CRG
Centre
de Regulació
Genòmica

CENTER FOR GENOMIC

GENOMIC

CENTER FOR GENOMIC REGULATION

Annual Report 2002-2003

SCIENTIFIC BOARD

CENTRE DE REGULACIÓ GENÒMICA

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Design by Estudi Freixes

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Scientific Structure CRG

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Transcriptional regulation and chromatin remodelling
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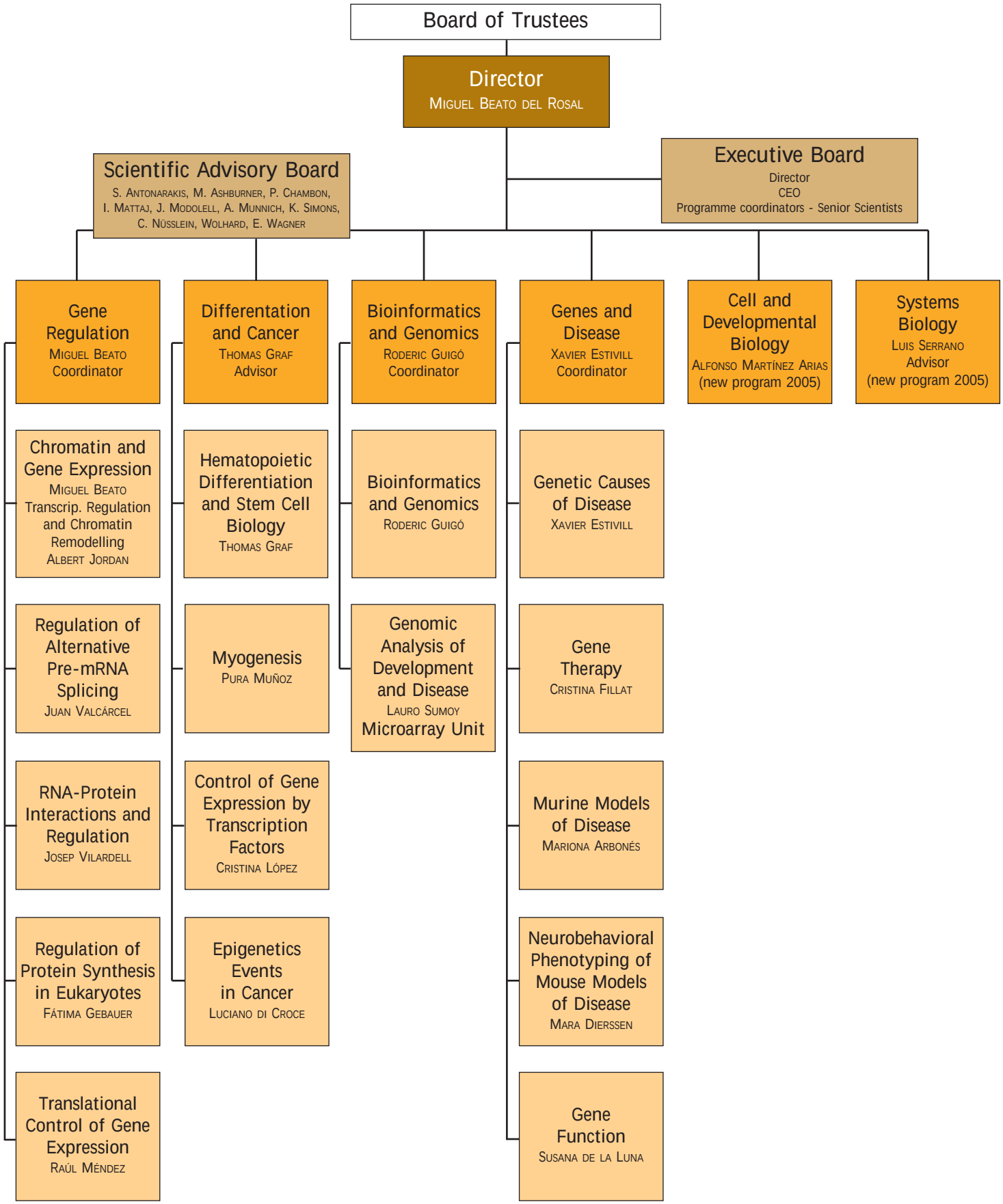
Scientific Structure CRG



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Introduction

In the first 15 months after the official inauguration of the CRG in October 2002, many important decisions have been taken that secure the future of the CRG project.

In terms of recruitment, the most important development has been the implantation of the first four groups of the programme Cell Differentiation and Cancer coordinated by Thomas Graf. The group of Pura Muñoz move to the CRG already December 2002 and is the largest group in the programme. The group is fully operative as they came from another research Institute in Barcelona. The group of Thomas Graf has started under the leadership of Florencio Varas, who came to the CRG at the beginning of 2003 with a position from the Ministry of Health. Thomas Graf has already obtained a position from the ICREA and has signed a preliminary contract with the CRG, which foresees his incorporation for the fall of 2004 or early 2005. The group of Cristina López Rodríguez started the spring of 2003 with a position from the programmed Ramón y Cajal, and is already working experimentally. Finally, our first foreign group leader, Luciano Di Croce from Milan, came to the CRG also in the spring of 2003 with a position from ICREA, and has already installed his lab and recruited the first collaborators. The programmes Gene Regulation and Genes and Disease have started a consolidation phase, without recruiting new group leaders, while the programme on Bioinformatics and Genomics still remains in an initial phase. The Microarray facility has been consolidated by completing the equipment and by the incorporation of new collaborators. It is now offering microarray analysis for scientists of the PRBB. In the summer of 2003 one of the group leaders of the programme, José Castresana, left the CRG to take a permanent position in the CSIC Institute of Molecular Biology in Barcelona.

At the end of 2003 the CRG encompassed 16 groups and 131 people, including 35 senior and postdoctoral scientists, 45 graduate students, 33 technicians and 18 administrative and support.

In the summer of 2003 the Department of Universities, Research and Information Society (DURSI) approved a four years financial plan (2003-2006) for the CRG that foresees the creation of a new programme on Cell and Developmental Biology and the expansion of the other four programmes to a final size of six groups each. This development will only be possible after completion of the new building of the Parc de Recerca Biomedica de Barcelona (PRBB), expected for the summer of 2005.

In view of the new programme the Scientific Advisory Board (SAB) was expanded to include two experts in Cell and Developmental Biology. Christianne Nuesllein-Volhard, from the Max-Plank Institute in Tübingen, and Joan Modolell, from the Center of Molecular Biology Severo Ochoa in Madrid, accepted to joint our SAB. During the spring of 2003 we announced in Nature the positions for the new programme: five group leaders and a coordinator. We have received nine applications for the coordinator positions and almost one hundred for the group leader positions. During its annual meeting in October, the SAB approved unanimously the decision of the CRG to make an offer to Alfonso Martínez Arias, from the University of Cambridge, UK, to act as the coordinator of the new programme. In addition the reorientation of the Bioinformatics and Genomic programme in the direction of Computational Biology or Systems Biology was also considered a positive development for the immediate future.

In the last year, the Executive Committee of the CRG has formulated the rules that will structure the scientific career at the CRG, which have been evaluated and completed during the annual meeting of the SAB. These rules form the basis for future contracts of new CRG scientists and attempt to combine flexibility with continuity. They envisage a strict periodic reviewing of all independent scientists and a continuous renewal by incorporation of young researchers.

The progress with the PRBB building is another very positive development. The construction was initiated and financed by a mixed Consortium of investors with a private majority. It was planned that we various Institutions will rent the required space. This strategy, of course, would have reduced our resources considerably. After long negotiations and just before the summer vacation 2003, the DURSI signed an agreement with the Consortium for buying some 7.000 m^2 of laboratory and office space for installing the CRG. This is the space we need for full development of the various programs of the CRG. Construction is proceeding according to schedule and we planed to move to the new building in the summer/fall of 2005.

The PRBB building will include a large animal facility (2.400 m^2) for experimentation with transgenic mice. There are plans to increase our capacity for creating and maintaining transgenic animals in the context of a coordinated Centre for Transgenic Animals (CAT) designed to fulfil the needs of the PRBB, the Parc Cientific of the University of Barcelona and the Hospital Clinic. These three institutions signed an agreement last September to build the Alianza Biomedica de Barcelona (ABB), which will allow the coordination of research plans and large infrastructure as well as the common use of all core facilities. The ABB is open for other universities and research institutions.

The integration with the other Institutions in the PRBB has been favoured by the CRG Friday Seminars with external speakers, which are regularly attended by scientists and students from the CEXS/UPF and the IMIM. Moreover the PRBB also organizes special Seminars, very appreciated by the CRG scientists.

Collaborations with other scientists from the CEXS/UPF and the IMIM are integrated within various networks funded by the Spanish government and by the government of Catalunya. In addition CRG scientists participate actively in advanced teaching of Biology students at the UPF, in particular in the Basic Research itinerary of the fifth year and in the International PhD programme on Basic Biomedical Research. Although this is a good start, one of the challenges for the immediate future is to improve these interactions by generating a natural system of relations and interactions among scientists in the PRBB. This task will be greatly facilitated when we all move to the new building.

Last January the CRG group leaders met for a one-day retreat near Barcelona. It was a very successful brainstorming that we want to repeat annually.

After the success of the first CRG Symposium in October 2002, the second CRG Symposium was held on October 11, 2003. It was organized by Luciano Di Croce, Thomas Graf and myself, and attracted more than one hundred colleagues, who came to listen to 14 experts in "Cell Reprogramming and Epigenetics". The symposium opened with a plenary lecture by John Gurdon, Cambridge UK, and was characterized by excellent talks full of new information and by lively scientific discussions.

As detailed in the reports of the individual groups, scientists at the GRG have been rather successful in obtaining financial support for their scientific projects. The total amount of competitive resources granted to CRG scientists from September 2003 to December 2003 exceeded 5 million €. Of particular relevance is the selection of the CRG as one of the nodes to set up a Genotyping Unit, which will focus on degenerative diseases of the nervous system and other complex genetic disorders. This Unit is the first step in developing a genotyping facility with the support of Genome Spain, which will make the CRG a very attractive place to centralize genetic studies in clinical and pharmacological research.

The first publications of results obtained in the CRG are starting to appear. By the end of 2003 there were 68 papers published or in press with authors affiliated at the CRG, and the average impact factor per paper was 8.2. However, this figure is not truly representative of the performance of scientists in the CRG as the results have been partly obtained in the institutions where the CRG scientist worked before joining the centre.

Much remains to be done in the next years, but I think we can look back at the past 15 months with a good feeling for what has been accomplished. This will not have been possible without the excellent help from the CRG administration team leaded by Marian Marrodan. We have also received continuous support from the CEXS/UPF, the IMIM, the PRBB and the DURSI. With this kind of stimulating help and the enthusiasm of the CRG scientists, I am confident that we can fulfill the original plan to make the CRG an outstanding biomedical research centre and the basic research unit of the PRBB.

Coordinator: Miguel Beato

GENE REGULATION

GENERAL COMMENTS

While planning the composition of the programme we made an attempt to have an equilibrated distribution of groups in three main areas of research on eukaryotic gene regulation, namely, transcription, RNA processing and translation. The end result of the recruitment process has been a concentration of four groups on the very timely area of RNA-mediated regulation, and a large group focussing on transcriptional control and chromatin remodelling.

This programme was the first to be established at the CRG. Two groups (Miguel Beato and Raúl Méndez) started already in the fall of 2001 in the premises of the Universitat Pompeu Fabra, while the other three groups (Fátima Gebauer, Juan Valcarcel and Josep Vilardell) joined the CRG in the late summer of 2002, after our relocation to the present laboratories. Initially, everybody had to fight with problems associated with starting in a new institute, and jet it is surprising how fast the groups began to work productively. Within less that 6 months virtually all the positions were filled, and several senior postdoctoral fellows have been incorporated with positions from the Ramón y Cajal programme. Now the laboratories are full of postdocs (16), graduate students (15) and technicians (6). There have also been 5 visiting scientist from abroad, that, along with the 5 groups leaders, the technician of the media kitchen, and a few undergraduate students, sum up to more than 50 people.

Given the space limitations, the size of the programme cannot grow until we move to the new building. Therefore during the next two years our task is to consolidate the groups and to stimulate the collaborations and crosstalks between them at all levels. To this end we have to further develop the available instruments and, possibly, develop new ones. Apart from the Lab meetings of the individual

groups, which are often attended by scientists from related groups, the programme has established a weekly Data Seminar. Since October 2002 each Monday at noon a scientist of the programme reports in front of a large crowd on the progress of his/her projects and on future plans. Often people from other programmes also attend the data seminars. Discussion is lively, though the participation of junior scientists could and must be improved. There is also a Journal Club each Wednesday noon. These seminars have contributed much to a better communication amongst scientists and have created a feeling of being part of a common undertaken. This feeling is reinforced by other social activities, like the popular beach volley ball championship.

It is also very important that scientists of the programme interact productively with scientists from other CRG programmes as well as with scientists from the other institutions within the PRBB. Collaborations are already strong with the Bioinformatics and Genomics programme. For instance, the group of Juan Valcarcel collaborates with the group of Roderic Guigó in attempts to identify sites for alternative splicing. My own 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. In this work there is also a participation of the Complex System group of the CEXS/UPF leaded by Ricard Solé. Cooperations with other scientists from the CEXS/UPF and the IMIM are the result of the participation of CRG scientists in various networks financed by the central government and by the government of Catalonia. In addition Gene Regulation scientists are actively involved in advance Biology teaching at the UPF, in particular in the Basic Research itinerary of the fifth graduate year and in the international PhD programme on Basic Biomedical Research.

In terms of funding, the groups have been quite successful. Although the CRG guarantees funding for supplies for the first two years, all groups have already succeeded in getting grants from governmental and private sources. Given the financial volume of the usual Spanish grants, this has required a strong effort from the group leaders. However, I hope that this effort does not have a detrimental effect on their future scientific productivity.

As can be seen in the detailed reports from each group, the first papers signed by scientists from the programme have been published. In most cases, however, a large part of the experimental work in these publications still originates from the former laboratories of the group leaders or from collaborations with other groups. It is only now that results generated in the CRG start to accumulate for new publications. We hope that a few of these projects will be already published when we prepare the 2004 CRG report.

Research Groups:

- Chromatin and gene expression (Miguel Beato)
  Transcriptional regulation and chromatin remodelling (Albert Jordan)
- Regulation of alternative pre-mRNA splicing (Juan Valcárcel)
- RNA-protein interactions and regulation (Josep Vilardell)
- Regulation of protein synthesis in eukaryotes (Fátima Gebauer)
- Translational control of gene expression (Raúl Méndez)

CHROMATIN AND GENE EXPRESSION

The group initiated its research activities at CRG in 2001.

Group Leader
MIGUEL BEATO

GROUP STRUCTURE

Staff Scientist:	ALBERT JORDAN
Postdoctoral Fellows:	CECILIA BALLARÉ
	MARÍA JESÚS MELIÁ
	ANTONIO RODRÍGUEZ-CAMPOS (till November 2003)
	GUILLERMO VICENT (since July 2003)
PhD Students:	THOMAS BECHTOLDT
	VERÓNICA CALVO
	JAUME CLAUSELL
Technicians:	SUSANNA FARAUDO
	SILVINA NACHT (since July 2003)
	NORA SPINEDI
Visitors:	LUCIANA ROCHA-VIEGAS
	GRISELDA VALLEJO
	PATRICIA SARAGÜETA

SUBGROUP: TRANSCRIPTIONAL REGULATION AND CHROMATIN REMODELLING

Incorporated to CRG on January 2002, with a Ramón y Cajal appointment to the group of Miguel Beato.

Sub-Group Leader
ALBERT JORDAN

SUBGROUP STRUCTURE

PhD Students:	IGNACIO QUILES
	MÓNICA SANCHO
	ALICIA SUBTIL
Technicians:	NORA SPINEDI

SUMMARY

The Chromatin and Gene Expression group is interested in understanding the mechanism of signal transduction and gene regulation in eukaryotes and uses as experimental model gene induction by steroid hormones. Regulation of transcription by glucocorticoids and progestins and crosstalk between estrogen and progesterone receptors and other signalling pathways have been the main lines of research. 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

A. Jordan, A. Rodríguez-Campos, G. Vicent, J. Clausell-Menero, S. Faraudo, S. Nacht

The group has previously shown that the precise nucleosomal organization of the mammary tumour virus (MMTV) promoter is essential for hormonal induction, mainly because it mediates the functional synergism between the hormone receptors and nuclear factor 1 (NF1). These findings were obtained in a cell-free chromatin assembly and transcription system and have been recently confirmed in the yeast Saccharomyces cerevisiae (1, 2). In all system tested, NF1 does not require a transactivation domain for synergism with the progestrone receptor (PR) on the MMTV promoter.

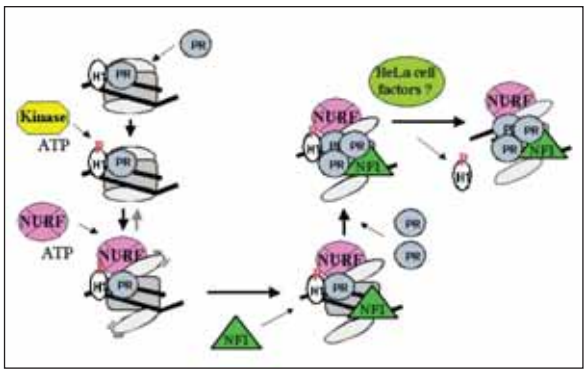


Figure 1. In preblastodermic Drosophila embryos PR recruits NURF to MMTV promoter. In other cells it may also recruit Swi / Snf-type complexes. Additional factors may be needed for H displacement.

The main finding of the last year has been the elucidation of the complex role of histone H1 in the induction process. We have shown that H1 binds asymmetrically to recombinant MMTV promoter nucleosomes, generating a more homogeneous population of positioned nucleosomes and making the DNA less accessible for nucleases and NF1 (3). However, due to its homogenous positioning, nucleosomes containing H1 exhibit a better binding of PR to the exposed HRE1 (3, 4). The same enhanced binding of PR is observed with MMTV minichromosomes containing histone H1, which are more efficiently transcribed in the presence of NF1 (5). As basal transcription and transcription in the presence of either PR or NF1 is repressed by H1, the end result is a higher synergism between the two factors (5). Incubation with PR enhances phosphorylation of H1, which has to leave the promoter prior to transcription initiation (Figure 1). This unexpected and complex role of histone H1 underlines the significance of chromatin structure in gene regulation. The main focus of this project in the immediate future is the elucidation of the structure of the activated MMTV promoter chromatin and how they are catalyzed, in particular modifications of the core histones and the role of various isoforms of histone H1 and HMG proteins.

2. Crosstalk between hormone receptors and other signalling pathways

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

In a previous collaboration with the group of Ferdinando Auricchio in Naples we found that progesterone can activate transiently the Src/Ras/Erk pathways via an interaction of PR with the estrogen receptor (ERα), and that this activation is essential for the proliferative response of breast cancer cell lines. In the last years 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 (Figure 2), and that both regions are required for progestin activation of the Src/Ras/Erk cascade (6). In the region located between ERID-I and ERID-II there is a proline cluster, which has the potential to interact with the SH3 domain of c-Src, but does not participate in activation of the Src/Ras/Erk cascade in breast cancer cells containing ERα (6). We are now trying to develop point mutants of PR with single amino acid exchanges 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 in transgenic mice.

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

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

In the context of a collaboration with the Department of Pathology od the Hospital del Mar, Barcelona, we are trying to define the patterns of genes regulated by estrogens and progestins via direct transcriptional control and via crosstalk with other signalling pathways in breast cancer cells. In addition we are investigating the interactions of PR and ER with the products of the BRCA genes and how this interaction modulates cell proliferation and apoptosis. For this type of studies we are using cDNA and oligonuleotide microarrays.

In a collaboration with the group of Adalí Pecci, 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 (7). We are now centered in understanding the molecular mechanism involved in the tissue-specificity of the apoptotic response. In collaboration with the group of Patricia Saragüeta, Buenos Aires, we are studying the reponse of endometrial stromal cells to estrogens and progesterone using cell biologial techniques and cDNA microarrays, with a focus on understanding the decidual reaction and the mechanism of blastocyst implantation.

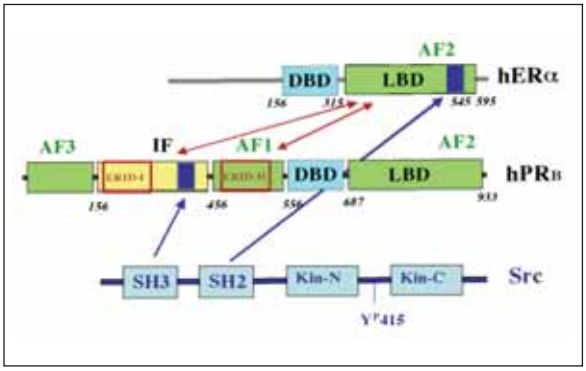


Figure 2. Proline cluster in hPR_B indicated in blue. The numers referr to the amino acid sequence. AF1 to 3 are the activation functions of the receptors. IF: inhibitory domain; DBD: DNA binding domain; LBD: ligand binding domain.

PUBLICATIONS

1. Prado F, Koop R, & Beato M. "Accurate chromatin organization of the MMTV promoter determines the nature of the synergism between transcription factors." J Biol Chem 277, 4911-4917. (2002)

2. Prado F, Vicent G, Cardalda C, & Beato M. "Differential role of the proline-rich domain of NF1-C splice variants in DNA binding and transactivation." J Biol Chem 277, 16383-16390. (2002)

3. Vicent GP, Meliá MJ, & Beato M. "Asymmetric binding of histone H1 stabilizes MMTV nucleosomes and the interaction of progesterone receptor with the exposed HRE." J Mol Biol 324, 501-517. (2002)

4. Vicent GP, Koop R, & Beato M. "Complex role of histone H1 in transactivation by progesterone receptor." J Steroid Bioche Molec Biol 83, 15-23. (2003)

5. Koop R, Di Croce L, & Beato M. "Histone H1 enhances synergistic activation of the MMTV promoter in chromatin." EMBO J 22, 588-599. (2003)

6. Ballare C, Uhrig M, Bechtold T, Sancho E, Di Domenico M, Migliaccio A, Auricchio F, & Beato M. "Two domains of progesterone receptor interact with estrogen receptor and are required for progesterone activation of the c-Src/Erk pathway in mammalian cells." Mol Cell Biol 23, 1994-2008. (2003)

7. Rocha-Viegas L, Vicent GP, Baranao JL, Pecci A & Beato M. "Steroid hormones induce bcl-X gene expression through direct activation of distal promoter P4."
J Biol Chem (in press)

Research Sub-Group

TRANSCRIPTIONAL REGULATION AND CHROMATIN REMODELLING

SUMMARY

Ovarian steroid hormones (estrogens and progestins) control growth and differentiation of breast normal and transformed epithelial cells by virtue of their interaction with specific intracellular receptors. Hormone receptors act directly in the nucleus over the chromatin organization and transcriptional activity of several cellular promoters. On the other hand, they have an indirect, less understood effect on cytoplasmic signal transduction pathways, affecting ultimately on gene expression, and consecuently on cell proliferation and apoptosis. Expression of the pS2 cellular gene responds to estrogen stimulation and is induced in about 50% of breast cancers. On the other hand, the gene for the 11ß-hydroxysteroid dehydrogenase (11ß-HSD) responds to progesterone. We aim to study the direct effects and those mediated by signal transduction pathways of hormone receptors on chromatin remodeling, transcription factors recruitment and transcriptional activity of pS2 and 11ß-HSD promoters in response to hormone stimulation. In parallel, we intend to study the Mouse Mammary Tumor Virus (MMTV) promoter, which is a model of transcriptional regulation by the progesterone receptor through chromatin changes. On the other hand, we have plans to study the Human Immunodeficiency Virus (HIV) promoter, which is stimulated through the activation of signal transduction pathways and promoter chromatin remodeling.

RESEARCH PROJECTS

1. Transcriptional regulation and chromatin remodeling of promoters responding to steroid hormones in breast cancer cells

A. Subtil, I. Quiles, N. Spinedi

We are interested on distinguishing between direct effects of nuclear hormone receptors and those mediated by signal transduction pathways on transcription of target genes. For this, we are currently constructing breast cancer-derived cell lines that express, under the control of an inducible promoter (ecdysone or Tet-OFF systems), forms of the progesterone receptor (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. Here, the MMTV promoter is used as a reporter to study the transcriptional effect of receptor variants. In addition, expression of tagged receptor will be used to perform proteomic studies of nuclear, cytoplasmatic and membrane-associated complexes containing PR in the absence or presence of hormone.

On the other hand, we have initiated the characterization of the 11ß-HSD promoter: its kinetics of hormonal-activation, identification of HRE sequences, nucleosome positioning and chromatin remodeling in response to hormones. We are going to use chromatin immunoprecipitation (ChIP) to study histone modifications, as well as the composition of associated chromatin remodeling complexes and transcriptional complexes.

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

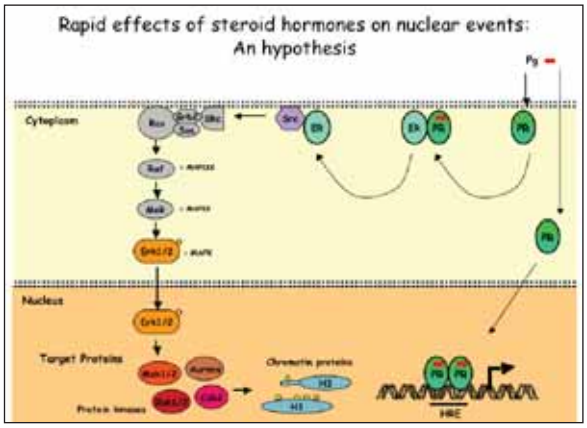
M. Sancho, A. Jordan

During the process of MMTV promoter activation by progesterone receptor, histone H1 is phosphorylated and leaves the promoter (Koop et al., 2003, EMBO

J. 22: 588). 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 remodeling, in addition to investigate H1 residues being phosphorylated and the responsible kinases. Taking advantage of these cell lines, we are also going to investigate the role of H1 variants on global gene expression by using microarrays.

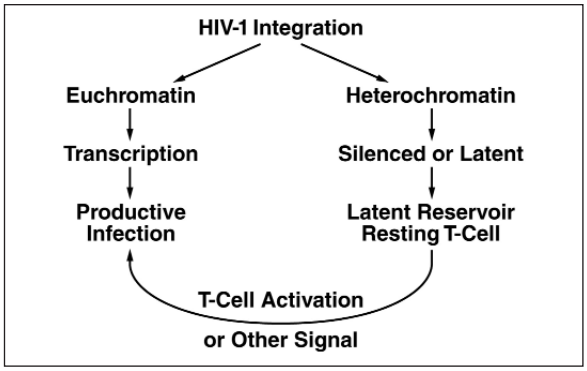
On the other hand, to go deeply into the process of MMTV activation in vivo, we have started two approaches to freeze the ordered recruitment of transcription factors and chromatin modifications induced by hormone addition: 1) RNA interference of NF1, a factor recruited to the nucleosome B of MMTV and necessary for the progress of activation; 2) integration in a breast cancer cell of MMTV promoters mutated at different regions (TATA, NF1 binding, HREs). For this, we are developing a method to integrate repeatedly different MMTV constructs into a same locus of the genome, based on the Cre/loxP technology.

A different project is to investigate the hypothetical global effects of hormonal activation on genomic chromatin. We are studying changes on H1 variants composition and phosphorylation in response to hormone treatment, in order to elucidate if H1 and chromatin are a general target of the rapid action of signal transduction pathways triggered by hormone receptor activation. We have also initiated the study of changes on histone H3 modifications (phosphorylation and acetylation).



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

HIV integrates at a multitude of sites without any clear preference in the human genome. The chromatin environment at the integration site influences the nucleosome structure of the viral promoter and consequently its basal and Tat-induced transcriptional activity (Jordan et al., 2001, EMBO J. 20: 1726), in a way that is independent of the degree of methylation of the proviral DNA (3). In this respect, we have shown that at low frequency integration occurs at regions of heterochromatin (i.e. pericentromeric) leading to promoter repression and to a state of viral latency that can be reactivated uppon T cell activiation (4). We plan to compare the chromatin structure of the proviral promoter when integrated in transcriptional-competent euchromatin or in repressed heterochromatin, as well as the involvement of H1 on promoter



activation. In addition, we plan to investigate the controversial role of glucocorticoids on HIV expression.

PUBLICATIONS

1. Uppsten M, Färnegårdh M, Jordan A, Ramaswamy S, Uhlin U. "Expression and Preliminary Crystallographic Studies of R1E, the Large Subunit of Ribonucleotide Reductase from Salmonella typhimurium." Acta Cryst D59: 1081-1083. (2003)

2. Uppsten M, Färnegårdh M, Jordan A, Eliasson R, Reichard P, Uhlin U. "Structure of the Large Subunit of class Ib Ribonucleotide Reductase from Salmonella typhimurium and its complexes with allosteric effectors." J Mol Biol 330: 87-97. (2003)

3. Pion M, Jordan A, Biancotto A, Dequiedt F, Gondois-Rey F, Rondeau S, Vigne R, Hejnar J, Verdin E, Hirsch I. "Transcriptional suppression of in vitro-integrated human immunodeficiency virus type 1 does not correlate with proviral DNA methylation." J Virol 77: 4025-4032. (2003)

4. Jordan A, Bisgrove D, Verdin E. "HIV reproducibly establishes a latent infection after acute infection of T cells in vitro." EMBO J 22: 1868-1877. (2003)

PATENTS

U.S. Patent Application No. US 2003/0157693 A1 (filled 18/12/02). "Cell lines with latent immunodeficiency virus and methods of use thereof.". Jordan A, Verdin E (The J. David Gladstone Institutes/UCSF)

REGULATION OF ALTERNATIVE PRE-mRNA SPLICING DURING CELL DIFFERENTIATION, DEVELOPMENT AND DISEASE

The lab was established in 1996 at the European Molecular Biology Laboratory (EMBL) in Heidelberg (Germany), and moved to the CRG in the summer of 2002.

Group Leader
JUAN VALCÁRCEL

GROUP STRUCTURE

Postdoctoral Fellows:	BRENDAN BELL
	CLAUDIA BEN-DOV
	DANIEL BILBAO
	JOSÉ MARÍA IZQUIERDO (until September 2003)
	VERONICA RAKER (since June 2003)
PhD Students:	MAFALDA ARAUJO
	NURIA MAJOS (since May 2003)
	LUIS SOARES
	LOUISE WOODLEY (until April 2003)
Diploma Student:	SUSANNE ESTERMAN (since October 2003)
Technicians:	CONCHI MARTÍNEZ
Visitor:	CARLES SUÑÉ

SUMMARY

We are interested in the molecular mechanisms that control alternative splicing during cell differentiation, development and disease. We study various pre-mRNAs and regulatory factors, including regulators of sex determination in Drosophila and of programmed cell death and tumor progression in mammalian cells. Several new lines of research were started after the lab moved to CRG, including the function of the Ewing's sarcoma protein (EWS) in RNA processing, the regulation of alternative splicing of the transcription factor TAF6 during apoptosis and the interplay between signal transduction pathways (e.g. Fas, TGFb signaling) and the activity of splicing regulators.

RESEARCH PROJECTS

1. Regulation of splicing and programmed cell death

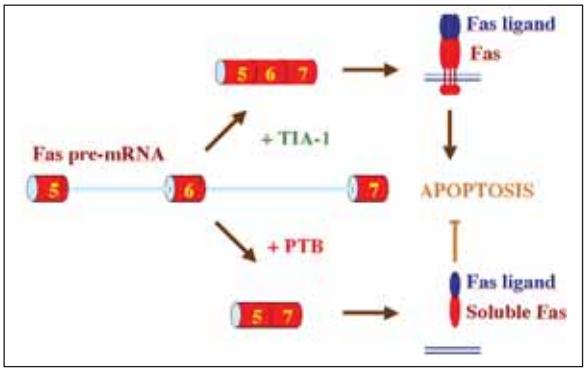


Figure 1. Regulation of Fas receptor alternative splicing. Inclusion or skipping of Fas pre-mRNA exon 6 generates mRNAs encoding, respectively, a membrane-bound form of the receptor that promotes apoptosis or a soluble form that inhibits programmed cell death. The splicing regulators TIA-1 and PTB play antagonistic roles in regulating exon 6 inclusion.

We previously reported that TIA-1, a protein that promotes apoptosis, can regulate splicing by promoting the assembly of U1 snRNP to weak 5' splice sites (ss) followed by uridine-rich sequences. We have investigated the mechanism by which TIA-1 regulates alternative splicing of the Fas receptor to generate isoforms that either promote or inhibit programmed cell death. We have found that TIA-1 and the Polypyrimidine Tract Binding protein (PTB) compete with each other for binding to a uridine-rich sequence in Fas exon 6. PTB inhibits association of the U2 Auxiliary Factor (U2AF) with the weak 3' splice site of intron 5 by interfering with stabilizing molecular interactions across exon 6. In contrast, binding of TIA-1 helps to relieve PTB inhibition and thereby promote exon 6 definition, thus revealing a function of TIA-1 distinct from its activity on 5' splice site recognition. Our results indicate that the balance between the activities of TIA-1 and PTB can be an important determinant of the control of programmed cell death (Figure 1).

Changes in the extent of exon 6 skipping occur during T lymphocyte maturation, and failure to undergo such changes results in autoimmune lymphoproliferative syndromes (ALPS). In collaboration with the lab of Joachim Roesler (Technical University Dresden), we have identified and characterized a mutation in an ALPS patient where duplication of the 3' ss AG preceding exon 6 causes complete skipping of the exon. The effect of this mutation is distinct from that of the same mutation in other pre-mRNAs, and appears to be linked to binding of a protein complex that competes with recognition of the 3' ss AG by U2AF35.

2. Function of the splicing factor U2AF and associated proteins

The splicing factor U2AF is important to initiate the assembly of splicing complexes on pre-mRNAs. The 65 Kda subunit of U2AF65 recognizes the pyridimine-rich stretch that precedes the 3' end of higher eukaryotic introns. The 35 Kda subunit recognizes the last two

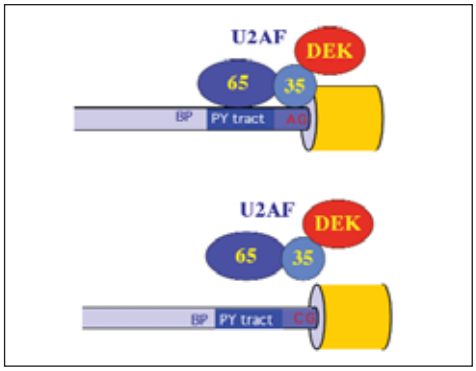


Figure 2. The protein DEK facilitates 3'splice site discrimination by U2AF. Sequences at 3'end of introns include the branch point (BP), polypyrimidine (Py)-tract and the AG dinucleotide that serves as the 3' splice site. The splicing factor U2AF consists of two subunits. The 65 Kda subunit recognizes the Py-tract, and the 35Kda subunit the AG. The protein DEK interacts with U2AF35 and enforces discrimination of the 3'splice site AG over other sequences (e.g. CG) by U2AF.

intronic nucleotides (AG). Our recent results indicate that the protein DEK, previously implicated in transcription and some forms of leukemia, can modulate the recognition of the 3' ss by U2AF (Figure 2).

Another U2AF65-associated factor, LUCA-15, was reported to have functions in tumor suppression and programmed cell death. We have identified genes involved in the control of these processes whose alternative splicing is regulated by LUCA-15, and have also observed differential modulation U2AF binding by LUCA-15 on different types of 3' ss.

3. Analysis of alternative splicing using DNA microarrays

In collaboration with Angela Relogio and Vladimir Benes at the EMBL Genomic Core Facility, oligonucleotide-based DNA microarrays were developed for gene expression profiling of alternatively spliced transcripts. These have been applied to a cell culture model of Hodgkin lymphoma. When different cell lines corresponding to different stages of progression of Hodgkin's lymphoma were studied, changes in the expression of splicing regulators and specific spliced isoforms correlated with tumor grade (Figure 3). These observations suggest that relevant events in tumor biology are based on regulation of expression of alternatively spliced isoforms. Changes in expression of some splicing regulators, including ectopic expression of neuron-specific RNA binding proteins of the NOVA family, as well as the increase in expression of the cdk2 substrate and U2 snRNP component SAP155, have been independently verified at the RNA and protein level. Our aim now is to use these data to obtain insights into the mechanisms underlying changes in alternative splicing involved in tumor progression.

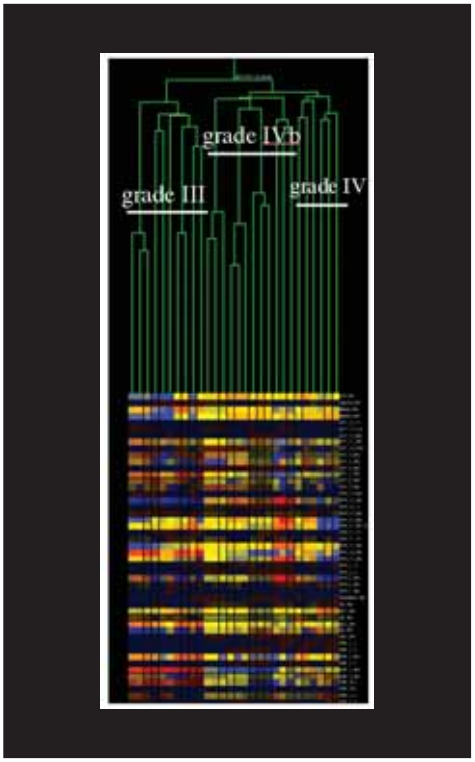


Figure 3. Microarray analysis of alternative splicing of RNAs from Hodgkin cell lines obtained from tumors at different stages of progression. Clustering analyses indicate that alternative splicing can be used as criteria to group the cell lines according to the stage of the tumor. This suggests a role for alternative splicing in tumor progression.

PUBLICATIONS

1. Förch P, Puig O, Martínez C, Séraphin B & Valcárcel J. "The splicing regulator TIA-1 interacts with U1-C to promote U1 snRNP recruitment to 5' splice sites." EMBO J 21, 6882-6892. (2002)

2. Förch P, Merendino L, Martínez C & Valcárcel J. "The U2 Auxiliary Factor U2AF65 can promote U1 snRNP recruitment to 5' splice sites." Bio J 372, 235-240. (2003)

3. Ortega A, Niksic M, Bachi A, Wilm M, Sánchez L, Hastie N, & Valcárcel J. "Biochemical function of Female-Lethal(2)D / Wilms' Tumor Suppressor-1 Associated proteins in alternative PRE-mRNA splicing." J Biol Chem 278, 3040-3047. (2003)

4. Förch P & Valcárcel J. "Splicing regulation in Drosophila sex determination." Prog Mol Subcell Biol 31, 127-151. (2003)

5. Banerjee H, Rahn A, Gawande B, Guth S, Valcárcel J & 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. (in press)

The publications above correspond to work carried out by the group while still at EMBL.

6. Bilbao D and Valcárcel J. "Getting to the heart of a splicing enhancer". Nature Struct Biol 10, 6-7. (2003)

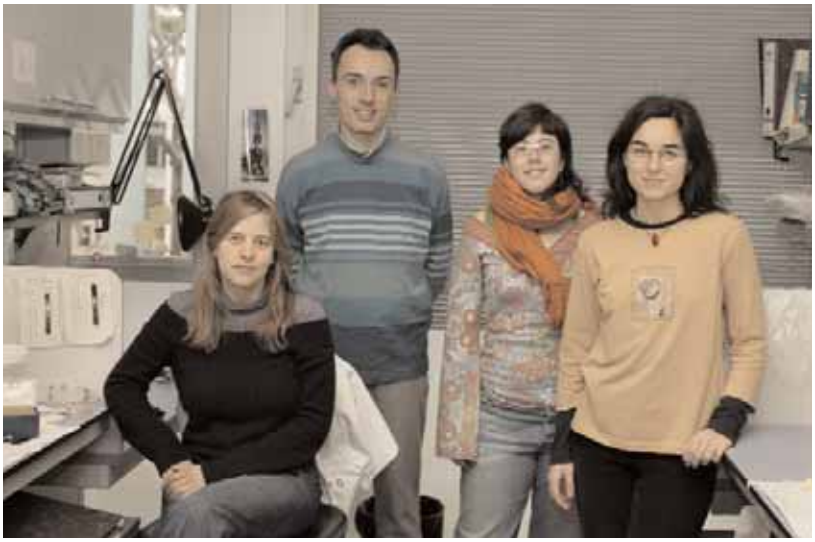
RNA-PROTEIN INTERACTIONS AND REGULATION

The group was created on June 2002, just after the CRG moved to its present location.

Group Leader
JOSEP VILARDELL

GROUP STRUCTURE

PhD Students:	MIREIA BRAGULAT
	SARA MACIAS
Technician:	JUDIT PEIX



SUMMARY

We are focused on the study of the molecular mechanisms of regulation of gene expression by RNA-protein interactions, using the model organism Saccharomyces cerevisiae 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 the environ of the 5' splice site (ss) can affect spliceosome assembly and splicing.

RESEARCH PROJECTS

1. Genetic screen to select mutants in regulation of splicing

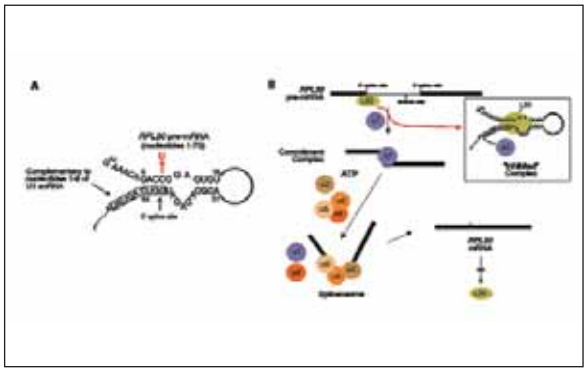
M. Bragulat

In order to understand how L30 inhibits splicing a genetic approach is being set up, with the use of two reporter genes (CUP1, HIS3, or LacZ) 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. We are using the T9 mutation (see figure) to predict the phenotype of the mutants we hope to select. In this approach, the lack of regulation will be needed for viability. We pursue the identification of activities involved in regulation of the splicing machinery. A first trial has produced already a number of mutants that preliminary data show as lacking regulation. They are being further characterized, while new screenings are being set.

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 approaches and biochemical purification procedures will be followed. We are pursuing psoralen cross-linkings with different synthetic RNAs, including an RPS17A – derived transcript as a positive control. For the biochemical purification we are following a TAP based approach, using the L30 orthologue from the archaebacterium Sulfolobus acidocaldarius, which can not be incorporated into yeast ribosomes but can regulate RPL30 splicing in vivo.



3. Sequence elements in the 5' splice site of RPL30

J. Peix, J. Vilardell

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. How then U4A can 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 suggest that the interaction between positions 3 and 4 of the 5' end of yeast introns is important in splicing.

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. 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.

PUBLICATIONS

1. Huang T, Vilardell J & Query, CC. "Pre-spliceosome formation in S.pombe requires a stable complex of SF1-U2AF^{59}-U2AF^{23}." EMBO J. 21 (20) 5516-5526. (2002)

REGULATION OF PROTEIN SYNTHESIS IN EUKARYOTES

Group Leader
FÁTIMA GEBAUER

GROUP STRUCTURE

Postdoctoral Researcher:	RAFAEL CUESTA
PhD Students:	IRINA ABAZA
	SOLENN PATALANO
Technician:	OLGA COLL

SUMMARY

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 the 5' and 3' UTRs of msl-2 pre-mRNA, which ultimately results in inhibition of msl-2 mRNA translation. During my stay in Matthías Hentze lab at EMBL and in collaboration with Marica Grskovic, we have found that translational repression requires additional factors that are nucleated by SXL in the 3´UTR of msl-2. After my establishment at CRG, we have isolated and identified a putative co-repressor. Experiments are ongoing to confirm its role in SXL-mediated translational regulation.

2. Translational regulation of early embryonic patterning in Drosophila

The establishment of the early antero-posterior and dorso-ventral axes in Drosophila is achieved by a series of translational control events affecting key patterning regulators. Two of these are the homeodomain protein Bicoid and the transmembrane receptor Toll. Expression of both Bicoid and Toll is activated at specific times in development by cytoplasmic polyadenylation of their respective maternal mRNAs. In order to study the translational regulation of Bicoid and Toll mRNAs, we have set up a cell-free system that recapitulates both cytoplasmic polyadenylation and translation of these transcripts. We are currently using this system to identify the cis-acting regulatory sequences responsible for translational control.

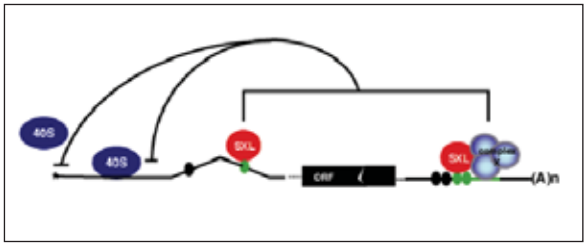


Figure 1. Translational repression by SXL. SXL binds to specific sites in both the 5´and 3´UTRs of msl-2 mRNA and recruits co-repressors to the 3´UTR in order to inhibit the stable association of the small ribosomal subunit with the mRNA.

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

$p27^{kip}$ is a cyclin-dependent kinase (cdk) inhibitor that blocks the mammalian cell cycle in G1 by binding to cyclin E/cdk2. Proper modulation of $p27^{kip}$ levels is essential for cell proliferation. One of the mechanisms that regulate the level of $p27^{kip}$ is the translational control of its mRNA. Translation of $p27^{kip}$ mRNA is driven by an internal ribosome entry site (IRES) whose activity changes during the cell cycle. Our aim is to identify factors that specifically regulate $p27^{kip}$ mRNA translation and that could be used as therapeutic targets in oncology. Our first approach consists of studying $p27^{kip}$ mRNA translation in cells (and cell extracts) synchronized in G1, S and G2/M.

PUBLICATIONS

Publications during this year are the result of previous work in Matthias Hentze´s lab (European Molecular Biology Laboratory, EMBL)

2. Bergamini G & Gebauer F. "Poly(A)-dependent cell-free translation systems from animal cells." In: Cell-free translation systems. Alexander S. Spirin (Ed.) Springer-Verlag Berlin Heidelberg, pp.79-88. (2002)

3. Grskovic M, Hentze MW & Gebauer F. "A co-repressor assembly nucleated by Sex-lethal in the 3´UTR mediates translational control of Drosophila msl-2 mRNA." EMBO J. 22: 5571-5581. (2003)

4. Gebauer F, Grskovic M & Hentze MW. "Drosophila sex-lethal inhibits the stable association of the 40S ribosomal subunit with msl-2 mRNA." Mol Cell, 11: 1397- 1404. (2003)

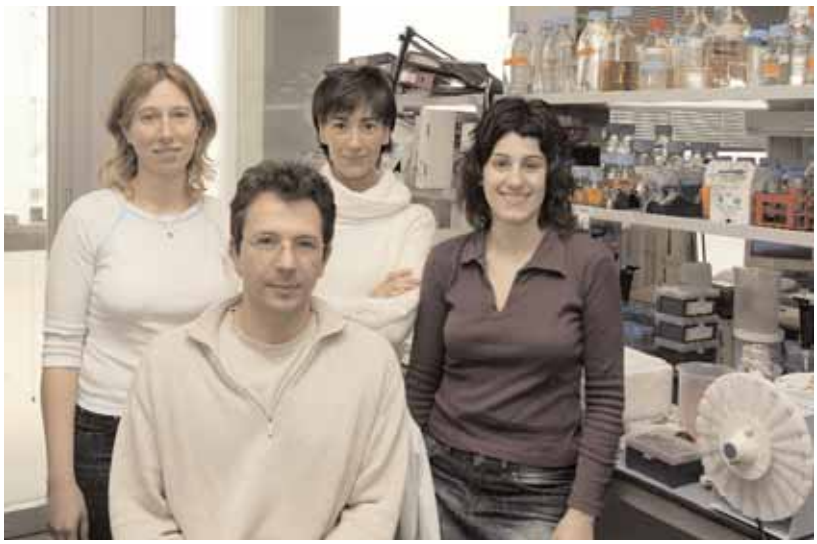
TRANSLATIONAL CONTROL OF GENE EXPRESSION

Our group was constituted in June 2002.

Group Leader
RAÚL MÉNDEZ

GROUP STRUCTURE

Postdoctoral Fellows:	MARÍA PIQUÉ
	JOSÉ MANUEL LÓPEZ
	ISABEL NOVOA (Ramón y Cajal, since June 2003)
Students:	CAROLINA ELISCOVICH (since May 2003)
	EULÀLIA BELLOC (since June 2003)



SUMMARY
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. Early development is programmed, at least in part, by maternally inherited mRNAs. These mRNAs are not translated en masse at any one time, or even at any one place - rather, their translation is specifically regulated by sequences located at the 3´-untranslated region (3´-UTR) of the mRNA and their binding proteins. Cytoplasmic polyadenylation is one the most important mechanisms for regulating translation during meiotic progression. We take a biochemical and molecular biological approach to identify the sequences and factors that control polyadenylation-induced translation in Xenopus oocytes.

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.

Vertebrate development is directed by maternally inherited mRNAs that are synthesized and stored during the very long period of oogenesis. Many maternal mRNAs are dormant in oocytes, and their mobilization into polysomes does not occur until very specific times during meiosis and early development (reviewed in Mendez and Richter, 2001, Nat Rev Mol Cell Biol 2: 521-529).

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 instigation of polyadenylation by this protein requires Aurora kinase, which is activated soon after oocytes are exposed to progesterone. Aurora phosphorylates CPEB serine 174 (Mendez et al., 2000, Nature 404: 302-307), which increases the affinity of CPEB for the cleavage and polyadenylation specificity factor (CPSF) (Mendez et al., 2000, Mol Cell 6: 1253-1259). CPSF, in turn, binds to the AAUAAA sequence, an interaction that is probably stabilized by CPEB, and recruits poly(A) polymerase (PAP) to the end of the mRNA (Mendez et al., 2000, Mol Cell 6: 1253-1259) (Fig 1).

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, (Stebbins-Boaz et al., 1999, Mol Cell 4: 1017-1027), a protein that interacts with CPEB as well as the cap binding protein eIF-4E.

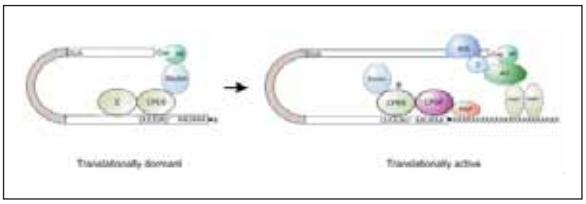
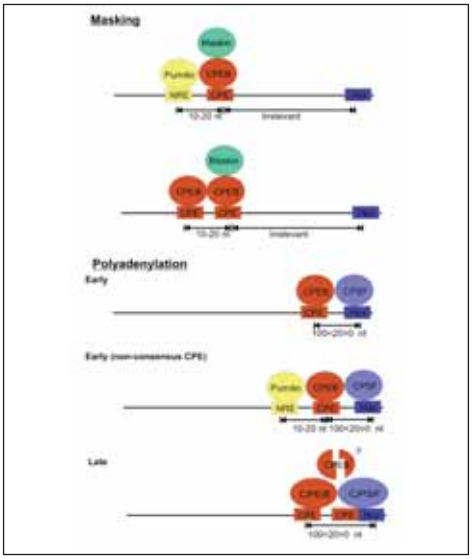


Figure 1
However, not all CPE-containing mRNAs are activated at the same time during cell cycle and polyadenylation is temporally regulated at several phases during meiosis and the embryonic development. Although phosphorylation of the trans-acting factors above described is partially responsible for the sequential activation of mRNA translation, this process is not yet well understood.



RESEARCH PROJECTS

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

To this end, we have performed extensive mutational analysis of the 3'-UTRs of the mRNAs encoding for the cyclins B1, B2, B3, B4 and B5.

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 (Fig. 2) 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 of Xkid and TPX2 mRNAs and its 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 (Groisman et al., 2000, Cell 103: 435-447).

Xkid chromokinesin and TPX2 are required for chromosome alignment and the organization of spindle poles and encoded by mRNAs containing putative CPEs.

Given the expression and localization profiles of Xkid and TPX2 as well as the phenotypical similarities between interfering CPEB activity and the activities of Xkid and TPX2 we hypothesize that CPEB could regulate temporarily and spatially the expression of both factors. Indeed, we have demonstrated that the 3'-UTRs of the mRNAs encoding for Xkid and TPX2 are cytoplasmically polyadenylated and mediate CPEB-regulated translation. In addition, the time and extent of activation is what we had predicted, based on the model depicted in figure 3, according to the distribution of NREs and CPEs in their 3'-UTRs. We are currently investigating whether the 3'-UTRs of Xkid and TPX2 mRNAs mediate spindle localized translation.

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 sequence of the cDNAs derived from this screening revealed that all the selected RNAs contained an hexanucleotide and at least one CPE, either consensus or non-consensus. Moreover, the direct testing of few random examples of the selected RNAs confirmed their cytoplasmic polyadenylation when tested in vivo, indicating that the method was very selective. Overall, we have obtained about 2000 different mRNAs that are translationaly regulated by cytoplasmic polyadenylation during meiosis. We have already sequenced some of them and we are in the process of sequencing all of them. Now that we have identified mRNAs that are polyadenylated during meiosis, our next step is to determine which of these mRNAs are required for cell cycle progression by injection of antisense RNA-pools.

Recently, "somatic" isoforms of CPEB have been identified in mice and It has been shown that cyclin B1 mRNA is polyadenylated in M phase and deadenylated in S phase (Groisman et al., 2002, EMBO J 21: 1833-1844). We are developing a screening to isolate mRNAs that are polyadenylated at any specific phase of the cell cycle in somatic cells.

4. Translational control of p53 mRNA in response to DNA damage

Irradiation of cells leads to a rapid increase in p53 protein biosynthesis even in the presence of transcriptional inhibition, suggesting that p53 biosynthesis is regulated at the translational level. p53 mRNA must be stored in an inactive state in actively dividing cells and translationaly activated during cell cycle arrest or in response to genotoxic agents, when p53 is expressed. However, cells derived from patients with acute myelogenous leukemia (AML) fail to activate p53 mRNA translation in response to χ-irradiation, suggesting that an element(s) implicated in the translational control of the mRNA encoding for p53 is defective.

This far, we have developed a model system of cultured cells where we detect endogenous p53 mRNA associated with polysomes (i.e., translationaly active) in response to MMS-induced DNA damage. Under these conditions, other cellular mRNAs are translationaly inactive and the cells are in the process of undergoing apoptosis, suggesting that indeed p53 mRNA is differentially translated under stress conditions. This translational control appears to be mediated by CPEB since the 3'-UTR of human p53 mRNA can mediate cytoplasmic polyadenylation and translational activation in response to CPEB phosphorylation when tested in *Xenopus* oocytes.

PUBLICATIONS

1. Mendez R, Barnard D & Richter JD. "Differential mRNA translation and meiotic progression require cdc2-mediated CPEB destruction." EMBO J. 21:1833-1844. (2002)

2. Groisman I, Huang YS, Mendez R, Cao Q & Richter JD. "Translational control of embryonic cell division by CPEB and Maskin". The Ribosome Vol. 66. LXVI 345-35. Cold Spring Harbor laboratory press. (2002)

3. Mendez R & Wells D. "Location, location, location: translational control in development and neurobiology". TRENDS in cell biol. 12(9): 407-409. (2002)

4. Sarkissian M, Mendez R & Richter JD. "Progesterone and Insulin Stimulation of CPEB-Dependent Polyadenylation is Regulated by Aurora A and Glycogen Synthase Kinase-3". Genes & Dev. 18: 48-61. (in press)

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

DIFFERENTIATION AND CANCER

Since its inception in 2002, the Programme has grown to host four groups, with Luciano di Croce joining last, at the beginning of 2003.

Research Groups:

- Hematopoietic differentiation and stem cell biology (Thomas Graf & Florencio Varas)
- Myogenesis (Pura Muñoz)
- Regulation of gene expression by NFAT5 during the immune response (Cristina López Rodríguez)
- Epigenetic events in cancer (Luciano Di Croce)

Considering that the individual laboratories were mostly engaged with ordering material and setting up their labs during the first half of 2003, it is not surprising that the science has not taken any major turns since the original description of the work in the inaugural CRG brochure (October 2002). All four groups work with cell lines and with mice, sharing their expertise in various technologies. Group leaders, postdocs and students now actively participate in the work in progress seminars of the Programme and in the Journal Club, organized by the Gene Regulation Programme. In addition, there are individual group seminars.

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 differentiation of hematopoietic cells.

HEMATOPOIETIC DIFFERENTIATION AND STEM CELL BIOLOGY

This group is still in between Albert Einstein College of Medicine, New York and CRG

Group Leader
THOMAS GRAF

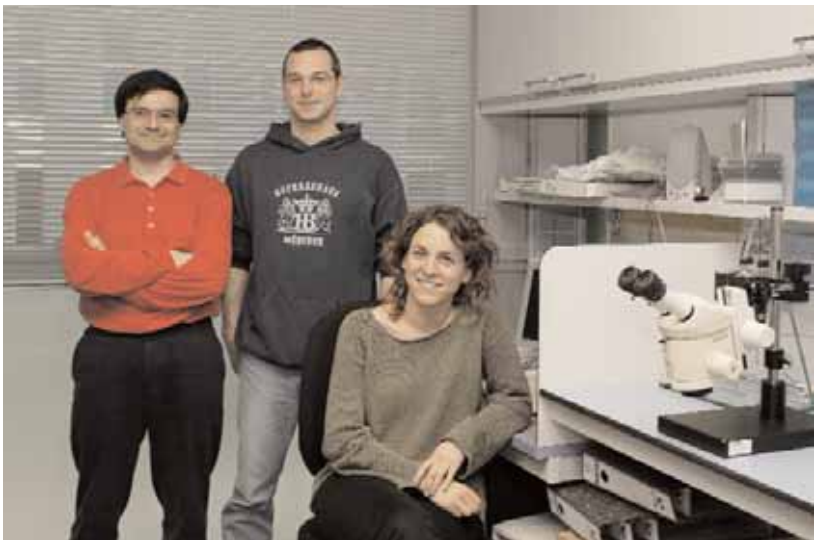
GROUP STRUCTURE

At Albert Einstein:

Postdoctoral Fellows:	MINYE
	RU FENG
	ANDRES CASTELLANOS
PhD Students:	HUAFENG XIE
	MATTHIAS STADTFELD
	CATHY LAIOSA
Technicians:	JINGHANG ZHANG

At the CRG:

Postdoctoral Fellows:	FLORENCIO VARAS
	ALEXIS SCHUBERT
Technicians:	LUISA IRENE DE ANDRÉS



SUMMARY

Broadly speaking we are interested in the pathways of blood cell differentiation and the role of transcription factors as well as in their ability to reprogram differentiated cells. We are also are interested in modeling fusion oncoprotein-induced leukemias in mice, and in particular, and in determining whether these proteins modulate the differentiation status of their target cells.

RESEARCH PROJECTS

1. Lineage priming in hematopoietic stem cells

Early models of hematopoiesis implied that hematopoietic stem cells are 'naïve' in that they express no lineage specific genes until commitment sets in. This view was shaken when it was shown that multipotent progenitor cell lines and enriched hematopoietic stem cells (HSCs) express combinations of myeloid and erythroid genes, such as lysozyme, myeloperoxidase and hemoglobin, at low levels. While highly suggestive, these previous studies suffered from the difficulty in assigning a homogeneous degree of purification to the stem cell population in question. Thus, it was always possible that the 30%, or so, of the purified stem cell population that contained transcripts for say, lysozyme, did not have actual functional stem cell activities, and that indeed this fraction was marked for terminal differentiation. To study this we used a Cre recombinase approach that allowed us to irreversibly label all cells that actually express lysozyme or expressed it at an earlier time in development.

The main conclusion from these studies is that fully functional stem cells can express the lysozyme gene, thus extending the 'lineage priming' concept to bona fide stem cells. They also indicate that lineage priming is a selective process, since this phenomenon was not found for two lymphoid genes studied. Our experiments raise the question whether the promiscuous expression of differentiation specific genes in stem cells is due to lineage specific transcription factors or to transcription factor-independent mechanisms.

2. Reprogramming hematopoietic cells by enforced transcription factor expression

An open question is which transcription factors determine whether a multipotent hematopoietic progenitor acquires either a lymphoid or a myeloid fate, a dichotomy occurring very early within the blood cell hierarchy. In attempts to answer this question we have chosen the approach used in our earlier work with chicken cell lines. This approach consists in the reprogramming of already differentiated ('committed') blood cells by enforced expression of specific transcription factors. Because work by others had shown that pro B cell lines can be converted into macrophages through activation of the Ras/Raf pathway, we chose to study B lymphocyte precursors from the bone marrow (here called 'B cells') by infecting them with retroviruses encoding transcription factors that are known to regulate macrophage specific genes.

We found that the bZip type transcription factor C/EBPα effectively induces a switch of these cells towards functional macrophages, and that PU.1 (an ETS family factor) can do this also, but weakly. Enforced expression of C/EBPα in a PU.1$^{-/-}$ pro-B cell line induced a rapid downregulation of CD19 without upregulation of Mac-1, suggesting that the two processes can be uncoupled and that myeloid gene activation requires endogenous PU.1. This is supported by the finding that co-expression of C/EBPα and PU.1 cooperated in inducing a switch in nearly all B cell precursors and that Mac-1

expression could be induced in double infected NIH3T3 fibroblasts. Downregulation of CD19 was found to be a consequence of PAX5 inactivation since C/EBPα, but not PU.1 or FOG, inhibited PAX5 induced activation of luciferase expression from a CD19 promoter reporter construct. These results demonstrate that B lineage cells can readily be reprogrammed to become macrophages by enforced expression of C/EBPα, leading to a remodeling of the B cell specific transcription factor network by two complementary mechanisms, one of which requires endogenous PU.1.

3. 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 again ‘translate’ expression of a hematopoietic restricted gene into an irreversible change in the DNA, again using the 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 revealed that essentially all hematopoietic stem cells express EYFP, while few tissues outside the blood cell system are labeled. Our current work focuses on endothelial cells, hepatocytes and skeletal muscle cells.

4. Creating a mouse model to study megakaryocyte differentiation and function

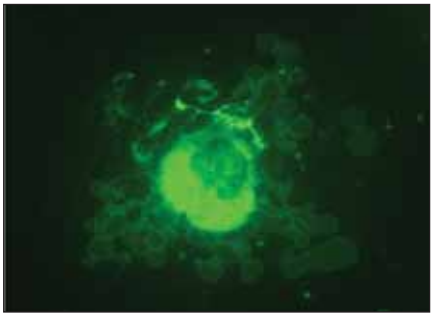
A poorly studied, but clinically important, lineage of blood cells are the one comprising megakaryocytes/platelets. To study this lineage we have introduced the EYFP gene into the platelet specific marker gene, GpIIB by homologous recombination. In this mouse, megakaryocytes and platelets are EYFP labeled, while no cells from other lineages express the reporter gene (Fig.1). To explore the lineage relationships between this lineage with myeloid and erythroid cells we crossed the line with the lysozyme EGFP and the globin ECFP mice that we developed earlier, yielding a mouse in which three lineages can be distinguished by their fluorescent colors.

5. T cell leukemogenesis induced by LMO2

Retroviruses are known to integrate within the viral 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. In collaboration with Christof von Kalle (Children’s Hospital, Cincinnati, Ohio), who discovered this, and Harris Goldstein (Albert Einstein College of Medicine), who developed a mouse model for human hematopoietic cells, we are now trying to model the leukemia in mice by expressing LMO2 and the gamma chain with retrovirus vectors.

6. Retrovirus insertions as a tool to discover genes that accelerate stem cell growth

Recent reports support the notion that the retroviral insertion sites into infected host cell DNA is less random then 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. This may constitute one of the contributing factors to the development of leukemias



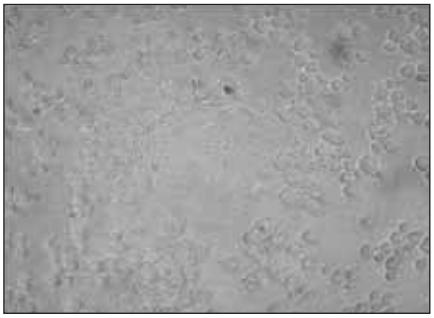


Fig.1. A, fluorescent microscopy image of a megakaryocyte labeled with membrane targeted EYFP. B, the same field, showing a bright field image

by insertional mutagenesis. To study whether retroviral insertions alter the repopulation potential of hematopoietic stem cells we will map retroviral integration sites in infected and transplanted stem cells.

PUBLICATIONS

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1. Graf T. "Making eosinophils through subtle shifts in transcription factor expression." J Exp Med 195, 43-47. (2002)

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3. Stadtfeld M, Varas F & Graf T. "Imaging fluorescent protein-labeled cells: A time-lapse approach to study hematopoietic differentiation." In: Developmental hematopoiesis, Humana Press (M. Baron, ed.) (in press)

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 Leader
CRISTINA LÓPEZ-RODRÍGUEZ

GROUP STRUCTURE

Technicians: ANA MARINA MOSQUERA

SUMMARY

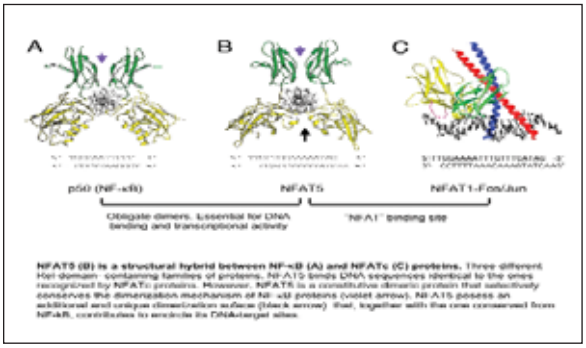
Our major interest is to elucidate the mechanisms by which transcription factors regulate gene expression. We use primarily immune cells as a model system to analyze the transcriptional control of cellular growth, proliferation and differentiation and how disturbance of these basic cellular processes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

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-kappaB) 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-kappaB). 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

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2. Jauliac S, López-Rodríguez C, Shaw LM, Rao A & Toker A. "The role of NFAT transcription factors in the regulation of integrin-mediated carcinoma invasion". Nature Cell Biol 4: 540-544. (2002)

MYOGENESIS

Our group started at CRG December 2002.

Group Leader
PURA MUÑOZ CÁNOVES

GROUP STRUCTURE

Postdoctoral Fellows:	MÒNICA SUELVES
Students:	FREDERIC LLUÍS
	BERNAT BAEZA-RAJA
	BERTA VIDAL
	VANESSA RUÍZ
Technicians:	MERCÈ JARDÍ
	ERIKA SERRANO

SUMMARY

The main interest of our group is to elucidate the mechanisms controlling myogenesis in vitro and in vivo, with an emphasis in skeletal muscle regeneration and inherited myopathies. Myogenesis is largely controlled by the basic helix-loop-helix (bHLH) family of muscle regulatory transcription factors (MRFs), including MyoD, Myf5, myogenin and MRF4, and by the myocyte-enhancer factor-2 (MEF2) family of proteins, which regulate the expression of muscle-specific genes, 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, continuing a research line initiated a few years ago, we are analysing signal transduction/trancription mechanisms activated in response to genotoxic stress. In summary, our laboratory is pursuing three main lines of research:

I. Signal transduction/transcription mechanisms controlling skeletal muscle differentiation in vitro.
II. Role of the plasminogen activation system in muscle regeneration and in inherited myopathies.
III. Mechanisms regulating the cellular response to genotoxic stress. To achieve these goals, we are using a combination of molecular, biochemical, cellular and transgenic mouse strategies.

RESEARCH PROJECTS

1. Myogenic regulatory factors (MRFs) as substrates for p38 MAPK

We have demonstrated that MRF4 binds p38 and is in vitro and in vivo phosphorylated by this kinase. We have identified serines 31 and 42 as p38 phosphorylation sites in MRF4, and demonstrated the functional consequences of MRF4 modification by p38 in myogenic differentiation.

We have demonstrated a biochemical cross-talk between p38 and NF-kB signaling pathways in myogenesis in vitro, being NF-κB a downstream affector of p38 during myoblast differentiation.

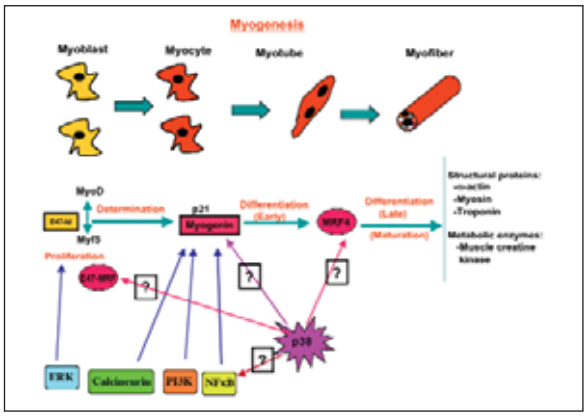

Figure 1. Possible phosphorylation targets of p38 MAPK that may account for the key role of this MAPK in myogenic differentiation.

2. Role of the plasminogen activation (PA) system in skeletal muscle regeneration in vivo

We had observed that the regeneration defect in uPA (urokinase plasminogen activator)-deficient mice was accompanied by a decreased recruitment of blood-derived monocytes and lymphocytes to the site of injury. We performed bone marrow transplantation experiments and observed that the restoration of the

normal inflammatory response in uPA-deficient mice results in the rescue of the muscle degeneration defect.

In contrast to uPA-deficient mice, we found that the onset of muscle regeneration following injury in plasminogen activator-inhibitor-1 (PAI-1)-deficient mice is more advanced than in wild-type mice, suggesting that the absence of PAI-1 is beneficial for skeletal muscle regeneration in vivo. The mechanisms underlying PAI-1 function in muscle regeneration are being analyzed at present.

To further analyze the function of the PA system in skeletal muscle regeneration, we are isolating satellite cells (skeletal muscle precursor cells) from single muscle fibers of wild type and PA-deficient mice. The differences in proliferation, fusion and differentiation rates among the satellite cell cultures from the different genotypes will be indicative of the role of each factor in myogenesis.

3. Role of the PA system in skeletal dystrophinopathies

We have generated double-mutant mice for uPA and mdx (the mouse model for Duchenne Muscle Dystrophy or DMD). mdx/uPA$^{-/-}$ mice show increased mortality and severe muscle degeneration, indicating that uPA is required for the ongoing regeneration of the mdx mouse. Importantly, defibrinogenation of mdx/uPA$^{-/-}$ mice restored the regeneration defect, demonstrating an inhibitory role for fibrin in mdx muscle regeneration.

4. Mechanisms regulating PA gene expression during the cellular response to genotoxic stress

Genotoxic agents such as ultraviolet (UV) light irradiation and alkylating carcinogens such as MNNG cause toxic and mutagenic DNA lesions, which eventually lead to cell killing. These agents also induce a cell-protective response by inducing the expression of DNA repair/transcription-related genes. We have previously shown that MNNG induces phosphorylation of p53 at Ser15, resulting in the stabilization of the protein, and in the induction of PAI-1 gene expression. We have now extended these studies by identifying ATM and ATR as the kinases responsible for p53 serine 15 phosphorylation in response to MNNG, and elucidated a signal transduction/transcription pathway for the activation of PAI-1 gene expression by alkylating carcinogens.

PUBLICATIONS

1. Doctoral Thesis presented by Maribel Parra at CRG (April 2003). Title: Regulation of uPA and PAI-1 gene expression by genotoxic agents. Thesis supervisor: Pura Muñoz-Cánoves. Maribel Parra is presently a Postdoctoral fellow at UCSF.

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EPIGENETICS EVENTS IN CANCER

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

Group Leader
Luciano Di Croce

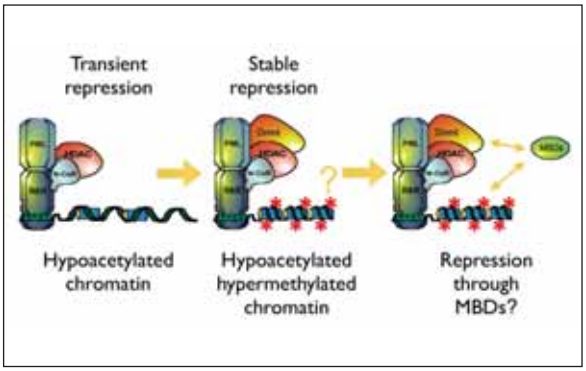
GROUP STRUCTURE

Postdoctoral:	Marcus Buschbeck
PhD Students:	Lluis Morey
	Raffaella Villa
Technicians:	Arantxa Gutierrez

SUMMARY
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

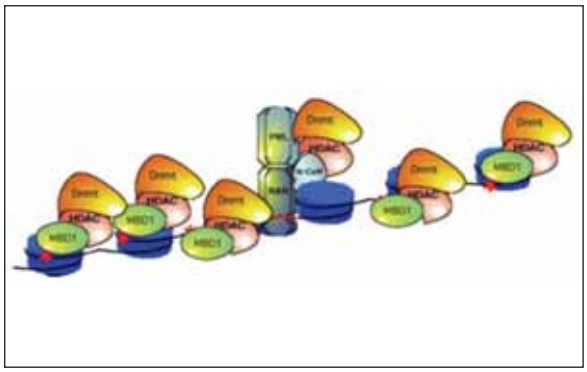
1.Biochemical link(s) between DNA methylation and transcriptional silencing



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-RARα 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-RARα 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-RARα hematopoietic differentiation block.

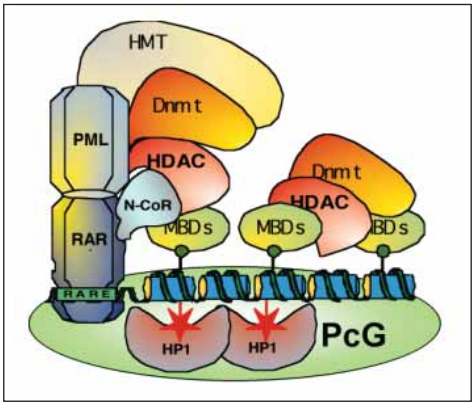
2. Role of epigenetic modification in cancer

We are also investigating the regulation of several PML-RAR target genes (such as p21, RARα, 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-RARα (de-)regulates gene transcription, and will allow us to discriminate which drugs (among several available) are more appropriate for a given set of repressed genes.



3. Histone tail modification and heterochromatin

Heterochromatin DNA is characterized by the presence of both a "closed" chromatin conformation and the presence of the Polycomb group (PcG) of proteins.



The recruitment of the PcG protein complexes, as well as their contribution to cancer progression, is also investigated, in collaboration with 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

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2. Di Croce L & Pelicci PG. "Tumour-associated hypermethylation: silencing E-cadherin expression enhances invasion and metastasis". Eur J Cancer (2003)

3. Koop R, Di Croce L & Beato M "Histone H1 enhances synergistic activation of the MMTV promoter in chromatin". EMBO J (2003)


SALA MICROSCÓPIA

Coordinator: Xavier Estivill

GENES AND DISEASE

With most of the human genome sequenced and with a large amount of information on the variability of the human genome in hand, the next step in the analysis of human genetic disorders consist of the identification of the function of genes with a potential important role in human disorders, the study of sequence and genomic variability, the phenotypic characterization of models of human disease, and the development of therapeutic approaches to cure diseases. The Genes and Disease (G&D) Programme has the goal to cover these topics for some disorders.

RESEARCH GROUPS:

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

Several disorders are studied by the five groups from different points of view. These include Down syndrome, anxiety disorders, eating disorders and hearing impairment. Specific work is focused on the understanding of the role of genes and proteins that are important for several phenotypic traits of Down syndrome. These include DYRK1A (Drosophila minibrain homolog), a serine/threonine kinase involved in neuronal development; DSCR1 (Down syndrome candidate region 1 or calcipressin 1), an inhibitor of calcineurin-mediated signaling pathways), BACE2 (ß-site APP cleaving enzyme 2), an aspartyl protease with APP ß-secretase activity, and TAB2-BP (TAB2 binding protein), a mediator of IL-1 signaling pathways, among other genes. For several genes and proteins functional studies have been developed, and a considerable amount of information has been generated by the groups of Susana de la Luna and Mariona Arbonés. The

understanding of the function of these genes and their potential implication for Down syndrome phenotypic consequences can also be addressed by modifying the levels of their expression at the cellular level in vitro, or in murine models in vivo. It is expected that those genes that are dosage sensitive will have critical consequences that can be explored by different phenotypical measures. Several groups of the programme have developed and evaluated murine models that either overexpress or disrupt human chromosome 21 genes. The group of Mariona Arbonés has generated murine models that have the Dscr1 and the Dyrk1A genes disrupted. Several interesting phenotypic consequences have been observed in these murine models, some of which give attractive insight into the potential function of the respective proteins and their role in Down syndrome. Cristina Fillat and Mara Dierssen have developed and analyzed several murine models that overexpress Down syndrome genes, in particular Dyrk1A and Bace2, showing features that reproduce some of the abnormalities that are present in Down syndrome patients. These models are being used for the development of therapeutic approaches that could correct some of the features involved in the mental retardation and other abnormalities. Similarly, for genes potentially involved in anxiety disorders, a murine model overexpressing the neurotrophin type 3 receptor (NTRK3) has been generated. The biochemical, neuropathological and behavioral characterization of TgNTRK3 indicates that increased dosage of NTRK3 alters synaptic plasticity in the LC by changing local trophic support, and produces abnormal function of catecholaminergic neurotransmitter systems leading to anxiety. Research in gene therapy by the group of Cristina Fillat is focused on pancreatic cancer, Down syndrome and Wiskott-Aldrich syndrome. The group is also working in the development of efficient gene delivery approaches for the local or systemic production of therapeutic proteins. The Gene Therapy group coordinates a network of groups of Catalonia that work in gene therapy. The group of Xavier Estivill is focusing efforts in the analysis of sequence and genomic variants of the human genome that could participate in the predisposition to common human disorders. The filed of analysis also involve the identification of genomic changes that have occured recently in the evolution of the human and other primates genome, which could have an important role in human behavior and psychiatric disease. Efforts of the programme have also been focused in setting up facilities for mouse behavioral studies. The group of Mara Dierssen has developed the technological facilities and scientific skills to perform neurobehavioral characterization of murine models developed in the programme and by other investigators. This group has developed strong links with other investigators at the UPF (Rafael Maldonado) and coordinates a Network supported by Catalan and Spanish research funding bodies. The G&D programme participates in several networks of centers or groups supported by the Instituto de Salud Carlos III – Fondo de Investigación Sanitaria (ISCIII-FIS). These networks include Clinical Genetics, Neuroscience, Hearing impairment, Psychiatric Genetics, and Cancer. The programme has initiated collaborations with several investigators of the UPF in the study of segmental duplications of the human genome and the study of their potential involvement in disease (Luis Pérez-Jurado) and evolution (Arcadi Navarro). Specific support for this project has been obtained from Genome Canada and Genome Spain.

The programme has recruited a scientist for the Transgenics Unit of the CRG. This unit will provide support to projects that need the development of transgenic and knock out mice models of the programme,

but also of the CRG and other groups of the PRBB. It is expected that the Transgenics Unit will be part of a resource for transgenics common to several research centers in Barcelona (the Barcelona Research Alliance). The G&D programme has been funded by the ISCIII-FIS to set up a Genotyping Unit. Although initially focused to the study of psychiatric disorders through the funding of the ISCIII-FIS to the Psychiatric Genetics Network coordinated by the programme, this unit will also be devoted to other complex genetic disorders. The support given by the ISCIII-FIS is for three years and covers equipment and personnel. We have already recruited two postdoctoral fellows and two technicians, and have acquired a 3730XL Sequencer, a Pyrosequencer and a Wave instrument. This genotyping facility will be reinforced with the support of Genome Spain to the National Center of Genotyping, allowing the acquisition of robotics, the implementation of LIMS systems and the expansion to genotyping of other complex disorders and pharmacogenomic projects.

National Genotyping Center – Barcelona Node:

Postdoctoral Fellow:

Mònica Bayès (Ramón y Cajal) since September 2003

Technicians:

Rafael de Cid (NIH)

Carles Arribas (FIS)

Anna Puig (FIS)

Transgenics Unit:

Technician:

Luís Sánchez Palazón

GENETIC CAUSES OF DISEASE

Group Leader
XAVIER ESTIVILL

GROUP STRUCTURE

Postdoctoral Fellows:	MÒNICA GRATACÒS
	MÒNICA BAYÈS since September 2003
	MIROSLAVA OGORELKOVA since October 2003
PhD Students:	LLUÍS ARMENGOL
	ESTER BALLANA
	NINA BOSCH
	CELIA CERRATO
	MONICA GUIDI
	JOSEP MARIA MERCADER
	MARTA RIBASÉS
Technicians:	MARTA MORELL
	ANNA CARRERAS
	IMMA PONSA
Research Assistant:	MARINA VENTAYOL
Visitors:	LISA RUSO
	SERGI VILLATORO

SUMMARY

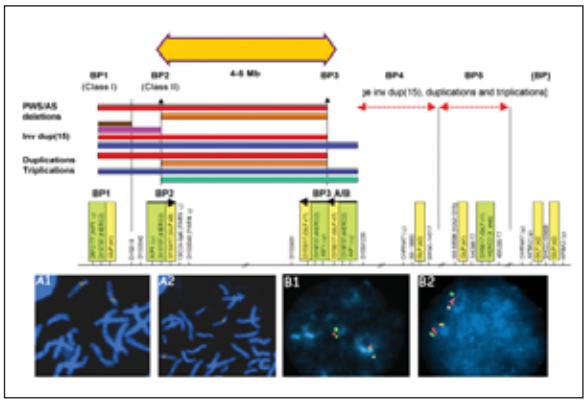
The group focuses its research in the identification of genetic factors involved in complex human disorders. These include eating disorders, hearing impairment, anxiety disorders, and mental retardation. Variability of the human genome at the nucleotide and genomic levels should contribute to most of the genetic factors that predispose to common disorders and that are responsible for specific traits in humans. Over the last five years the group has contributed to the identification of genetic variants that are involved in the predisposition to hearing impairment, anxiety disorders and anorexia. The group has set up the analytical basis for the study of human genome diversity throughout genotyping nucleotide variants and genomic changes that are specific of humans and have participated in evolution of the hominoid species.

RESEARCH PROJECTS

1. Segmental duplications, variability and human disease

M. Gratacòs, M. Bayès, Ll. Armengol, I. Ponsa, N. Bosch, C. Cerrato

One of the most surprising observations to arise from the analysis of the human genome sequence has been the discovery that about 5% of its content has arisen through recently occurring large-scale segmental duplication, leading to long stretches of nearly identical DNA being present at two or more sites in the genome. Most of these duplication events have occurred only since primates evolved and some are specific to the human genome, leading to evolution of hominoid specific genes. Segmental duplications can be 'hot spots' or predisposition sites for the occurrence of non-allelic homologous recombination or unequal crossing-over leading to genomic mutations such as deletion, duplication, inversion, or translocation. While these regions of the genome are so important to the biology of chromosomes, in some cases they are still not accurately represented in genome sequence assemblies. With the majority of the human genome sequence known, it is only now that we can systematically and fully investigate the role of segmental duplications in disease. The work of our group has led to the identification of a common inversion on human chromosome 15q11-q13, which is involved in the predisposition to Angelman syndrome. This work is now being extended to Prader-Willi syndrome and to autism. Preliminary results confirm the inversion in the fathers of cases of 15q11-q13 deletions, and the presence of microdeletions of this region in cases of autism.



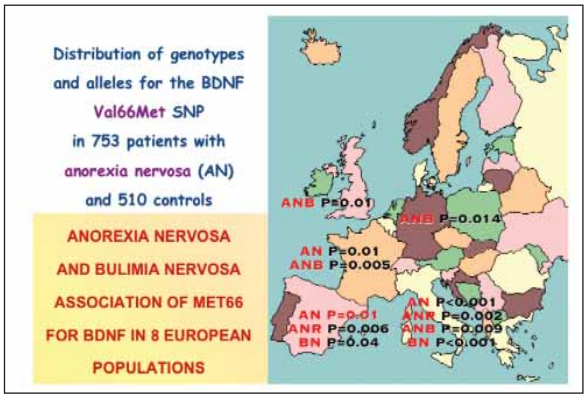
We have observed that some chromosome regions involved in genomic disorders are shuffled in orientation and order in the mouse genome and that regions flanked by segmental duplications are often polymorphic. We have compared the human and mouse genome sequences and demonstrated that recent segmental duplications correlate with breaks of synteny between these two species. We also observed that several primary regions involved in human genomic disorders show changes in the order or the orientation of mouse/human synteny segments, were often flanked by segmental duplications in the human sequence. Moreover, we have found that

53% of all evolutionary rearrangement breakpoints associate with segmental duplications, as compared to 18% expected in a random location of breaks along the chromosome. Our data suggest that segmental duplications have participated in the recent evolution of the human genome, as driving forces for evolutionary rearrangements, chromosome structure polymorphisms, and genomic disorders.

The group has focused its attention to three regions of the human genome that contain segmental duplications potentially involved in human disease. These include 15q11-q13, 15q24-25 and 8p23.1. Specific work is being performed in these regions with the characterization of the organization of segmental duplications, content of genes and identification of variability in copy number of gene sequences. We have initiated the study of other regions containing segmental duplications and their potential relationship with human disease. A complete BAC collection covering the whole human genome (32K) is available for molecular cytogenetic studies and will be used during the next years for the development of microarrays for comparative genome hybridization studies.

2. Genetic factors involved in psychiatric disorders

M. Gratacòs, M. Ribasés, R. de Cid, J.M. Mercader, M. Guidi



The availability of hundreds of thousand single nucleotide variants (SNPs) in the human genome sequence facilitates the analysis of the genetic predisposition to disease. We have been studying several human diseases, including eating disorders and hearing impairment. We have identified that two 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 of in 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. This is the first unequivocally identification of a gene in the pathophysiology of eating disorders in different populations, and indicates a participation of BDNF as a susceptibility factor to eating behavior alterations. This is the first significant association between genetic variants and eating disorders detected in several populations.

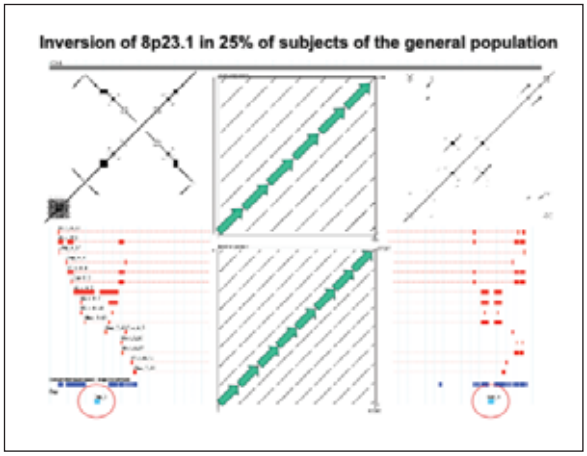
During this period we have also organized a genotyping facility (supported by the Instituto de Salud Carlos III Fondo de Investigaciones Sanitarias), mainly focused to psychiatric disorders (http://rgpg.net). The RGPG (Red de Grupos de Psiquiatría Genética)network contains 11 groups, two of them having large scale genotyping facilities. During the first year of funding the two genotyping centers have developed the genotyping cores and have started to design specific "plexes" for genetic pathways potentially involved in mental disorders. The RGPG will be the core for the development of the National Genotyping Center supported by Genome Spain.

3. Genetic factors that predispose to hearing impairment

E. Ballana, M. Ventayol, R. De Cid

Three genes (GJB2, GJB3 and GJB6) encode 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. During the 2003 year the group has been supported by the Instituto Carlos III / Fondo de Investigaciones Sanitarias under a Network of research groups. 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 is inverted in a significant proportion (about 25%) of individuals of the general population. In addition to this common inversion, chromosome 8p23 hemizygosity and duplication are involved in several disorders. The positive linkage region associated with the A1555G deafness phenotype was described around markers D8S277-D8S1819, located telomeric with respect to the inverted region. The presence of segmental duplications, which mediate the 8p23.1 inversion, are next to this region may be a confounding factor in linkage studies and could participate in the ambiguous results obtained in different genotyping studies in families with the A1555G mutation. We have performed an in silico characterization of this genomic region and have found that



flanking the inversion there are two pairs of segmental duplications with a complex organization. This genomic organization results in a mirror image defined by the duplicons, enabling the 8p23 region to undergo different rearrangements. 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, distinct 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 we have started the study of other regions with LOD score higher than 1.5 in the linkage studies performed. We will perform a finest mapping of these regions, typing additional markers, to determine the contribution of each one as a modifying factor for the mitochondrial A1555G mutation.

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2. Scherer S, ..., Armengol L, Pujana MA, Estivill X, et al. "Human Chromosome 7:DNA sequence and biology." Science 300:767-772. (2003)

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10. Dounady F, Snoeckx R, Pfister M, ..., Ballana E, Estivill X et al. "Non-muscle myosin heavy chain gene MYH14 is expressed in cochlea and mutated in patients affected by autosomal dominant hearing impairment (DFNA4)." Am J Hum Genet. (in press)

GENE THERAPY

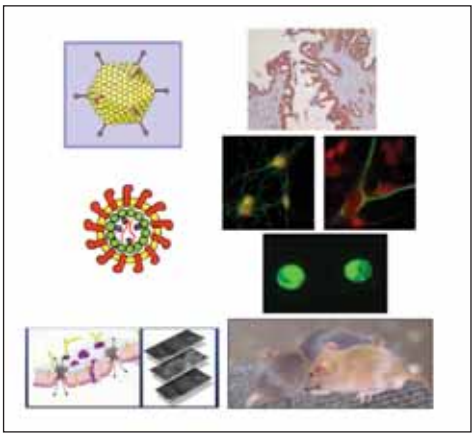
Group Leader
CRISTINA FILLAT

GROUP STRUCTURE

PhD Students: ANNA CASCANTE
MERITXELL HUCH
JON ORTIZ
LAURA GARCIA
DANIEL ABATE DAGA

Technicians: NÚRIA ANDREU

SUMMARY
Gene therapy is an emerging field that holds the promise of treating a wide variety of diseases. However, before this can be achieved, successful vector systems must be developed to deliver therapeutic genes and successful preclinical studies in animals models need to be carried out. Moreover a broad understanding of the disease pathology is required to be able to design candidate gene transfer approaches. The group is interested in understanding the pathophysiology and molecular aspects of Pancreatic cancer, Down syndrome and Wiskott-Aldrich syndrome disorders and more importantly is interested in the development of optimal gene therapy approaches.



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.

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2. Fillat C, Carrió M, Cascante A, Sangro B. "Suicide gene therapy mediated by the Herpes simplex virus thymidine kinase/ganciclovir system. Fifteen years of application." Current Gene Ther 3:13-26. (2003)

3. Andreu N, Pujol-Moix N, Martínez-Lostao L, Oset M, Muñiz-Díaz E, Estivill X, Volpini V, Fillat C. "Wiskott-Aldrich syndrome in a female with skewed X-chromosome inactivation." Blood Cells Mol Dis 31:332-337. (2003)

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Publications-book chapters

1. 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. in press (2003)

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MURINE MODELS OF DISEASE

The research group started in January 2002 with the incorporation of the group leader at the CRG.

Group Leader
MARIONA ARBONÉS

GROUP STRUCTURE

Postdoctoral Fellows:	EULÀLIA MARTÍ
PhD Students:	ARIADNA LAGUNA (since July 2003)
	SILVIA PORTA
Technicians:	ERIKA RAMÍREZ

SUMMARY
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.

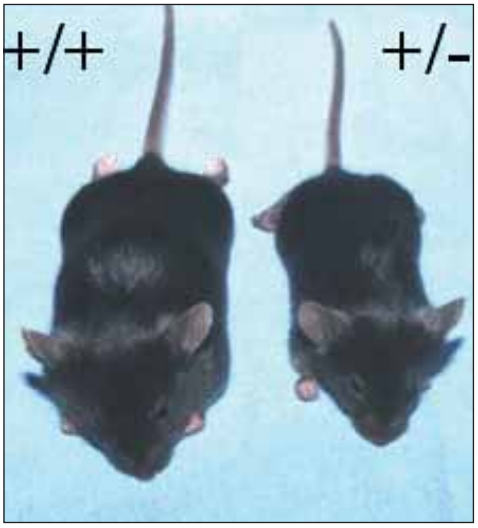
RESEARCH PROJECTS

1. DYRK1A
A. Laguna

DYRK1A is the mammalian homologue of minibrain, a serine/threonine kinase in Drosophila melanogaster involved in neurogenesis.

We have shown that targeted mutation of a Dyrk1A allele in the mouse causes haploinsufficiency characterized by reduction of the body size, developmental delay and behavioral alterations affecting specific domains. Brains of mice hemizygous for the mutation (Dyrk1A$^{+/-}$ mice) are decreased in size in a region-specific manner although the general cytoarchitecture is not altered. The phenotype of Dyrk1A$^{+/-}$ mice suggest that dose reduction of DYRK1A could be the cause of the microcephaly and metal retardation described in partial monosomy 21 patients.

In collaboration with the group lead by M. Dierssen (CRG) we are conducting experiments trying to identify the anatomical and neurochemical alterations that lead to the behavioural changes shown by Dyrk1A$^{+/-}$ mice.
In the context to identify neurone populations that may be sensible to Dyrk1A dose reduction, we have analysed



retinas from Dyrk1A$^{+/-}$ mice. In close analogy to what we observed in the brain, those retinas show normal cytoarchitecture but a decreased number of some types of neurones and an alter morphology of their neuronal processes. These observations clearly suggest a role of Dyrk1A in neurogenesis and differentiation of specific neurone populations. Since Dyrk1A is highly expressed during retinogenesis we are planning to use the retina as a model system to define the role/s of Dyrk1A in central nervous system development.

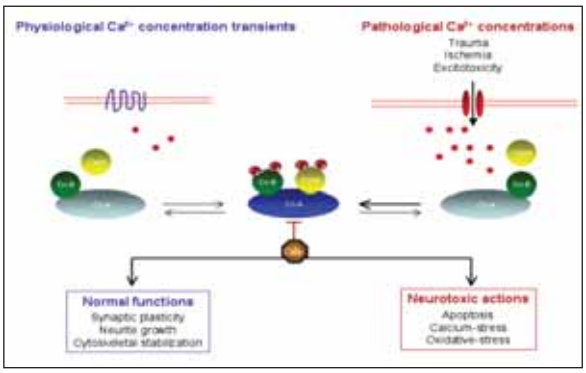
2. DSCR1/Calcipressin 1
E. Martí, S. Porta

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 regulates a variety of responses in different organs and tissues including brain and skeletal muscle where CALP1 is highly expressed.

We are interested in identify physiological and pathological processes that may be regulated by CALP1. We begin studying well known calcineurin mediated responses in skeletal muscle and in the nervous system.

CaN signalling in skeletal muscle is involved in the regulation of myofiber specific genes associated with the slow twitch phenotype as well as in different processes that are relevant for muscle regeneration. To determine whether or not CALP1 is regulating these processes we are analyzing a mouse model with a null Dscr1 null mutation.

In the central nervous system CaN participates in different physiological functions including neurite elongation and synaptic plasticity. However, sustained activation of CaN activity by calcium overload is detrimental to neurones. The involvement of CaN in the physiopathology of ischemia has been proposed since hypoxia/ischemia leads to sustained depolarization and calcium entry into neurons. On the other hand, the fact that CALP1 expression is upregulated by calcium in a CaN dependent manner suggests that CALP1 could be relevant in situation of maintained high intracellular calcium concentrations. We hypothesise that CALP1 could act as a neuroprotector in ischemia and in excitotoxic processes leading to neuronal death. To address this question we start analysing the susceptibility of neurones lacking CALP1 to glutamate-mediated excitotoxicity.



PUBLICATIONS

Publications correspond to work carried out by the group while still at IRO.

1. Fotaki V, Dierssen M, Alcantara S, Martinez S, Marti E, Casas C, Visa J, Soriano E, Estivill X, Arbones ML. "Dyrk1A haploinsufficiency affects viability and causes developmental delay and abnormal brain morphology in mice." Mol Cell Biol 22:6636-47. (2002)

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3. Marti E, Altafaj X, Dierssen M, de la Luna S, Fotaki V, Alvarez M, Perez-Riba M, Ferrer I, Estivill X. "Dyrk1A expression pattern supports specific roles of this kinase in the adult central nervous system." Brain Res 964:250-63. (2003)

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NEUROBEHAVIORAL PHENOTYPING OF MOUSE MODELS OF DISEASE

Group Leader
MARA DIERSSEN

GROUP STRUCTURE

PhD Fellows:
MARÍA MARTÍNEZ DE LAGRÁN CABREDO
IGNASI SAHÚN ABIZANDA
ALEJANDRO AMADOR ARJONA
XAVIER GALLEGO MORENO
GLORIA ARQUÉ FUSTER

Technicians:
NOELIA FERNÁNDEZ

SUMMARY

The research group began its research activities in 2002 with the creation of the Genes and Disease Programme lead by Dr Xavier Estivill, as a part of the Genomic Regulation Center. The overall goal of our research is the understanding of the genetic substrates regulating the expression of complex behavioral traits. We investigate specific links between cognitive impairments memory disorders in Down syndrome and behavioral and neuromorphological deficits in mouse models of this disease. We are also currently working on mouse behavioral traits relevant to panic and to anxiety. We are actively collaborating with research groups from Genes and Disease and Differentiation and Cancer Programs, with other group 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

Our research group has made important contributions to the understanding of the pathogenesis of mental retardation in Down syndrome. In collaboration with J. de Felipe in the Cajal Institute (Madrid) we have reported for the first time the phenotypic changes in the pyramidal neuron of the cerebral cortex and the associated dendritic pathology and the impact of environmental manipulations on this phenotype in a mouse model of Down syndrome, Ts65Dn. We have also studied the genetic dependence of this phenotype and the implication of Dyrk1A in mice with reduced dose for this gene (see Fig 1). 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.

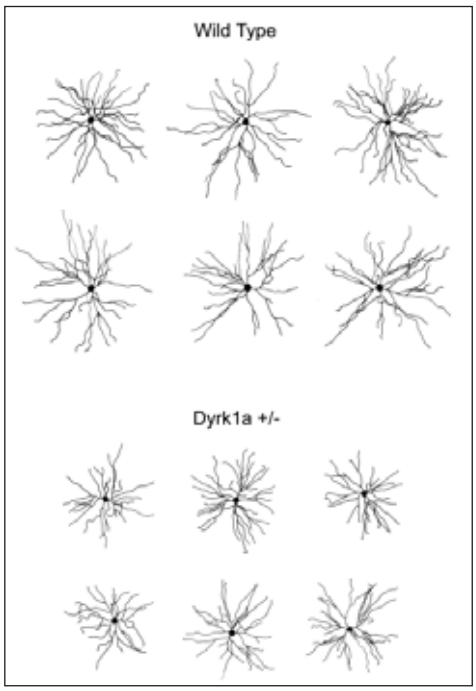

Figure 1. Down syndrome phenotypes in pyramidal neurons of the Cerebral Cortex.

2. Panic/Anxiety

I. Sahún, A. Amador, X. Gallego

We have characterized a mouse model of overexpression of NTRK3, the NT-3 receptor, proposed for panic disorder from the neurobehavioral, neurochemical and neuromorphological point of view. We have proposed a neurodevelopmental hypothesis for the pathogenesis of panic disorder.

In collaboration with Dr. R. Maldonado (Pompeu Fabra University) we will 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, treadmill, running wheel, stereotaxia, chronic mild stress b/ Neuromorphological: stereological analysis with the CAST grid (optic dissector and Cavalieri method).

4. OTHER

Jaime Blanco Award in Down syndrome research, 2002

Ramón Trías Fargas Award in Down syndrome research, 2003

M. Dierssen has been named President of the Internation Behavioral Genetics Society (2003-2005)

PUBLICATIONS

1. Fountoulakis M, Juranville JF, Dierssen M, Lubec G. "Proteomic analysis of the fetal brain." Proteomics 2:1547-76. (2002)

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4. Dierssen M, Benavides-Piccione R, Martínez-Cué C, Estivill X, Flórez J, Elston GN, DeFelipe J. "Alterations of neocortical pyramidal cell phenotype in the Ts65Dn mouse model of Down syndrome: effects of environmental enrichment." Cerebral Cortex 13:758-64. (2003)

5. Martí E, Altafaj X, Dierssen M, de la Luna S, Fotaki V, Alvarez M, Pérez-Riba M, Ferrer I, Estivill X. "Dyrk1a expression pattern supports specific roles of this kinase in the adult central nervous system." Brain Res 964:250-63. (2003)

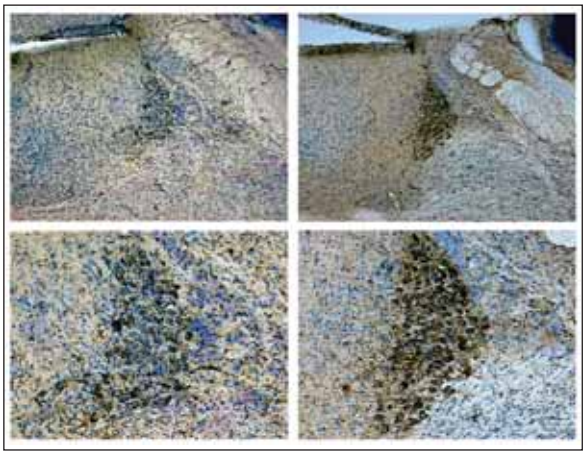

Figure 2. Neurotrophic Effect on Locus Coeruleus noradrenergic neurons after overexpression of NTRK3.

6. 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." J Behav Neurosci (in press)

7. 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 Disease 15: 132-142. (in press)

Publications-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. in press (2003)

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. The group members were recruited along the first semester of 2002.

Group Leader
SUSANA DE LA LUNA

GROUP STRUCTURE

PhD-Students:	Mónica Alvarez
	Sergi Aranda
	Lali Genescà
Technician:	Alicia Raya

SUMMARY

Research in the past few years has revealed that a number of human chromosome 21 (HSA21) genes are overexpressed in Down syndrome by, at least, 50% due to gene dosage. Because of the complexity of the Down syndrome phenotype, it is very likely that the increased expression leads to perturbations in a great variety of biological pathways. Furthermore, it is predictable that many HSA21 genes can interact functionally with each other within particular signalling pathways. Understanding the functional roles of the overexpressed genes will 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

Mónica Alvarez, Sergi Aranda

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.

The subcellular localization of DYRK1A is an intriguing aspect of this molecule since it is present both in the nuclei and the cytosol of different cell types including neurons. As it is clear that the nucleocytoplasmic transport of cell signalling related molecules is a very significant and effective process to modulate their activities, we are specially interested in finding out which mechanisms control DYRK1A subcellular localization. In this sense, we have contributed to the description of the nuclear localization signals that direct DYRK1A to the nucleus. DYRK1A accumulates in nuclear speckles that we have identified as the splicing factor compartment or SFC (Figure 1). DYRK1A manages to do so by using a completely novel speckle-targeting signal corresponding to the histidine-rich region at its C-terminus. This targeting signal is also present in cyclin T1, another SFC-accumulating protein.

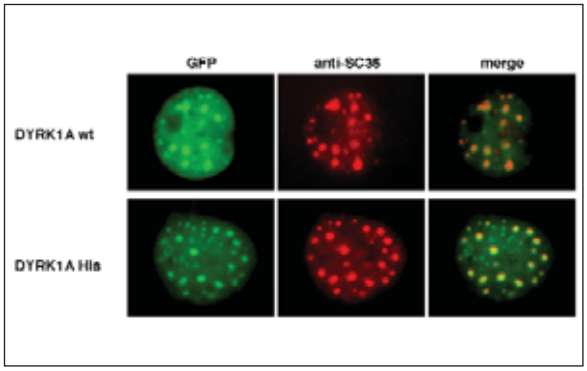

Figure 1. DYRK1A accumulates in nuclear speckles through its histidine rich region. Co-localization of GFP fusions of DYRK1A full-lenght (wt) and the histidine-rich segment (His) with SC-35 marker is shown.

Moreover, DYRK1A overexpression induces the disassembly of the SFC suggesting a potential role for this kinase in regulating splicing.

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. 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 C-terminus of the protein kinase TAK1.

TAK1 is a member of the MAPKKK family that functions in the signaling pathway triggered by TGF-b superfamily members. TAK1 has also been shown to participate in mesoderm induction and patterning mediated by BMP in Xenopus laevis embryos and to be involved in the IL-1 signaling pathway. Two TAK1 binding proteins have been identified, TAB1 and TAB2, that work as positive effectors of the kinase. TAB2 is also capable of interacting with TRAF6 and it has been proposed to be a key component of the IL-1 signal transduction pathway by linking TAK1, IRAK and TRAF6.

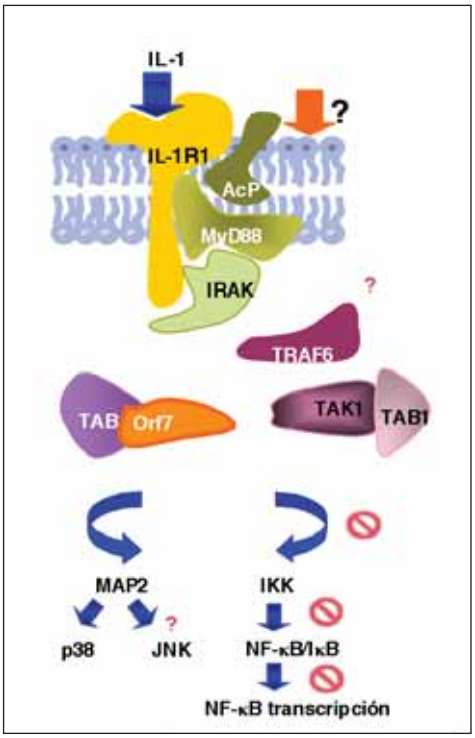

Figure 2. Schematic representation of the interaction of C21orf7 ORFs with the IL-1 signalling pathway

Due to the high degree of similarity between the C-terminus of C21orf7 ORFs with the C-terminus of TAK1, we raise the hypothesis that these proteins might work as TAB2 interacting proteins (Figure 2). The interaction could lead to a sequestration of TAB2 resulting in an inability for the formation of TAK1-TRAF6 complexes and the subsequent inhibition of NF-kB activation. C21orf7 ORFs may work then as intracellular antagonists of the inflammatory response mediated by IL-1.

PUBLICATIONS

1. Alvarez M, Estivill X & de la Luna S. "DYRK1A accumulates in splicing speckles through a novel targeting signal and induces speckle disassembly." J Cell Sci 116: 3099-3107. (2003)

The publications below correspond to work carried out by group leader while still part of the HSA21/Down syndrome research group at IRO.

2. Martí E, Altafaj X, Dierssen M, Alvarez M, de la Luna S, Fotaki V, Perez-Riba M, Ferrer I & Estivill X. "Dyrk1A expression pattern supports specific roles of this kinase in the adult central nervous system." Brain Res 964: 250-263. (2003)

3. Genesca L, Aubareda A, Fuentes JJ, Estivill X, de la Luna S & Perez-Riba M. "Phosphorylation of the FLISPP motif of calcipressin 1 affects calcineurin inhibition and calcipressin stabilization." Biochem J 374: 567-575. (2003)

4. Hilton JF, Christensen KE, Watkins D, Raby BA, Renaud Y, de la Luna S, Estivill X, MacKenzie RE, Hudson TJ & Rosenblatt DS. "The molecular basis of glutamate formiminotransferase deficiency." Human Mutation 22: 67-73. (2003)


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Coordinator: Roderic Guigó

BIOINFORMATICS AND GENOMICS

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. The exact contents of programme are still in the process of being defined. In particular, we are considering the possibility of a larger program, which would also include a number of research groups in System Biology. Space for new groups, however, is currently scarce, and we don't plan for the programme to expand until the new CRG facilities became available in a year from now. Currently, the programme has two active research lines in Genome Bioinformatics and Microarrays, since Jose Castresana, who was leading the Molecular Evolution group, has left CRG, after obtaining a senior research position at the CSIC (The Spanish Research Council). We congratulate Jose Castresana for this achievement.

The two active groups, and José Castresana's group 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 group, 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)

BIOINFORMATICS AND GENOMICS

(join group with GRIB, IMIM-UPF)

Group Leader
RODERIC GUIGÓ

GROUP STRUCTURE

Research Associate:	Mar Albà (UPF)
Postdoctoral Fellows:	Robert Castelo (UPF)
	Jan Jaap Wesselink (IMIM)
	Eduardo Eyras (UPF)
PhD-Students:	Josep F. Abril (UPF)
	Genís Parra (IMIM)
	Sergi Castellano (UPF)
	Enrique Blanco (UPC)
	Charles Chapple (UPF)
	Nicolás Bellora (UPF)
	Noura Dabbouseh (CRG)
Technicians:	Oscar González (IMIM)
	Francisco Câmara (IMIM)

SUMMARY

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. geneid has been used in the annotation pipeline of Dyctiostelium discoideum (Glökner et al., 2002). And of Tetraodon nigroviridis (Jaillon et al., submitted). 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 Insitute. In particular, we are collaborating with the Whitehad Institute from the Massachussets Institute of Technology to train geneid for the fungal genomes in this high-priority list.

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 eukaryotic selenoproteins (Castellano et al., 2004). A recent discovery further challenges these assumptions (work in progress).

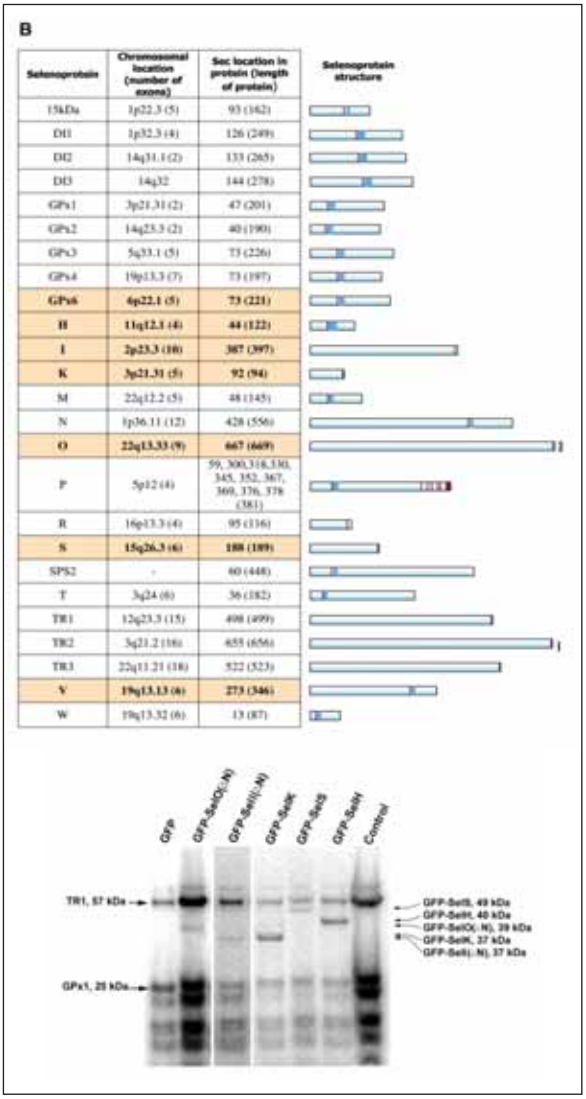


Figure 1. Identification of novel mammalian selenoproteins (in orange). Computational prediction followed by experimental verification. Taken from Kryukov et al. (2003)

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 identifiy sequence signals involved in the regulation of splicing. We are developing this line of research in close collaboration with Juan Valcárcel group.

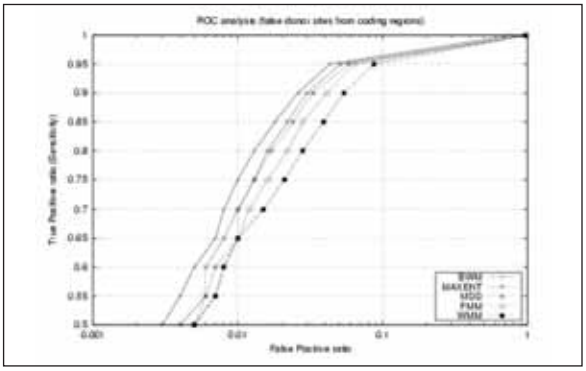


Figure 2. Comparative ROC of different methods to predict donor sites. The methods are ordered according to accuracy: BWM, MAXENT (Yeo and Burge, 2003), MDD (Burge and Karlin, 1997), FMM (First Order Markov Model) and WMM (Weigth Matrix Model)

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 also been applied to the analysis of the rat genome (Rat Sequencing Consortium, 2004)

5. Recognition of Promoter Regions

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

PUBLICATIONS

1. Abril JF, Guigó R & Wiehe T. "gff2aplot: Plotting sequence comparisons." Bioinformatics 19:2477-2479 (2003)

2. Guigó, R & T. Wiehe, T. "Gene Prediction Accuracy in Large DNA Sequences." In M.Y. Galperin and E.V. Koonin, editors: Frontiers in Computational Genomics. Chapter 1. (Functional Genomics Series, Volume 3) Caister Academic Press (UK) (2003)

3. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigó R & Gladyshev VN. "Characterization of mammalian selenoproteomes." Science 300(5624):1439-1443. (2003)

4. Beltran S, Blanco E, Serras F, Pèrez-Villamil B, Guigó R, Artavanis-Tsakonas S & Corominas M. "Transcriptional network controlled by the trithorax-group gene ash2 in Drosophila melanogaster." Proc Nat Acad Sci 100(6):3293-3298. (2003)

5. Guigó R, Dermitzakis ET, Agarwal P, Ponting CP, Parra G, Reymond A, Abril JF, Keibler E, Lyle R, Ucla C, Antonarakis SE & Brent MR. "Comparison of mouse and human genomes followed by experimental verification yields an estimated 1,019 additional genes." Proc Nat Acad Sci 100(3):1140-1145. (2003)

6. Parra G, Agarwal P, Abril JF, Wiehe T, Fickett JW & Guigó R. "Comparative gene prediction in human and mouse." Genome Research 13(1):108-117. (2003)

7. Blanco E, Parra G & Guigó R. "Using geneid to Identify Genes." In A. D. Baxevanis and D. B. Davison, chief editors: Current Protocols in Bioinformatics. Volume 1, Unit 4.3. John Wiley & Sons Inc., NY (2002)

8. Waterston R et al. (including Abril JF, Parra G & Guigó R). "Initial sequencing and comparative analysis of the mouse genome." Nature 420(6915):520-562. (2002)

9. Holt RA et al (including Abril JF & Guigó R). "The Genome Sequence of the Malaria Mosquito Anopheles gambiae." Science 298(5591):129-149. (2002)

10. Guigó R & D. Gusfield editors. "Algorithms in Bioinformatics." Proceedings of the Second International Workshop, WABI 2002. (Lecture Notes in Computer Science, Volume 2452) Springer-Verlag, Berlin Heidelberg. (2002)

11. Glökner G, Eichinger L, Szafranski K, Pachebat JA, Bankier AT, Dear PH, Lehmann R, Baumgart C, Parra G, Abril JF, Guigó R, Kumpf K, Tunggal B, the Dictyostelium Genome Sequencing Consortium, Cox E, Quail MA, Platzer M, Rosenthal A & Noegel AA. "Sequence and Analysis of Chromosome 2 of Dictyostelium discoideum." Nature 418(6893):79-85. (2002)

GENOMIC ANALYSIS OF DEVELOPMENT AND DISEASE

Group Leader
LAURO SUMOY

GROUP STRUCTURE

PhD-Student: LAURA CARIM

Microarray Unit

Senior Technicians: JUANJO LOZANO
BELEN MIÑANA

Technician: DAVID OTERO

Guest members: EVA GONZALEZ (PRBB, L. Perez Jurado - UPF)
ANNALISA SAETTA (Erasmus, M. Beato - CRG)

SUMMARY

The group is interested in the study of gene regulation at the transcriptional level 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 and to understand the function of these newly characterized genes through inference from gene expression profile data.

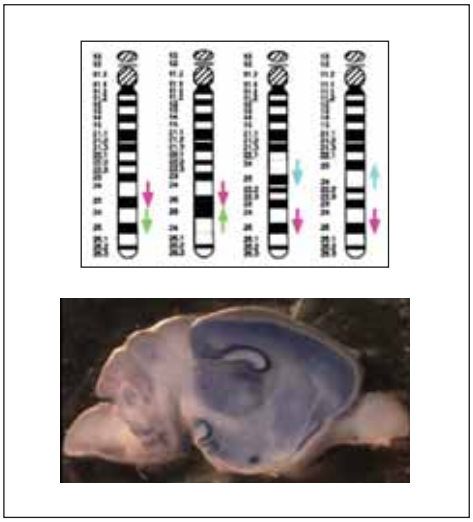
Past research experience had focused on the study of homeobox gene regulation during limb development and axis formation in vertebrate embryogenesis. More recently our area of research shifted to the characterization of genes implicated in complex psychiatric human diseases such as panic and anxiety disorder, through the isolation and sequencing of cDNAs from specific chromosomal regions by in silico selection using EST database searching and clustering. With the finishing of the sequence of the human genome our main goal has become to assign function to unknown genes and to establish regulatory relationships between unknown genes and known genetic pathways.

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, including homeobox gene regulation during embryonic development, genetic mechanisms causing predisposition to develop panic and anxiety disorder associated to the duplication of a large genomic region on human chromosome 15, and the study of gene regulation and of the function of novel genes involved in breast, prostate and bladder cancer.

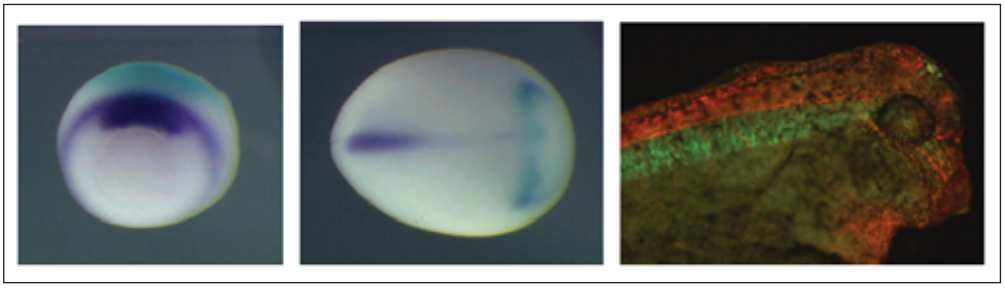
RESEARCH PROJECTS

1. Developmental biology of body axis formation and neural differentiation

We wish to study vertebrate specific gene function (by vertebrate specific genes or acquisition of novel functionality by ancestral genes). In particular we focus on the study, through contemporary genomic methodologies as well as more classical molecular, cell and developmental biology approaches, of genes involved in the generation of structures such as the vertebrate primary body axis, the central nervous system, sensory organs and appendages.



Current research is primarily focused on LERN1, 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 centered on genes from 15q24-q26. With relevance to the disease, we have found that LERN1 is expressed in the adult limbic system and shown it



maps within the duplication. 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.

There is a continued collaboration with Dr. Miranda Gomperts, Wellcome-CRC Institute, Cambridge, UK, to study the regulation of Xnot/flh homeobox gene in notochord and pineal gland in Xenopus and zebrafish. Fruit of this work has been the description of a gastrulation requirement for Xnot in amphibian development and the generation of pineal gland specific gfp reporter constructs that were used in the study of CNS laterality. The aim is to understand the function of this gene by screening for transcriptional targets and to use cross-species comparisons to delineate regulatory mechanisms common to all vertebrates.

2. Meta-analysis of genomic of changes in mRNA expression and DNA copy number. Application to the study of disease

Through participation in several large scale projects involved in the study of diseases such as cancer, 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.

Specifically, we are involved in projects focusing on breast cancer (collaboration with Dr. Miguel Beato, CRG), bladder and prostate cancer (collaboration with Dr. Antonio Alcaraz, Fundació Puigvert) and genomic disorders mediated by homologous recombination events between segmented duplications (collaboration with Dr. Xavier Estivill, CRG, and Luis Perez Jurado, UPF).

PUBLICATIONS

1. Pan Z-Z, Kronenberg MS, Huang D-Y, Sumoy L, Rogina B, Lichtler AC, Upholt WB. "Msx2 expression in the apical ectodermal ridge is regulated by a Msx2 and Dlx5 binding site." Biochem Biophys Res Commun 290(3):955-61. (2002)

2. Carim-Todd L, Escarceller M, Estivill X, Sumoy L. "LRRN6A/LERN1 (Leucine-rich Repeat Neuronal protein 1), a novel gene with enriched expression in limbic system and neocortex." Eur J Neurosci 18:3167-3182. (2003)

3. Concha ML, Russell C, Regan JC, Tawk M, Sidi S, Gilmour DT, Kapsimali M, Sumoy L, Goldstone K, Amaya E, Kimelman D, Nicolson T, Gründer S, Gomperts M, Clarke JDW, Wilson SW. "Local tissue interactions across the dorsal midline of the forebrain establish CNS laterality." Neuron 39(3):423-38. (2003)

4. 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 (in press)

Research Group
MICROARRAY CORE FACILITY

SUMMARY

The Microarray Laboratory at CRG started its activities with the aim to set up DNA chip based methodologies at the CRG. It centers its activities in research projects that use microarrays, and in providing services to scientists in its immediate surroundings which are now available to the community at established rates.

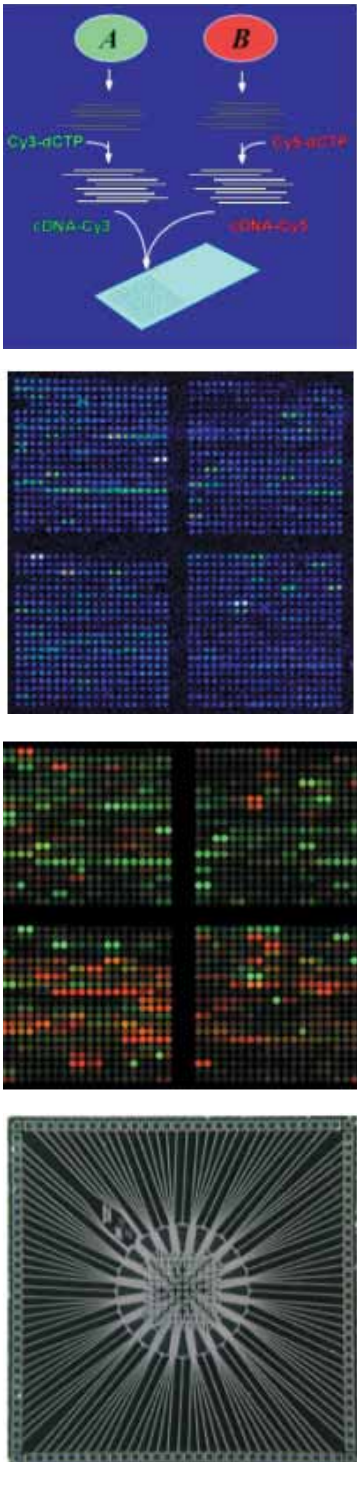
RESEARCH PROJECTS

Microarray technology development

We are committed to developing and optimizing procedures for the design, fabrication, hybridization, processing and analysis of data generated from spotted DNA microarrays (cDNA microarrays for expression profiling, genomic microarrays for detection of aneuploidies though comparative genomic hybridization, and diagnostics applications).

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.

In addition we have set up automated image data acquisition, pre-processing, filtering, normalization and quality controls for microarray experiments. Some of these tools have been implemented in web accessible form and are being shared with other microarray laboratories in Barcelona. We plan to include a shared 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).



We have already used microarrays to study gene expression using whole genome arrays (yeast, human, rat, maize and mouse) and customized targeted

small arrays (pancreas, breast cancer and neural). Of relevance, the core facility generated expression profiling results that lead to the discovery that Rpd3 histone deacetylase recruits Hog1 MAPK to promoters in response to osmotic stress in yeast (de Nadal et al, 2004).

One of our main goals will be to set up array comparative genomic hybridization protocols for the study of genome wide changes in DNA copy number. Currently planned applications include diagnostics of breast, prostate and bladder cancer, and genomic mutations mediated by segmental duplications through participation in collaborative projects.

PUBLICATIONS

1. Muckenthaler M, Roy CN, Custodio AO, Minana B, deGraaf J, Montross LK, Andrews NC, Hentze MW. "Regulatory defects in liver and intestine implicate abnormal hepcidin and Cybrd1 expression in mouse hemochromatosis." Nat Genet 34(1):102-107. (2003)

2. Sanchez-Carbayo M, Saint F, Lozano JJ, Viale A, Cordon-Cardo C. "Comparison of gene expression profiles in laser-microdissected, nonembedded, and OCT-embedded tumor samples by oligonucleotide microarray analysis." Clin Chem 49(12): 2096-100. (2003)

3. Sanchez-Carbayo M, Socci ND, Lozano JJ, Li W, Charytonowicz E, Belbin TJ, Prystowsky MB, Ortiz AR, Childs G, Cordon-Cardo C. "Gene discovery in bladder cancer progression using cDNA microarrays." Am J Pathol 163(2): 505-16. (2003)

4. 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 427:370-374. (in press)

II SYMPOSIUM OF THE CENTRE FOR GENOMIC REGULATION.
CELL REPROGRAMMING AND EPIGENETICS.

Barcelona, October 11, 2003

The second annual Symposium of the Centre for Genomic Regulation has versed about "Cell Reprogramming and Epigenetics".

The organizers of this event, held in the Conference Room of the Centre for Genomic Regulation, were Dr. Luciano Di Croce, Dr. Thomas Graf and Dr. Miguel Beato del Rosal. The host was the programme on "Differentiation and Cancer", one of the five programmes of the Centre for Genomic Regulation, coordinated by Dr. Thomas Graf.

The aim of this symposium was gathering top-level researchers from around the world (Europeans, Asians, and Americans) in the field of differentiation and cell reprogramming, who work with different experimental models and deal with different aspects of the same problem.

The main subject of the meeting is one of the most relevant topics of current Biomedicine: "Reprogramming one type of cell into another". This is what happens during the regeneration of tissues in amphibious: when you cut off an extremity to any of these creatures, another one grows up. The possibility to clone animals is just one of the multiple applications resulting from knowing that cell differentiation is an epigenetic and reversible process and, therefore, dirigible (for example, Dolly's cloning). Another application of clinical value is the possibility of generate, in cultures, several types of cells that can be used to replace damaged tissues in certain degenerative diseases as Parkinson disease or diabetes.

Invited speakers presented results of high scientific quality, in some cases really exceptional in each of the areas selected, especially in the discussion of the most controversial themes.

One of the invited speakers was not able to attend the symposium due to unforeseen causes, but Dr. Thomas Graf, organiser of the symposium, replaced him since he is widely acknowledged as one of the most expert researchers in this area world wide.

John Gurdon (Keynote Lecture)
He made a historical and current review of the topic and showed his last experiments on frogs.

Brockes & Izpisua
They explained the transdifferentiation in models of amphibious and mice.

Akashi, Rossi & Graff
They talked about the topic of transdifferentiation related to blood cells

Smith & Mueller
They explained the differentiation of mother cells in cell cultures and in mice

Kadam, Li & Reik
They proposed mechanisms of cell reprogramming at a molecular level (chromatin).

Stadtfeld, Ballestar & Brunmeir
Short talks about the topic of the symposium.

The 120 seats of the Conference Room were taken up, the symposium was attended by European and American doctoral and postdoctoral students, who participated actively in the discussions of the presented lectures.

Conclusions
This Symposium was an important chance to discuss the most delicate points of the topic of cell transdifferentiation, epigenetics and animal cloning.

Particularly, for the first time we achieved to gather the best leaders around the world in the field of epigenetics. This allowed the exchange of knowledge amongst these experts and, the most important thing, this knowledge was transmitted to the young researchers that begin their careers in the field of epigenetics. More specifically, experts tried to prove with experimental data and new ideas that it is possible to modify the predetermined epigenetic link in cells, aiming to derive a type of cell from another one (for example, obtaining muscular cells from blood cells).

The topic of cell reprogramming and epigenetics is not only greatly interesting for scientists working on basic research of development and cancer, but also for clinical researchers working with diseases in which destruction of tissues can be found (as for example, muscular dystrophy).

Taking into account the hopes arisen due to the therapeutic potential of embryonic and adult mother cells, together with cloning and regenerative medicine, we reiterate that our symposium was an essential event that allowed a deep reflection and an exchange of scientific results amongst worldwide leaders and participants.

Appendix 2

CRG SEMINARS

September 2002 - December 2003

06.09.02
Koldo Aurrekoetxea, Departamento Biologia Molecular y Celular, Madrid
"Signaling and transcriptional mediators of the mouse mammary tumor virus in B cells"

09.09.02
Yoshitaka Nagahama, National Institute of Basis Biology, Okazaki
"Molecular mechanisms of sex determination and gonadal differentiation in fish"

19.09.02
Mike Edel, Netherland Cancer Institute, Amsterdam
"Identification of novel genes involved in cell cycle regulation using function genomic screens in mammalian cells".

08.11.02
Javier Martinez, Max-Plank Institut for Biophysical Chemistry, Gottingen
"A human in vitro system for RNA interference"

14.11.02
Research Leaders of Affymetrix and Medplant Genetics
"Genechip applications in biomedicine"

19.11.02
Elena Bosch, Leicester Universty
"Y cromosome dynamics; an evolutionary approach to the evolutionary approach to the investigation of pathogenic and non pathogenic rearrangements"

21.11.02
Francisco Antequera, Inst. Microbiología Bioquímica, CSIC, Universidad de Salamanca
"CpG island as promoters and DNA replication origins in the mammalian genome"

22.11.02
Ariel Ruiz Altaba, Skirball Institute for Biomolecular Medicine, New York
"Hedgehog-Gli function in brain development: tumors, embryos and stem cells"

22.11.02
Sophie Bonal, French Institute of Health and Medical Research, Toulouse
"hnRNP A1 promotes translation of the fuman fibroblast growth factor 2 (FGF2) mRNA.

05.12.02
Ferdinando Auricchio, Universidad de Nápoles
"Signaling pathway activation by steroid hormones"

23.12.02
Elena Rivas, Washington University, Saint Louis
"RNA genefinding using probabilistic and phylogenetic comparative methods"

16.01.03
Cornelia de Moor, School of Biomedical Sciences, Queen's Medical Centre, Nottingham
"Translational control in oocyte development"

17.01.03
Jamal Tazi– Institut de Génétique Molecularie, CNRS, Montpellier
"Phosphorylation –dependent and independent- regulation of SR protein splicing factors"

21.02.03
José Ramón Naranjo del Centro Nacional de Biotecnología – CSIC –Madrid
"Transcpritional repressor dream: functional analysis in vivo using dominant negative mutants."

28.02.03
Ferrán Azorín- Centre d'Investigació i Desenvolupament "CID" – CSIC Barcelona
"Structure and properties of centrometric chromatin: contribution of single-stranded nucleic acids

07.03.03
Edouard Bertrand – Institut de Génétique Moléculaire – CNRS, Montpellier
"RNA trafficking: insights from in vivo imaging of single molecules"

14.03.03
Cayetano González – EMBL, Heidelberg
"Chromatin and spindle assembly"

11.04.03
Joaquin Dopazo – CNIO, Madrid
"DNA arrays data analysis:genomic perspective"

25.04.03
Encarna Martínez-Salas – Centro de Biología Molecular "Severo Ochoa" CSIC-UAM , Madrid
"Functional interactions in IRES-driven initiation of translation"

09.05.03
Luis Serrano – EMBL, Heidelberg
"Protein Design and amyloidoses"

09.05.03
Isabelle Vernos – EMBL, Heidelberg
"Mechanism of mitotic spindle formation"

23.05.03
Javier Cáceres – Western General Hospital, Edinburgh
"Multiple roles of arginine/serine rich splicing factors in mRNA processing"

27.05.03
Vivek Malhotra, University of California, San Diego
"The Golgi apparatus: formation of transport carriers and regulation of entry into mitosis"

30.05.03
Ralf-Peter Jansen, University of Munich
"Nuclear and cytoplasmic steps during yeast RNA trafficking"

06.06.03
James Fickett, Worldwide Director of Bioinformatics, AstraZeneca
"Systems biology: early application in drug discovery"

17.06.03
Greg Matera, Case Western Reserve University, Cleveland, Ohio
"Cajal bodies, snRNP biogenesis and spinal muscular atrophy"

20.06.03
Alfonso Martínez Arias, University of Cambridge
"Signal integration by interactions between Wnt and Notch during cell fate specification and growth"

27.06.03
Peter Sicinski, Harvard Medical School, Boston
"Cell cycle machinery in development and in oncogenesis"

30.06.03
Lynne E. Maquat, University of Rochester
"Nonsense-mediated mRNA decay in mammalian cells: evidence for a pioneer round of translation and factors involved in decay".

03.07.03
Minoru Ko, National Institute on Aging, Baltimore
"Embryogenomics of mouse early embryos and stem cells"

10.07.03
Juan Mata, Wellcome Trust Sanger Institute, Cambridge
"Transcription and differentiation in fission yeast"

11.07.03
Robert Rhoads, Louisiana State University, Shreveport
"Caps and worms: studying initiation of protein synthesis in caenorhabditis elegans".

18.07.03
Juan Carlos Izpisua, the Salt Institute for Biological Sutides, La Jolla
"Molecular basis of body asimetry"

09.09.03
Masahiro Okamoto, Kyushu University, Fukuoka
"Development of system identification technique by using time course data of expression profile"

26.09.03
José Luis Jorcano, CIEMAT, Madrid
"The skin: a model system for cellular and gene therapies."

03.10.03
Federico Mayor Zaragoza, Centro de Biología Molecular Severo Ochoa, Madrid
"G protein-coupled receptor kineases (GRKs): mechanisms of regulation & functional implications."

07.10.03
Luis F. Parada, UT Southwestern Medical Center, Dallas
"The role of insulin family signalling in male sexual differentiation."

28.10.03
Yusuke Nakamura, The University of Tokyo
"High- throughtput SNP analisis for isolation of genes susceptible to common diseases."

06.11.03
Anjana Rao, Dep. Of Pathology, Harvard Medical School, Boston, USA
"Gene regulation by NFAT."

07.11.03
Grace Pavlath, Emory University, Atlanta, USA
"NFAT mediated regulation of muscle cell drive."

11.11.03
Flora De Pablo, CIB, CSIC, Madrid
"Proinsulin gene regulation and function in early development."

11.11.03
Thomas J. Hudson, McGill University & Genome Quebec Innovation Centre Montreal
"SNPs, Haplotypes and Common Diseases."

14.11.03
Manuel Perucho, The Burnham Institute, La Jolla Cancer Research Center
"Genetics supersedes epigenetics in colon cancer phenotype."

21.11.03
Alberto Muñoz, Instituto Superior de Investigaciones Biomédicas, Madrid
"Vitamin D effects and regulation in human colon cancer."

18.12.03
Ian Mohr, NYU School of Medicine, USA
"Control of translation in herpesvirus infected cells."

Appendix 3

GRANTS

The grants that the CRG has obtained from September 2002 until the end of 2003 are the following:

ORGANISM	AMOUNT (€)
MINISTERIO DE CIENCIA Y TECNOLOGÍA	2.495.909,88
FUNDACIÓN DESARROLLO INVESTIGACIÓN EN GENÓMICA Y PROTEÓMICA	863.383,38
EUROPEAN COMISSION	830.992,79
MINISTERIO DE SANIDAD Y CONSUMO (Instituto Carlos III)	650.434,21
FUNDACIÓ MARATÓ TV3	154.271,59
HUMAN FRONTIERS SCIENCE	145.896,35
FUNDACIÓ LA CAIXA	124.994,60
FUNDACIÓN CIENTÍFICA DE LA ASOCIACIÓN ESPAÑOLA CONTRA CANCER	99.133,21
DURSI RECERCA	98.439,22
BUNDESMINISTERIUM BILDUNG FORSCHUNG (MAX PLANK)	72.000,00
TITAN PHARMACEUTICALS INC.	58.794,96
NATIONAL INSTITUTE OF HEALTH	56.100,52
FONDATION JEROME LEJEUNE	49.000,00
ALMIRALL PRODESFARMA, S.A.	34.500,00
THE LEUKEMIA & LIMPHOMA SOCIETY	25.569,86
SEVERAL GRANTS	9.844,75
NOVARTIS FARMACEUTICA, S.A.	6.807,22
PFIZER, S.A.	6.010,12
LILLY, S.A.	5.203,76
AGAUR	2.554,25
FAES FARMA, S.A.	2.000,00
BRISTOL MYERS SQUIBB	1.803,04
PANLAB, S.L.	900,00
REAL PATRONATO SOBRE DISCAPACITADOS	450,00
SOCIEDAD ESPAÑOLA BIOQUÍMICA	350,00
TOTAL AMOUNT:	5.810.968,71