (in press)

GENES AND DISEASE

Neurobehavioral Phenotyping of Mouse Models of Disease

Group structure

Group Leader
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Nohelia Fernández
Carla Obradors

The group is interested in identifying contributing genes involved in the pathophysiology of two important human complex genetic disorders: Down syndrome and panic disorder. As experimental approach we use loss-of-function and gain-of-function in vivo models systems to elucidate the physiological function of candidate proteins and the effects caused by changes in their dosage. Our research is multidisciplinary, using anatomical, neurochemical, neurobehavioral and neuroimaging techniques in order to correlate structural and functional parameters at various levels, from cells to the whole organism. This includes studies of mechanisms of simple and complex learning, emotionally driven behaviour, environmental influences on outcome, and the development and testing of pharmacological interventions. We are actively collaborating with research groups from Genes and Disease and Differentiation and Cancer Programs, with other groups in the PRBB and other Spanish and European research groups. Our group is member of the European DANA Alliance for the brain.

RESEARCH PROJECTS

1. Down syndrome

M. Martínez de Lagrán, G. Arqué

The neuropathological processes underlying Down syndrome (DS) mental retardation and their genetic dependence remain an open question. The crucial point is to define how does an excess of normal gene products in interaction with the environment direct and constrain neural maturation, and how does this abnormal development translate into cognition and behaviour. We use a global model of the disease the trisomy 16 mouse model Ts65Dn (Ts65Dn) to study the pathogenetic mechanism of mental retardation, and to assay pharmacological and non-pharmacological interventions.

We also explore the role of specific candidate genes using conventional transgenic and gene targeted ("knock out") mice of single candidate genes generated in our Program. These include DYRK1A (Drosophila minibrain homolog), a serine/threonine kinase involved in neuronal development; DSCR1 (Calcipressin 1), an inhibitor of calcineurin-mediated signalling pathways, and BACE2 (ß-site APP cleaving enzyme 2), an aspartyl protease with APP ß-secretase activity.

We have been able to recreate certain conditions that result in abnormal learning capabilities in DS and to demonstrate that experience-dependent plasticity is impaired in shaping the three-dimensional architecture of neurons in the frontal cortex of Ts65Dn mice using environmental manipulations as a paradigm. Our research in the last years has bring to light some interesting and unsuspected culprits that will give us entry points into novel biological pathways. The phenotyping screens have identified a major dosage dependent effect for some of these genes in neurobehavioral development and in the formation of new memories. Moreover, we have determined synaptic transmission alterations are underlying features in some of these models as demonstrated by means of ultrastructural, pharmacological and microdyalisis experiments. We also explore the formation and maintenance of memories, the formation of activity-

dependent neural connections and the molecular changes underlying activity-dependent neural plasticity at the structural and molecular levels, in primary cerebral cortex cultures.

Moreover, we have continued the analysis of the synaptic features in the hippocampus of Ts65Dn mouse (collaboration with DC Davies, U.K.) and we will analyze the spinal cord development in TgDyrk1A mice (collaboration with Anna Casanovas, University of Lleida). Our work has shed new light on the possible mechanisms underlying the cognitive deficits and the defects in neural plasticity of Down syndrome. Besides we have observed significant changes in age-associated neurobehavioral and neuromorphological aspects in a murine model of overexpression of Dyrk1A (TgDyrk1A), a candidate gene for Down syndrome. Besides, we continue the characterization of transgenic and knockout models for Dyrk1A, DSCR1 and BACE2.

2. Panic/Anxiety

I. Sahún, A. Amador, X. Gallego

A second research line in our group is aimed at identifying genetic causative and vulnerability factors underlying anxiety-related behaviour and that could predict the onset of panic disorder. To this aim we use genetically modified animal models that help to elucidate mechanisms that may be acting in humans. Our current project is focused on the biochemical changes that occur in the CNS during development that are determinant of emotionality-related behaviors and to the development of therapeutic strategies that may overcome and/or prevent the brain alterations leading to panic attacks. Our interest is also aimed at elucidate the deficits of specific neurotransmitter systems that possibly underlie the inability of persons with anxiety disorders to correctly identify the fear-related information and the possible common neurobiological pathways responsible for co-morbid processes. We have raised the possibility that NTRK3 can exert a major role in anxiety disorders based on the observation that its overdosage leads to an increased anxiety-like behavior and panic reaction, possibly due to the trophic effect attained on the catecholaminergic nuclei. Now we evaluate the mechanism of this effect studying if noradrenergic neuron neurogenesis, survival or plasticity can be affected. To this end we will evaluate the functional and structural modulations that occur upon application of specific neuromodulators or non-pharmacological stimuli that may regulate overall structure and plastic features of neurons and synapses. Finally we explore specific factors affecting the course of the disease in particular those that are associated with the regulatory capacity of the stress system. We have also initiated the study of the role of other candidate genes (nicotinic receptors) for which murine models have already been generated in our Program.

We have proposed a neurodevelopmental hypothesis for the pathogenesis of panic disorder.
In collaboration with Dr. R. Maldonado (Pompeu Fabra

Figure 1.
Down syndrome phenotypes in piramidal neurons of the Cerebral Cortex
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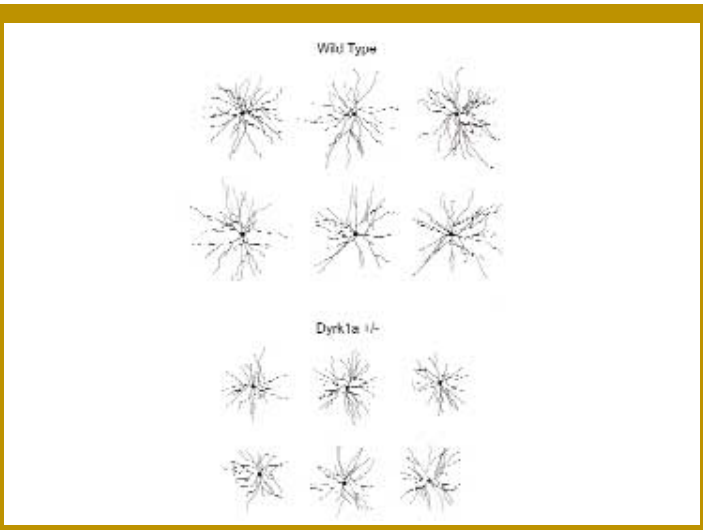

Figure 2.
Neurotrophic Effect on Locus Coeruleus noradrenergic neurons after over expression of NTRK3.
.

University) we have initiate a project for studying the implication of NTRK3 in processes comorbid to panic disorder, such as predisposition to substance abuse. Also we will analyze the predisposition to stress (collaboration with Dr. A. Armario, Autonomous University of Barcelona). New models overexpressing nicotinic receptors A3 (CHRNA3), A5 (CHRNA5) y B4 (CHRNB4) are now available.

3. Technical development

N. Fernández

We have set up new techniques: a/ Neurobehavioral: radial arm maze, fear conditioning, startle response, pre-pulse inhibition, Y-maze b/ Activity-dependent neuritogenesis and synaptic connectivity in primary cultures

PUBLICATIONS

- Dierssen M, Ramakers G
  "Animal models of mental retardation."
  Genes Brain Behav (in press)
- Benavides-Piccione R, Elston GN, Arbonés ML, Fotaki V, Estivill X, DeFelipe J, Dierssen M
  "Alterations in the phenotype of neocortical pyramidal cells in the DYRK1A+/- mouse."
  Neurobiol of Disease (in press)
- Dierssen M
  "Genetic and environmental factors in the maldevelopment of the cerebral cortex in Down syndrome."
  Amino Acids (in press)
- Ayberk Kurt M, Ilker Kafa M, Dierssen M, Davies DC
  "Deficits of neuronal density in CA1 and synaptic density in the dentate gyrus, CA3 and CA1, in a mouse model of Down syndrome."
  Brain Res, 1022(1-2), 101-9 (2004)
- Benavides-Piccione R, Ballesteros I, Martínez de Lagrán M, Elston G, Estivill X, Fillat C, DeFelipe J, Dierssen M
  "On dendrites in Down syndrome and DS murine models: a spiny way to learn."
  Prog Neurobiol, 74, 111-126 (2004)
- Fotaki V, Martínez de Lagrán M, Estivill X, Arbonés M, Dierssen M
  "Haploinsufficiency of Dyrk1A in mice leads to specific alterations in the development and regulation of motor activity."
  Behav Neurosci, 118, 815-21 (2004)
- Martínez de Lagrán M, Altafaj X, Gallego X, Martí E, Estivill X, Sahún I, Fillat C, Dierssen M
  "Motor phenotypic alterations in TgDyrk1A transgenic mice implicate DYRK1A in Down syndrome motor dysfunction."
  Neurobiol Dis, 15, 132-142 (2004)

Book chapters

- Dierssen M, Benavides-Piccione R, Martínez-Cué C, Estivill X, Baamonde C, Fillat C, Martínez de Lagrán M, Altafaj X, Flórez J, Elston G, DeFelipe J
  "Genotype–phenotype neural correlates in trisomy 21."
  In: The Adult with Down Syndrome. A new challenge for society. Edited by Jean A. Rondal, Alberto Rasore-Quartino and Salvatore Soresi. Whurr Publishers, London (2004)

GENES AND DISEASE

Gene function

The group was formally created at the beginning of 2002 and it started running at the Institut de Recerca Oncològica-IRO until the move at CRG in July 2002.

Group structure

Group Leader
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Eulàlia Salichs

Technician
Alicia Raya

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

RESEARCH PROJECTS

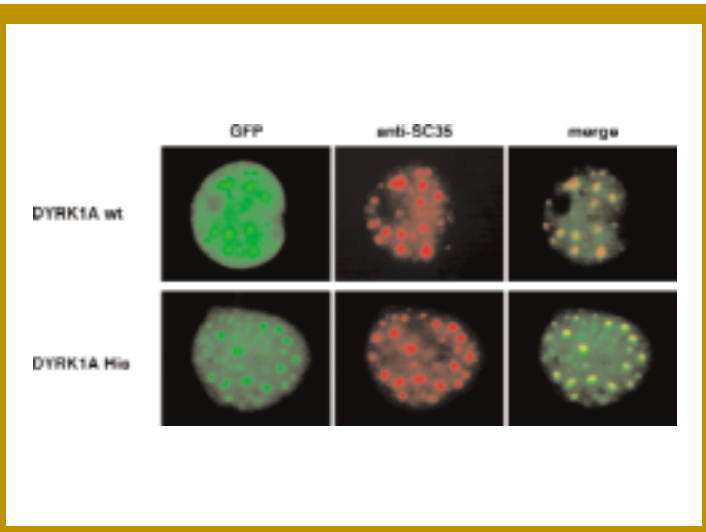
1. DYRK1A: control of cell proliferation

Mónica Alvarez, Sergi Aranda, Eulàlia Salichs

DYRK1A is one of the HSA21 genes for which changes in gene doses result in neuropathological alterations as it become evident from the analysis of the phenotypes shown by mouse transgenic mice in which the gene is either overexpressed or has been deleted. However, a clear role for the encoded kinase in different cellular processes and signal transduction pathways has not been defined yet. We intend to study DYRK1A from the molecular and cellular points of view.

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. In the DYRK subfamily, the lower eukaryotic members, such as Yak1p and Pom1p in yeast and YakA in *Dyctiostelium* have been associated with pathways controlling growth and development. Studies with null alleles for the *C. elegans* DYRK genes, *mbk-1 and mbk-2* have shown no apparent phenotypes for *mbk-1* and lack of viability for *mbk-2* mutants. Mbk-2 has been postulated as a candidate master regulator of maternal-protein degradation. Comparative analysis of the fly genomic sequence identify three DYRK members: *minibrain*, involved in postembryonic neurogenesis, dDYRK2 the putative product of the smell-impaired gene and

Figure 1.
DYRK1A accumulates in nuclear speckles through its histidine rich region. Co-localization of GFP fusions of DYRK1A full-length (wt) and the histidine-rich segment (His) with SC-35 marker is shown.



dDYRK3, not described yet. Finally, five members of the DYRK subfamily exist in mammals, DYRK1A, DYRK1B, DYRK2, DYRK3 and DYRK4 that share a high degree of conservation in the catalytic domain, but are very divergent in their N- and C-terminal domains. Although rather limited information is available for all these kinases, in general terms, all DYRKs might perform functional roles related to cell proliferation and/or differentiation.

Two are the main reasons for choosing DYRK1A as our target molecule to be used as a tool to learn more about how the cells interpret the external cues and take decisions related to cell proliferation/differentiation. On one side, the growth-related phenotypes shown by the Dyrk1A +/- mice (available in the Program), and on the other, the fact that the DYRK1A substrates list consists of both cytosolic and nuclear proteins, transcription factors included. The latter might point to DYRK1A acting as a crossroads for different signalling pathways.

We have started by studying the subcellular localization of DYRK1A, an intriguing aspect of this kinase since it is present in the nucleus and/or the cytosol of different cell types including neurones. Controlling nucleocytoplasmic transport is now considered a common means by which signalling molecules can be modulated. We have contributed to the description of the nuclear localization signals that direct DYRK1A to the nucleus and identify the splicing factor compartment as the subnuclear structure where the kinase accumulates. DYRK1A manages to do so by using a completely novel speckle-targeting signal corresponding to the histidine-rich region at its C-terminus. No other DYRK protein is targeted to the nuclear speckles. Moreover, we have learnt that DYRK1A is able to shuttle between the nucleus and the cytosol. This shuttling activity, shared by DYRK1B, could represent a mechanism to establish spatial separation from the kinase targets or activators and thus act as a potent regulator of DYRK1A function. Efforts are directed to find out how the shuttling activity is controlled.

In a complete different approach, the full-length sequence of DYRK1A has been used as a bait to search for interacting partners in a human fetal brain library by using a yeast two-hybrid screening. Several candidates are now being explored to confirm the detected interactions.

2. C21orf7

Eulàlia Genescà

C21orf7 has been isolated during a screening of novel genes mapping on HSA21. However, no functional role has been associated to the encoded protein products. Alternative splicing events lead to the expression of two open reading frames, ORF242 (242 amino acids) and ORF142 (142 amino acids), that are identical in their 119 C-terminal end amino acids. This segment is strongly similar to the

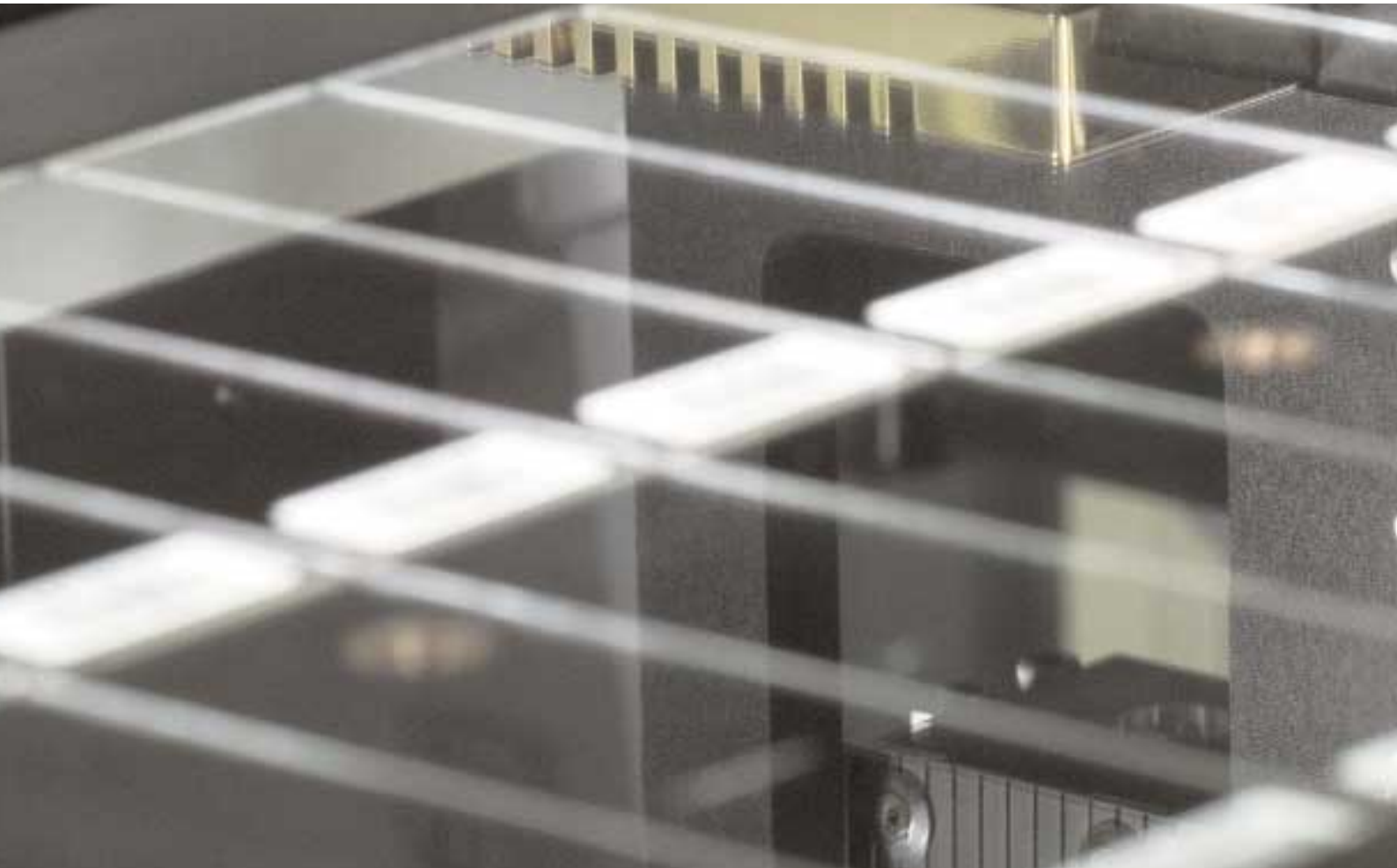
Figure 2.
Schematic representation of the interaction of C21orf7 ORFs with the IL-1 signalling pathway
.

C-terminus of the protein kinase TAK1.

TAK1, a member of the MAPK family, is activated by several stimuli such as interleukin-1 and Wnt, among others. TAK1 regulates various downstream signalling proteins including the MAPKs p38 and JNK and NF-kappaB, crucial players of many cellular activities. TAK1 participates in these pathways through the formation of signalling complexes in which several TAK1 binding proteins such as TAB1 (TAK1 N-terminal interactor), TAB2 or TAB3 (TAK1 C-terminal interactors) are present (Figure 2). Based on to the high degree of similarity between the C-terminus of *C21orf7* ORFs and the C-terminus of TAK1, we are trying to test whether these proteins work as intracellular modulators of TAK1-dependent signaling pathways

Bioinformatics and Genomics

Coordinator: *Roderic Guigó*



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

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

Research Groups:

1. **Bioinformatics and Genomics**
   (Roderic Guigó, join group with GRIB (IMIM, UPF))

2. **Genomic Analysis of Development and Disease**
   (Lauro Sumoy)
   - Microarray Unit

BIOINFORMATICS AND GENOMICS

Bioinformatics and Genomics

(join group with GRIB, IMIM-UPF)

Group structure

Group Leader
Roderic Guigó

Research Associate
Eduardo Eyras

Postdoctoral Fellows
Robert Castelo
Jan Jaap Wesselink
France Denoeud
Tyler Alioto
Nuria Lopez

Students
Josep F. Abril
Enrique Blanco
Charles Chapple

Technicians
Oscar González
Francisco Câmara
Julien Lagarde

Research in the Genome Bioinformatics group focuses in the problem of eukaryotic gene identification. Our group is both involved in 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 a number of eukaryotic genomes: human, mouse, rat, fly, mosquito, and slime mold

RESEARCH PROJECTS

1. Gene Prediction

We are working in the development of geneid, an "ab initio" gene prediction program. Recently geneid has been used in the annotation pipeline of *Tetraodon nigroviridis* (Jaillon et al., 2004). We are in the process of implementing versions of geneid for a number of eukariotic genomes according to the priorities set at the National Human Genome Research Institute. In particular, we are collaborating with the Whitehead Institute from the Massachussetts Institute of Technology to train geneid for the fungal genomes in this high-priority list, and with Genoscope in the analysis of the Paramecium genome.

2. Prediction of Selenoproteins

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

Figure 1.
Identification of novel mammalian selenoproteins (in orange).
Computational prediction followed by experimental verification. Taken from Kryukov et al. (2003)
.

eukaryotic selenoproteins (Castellano et al., 2004). The analyIsis of the *T. nigroviridis* genome discovery further challenged these assumptions (work in progress).

3. Splicing

We have developed a new method (BWM) based on Bayesian networks to improve the identification of splice signals (Castelo and Guigó, submitted). The methods appear to improve over previously existing methods (figure 2). We are also using comparative analysis of genomes to identify sequence signals involved in the regulation of splicing. We are developing this line of research in close collaboration with Juan Valcárcel group.

4. Comparative Genomics

We are particularly interested in using comparative analysis of genomes to improve gene prediction. In this regard, we have developed SGP-2 a comparative gene prediction program (Parra et al., 2003). This program was used in the context of the comparative analysis of the mouse genome (Waterston et al., 2003)—a project in whose leadership we participated. The application of SGP-2 has lead to the identification of previously unknown human genes (Guigó et al., 2003). SGP-2 has recently been applied to the analysis of the rat (Rat Genome Sequencing Consortium, 2004) and chicken genomes (Chicken Genome Sequencing Consortium, 2004). In the table below the comparative accuracy of SGP-2 in the chicken genome.

5. Recognition of Promoter Regions

In collaboration with Xavier Messeguer from the Universitat Politècnica de Catalunya, we have initiated a research line on algorithmcs for promoter recognition. We have a preliminary meta-alignment tool, and we are in the process of testing it in real experimental data.

6. ENCODE Project

The National Human Genome Research Institute (NHGRI) launched a public research consortium named ENCODE, the Encyclopaedia Of DNA Elements, in September 2003, to carry out a project to identify all functional elements in the human genome sequence (The ENCODE consortium, 2004). In its pilot phase the project is aiming to characterize all functional elements in 1% of the human genome. Within ENCODE, we are leading the GENCODE consortium with the goal of identifying all protein coding genes in the ENCODE regions. In the first phase of our approach we have established a collaboration with the HAVANA group at the Sanger Institute to provide a reference annotation of the known coding genes in the ENCODE regions. This annotation will be released at a Gene Prediction Workshop to be held at the Sanger Institute in May 2005.

Figure 2.
Comparative ROC of different methods to predict donor sites. The methods are ordered according to accuracy: BWM, MAXENT (Yeo and Burge, 2003), MDD (Burge and Karlin, 1997), FMM (First Order Markov Model) and WMM (Weigth Matrix Model)

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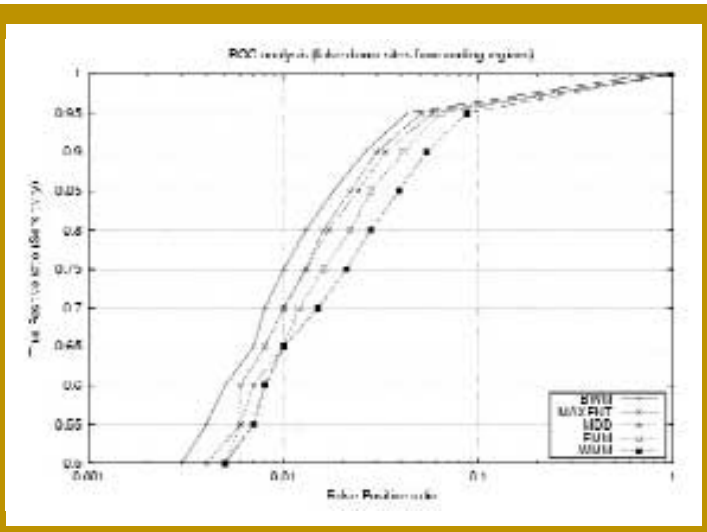


Figure 3.
Sensivity of gene predictions as mesured by comparison to ORF-containing cDNAs. Numbers are the percentage of coding exons from the cDNA-based models found by the three prediction systems. The sensivity numbers are quoted at two levels: exact exon prediction and >80% coverage of the cDNA exon
.

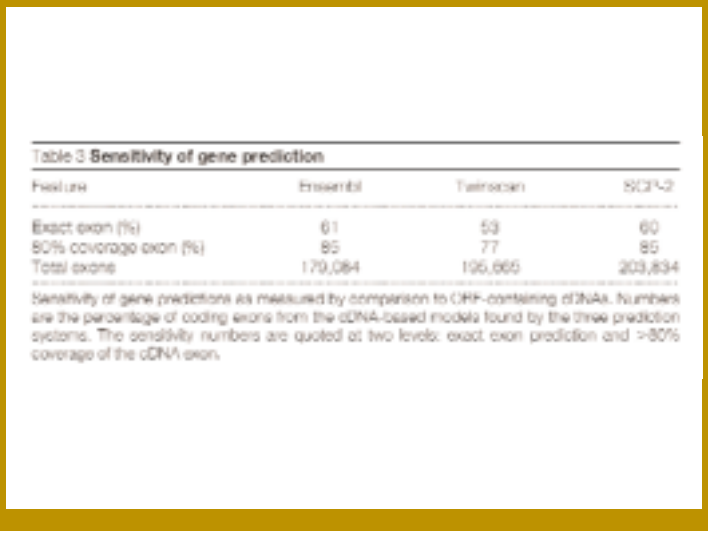


Table 3 **Sensitivity of gene prediction**

Feature	Ensembl	Twinscan	SGP1-2
Exact exon (%)	61	59	60
80% coverage exon (%)	85	77	85
Total exons	179,084	196,665	203,834

Sensitivity of gene predictions as measured by comparison to ORF-containing cDNAs. Numbers are the percentage of coding exons from the cDNA-based models found by the three prediction systems. The sensitivity numbers are quoted at two levels: exact exon prediction and >80% coverage of the cDNA exon.

PUBLICATIONS

- Wahl MB, Caldwell RB, Kierzek AM, Arakawa H, Eyras E, Hubner N, Jung C, Soeldenwagner M, Cervelli M, Wang Y-D, Liebscher V and Buerstedde J-M
  "Evaluation of the chicken transcriptome by SAGE of B cells and the DT40 cell line." BMC Genomics, 5, 98 (21 December 2004)
- International Chicken Genome Sequencing Consortium (including Eyras E, Castelo R, Abril JF, Castellano S, CÃ¢mara F, Parra G and Guigó R)
  "Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution."
  Nature, 432(7018), 695-716 (2004)
- The ENCODE Project Consortium (including Denoeud F, Lagarde J, Wesselink JJ, Castelo R, Eyras E and Guigó R)
  "The ENCODE (ENCyclopedia Of DNA Elements) Project."
  Science, 306(5696), 636-640 (2004)
- Jaillon O et al (including Castellano S, Parra G, Chapple C and Guigó R)
  "Genome duplication in the teleost fish Tetraodon nigroviridis reveals the early vertebrate proto-karyotype."
  Nature, 431(7011), 946-957 (2004)
- Castresana J, Guigó R and Albà M
  "Clustering of Genes Coding for DNA Binding Proteins in a Region of Atypical Evolution of the Human Genome."
  J Mol Evol, 59(1), 72-79 (2004)
- Castelo R and Guigó R
  "Splice Site Identification by idlBNs."
  Bioinformatics, 20(Suppl1), I69-I76 (2004)
- Brent MR and Guigó R
  "Recent advances in gene structure prediction."
  Curr Opin Struc Biol, 14(3), 264-272 (2004)
- Albà M and Guigó R
  "Comparative analysis of amino acid repeats in rodents and humans."
  Genome Res, 14(4), 549-554 (2004)
- Rat Genome Sequencing Project Consortium (including Camara F, Albà M, Abril JF, Eyras E and Guigó R)
  "Genome sequence of the brown Norway rat yields insights into mammalian evolution."
  Nature, 428(6982), 493-521 (2004)
- Castellano S, Novoselov SV, Kryukov GV, Lescure A, Blanco E, Krol A, Gladyshev VN and Guigó R
  "Reconsidering the evolution of eukaryotic selenoproteins: a novel non-mammalian family with scattered phylogenetic distribution."
  EMBO Reports, 5(1), 71-77 (2004)
- Castresana J, Guigó R and Alba M
  "Binding proteins in a region of atypical evolution of the human genome."
  J Mol Evol, 59, 72-79 (2004)
- Abril JF, Castellano S and Guigó R
  "Comparative gene prediction"
  Humana Press (in press)

Book chapters

- Blanco E and Guigó R
  "Predictive Methods using DNA Sequences."
  In: A.D. Baxevanis and B.F.F. Oullette, editors: Bioinformatics: A Practical Guide to the Analysis of

Genes and Proteins. 3rd Edition. Chapter 5.
John Wiley & Sons Inc., New York (2004)

- Dictionary of Bioinformatics and Computational Biology.
  (Guigó R contributor)
  Hancock JM and Zvelebil MJ editors. Wiley-Liss (2004)
  [Description]
- Castelo R and Perlman MD
  "Learning Essential Graph Markov Models from Data."
  In Gamez JA, Moral S and Salmeron A editors: Advances in Bayesian Networks. Chapter 14, Pp:255-270. (Series: Studies in Fuzziness and Soft Computing, Volume 146)
  Springer-Verlag, Berlin, Germany (2004)

BIOINFORMATICS AND GENOMICS

Genomic Analysis of Development and Disease Associated Technological Unit: Microarray Laboratory

Group structure

Group Leader
Lauro Sumoy

Postdoctoral Fellow
Ana Andrés

Microarray Core Facility

Bioinformatician
Juanjo Lozano

Senior Technician
Belen Miñana

Technician
David Otero

Guest or associated members

Senior Technician
Eva Gonzalez (PRBB, L. Perez Jurado - UPF)

Technician
Manuel García (CRG, X. Estivill)

Informatician
Gregorio Gomez (PRBB, P. Real – IMIM)

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

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

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

The laboratory has two main areas of research: first, an independent basic line of research centred on the functional analysis of the LRRN6A gene; secondly, technological research activities arising from the involvement of the microarray core facility in many different collaborative projects.

RESEARCH PROJECTS

1. Functional genomic analysis of the LRRN6A gene in neural differentiation and axonal regeneration

LRRN6A is a novel gene with a hypothesized function in axon path-finding during development and in neuronal plasticity in the adult. This gene was found in the context of the study of the 15q24-q26 region of the human genome (DUP25) associated to panic and anxiety syndrome and joint laxity that lead to exhaustive characterization of cDNAs and construction of a transcript map of the region. We have undertaken functional studies centred on genes from 15q24-q26 and chose the LRRN6A gene for further study. With relevance to the disease, we have found that LRRN6A is expressed in the adult limbic system and shown it maps within the duplication. Recent findings involve LRRN6A in a signalling pathway which inhibits axon regeneration in damaged nerves in response to myelin. With secured external funding, we are continuing to characterize this gene and to use molecular biology and genomics tools to study its function and to test its involvement in duplication associated pathologies.

PUBLICATIONS

- *Muñoz X, Sumoy L, Ramírez-Lorca R, Villar J, García de Frutos P, Sala N
  "Human Vitamin K-Dependent GAS6: Gene Structure, Allelic Variation and Association with Stroke."
  Hum Mutat 23(5), 506-12 (2004)

*work derived from prior collaborations

MICROARRAY UNIT

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

TECHNOLOGY DEVELOPMENT PROJECTS

1. Microarray technology

We are committed to developing and optimizing procedures for the design, fabrication, hybridization, processing and analysis of data generated from two colour DNA microarrays.

We are involved in projects focusing on breast cancer (collaboration with Dr. Miguel Beato, CRG; Dr. Francesc Solé, Hospital del Mar-IMAS), bladder and prostate cancer (collaboration Dr. Antonio Alcaraz, Fundació Puigvert), genome structure variation in control population and genomic disorders mediated by homologous recombination events between segmented duplications (collaboration with Drs. Xavier Estivill, CRG, and Luis Perez Jurado, UPF), and variation in splicing (collaboration with Drs. Roderic Guigó and Juan Valcarcel, CRG). There is an ongoing project to develop standardized procedures for amplification of RNA from small sample amounts, and to validate and compare different labelling methodologies (collaboration with Dr. Paco Real, IMIM). We are testing procedures for CHIP on chip to detect differences in binding of transcription factors and chromatin components to DNA (collaborations with Dr. Jorge Ferrer, IDIBAPS; Dr. Antonio García de Herreros, UPF; Dr. Anna Bigas, IRO). Currently planned applications include diagnostics of breast, prostate and bladder cancer, and genomic mutations mediated by segmental duplications through participation in collaborative projects. We have also developed tiling path BAC arrays for human chromosomes 15 and 22.

2. Microarray bioinformatics

We have set up automated image data acquisition, pre-processing, filtering, normalization and quality control Web based software for analysis of microarray experiments. We are implementing an Oracle based database for direct data browsing by service users. In the future, web tools for advanced data set classification, clustering, grouping, discriminant, factor analysis and data mining purposes, already under development (Sanchez-

Corbayo et al, 2003). This also includes capabilities for analysis of other types of microarrays data such as Affymetrix.

We are directly involved in providing support for the analysis of CGH data from large cancer datasets (collaborations with Dr. Paco Real, IMIM; Dr. Rosa Miró, UAB) and for annotation of alternative splicing microarrays (collaboration with Dr. Juan Valcarcel, CRG).

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

SERVICES

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

MICROARRAY-RELATED PUBLICATIONS FROM LABORATORY MEMBERS

- De Nadal E, Zapater M, Alepuz PM, Sumoy L, Mas G, Posas F
  "The Hog1 MAP kinase recruits the Rpd3 histone deacetylase to activate osmoresponsive genes."
  Nature, 427(6972), 370-374 (2004)
- Muckenthaler MU, Rodrigues P, Macedo MG, Minana B, Brennan K, Cardoso EM, Hentze MW, de Sousa M
  "Molecular analysis of iron overload in beta2-microglobulin-deficient mice."
  Blood Cell Mol Dis, 33(2), 125-31 (2004)
- Bonnah RA, Muckenthaler MU, Carlson H, Minana B, Enns CA, Hentze MW, So M.
  "Expression of epithelial cell iron-related genes upon infection by Neisseria meningitidis."
  Cell Microbiol, 6(5), 473-84 (2004)

PUBLICATIONS BY CORE FACILITY USERS

- Martell M, Briones C, de Vicente A, Piron M, Esteban JI, Esteban R, Guardia J, Gomez J
  "Structural analysis of hepatitis C RNA genome using DNA microarrays."
  Nucleic Acids Res, 32(11), e90 (2004)

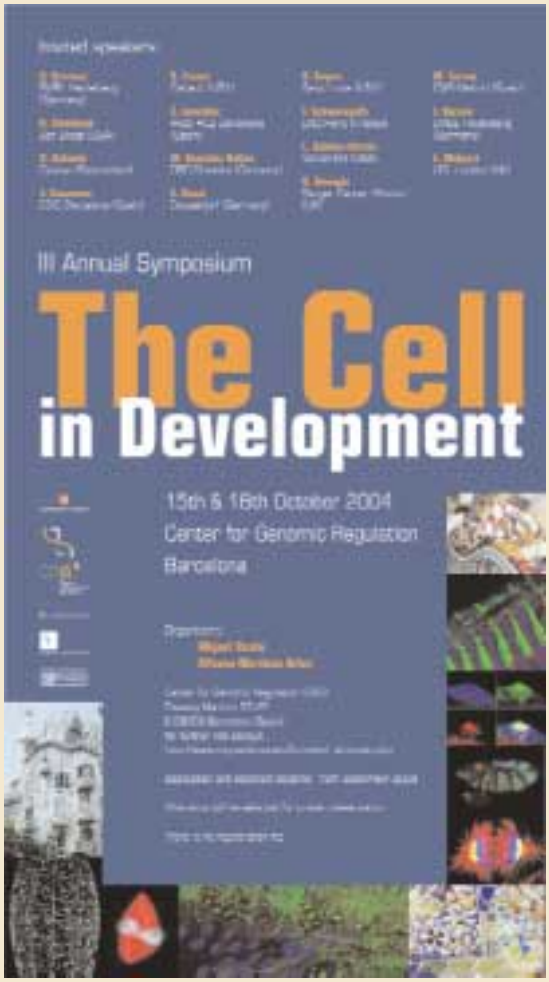
Appendix

Appendix 1

III Annual Symposium of the Center for Genomic Regulation "The Cell in Development"

The third CRG Symposium, entitled "The Cell in Development", was held on 15 and 16 October 2004 in Barcelona. The main objective of this Symposium was gathering top-level scientists of modern Biology to discuss in public forefront topics on Developmental Biology and the interactions with Cell Biology.

The understanding of the mechanisms that are involved and regulate the generation of cell types from stem cells and the organization of these cell types in tissues and organs is essential in modern Biology. The reason is simple, since, apart from establishing an important basis for the comprehension of the generation of organisms, these studies provide an important platform for the analysis of pathological processes. During the last twenty years there have been very important advances in our appreciation on how organisms are build in, in terms of transcriptional programmes that regulate the generation of different cell types. However, these studies are only the previous stage to answer questions referred to spatial organization of cells, which is the basis of organisms. During the last two or three years, the interest in replying this question has revealed important connections between Cell Biology and Developmental Biology. Topics like the organization of different molecular processes in cells; the spatial control of cellular splicing and the regulation of proliferation or cellular movement have become the epicentre of the studies in Developmental Biology. This has opened an important dialogue, intellectually fruitful, between developmental and cell biologists. The objective of the CRG Symposium in 2004 was to provide an open forum for the discussion between biologists from these two fields. Barcelona and its environment have a very important concentration of biologists with similar interests in this area who had the occasion to participate in high-level discussions about leading research topics.



The symposium had excellent presentations, intense discussions and went fluently. During the first afternoon, presentations versed about basic cellular processes (cellular splicing and cytoskeleton activity) and their incidence in developmental processes. The session finished with a keynote talk by professor Lewis Wolpert (University College, London, UK) who reviewed the role that biology currently plays and has played in developmental biology. The second day went on with the topic of basic cellular processes; this time focused on vesicle and membrane traffic, and gradually moved to most multicellular processes, as for example intercellular signalling and transcription regulation in cellular fields. The session and the symposium finished with a second keynote talk by professor Dennis Duboule (University of Geneva, Switzerland) about the way in which regulating elements of transcription make up the space and time in the construction of the extremities of vertebrates.

The Symposium had a large number of participants (more than 180 people), most of them from overseas. In this sense, the activity contributed to the internationalization of research in Catalonia and the rest of Spain and meant a high-level training for young investigators. The contents and format of the Symposium clearly served as an adequate forum that contributed to the consecution of these objectives. Another important element of the program that contributed to the accomplishment of this objective was the set of short presentations by young people from Barcelona and its surroundings.

Summing-up, the idea of gathering cell and developmental biologists, estimated as very important in these moments in Biology, was a great catalyser and the Symposium was a success, which was reflected as well in the number of possible collaborations arisen.

MINI-SYMPOSIUM

"Cancer, Transcription and Development"

Dr. Thomas Graf is one of the most prestigious scientists internationally with recognized influence due to his studies in Oncology, essentially about leukaemia. It is worth to mention his studies contributing to the elucidation of the differentiation pathways of hematopoietic lineages and the modulator role performed by the differential expression of diverse transcription factors in these processes. As a recent example of the strength and excellence of his investigation task we could mention his last scientific study published on May 28th 2004 in *Cell*, entitled "Stepwise reprogramming of B cells into macrophages" (Xie H et al.).

Since he got his PhD in 1969 by the University of Tübingen (Germany), his steady and extensive professional career has been developed basically between Europe and USA. His investigating activity has been always performed at elite Institutions, as the Max Planck Institute of Virology (Tübingen, Germany), the Duke University (North Carolina, USA), the German Cancer Research Center and the European Molecular Biology Laboratory (Heidelberg, Germany) and, most recently, the Albert Einstein College of Medicine (New York, USA). Throughout his career, he has established numerous and fruitful collaborations with eminent scientists that have been reflected in publications in journals of international prestige. We should mention as well his outstanding teaching task with a decisive influence in the training of young scientists who later become Group Leaders.

Dr. Thomas Graf is currently the coordinator of the Differentiation and Cancer Programme at the Center for Genomic Regulation (CRG) in Barcelona, where he has as well his own research group about Hematopoietic Differentiation and Stem Cell Biology. Due to the reasons briefly exposed above, the CRG hosted a one-day scientific meeting to honour the scientific career of Dr. Graf, practically coinciding with his 60th birthday. This meeting, open to the audience interested in attending, was held on November 15th and was entitled "Cancer, Transcription and Development".

This event gathered in Barcelona some of the most insigne scientists studying the role transcription factors in cancer and development, mainly of the hematopoietic system, to expose and discuss their last research studies. Some of the attendants were:

- Dr. Daniel Tenen (Boston, USA).
- Dr. Hartmut Beug (Vienna, Austria).
- Dr. Douglas Engel (Ann Harbor, USA).
- Dr. Jonathan Frampton (Birmingham, UK)
- Dr. Achim Leutz (Berlin, Germany).
- Dr. Kelly McNagny (Vancouver, Canada).
- Dr. Claus Nerlov (Monterotondo, Italia).
- Dr. Scott Ness (Albuquerque, USA).
- Dr. Fabio Rossi (Vancouver, Canada).
- Dr. Michael Sieweke (Marseille, France).

This event was organized by Nelly McNagny (Vancouver) and Florencio Varas (CRG) and was attended by more than 100 people.

Appendix 2

CRG SEMINARS 2004

CRG SEMINARS

DATE	SPEAKER	AFFILIATION	SEMINAR TITLE
29/10/04	**Aldea, Martí**	Dept. Ciències Mèdiques Bàsiques, Universitat de Lleida, Spain	"Mechanisms of spatial control of cell cycle entry"
8/10/04	**Sallenave, Jean-Michel**	Rayne Laboratories-MRC Centre for Inflammation Research, Edinburgh University Medical School, UK	"Models of lung infections in mice: use of adenovirus vectors for therapeutic and vaccination strategies"
30/9/04	**Agarwal, Pankaj**	Manager, Bioinformatics Sciences, GlaxoSmithKline, USA	"Omics to Pathways: Interpreting gene lists using literature-based protein interaction networks"
27/9/04	**Weissenbach, Jean**	Genoscope and CNRS UMR8090, Evry, France	"Genome analysis of the pufferfish Tetraodon nigroviridis sheds new light on vertebrate genome evolution"
17/9/04	**Hancock, John**	Bioinformatics, MRC Mammalian Genetics Unit, Harwell, Oxfordshire, UK	"Simple protein sequences"
16/9/04	**Jenuwein, Thomas**	IMP, Vienna, Austria	"The indexing potential of histone lysine methylation"
3/9/04	**Blanchard, Jean-Marie**	Institut de Génétique Moléculaire de Montpellier, UMR 5535 CNRS-Université Montpellier 2, Institut Montpelliérain de Biologie (IFR 122), Montpellier, France	"Transcription, chromatin remodelling and cell cycle control"
23/7/04	**Speed, Terry**	Division of Genetics and Bioinformatics, The Walter & Eliza Hall Institute of Medical Research, Victoria, Australia	"Incorporating dependence into models for DNA motifs"
13/7/04	**Black, Doug**	Dept. of Microbiology, and Molecular Genetics, Howard Hughes Medical Institute, UCLA, Los Angeles, USA	"Alternative splicing and the regulation of neuronal gene expression"
9/7/04	**Yélamos, José**	Dpto. Bioquímica, Biología Molecular B e Inmunología, Facultad de Medicina, Universidad de Murcia, Spain	"Poly(ADP-ribose)polymerase-dependent signaling pathways in the inflammatory response"
18/6/04	**Seoane, Joan**	Institut de Recerca Vall d'Hebron-ICREA, Barcelona, Spain	"Mechanisms of TGFb-mediated inhibition of cell proliferation. Relevance in oncogenesis"
4/6/04	**Helin, Kristian**	Division of Experimental Oncology, European Institute of Oncology, Milan, Italy	"Regulation of cell proliferation and cancer by E2F and E2F target genes"
3/6/04	**Samso, Montserrat**	Brigham and Women's Hospital, Harvard Medical School, Boston, USA	"Cryo-electron microscopy and 3D reconstruction of dynein and the ryanodine receptor"
1/6/04	**Zotter, Angelika**	Dept. of Cell Biology & Genetics, Erasmus Medical Center, Rotterdam, The Netherlands	"Dynamics of Nucleotide Excision Repair in living mammalian cells"

21/5/04	**Lührmann, Reinhard**	Max Planck Institute for Biophysical Chemistry, Göttingen, Germany	"Structural dynamics and function of the mammalian spliceosome"
7/5/04	**Bickmore, Wendy**	MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom	"Relating higher-order chromatin structure and nuclear organisation to gene expression"
23/4/04	**Moreno, Sergio**	Cancer Research Institute, CSIC, Universidad de Salamanca, Spain	"mRNA stability and translational control: two ways of keeping the cell cycle regulators under control"
2/4/04	**Osborne, Howard-Beverly**	CNRS UMR 6061 Génétique et Développement-Regulation of Gene Expression Group, Faculté de Médecine, Université de Rennes 1, Rennes, France	"Post-transcriptional regulation and somatic segmentation ini the Xenopus laevis embryos"
1/4/04	**López-Schier, Hernán**	Laboratory of Sensory Neuroscience, The Rockefeller University, New York, USA	"Planar cell polarity in a remodelling organ, the case of the lateral line in the zebrafish"
31/3/04	**Sharpe, James**	MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom	"3D imaging and computer modelling: New integrated approaches for studying gene networks in development"
30/3/04	**Tze Leon Ng, Julian**	Dept. of Biological Sciences, Stanford University, California, USA	"Regulating the cytoskeleton during nerve growth cone motility"
19/3/04	**Dimitrov, Stefan**	Unité INSERM 309, Institut Albert Bonniot, Domaine de la Merci, La Tronche cedex, France	"Mitotic chromosome structure, remodelling and histone H3 phosphorylation"
5/3/04	**Ribas de Pouplana, Lluís**	Investigador ICREA, Parc Científic de Barcelona, Spain	"The genetic code apparatus, where did it come from and how can we exploit it"
25/2/04	**Moreno, Eduardo**	University of Zurich, Institute of Molecular Biology, Switzerland	"The competitive nature of cells"
24/2/04	**García García, Mª Jesús**	Laboratory of Dr. Kathryn V. Anderson, Sloan Kettering Institute, New York, USA	"Mouse gastrulation: lessons from ENU mutagenesis
13/2/04	**Aguilar, Andrés**	Dpto.Genética, Facultad de Biología, Universidad de Sevilla, Spain	"A role for the nascent mRNA in the connection between transcription, mRNA metabolism and DNA recombination"
30/1/04	**Enjuanes, Luis**	CNB, CSIC, Madrid, Spain	"Coronavirus reverse genetics and virus vector design"
16/1/04	**Zavolan, Mihaela**	Biozentrum der Universitaet Basel&Swiss Institute of Bioinformatics (SIB), Switzerland	"From finding splice variants to finding regulatory signals"
9/1/04	**Nagamine, Yoshikuni**	Friedrich Miescher Institute for Biomedical Research, Switzerland	"Regulation of Urokinase mRNA Stability by AU-rich Element-Binding Proteins HuR and RHAU"

PROGRAMME SEMINARS 2004

GENE REGULATION

DATE	SPEAKER	AFFILIATION	SEMINAR TITLE
22/12/04	**Ceron, Julian**	MGH Cancer Center - Harvard Medical School - Charlestown - Massachusetts	"Functional genomics in C elegans: The use of RNAi libraries in dissecting cancer pathways"
20/12/04	**Aza-Blanc, Pedro**	Genomic Institute - Novartis Research Foudation -	"Pathway analysis via genome-scale RNAi-based loss-of function: from HT technology to biological data"
19/10/04	**van Room, Anne-Marie M.**	Department Biophysical Structural Chemistry - Leiden University - The Netherlands	"Schistosoma mansoni: Structural and biophysical aspects of Lewis X-antibody interactions"
26/7/04	**Loza-Coll, Mariano**	Department of Medical Biophysics - University of Toronto - Canada	"Regulation of anoikis in intestinal epithelial cells by the tyrosine kinase Src"
12/7/04	**Hartman, Britta**	Dept. Cell Biology - Biozemtrum - University of Basel	"Nuclear Interpretation of Dpp Signalling in Drosophila"
16/1/04	**Korn, Bernhard**	Deutsches Ressourcenzentrum Für Genomforschung - RZPD - Heidelberg	"Experimental verification of predicted variants of human transcripts"

DIFFERENTIATION AND CANCER

DATE	SPEAKER	AFFILIATION	SEMINAR TITLE
25/11/04	**Terranova, Remi**	Lymphocyte Development Group, MRC, Clinical Sciences Centre, ICSM, Hammersmith Hospital, London, U.K.	"An epigenetic analysis of muscle reprogramming and differentiation".
28/10/04	**Domínguez, M. Isabel**	Hematology-Oncology Section, Boston University School of Medicine, USA	"Protein kinase CK2 is required for dorsal axis formation in Xenopus embryos".
21/10/04	**Quina, Ana Sofía**	Instituto de Medicina Molecular, Lisboa, Gulbenkian Institute of Science, Oeiras, Portugal	"New insights into telomere organization in human cells".
9/9/04	**Perdiguero, Eusebio**	Signaling and Cell Cycle Group, Molecular Oncology Programme, CNIO, Madrid, Spain	"New roles for the p38 pathway in Xenopus oocyte maturation or The never ending Cdc25C regulators".
10/6/04	**Mann, Christopher**	Randall Centre for Molecular Mechanism of Cell Function, Kings College London, U.K.	"Duchenne muscular dystrophy: antisense oligonucleotide therapy and satellite cell behaviour".

GENES AND DISEASE

DATE	SPEAKER	AFFILIATION	SEMINAR TITLE
21/12/04	**Raphael, Prof. Yehoash**	The University of Michigan Medical School, USA	"Math1 expression influences phenotype on non-sensory cochlear cells."
20/12/04	**M Martin, Prof. Donna**	Pediatrics and Human Genetics Department, The University of Michigan Medical Center, USA	"The role of Pitx2 in CNS neuronal differentiation."
23/11/04	**Cavaille, Jérôme**	"Lab,de Biologie Moléculaire des Eucaryotes Université Paul Sabatier - Toulouse , France "	"Imprinted small RNA genes."
26/7/04	**Pozas, Esther**	Division of Molecular Neurobiology, Department of Neuroscience, Karolinska Institute, Stockholm-Sweden	"GDNF and other neurotrophics factors in the development of the telencephalon"
22/6/04	**Millet, Oscar**	Plataforma de RMN de Biomolècules, Parc Científic de Barcelona	"The energetic cost of domain reorientation in maltose binding protein."
7/6/04	**Caceres, Dr. Mario**	Dpt of Human Genetics, Emory University School of Medicine, Atlanta	"What makes us humans? Gene expression differences in the brain between humans and chimpanzees."
13/4/04	**Pérez-Enciso, Miquel**	ICREA Professor Dept. Ciència Animal i dels Aliments. Universitat Autònoma de Barcelona	"Genetical Genomics: Promises and Pitfalls."
9/2/04	**Besser, Daniel**	Lab. of Molecular Vertebrate Embryology, The Rockefeller University, New York	"TGF-beta signaling in human embryonic stem cells."

BIOINFORMATICS AND GENOMICS

DATE	SPEAKER	AFFILIATION	SEMINAR TITLE
4/10/04	**Ranganathan, Shoba**	Biotechnology Research Institute, Macquarie University, Sydney, Australia	"Alternative Splicing: bridging the gap between the genome and the transcriptome".
26/7/04	**Ruvinsky, Anatoly**	Genetics SRSA, Faculty of the Sciences, University of New England, Australia	"Natural selection of exonic ends and frequencies of codon usage as a factor affecting exon-intron structure of eukaryotic genes".
3/6/04	**Korf, Ian**	Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, U.K.	"Gene prediction in novel genomes & 3'end formation in C. Elegans".
19/5/04	**Cristina i Pau, Xavier**	Applied Biosystems - Technical Seminar	"New Microarrays for study gene expression".
15/3/04	**Hatzigeorgiou, Artemis-Georgia**	Ass.Professor, Dep. of Genetics, School of Medicine, University of Pennsylvania, USA	"miRNA genes and their targets".
19/2/04	**Alioto, Tyler**	Ngai Lab., Department of Molecular & Cell Biology, Berkely, CA, USA	"The odorant receptor gene repertoire of zebrafish".

Appendix 3

GRANTS

The grants that the CRG has obtained from 1st January to 31st December 2004 are the following:

ORGANISM	AMOUNT (€)
MINISTERIO CIENCIA TECNOLOGIA	1.264.293,34
MINISTERIO SANIDAD Y CONSUMO	786.963,13
EUROPEAN COMISSION	624.814,62
FUNDACION DESARROLLLO INVEST GENOMICA PROTEOMICA	623.620,69
FUNDACIO MARATO TV3	181.975,93
FUNDACIO LA CAIXA	142.319,00
MUSCULAR DYSTROPHY	119.154,81
FUNDACION CIENTIFICA DE LA ASOCIACION ESPAÑOLA CONTRA EL CÁNCER	100.987,49
AGENCIA GESTIO D'AJUTS UNIVERSITARIS	97.451,35
FONDATION JEROME LEJEUNE	97.000,00
DURSI RECERCA	61.628,72
BUNDESMINISTERIUM BILDUNG	53.000,00
NOVARTIS FARMACEUTICA, S.A.	50.000,00
ASSOCIATION FRANÇAISE CONTRE LES MYOPATHIES	20.000,00
NATIONAL INSTITUTE OF HEALTH	12.757,00
GRANTS (MISCELLANEOUS)	3.469,26
THE COMPANY OF BIOLOGISTS Ltd	2.988,51
EMBO EUROPEAN MOLECULAR BIOLOGY ORGANISATION	2.432,00
TOTAL AMOUNT	**4.244.855,85**

Appendix 4

Honors and prizes of CRG scientists during 2004

Juan Valcárcel
2004 Elected member of EMBO

Luciano Di Croce
2004 "Vincenzo Caglioti Prize" in biological Sciences, "Academia Nacionale dei Lincei", Italy

Cristina Fillat
2004 Award for an outstanding achievement. 9th World Congress on Advances in Oncology and 7th International Symposium on Molecular Medicine, Creta, Greece

Mara Dierssen
President of the International Behavioral Genetics Society (2003-2005)

CRG

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de Regulació
Genòmica

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