CRG
Centre de Regulació Genòmica

Annual Report 2004-2005

SCIENTIFIC BOARD

CENTRE FOR GENOMIC REGULATION

Annual Report 2005

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Centre for Genomic Regualtion

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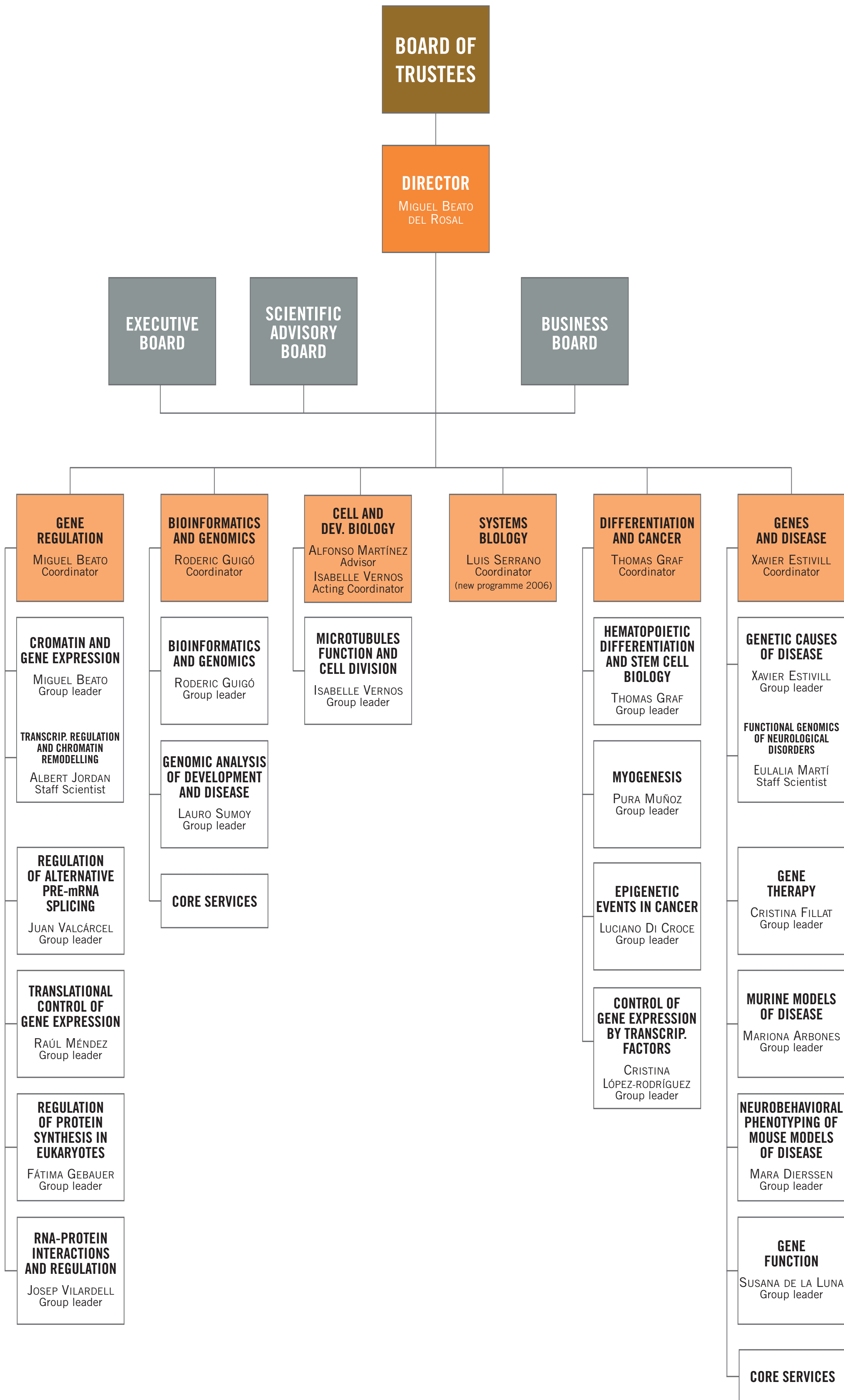
SCIENTIFIC STRUCTURE

Centre for Genomic Regualtion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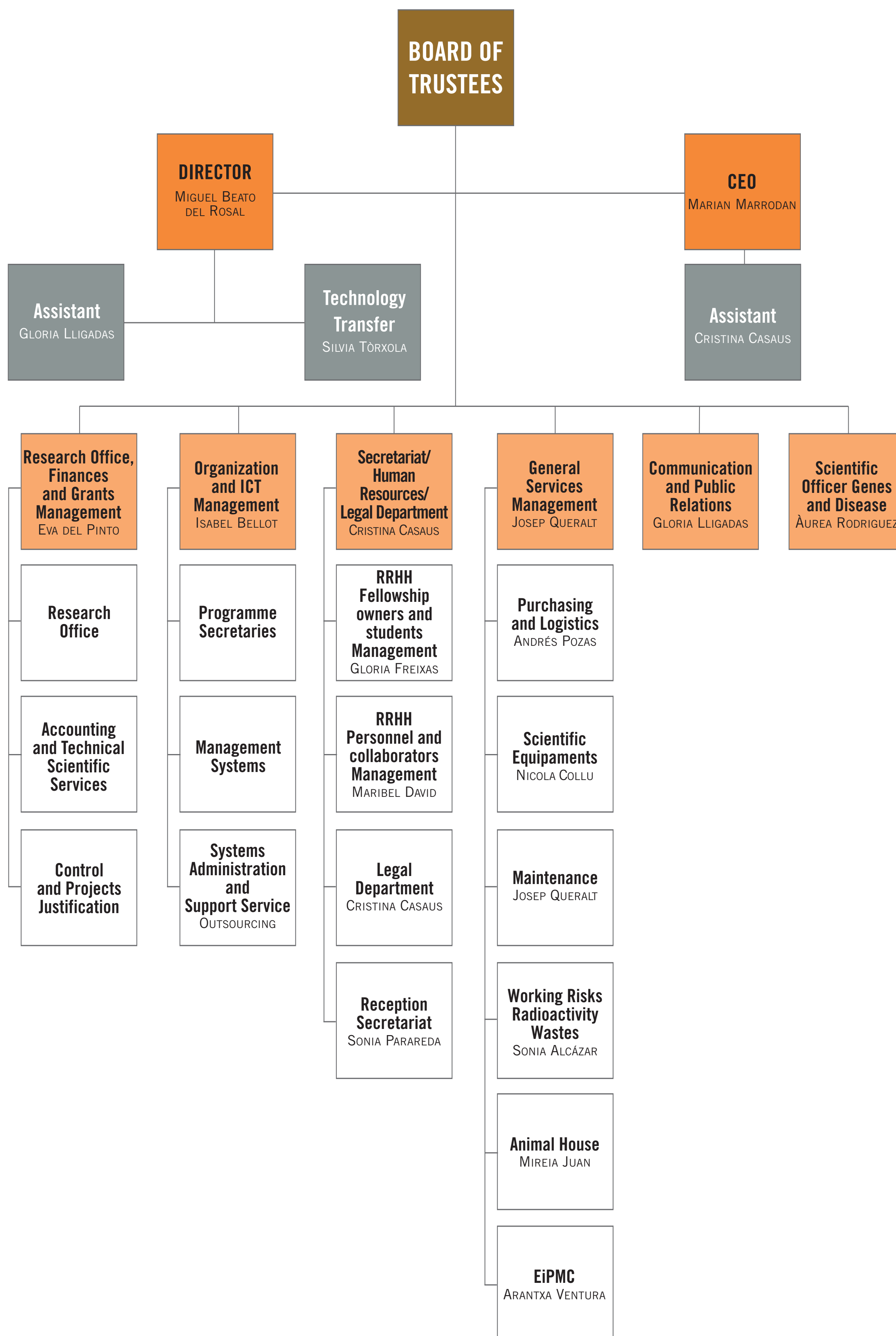

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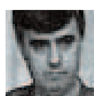

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General Manager, Fundación BBVA

CRG

LABORATORIS

YEAR RETROSPECT

Director of the CRG: Miguel Beato del Rosal

During the past year the CRG has continued with its consolidation in terms of established research groups and core facilities. Our envisaged growth has been made impossible by the fact that the moving to the new building of the PRBB has been delayed again to the beginning of 2006. The main achievements of the past year have been the formulation and negotiation of an institutional partnership agreement with the European Molecular Biology Laboratory (EMBL), for the creation of an *EMBL-CRG Research Unit in the field of Systems Biology*. In order to complete de panel of experts in the area of Systems Biology, Marc Vidal, from the Dana-Farber Cancer Institute, Boston, Massachusetts, has been nominated as the tenth member of the CRG Scientific Advisory Board. Moreover the foundations for the two new programmes have been established and with the help of the CRG Business Board, we have recruited a Project Manager dedicated to technology transfer issues.

In January a new contract was signed with the Department of Universities, Research and Information Society (DURSI) of the Catalan Government that provides a framework for our development until the end of 2006. By that time our contract with the Department of Health (DS) is also expiring. There is a consensus that early next year a new contract covering a longer time period, ideally for 8 years, should be elaborated and signed with DURSI and DS, in order to allow the CRG middle term financial planning.

Plans to establish an institutional partnership between EMBL and CRG with the objective to create an *EMBL-CRG Research Unit in Systems Biology* (ECRUSB) have advanced to the point that an agreement with the Spanish Ministry of Education and Science (MEC) is in sight. In several meetings of CRG scientists with representatives of MEC and DURSI, as well as with Iain Mattaj, Director General of EMBL, and the Spanish representative in the EMBL Council, a text was elaborated that envisages a nine years partnership, with the possibility for an extension after external evaluation. The decision was based on the recognition that the CRG is the only Spanish institution in the area of biological research that fulfils the criteria expected from EMBL in terms of scientific organization, recruitment and mobility of group leaders, scientific advisory board and external evaluation, as well as international character. Ad hoc resources from the Spanish MEC will cover the cost of installation and functioning of the ECRUSB, which will operate in close connection with the EMBL and will be directed by Luis Serrano, the acting coordinator of the CRG Systems Biology programme. We expect the agreement to be signed early 2006.

The four established programmes have intensified their activity within the limitations of the available space. Ties and collaborations within the groups of each programme and across the programmes have increased considerably. The programmes have initiated the search for group leader candidates, who will join the CRG after the moving to the PRBB building. Following the announcement in Nature, a large number of scientists applied for the open group leader positions. More than half of candidates were foreigners, underlining the international recognition of the CRG as an attractive centre for basic biomedical research. A small group of candidates were selected for seminars and interviews, as the result of which offers has been made to eight scientists, whose names and research plans will be mentioned in the introduction to the various programmes.

In an attempt to assimilate the CRG structure to that of EMBL and also to facilitate selection of a new Director in due term, the CRG Executive Committee and the Board of Trustees approved the creation after the scientific evaluation in 2007 of the *Director's Research Group* without a formal programme affiliation. This decision implies that the Programme Coordinator position and his Senior Scientist position in the *Gene Regulation* programme will be free in the near future. The selection of the Programme Coordinator will be carried out under the supervision of the Scientific Advisory Board. The establishment of a *Proteomics facility* associated to the programme is planed for 2007.

The advisor of the programme *Cell Differentiation and Cancer*, Thomas Graf, has accepted to sign a contract with ICREA and with the CRG committing himself to move to Barcelona next September, when the animal facility is expected to be fully functional. The programme has started the search for new group leaders who will start after the moving to the PRBB building and has established the core of the *FACS facility*.

The *Genes and Disease* programme has continued to develop its ties within the CRG and with the clinical research groups particularly in the area of psychiatric disorders and mental retardation. Members of the programme participate in a number of research networks at national and international level. The *Genotyping facility* established with the support of Genoma España has continued its development and is now providing services for several groups within the PRBB and also for external scientists. The *Transgenic facility* has started to offer services for the creation of transgenic and knockout mice strains.

The advisor of the *Bioinformatics & Genomics* programme, Roderic Guigó, had been working on a part time contract with the CRG. Since October 2005 he has a full contract as programme coordinator with the CRG. He is also in charge of the Barcelona node of the Spanish *Virtual Institute for Bioinformatics (IVB)*, which was created by Genoma España and has another node in Madrid. The IVB will provide bioinformatics expertise to the scientific community. The *Microarray unit* continues to expand and provides services and advice to many groups within the CRG/PRBB and from other Barcelona research centres.

With the help of the external advisor Alfonso Martínez Arias the *Cell and Developmental Biology* programme managed to recruit Isabelle Vernos from EMBL, who obtained an ICREA position and started her group in the summer. Coinciding with her moving to Barcelona, Isabelle was elected member of EMBO. Hernan Lopez-Schier from Rockefeller University has accepted an offer of the CRG as group leader and will come to the CRG when the PRBB building is finished. Isabelle has also taken the responsibility of organizing the *Advanced Light Microscopy facility*. Following the appearance of an ad in Nature several candidates were interviewed and an offer has been made to an excellent candidate.

The plans for the establishment of the *Systems Biology* programme are coordinated by Luis Serrano, who signed a contract with ICREA and with the CRG last fall and has initiated the recruitment of group leaders. A small group of 6 candidates was selected and attended a mini-symposium February 11/12 in Heidelberg, where they were interviewed by a searching committee including scientists from the CRG

and from EMBL. Two candidates were selected and received an offer. The first was James Sharpe from MRC in Edinburgh, who has accepted to be a senior group leader of the CRG, and the second was Mark Isalan from the EMBL in Heidelberg, who will be the first group of the EMBL-CRG unit. Both candidates will come to the CRG when we move to the new building of the PRBB. Recruiting will continue next year with the intention to recruit at least two additional groups for the EMBL-CRG Unit. The CRG has decided to change the pre-doctoral fellowships during the third and fourth year to full contracts with the standard social benefits. A procedure similar to that in use at EMBL has been established for following the progress of the thesis research projects that also defines the composition of the Thesis Committees and how they operate.

Following a recommendation of the Business Board the CRG recruited Silvia Tórtola as a Project Manager in charge of detecting potential applications of the CRG research and promoting the protection of the intellectual property and the relationship with biotech or pharma companies. Silvia started in September and since then has been very proactive in learning about the science in the CRG and established initial contacts with EMBL from Heidelberg and Cancer Research Technology from the UK. Representatives of the two companies visited the CRG and are negotiating a TT agreement. Moreover Silvia has initiated the procedures for protection of two discoveries at the Spanish and European level.

Following an evaluation by the consulting company Konsac, the organization of the CRG has been changed in order to be prepared for the expansion of the institute expected next year. Five main areas have been defined for which responsible persons have been nominated:

- Research Office, Finances and Grants
- Organization and Information and Communication Technologies (ICT)
- Secretariat / Personnel / Legal Department
- General Services: purchasing and logistics, scientific equipments, maintenance, working risks, radioactivity and wastes, animal house, sterilization, etc.
- Communication and Public Relations

Since September the new integrated management system ORACLE is in full operation.

To make the activities of the CRG comprehensible for the general public, we have started a project with a science journalist and a design team for publishing a popular version of the main scientific projects of the centre in form of an intriguing story. We expect to have it finished next spring and to distribute it during the inauguration of the PRBB building. If the project is successful we plan to edit every year a short popular report focused on specific scientific achievements of the CRG scientists.

Apart from the Data Seminars and Journal Clubs organized weekly by the individual programmes, and from the monthly Faculty meetings of all group leaders and staff scientists, in January 21/22 the CRG group leaders met for the third time in the context of a two-days retreat near Barcelona. The meeting was also attended by the three programme advisors who are not yet established in Barcelona, Alfonso Martínez-Arias, Luis Serrano and Thomas Graf.

The main problems presently faced by the biomedical research community were discussed and the potential contributions of the CRG were debated in this context. The conclusion was that the CRG should focus on epigenetics, computational biology, imaging and medical genetics. Moreover the CRG should establish within the PRBB state-of-the-art core facilities in these areas as well as in genomics and proteomics. These conclusions were further elaborated in a brainstorming meeting of all group leaders of the CRG on July 4th. After long and thorough discussion we agreed on three interconnected main lines: i) Epigenetics, that groups three programmes (Gene Regulation, Cell Differentiation and Cancer, and Cell and Developmental Biology), ii) Computational Biology, that groups two programmes (Bioinformatics and Genomics and Systems Biology), and iii) Mechanisms of Disease that focus on the Gene and Disease programmes and should be oriented towards more molecular ad cell biological mechanisms as well as to mathematical models through new groups and collaborations with the other programs.

On January 28 the first meeting of the Hormone Dependent Cancer Network (CAHODE) financed by the DURSI took place at the CRG with the participation of several speakers from the Barcelona area and invited speakers from France, Austria and The Netherlands.

From March 14 to 20 the first Brain Awareness Week, coordinated by Mara Dierssen took place at the CRG with more than 350 participants and a plethora of activities.

On October 7, the CRG hosted the first meeting of the Iberic Chapter of the EMBL Alumni Association, with excellent talks by Peter Becker, Munich, and Angus Lamond, Dundee. The act closed with a conference by Andreu Mas-Colell former Universities, Research and Information Society Minister of the Catalan Government.

The *fourth CRG Symposium* was held on December 2/3, 2005. It was organized by Xavier Estivill, and attracted many colleagues, who came to listen to the 20 invited speakers. The speakers gave excellent talks on the topic of the meeting "*Connecting the Genome with Disease*", which were followed by lively scientific discussions. As usual the attendance was free and more than 200 people participated.

The construction of the *PRBB building* has progressed, though more slowly than originally planned. Coordination with the other institutions and the definition of the various labs, meeting rooms and services as well as of the equipment has required a strong effort of both scientists and the administration of the CRG. The plans are now that the building will be finished and ready for moving in early 2006.

According to the agreement signed with the UPF in 2004, to become a University Associated Institute, during 2005 CRG scientists participated in advanced teaching of Biology students at the UPF, in particular in the fifth year of the Basic Research itinerary and in the International PhD programme on Basic Biomedical Research. The CRG contributes to financing the Journal subscriptions of the UPF library.

Integration with the other Institutions in the PRBB has been favoured by the 54 *Friday Seminars* with foreign speakers organized by the CRG, which are regularly attended by scientists and students from the CEXS/UPF and the IMIM. In addition the various programmes of

CRG organized over 100 Seminars, 28 of them with external speakers, which were also very appreciated by the other PRBB colleagues.

Numbers. By the end of 2005 the CRG had 17 research groups and a total of 168 people, including 50 senior and postdoctoral scientists, 56 graduate students, 35 technicians and 27 administrative and support. Relative to 2005, this represents an increment of 14 persons in the number of people working at the CRG.

As detailed in the reports of the individual groups and in the Appendix 3, scientists at the CRG continue to be successful in obtaining *external grants* and financial support for their scientific projects. The total amount of competitive resources granted to CRG scientists in 2005 exceeded 4.6 million.

During the last year there were 80 *publications* published or in press with authors affiliated at the CRG, and the average impact factor per paper was 7.641.

Retrospectively, 2005 has been a bit more difficult than the previous ones. The provisional labs of the CRG as well as the offices of the administrative area are extremely crowded. This, along with the need to define the labs in the new building, has created considerable problems and slowed down the scientific output of the groups. Nevertheless, the CRG has continued to grow and has consolidated its scientific reputation as an emergence centre of excellence in Biomedical research. I want to thank all the scientists and the administration for the enormous effort they have made to continue working with very high standards despite the difficulties imposed by space limitations and planning constrains. The CRG needs urgently to expand in the new building in order to develop the research programmes and to establish state-of-the-art core facilities, specially the animal house. The plans for recruitment give reason to hope that, after moving to the new building, we will continue the development of our scientific productivity along the expected lines.

GENE REGULATION

Coordinator: Miguel Beato

The programme is still composed of the same five groups (Fátima Gebauer, Raúl Méndez, Juan Valcárcel, Josep Vilardell, Miguel Beato) that have been operative since 2002, but plans for incorporating two additional groups were developed in 2005. A Senior Group Leader position has been offered to Ramin Shiekhattar from the Wistar Institute in Philadelphia, USA. Ramin is an expert in the biochemistry of multi-subunit complexes with a special interest in micro RNA biogenesis. He will bring to the CRG a well-developed technology for studying molecular machines and ample know-how in proteomics. Four candidates for the junior Group Leader position available in the programme were selected and interviews were planned for 2006.

The programme has also decided to establish a *Proteomics facility*. The planning for this facility, which should be functional in 2007, has been assigned to Juan Valcarcel. The development of the facility will be coordinated with the actual proteomic facility of the UPF/CEXS.

Since the fall this programme holds its Data Club and Journal Club meetings together with the Differentiation and Cancer programme.

From May 12 to 14, the Centre for Developmental Biology of Andalusia (CABD), in Seville, organized a Symposium with the participation of Christiane Nüsslein-Volhard, member of the CRG SAB. In the context of the Symposium, the University Pablo de Olavide invested Miguel Beato as *Dr. Honoris Causa*.

In September 15 the CRG hosted a mini-symposium on “Histone H1: Why so many isoforms?” organized by Albert Jordan and Miguel Beato, with the participation of several European invited speakers.

GENE REGULATION

Chromatin and Gene Expression

Group Leader
Miguel Beato del Rosal

Postdoctoral Fellows
Cecilia Ballaré
Mike Edel
María Jesús Meliá
Guillermo Vicent (Ramón y Cajal)

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

Technician/s
Jofre Font
Silvina Nacht
Nora Spinedi (till May 2005)

Visitors
Patricia Saragüeta

Subgroup
Transcriptional Regulation and Chromatin Remodelling

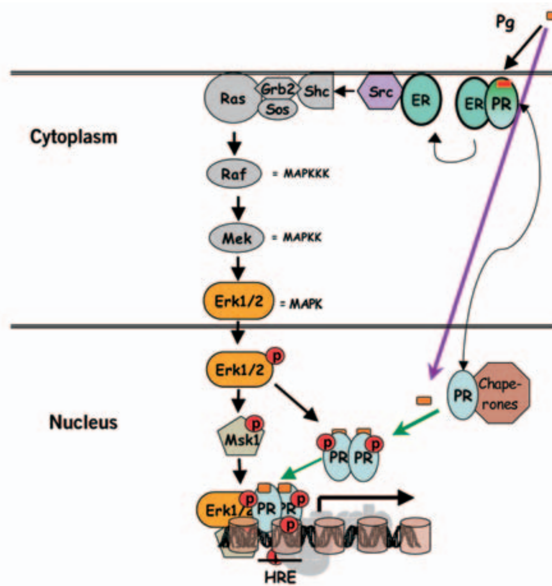
Subgroup structure

Staff Scientist
Albert Jordan (subgroup leader)

PhD Students
Ignacio Quiles
Mónica Sancho
Alicia Subtil
Edurne Gallastegui

SUMMARY

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



RESEARCH PROJECTS

1. Crosstalk between hormone receptors and other signalling pathways

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

We have reported that progestins can activate transiently the Src/Ras/Erk pathways (Migliaccio *et al*. EMBO J 17, 2008, 1998) via an interaction of two domains the progesterone receptor (PR) with the estrogen receptor alpha (ER?), and that Erk kinase activation is essential for the proliferative response of breast cancer cell lines (Ballaré *et al*. Mol Cell Biol 23, 1994, 2003; Migliaccio *et al,* 2005). We have now identified the amino acids implicated in the interaction between PR and ER?, and have generated PR mutants with defects in ERID-I useful for the study the role of the progestin activation of the Src/Ras/Erk cascade in cultured cells. We are using new cell lines constitutively expressing PR mutants to study the role of various PR domains on chromatin dynamics during progesterone induction of gene expression.

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

2. Regulation of MMTV transcription in the chromatin context

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

After clarifying the complex role of nucleosome positioning and histone H1 in the induction process, the group has studied the structural changes accompanying activation of MMTV promoter chromatin and how they are catalyzed. Within 5 minutes of progestin addition to breast cancer cells carrying an integrated copy of the MMTV promoter, a ternary complex of PR and two activated kinases, pErk1/2 and pMsk1, is recruited to the promoter and leads to phosphorylation of histone H3 at serine 10 and dissociation of HP1g from the promoter (Figure 2, Vicent *et al*, submitted). This is a prerequisite for recruitment of ATP-dependent chromatin remodelling complexes histone modifying enzymes. Shortly thereafter we detect the displacement of histones H2A and H2B from the promoter nucleosome containing the HREs but not from the adjacent nucleosomes (Figure 2). The remodelled nucleosome is stabilized by binding of NF1, which ultimately enables access of PR to previously masked HREs.

On nucleosomes assembled with recombinant histones, SWI/SNF catalyzes displacement of H2A/H2B dimers from MMTV promoter but not from positioned nucleosomes containing ribosomal promoter DNA (Vicent *et al*. Mol Cell 16,

Figure 1. *Model for crosstalk between membrane and nuclear PR*. When progestins (Pg) reach a target cells, they bind first a complex of PR and ER on the inner side of the cell membrane and subsequently the nuclear PR-chaperones complex, leading to its dissociation and the formation of PR homodimer. The membrane PR/ER complex activates the Src/Ras/Erk cascade via an interaction of ER with Src. The activated Erk translocates to the nucleus where it phosphorylates the homodimer of PR and Msk1, leading to the formation of a ternary complex that interacts with target genes in chromatin.

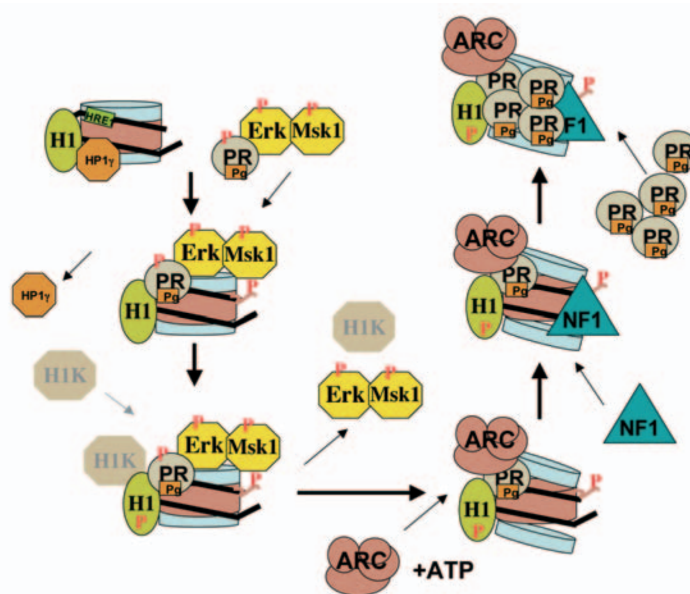


Figure 2. *Model for the initial steps in the progesterone activation of the MMTV promoter.* The complex of activated PR/Erk/Msk1 binds the accessible HRE1 and phosphorylates histone H3 at serine10, leading to the dissociation of HP1g. Another kinase (indicated by H1K) phosphrylates histone H1, a prerequisite for ATP-dependent remodelling by a complex (ARC) that binds the activated PR on the target nucleosome. Remodelling involves dissociation of H2A/H2B dimers and is stabilized by NF1 binding, enabling recruitment of additional PR to the previously masked HREs.

439, 2004), suggesting the existence of topological information in the DNA sequence that determines the outcome of the remodelling process. We are extending these studies to other nucleosomes in order to understand the properties of the DNA sequence responsible for the differential responses to SWI/SNF action.

We are also studying the role of various linker histone isoforms on the remodelling and transcription of MMTV minichromosomes assembled on *Drosophila* embryo extracts, and are using this systems to investigate the role of core histone variants and histone modifications on MMTV chromatin function.

3. Role of steroid hormones in breast cancer and endometrial physiology
M.J. Meliá, V. Calvo, M. Edel, P. Saragüeta

In collaboration with the Departments of Pathology and Oncology of the Hospital del Mar, and with Belen Miñana of the Microarray Unit of the CRG, we are studying the gene networks regulated by estrogens and progestins in breast cancer cells and in primary tumour material. This information will be correlated with the effect of kinase inhibitors and with the analysis of SNPs and epigenetic marks in the promoters of the relevant genes studied by ChIP-on-chip.

In collaboration with the group of Adalí Pecci, University of Buenos Aires, we have identified the HREs responsible for progesterone induction of the *bcl-X* gene in mammary epithelial cells (Rocha-Viegas *et al.*, J Biol Chem 279, 9831, 2004). We are now analyzing the molecular mechanism involved in the tissue-specificity of the apoptotic response to hormones, focusing on the differential effect of glucocorticoids in thymocytes, where they induced apoptosis, and in mammary epithelial cells, where they prevent apoptosis. We find that this difference is mediated by selective functional interactions with members of the STAT5 family of transcription factors (Rocha-Viegas *et al*, submitted).

In collaboration with the group of Patricia Saragüeta, CONYCET, Buenos Aires, we have defined the differential response of endometrial stromal cells to progestins alone or in combination with estrogens. These cells have very low amount of PR and are unable to mediate transcriptional regulation of progesterone reporter genes, thus allowing dissociation of the non-genomic and the genomic effects of progestins. We found that very low concentrations of progestins induce cell proliferation via activation of the crosstalk of PR with ERß and the mitogenic kinase cascades Src/Ras/Erk and PI3K/Akt (G. Vallejo *et al*, 2005). PR and activated pErk co-localize in the nucleus of activated cells (Figure 3).

We are also studying the role small GTPases in the angiogenic response of endothelial cells to estrogens, as well as the role of C/EBPb in progesterone-mediated cell cycle arrest in breast cancer cells via regulation of the $p21^{Cip1/Waf1}$ gene expression.

Research Subgroup

Transcriptional Regulation and Chromatin Remodelling

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

1. Transcriptional regulation and chromatin remodelling of promoters responding to steroid hormones in breast cancer cells. Distinguishing between direct effects and those mediated by signal transduction pathways.

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

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

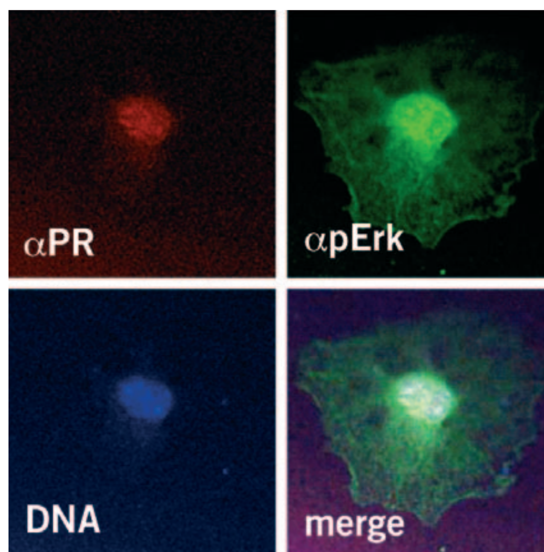


Figure 3. *Nuclear translocation of PR and Erk1-2.* Endometrial stromal cells treated with 10 pM R5020 for 5-10 min, were fixed, permeabilized and incubated with polyclonal antibody to hPR (aPR), or antibodies to phospho-Erk1-2 (apErk), and with Topro-3 Iodine to stain nucleic acids.

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

During the process of MMTV promoter activation by PR in *Drosophila* extracts, histone H1 is phosphorylated and leaves the promoter (Koop *et al*, EMBO J 22, 588, 2003). At least six H1 variants exist in mammalian somatic cells that bind to the nucleosome core particles and linker DNA. We are now developing RNA interference to create stable breast cancer cell lines lacking expression of each of the H1 variants specifically. With these cells we plan to investigate the role of each variant on MMTV promoter repression, activation and chromatin remodelling, as well as on global

gene expression by using microarrays. In parallel, we are developing specific antibodies for H1 isoforms 1 to 5 that will be used on ChIP experiments devoted to determine the presence of each form in several target promoters, as well as at a genome level using promoter microarray (chip-on-ChIP).

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

Finally, we are also studying the influence of chromatin at the integration site on the transcriptional activity of the HIV promoter. HIV integrates at a multitude of sites without any clear preference in the human genome. The chromatin environment at the integration site influences the nucleosome structure of the viral promoter and consequently its basal and Tat-induced transcriptional activity (Jordan *et al*, EMBO J 20, 1726, 2001), in a way that is independent of the degree of methylation of the proviral DNA (Pion *et al*, J Virol 77, 4025, 2003). In this respect, we have shown that at low frequency integration occurs at regions of heterochromatin (i.e. pericentromeric) leading to promoter repression and to a state of viral latency that can be reactivated upon T cell activation (Jordan *et a*l, EMBO J 22, 1868, 2003). 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.

PUBLICATIONS 2005

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GENE REGULATION

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

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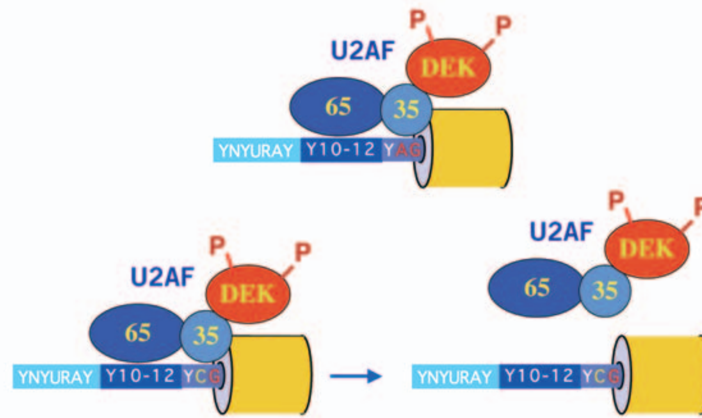
SUMMARY

We investigate how alternative splicing of mRNA precursors is regulated in different cell types or under different conditions. We focus on three questions: How are splice sites identified? How are splice sites regulated? How do cells program alternative splicing decisions in multiple genes? During 2005 we have made progress to understand how the protein DEK proof-reads 3' splice site recognition, to understand how mutations leading to disease can lead to aberrant splicing, to define factors and mechanisms that control alternative splicing of the Fas and Pax-6 genes and to use splicing-sensitive microarrays to study tissue-specific and disease-specific programs of splicing regulation, as well as their interface with signal transduction pathways.



RESEARCH PROJECTS

1. Splice site recognition

3' splice sites are initially recognized by the 65 and 35 Kda subunits of the splicing factor U2AF, which bind –respectively- to the pyrimidine-rich tract and AG dinucleotide present at the 3' end of the intron. We have found that the chromatin-associated protein DEK, previously implicated in transcription regulation, enforces specificity of 3' splice site recognition by U2AF: DEK interacts with U2AF35 and displaces the U2AF complex from pyrimidine-rich tracts not followed by the consensus 3' splice site AG (Figure 1). Surprisingly, DEK depletion causes inhibition of splicing without affecting the formation of splicing complexes, suggesting that absence of proof-reading of the early U2AF-3' splice site interaction has delayed consequences for proper function of the spliceosome. Phosphorylation of two serine residues at the amino-terminus of DEK is important for interaction with U2AF35, for enforced AG discrimination by U2AF and for the function of DEK in splicing. DEK is overexpressed in certain cancers and autoimmune conditions and translocations involving the DEK gene are characteristic of some forms of acute lymphocytic leukaemia. We plan to investigate how alterations in DEK expression affect alternative splicing.

We have also investigated how mutations leading to Autoimmune Lymphoproliferative Syndrome, Spinal Muscular Atrophy and Cystic Fibrosis affect recognition of splice sites by the splicing machinery. Our results indicate that different mutations affect distinct steps in splice site recognition, often different from those targeted by regulatory factors under physiological conditions.

Figure 1. Proof-reading of 3' splice site recognition by DEK. Recognition of the polypyrimidine-tract (Y10-12) and 3' splice site AG by the 65 and 35 Kda subunits of U2AF is an important early step in splice site identification. Phosphorylation of DEK at serines 19 and 32 allows interaction with U2AF35 and this causes the release of U2AF from pyrimidine-tracts not followed by consensus 3' ss.

2. Mechanisms of alternative splicing regulation

We are focusing on two alternative splicing events important for cell proliferation, differentiation and apoptosis. Alternative splicing of the Fas receptor generates a membrane-bound form of the Fas receptor that promotes apoptosis or a soluble form that prevents it (Figure 2). Through a combination of sequence mapping, biochemical assays and educated guesses we have identified five factors that influence Fas alternative splicing decisions. These include the apoptosis-promoting factort TIA-1, the well studied splicing regulator PTB and its cofactor Raver-1, SPF45 (previously described by our lab as a second-step splicing factor implicated in sex-specific splicing in Drosophila) and the products of the Ewing sarcoma oncogene (EWS) and the tumor suppressor LUCA-15/RBM5 (Figure 2). Analysis of the mechanisms of regulation indicate that different regulators target distinct steps of the splicing process. For example, we found that TIA-1 promotes binding of U1 snRNP to the 5´ splice site of exon 6, which in turn –through the process of exon definition- promotes the association of U2AF with the 3' splice site upstream of exon 6. PTB antagonizes this effect through an exonic silencer sequence by interfering with the molecular interactions that lead to exon definition. This effect of PTB –or similarly for its cofactor Raver-1- can be mimicked by tethering these proteins through an heterologous RNA-protein

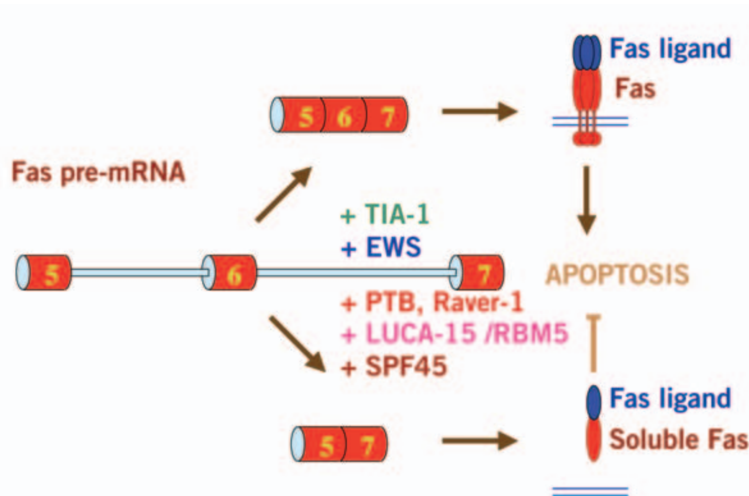


Figure 2. Factors regulating Fas alternative splicing. Fas exon 6 can be included or skipped to generate mRNAs encoding either the membrane-bound form of the receptor that promotes apoptosis or a soluble form that prevents programmed cell death. Indicated are protein factors that we have found to regulate this choice by either promoting exon 6 inclusion (TIA-1, EWS) or skipping (PTB, Raver-1, LUCA-15 and SPF45).

interaction, which is allowing us to finely map the minimal domains in PTB and Raver-1 that promote exon skipping. Different cis-acting elements mediate the effects of EWS, SPF45 and LUCA-15. Our results indicate that some of their regulatory effects take place after the exon has been defined but before spliceosomes are assembled on the adjacent introns, suggesting the targeting of interactions occurring in the transition between exon definition and intron definition.

Alternative splicing of the gene Pax 6 generates transcription factors of different target specificity that promote either proliferation or differentiation in a variety of developmental situations. For example, a mutation near the 3' splice site of exon 5a affects the ratio between isoforms that include or skip this exon and this change leads to aniridia, a defect eye development. We have identified TIA-1, LUCA15/BRM5 and SPF45 as factors that regulate this splicing event, as well as a network of molecular interactions that can explain the effects of aniridia-causing mutations.

3. Cellular programs of splicing regulation

In contrast with transcriptional regulation, the number of regulatory factors and in particular of tissue-specific factors is very limited in mammalian cells. This has led to the proposal that programs of splicing regulation are based on establishing cell type-specific differences in the levels of expression of general factors. Deciphering this *code* (and therefore predicting the functional read out of a primary transcript in a particular cell type), however, is not yet possible. Our group has approached this question by using microarrays designed to detect changes in alternative splicing of multiple genes and in parallel detect changes in expression of regulatory factors, with the aim of establishing correlations between the expression of regulators and splice site choices. We have applied this array design to the analysis of 1) RNAs from different tissues and 2) RNAs from cells derived from Hodgkin lymphoma tissues at different stages of tumor progression. Initial results have unraveled correlations between global changes in alternative splicing and tumor progression, as well as ectopic expression of neuron-specific splicing regulatory factors in high grade lymphoma cell lines and tumors, which is linked to neuron-specific splice site choices in these cells.

A third project involving microarrays focuses on analyzing the impact of activation of signaling cascades (e.g. insulin, wingless) on alternative splicing (a largely unexplored territory) in Drosophila cells. We have identified events regulated by these signals and are in the process of investigating cis-acting sequences that mediate these effects.

Collaborations

- With the group of Roderic Guigó (CRG) on projects related to computational prediction and modelling of alternative splicing.
- With the group with Maria-Carmo Fonseca

(University of Lisbon) on changes in alternative splicing occurring in cellular models of muscular dystrophies.

- With the group of Reinhard Lührmann (MPI, Göttingen) on proteomic and structural characterization of regulatory complexes.
- With the group of Michael Sattler (EMBL, Heidelberg) on structural characterization of DEK-U2AF interactions.
- With the group of Don Rio (University of California at Berkeley) on splicing-sensitive microarrays in Drosophila.
- With the group of Joachim Roesler (Technical University, Dresden) on mutations affecting alternative splicing of Fas that lead to ALPS syndrome.
- With the group of Lauro Sumoy (CRG) on splicing microarray data analysis.

PUBLICATIONS

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GENE REGULATION

RNA-Protein Interactions and Regulation

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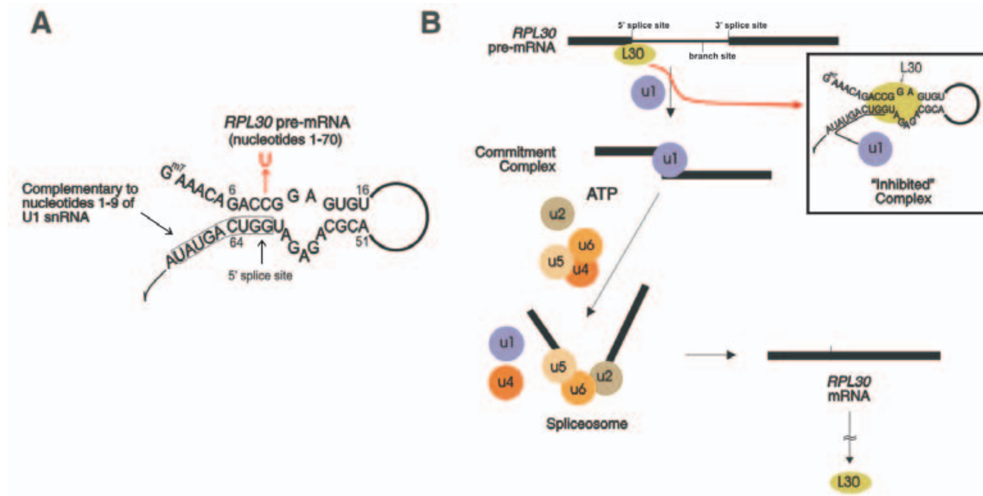
SUMMARY

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

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



RESEARCH PROJECTS

1. Genetic screen to select mutants in regulation of splicing
M. Bragulat

Employing refined screening methods we have isolated mutants that alter L30 regulation of splicing in either way. Some mutants display a phenotype in which L30 can not regulate splicing of a target transcript. Others behave in the opposite way, in which L30 can regulate splicing of a transcript with a mutation that blocks L30 inhibition in wild type cells. One of the latter mutations is located in the gen *STO1*, encoding the large subunit of the cap binding complex (CBC), Cbp80. CBC has been shown to be required for the proper stability of U1 snRNP bound to the pre-mRNA, and we are investigating how this can affect L30 regulation.

2. Characterization of the "inhibited" complex
S. Macias

As shown in the figure, during L30 regulation of splicing a new complex is formed. We are interested on the nature of this complex, its components and their interactions. Cross-linking and western approaches together with biochemical purification procedures are being followed. We have generated interesting data showing that U1 RNA can be crosslinked to the 5' SS and by co-IPs with extracts with tagged splicing factors a picture of the regulation mechanism is becoming clear, suggesting that L30 is likely to disrupt the crosstalk between splicing signals. We are trying to refine the particular step that is being altered by L30, with data pointing to the stable association of U2 snRNP to the transcript, after U1 snRNP has committed the substrate to splicing.

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

3. Genetic interactions in positions 3 and 4 of the intron
J. Vilardell (in collaboration with Charles Query (Albert Einstein College, NY; and Magda Konarska, Rockefeller University).

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

4. Other *S. cerevisiae* genes with regulated splicing
E. Muñoz y S. Ramundo

To what extent splicing regulation plays a role in the control of gene expression in *Saccharomyces*? Using bioinformatics, several studies have been, and are being, performed. We are looking at the possible folding around 5' splice site

regions, and putative alternate 3’SS. We include filogenetic comparisons in our studies. A putative novel case of alternative 3’SS has been uncovered, and others show discrepancies with the annotated sequences in our experimental conditions.

PUBLICATIONS

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Molecular Cell, 21, 543-553 (in press)

GENE REGULATION

Regulation of protein synthesis in eukaryotes

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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). We study these mechanisms of translational control under three different biological contexts: X-chromosome dosage compensation, early embryonic patterning and cell cycle progression.

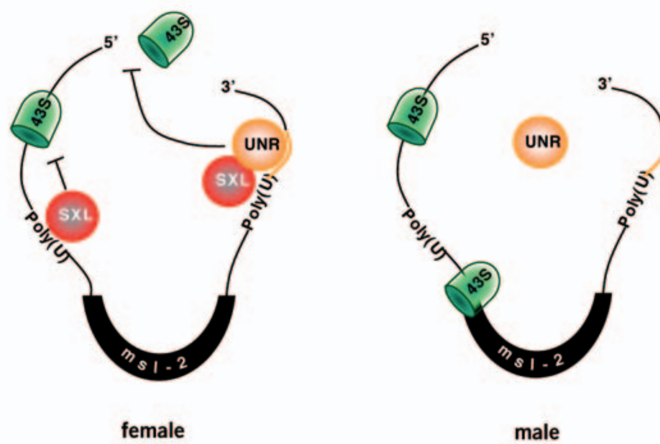

Figure 1. Translational control of msl-2 mRNA. SXL binds to uridinerich stretches in the 5' and 3' UTRs of msl-2. 3'-bound SXL recruits UNR, and the complex inhibits the association of the 43S pre-initiation complex with the mRNA. SXL bound to the 5' UTR inhibits the scanning of those 43S complexes that may have escaped the 3'-mediated control.

RESEARCH PROJECTS

Translational control of dosage compensation

Dosage compensation in *Drosophila* is achieved by hypertranscription of the male X chromosome via the action of a ribonucleoprotein complex known as the MSL (for male specific lethal). This process is inhibited in female flies primarily because the expression of a critical MSL subunit, the protein MSL-2, is repressed. Sex-lethal (SXL), a female-specific RNA-binding protein, binds to stretches of uridines present in the 5' and 3' UTRs of *msl-2* pre-mRNA, which ultimately results in inhibition of *msl-2* mRNA translation by a "fail-safe" mechanism: SXL bound to the 3' UTR inhibits the recruitment of the small ribosomal subunit, while SXL bound to the 5' UTR inhibits the scanning of those subunits that presumably have escaped the first control (Figure 1). Translational repression requires an additional factor that is recruited by SXL to the 3´UTR of *msl-2*. We have identified this factor as the *Drosophila* homolog of mammalian Upstream of N-ras (UNR). UNR is an ubiquitous protein present in both male and female flies. However, UNR binds to msl-2 mRNA only in females because binding of UNR depends on the previous binding of SXL. Our data shows that SXL provides a sex-specific function to UNR, and suggests that UNR is a novel regulator of dosage compensation in *Drosophila*.

Translational regulation of early embryonic patterning

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

Regulation of p27^{kip} mRNA translation

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

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Genes Dev. 20, 368-379 (in press)
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Cell, 122, 529-540 (2005)

GENE REGULATION

Translational Control of Gene Expression

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Technician
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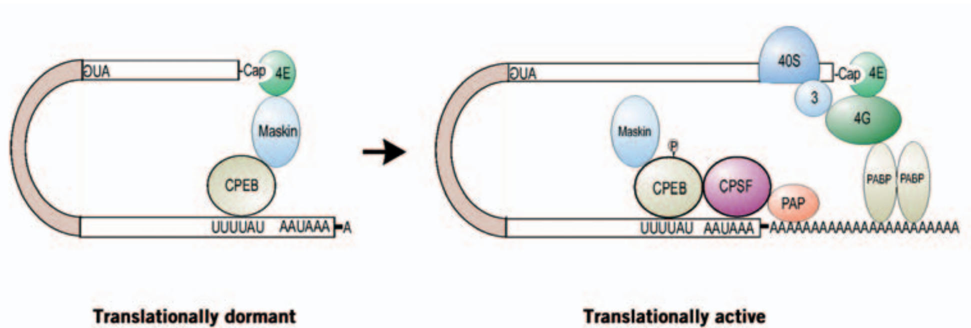
SUMMARY

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

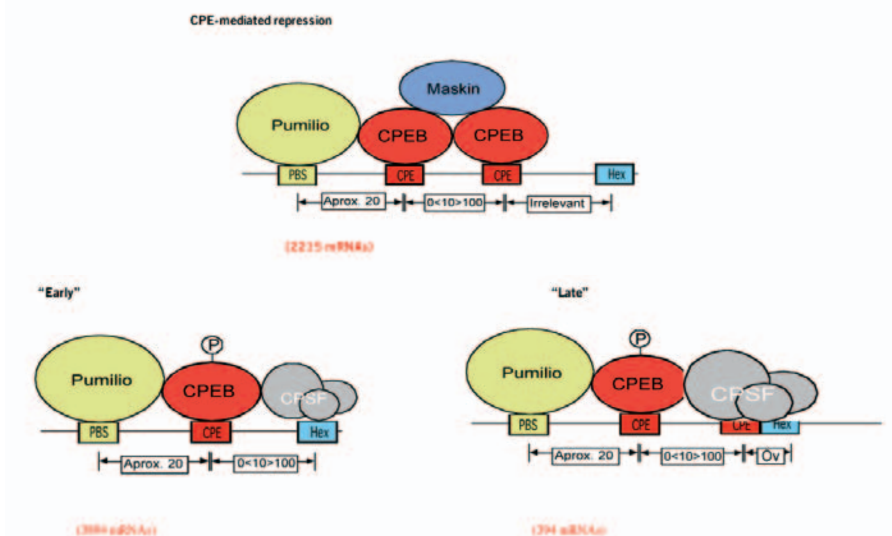


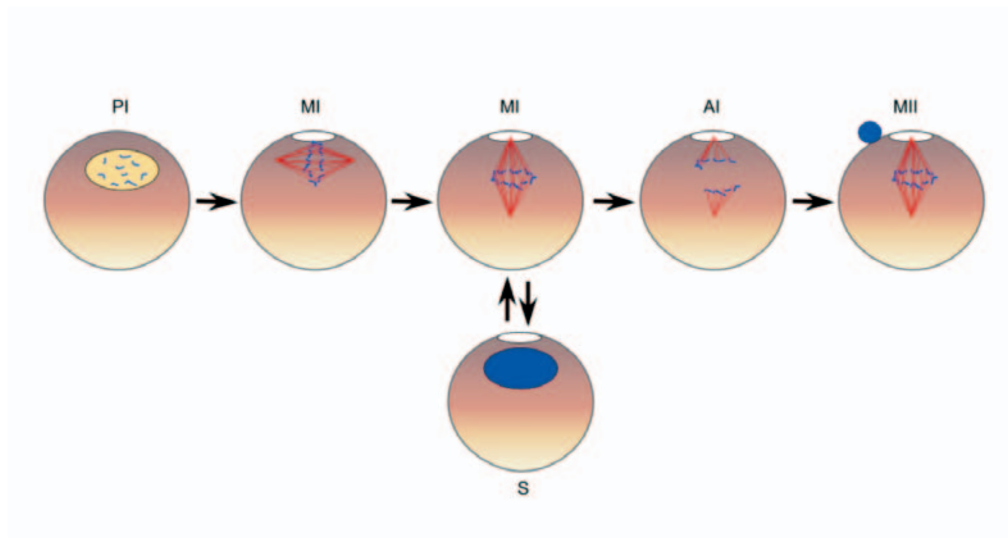
RESEARCH PROJECTS

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

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

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

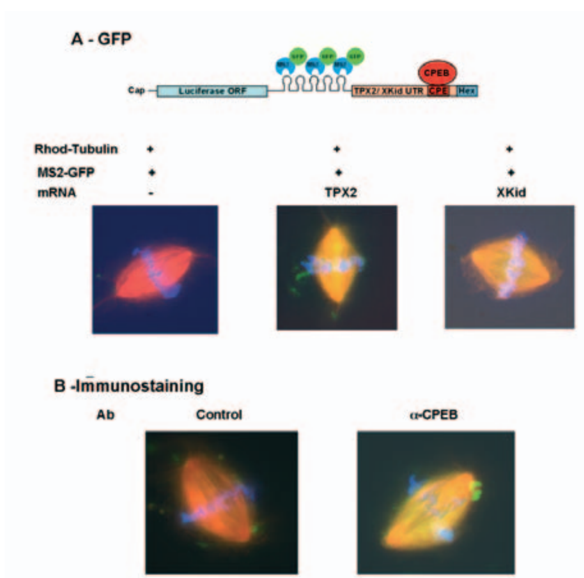




new mRNAs regulated by cytoplasmic polyadenylation and to predict their time and extent of activation.

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

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

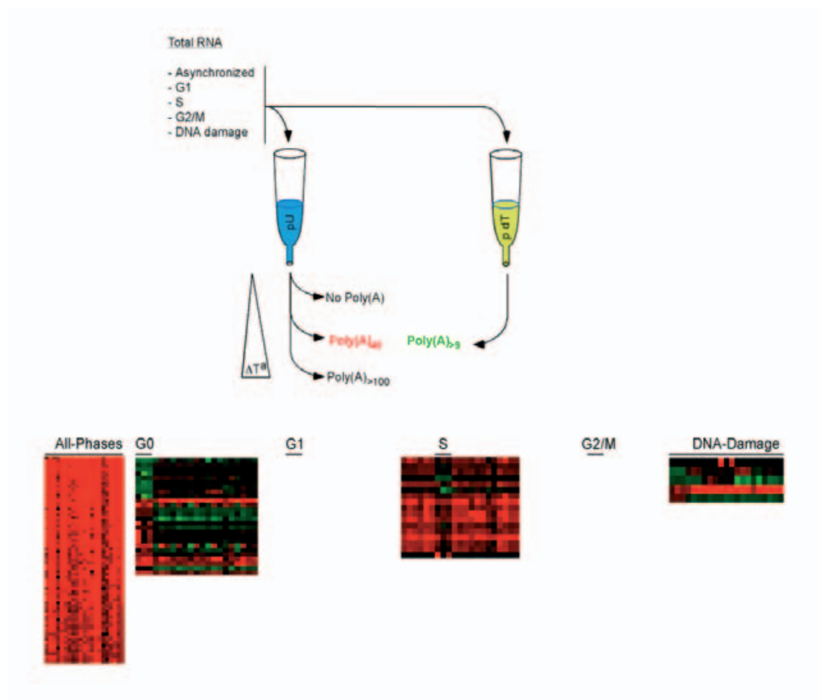


Based on the combination of cis-acting elements defined above. We have identified maternal mRNAs, encoding for proteins that regulate spindle formation, which are polyadenylated in response to progesterone and targets for CPEB-regulated translation. In collaboration with Isabelle Vernos group (EMBL/CRG)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

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

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

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



have characterized in detail a CPE-regulated mRNA encoding for a Zinc finger protein that, in turn, induces deadenylation of ARE/CPE containing mRNAs and regulates metaphase arrest.

4. Translational control of mitotic cell cycle

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

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

PUBLICATIONS

Book chapter

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

LEICA 300 FX

DIFFERENTIATION AND CANCER

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

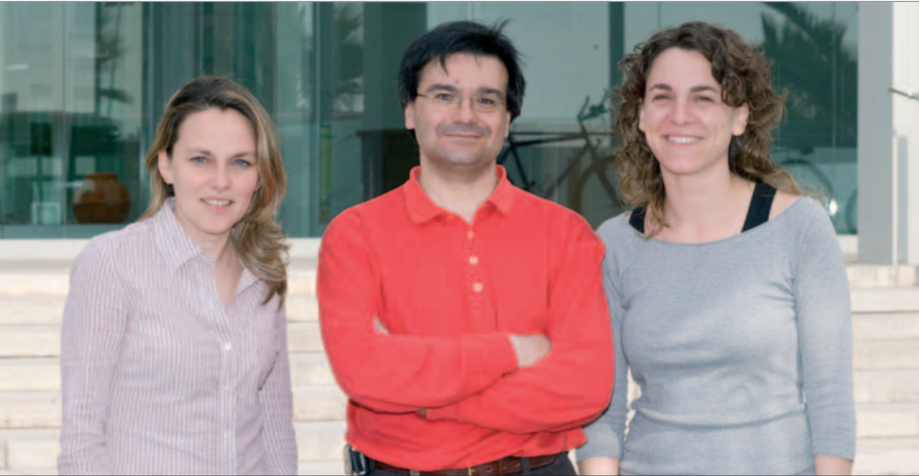
Since its inception early in 2002, the programme hosts four groups headed by Pura Muñoz, Thomas Graf, Cristina López-Rodríguez and Luciano di Croce. An additional group will join the programme in 2006, when the CRG moves to the new PRBB building.

Research within the Programme covers the areas of:

- Transcription factors in the development and function of specialized cells, in particular, blood and muscle cells (Pura Muñoz)
- Adult stem cells, plasticity and tissue regeneration (Thomas Graf)
- Control of gene expression by the NFAT5 transcription factor (Cristina López-Rodríguez)
- Epigenetic events in leukemia and the reprogramming of hematopoietic cells (Luciano Di Croce)

All groups work with mammalian cell lines and with mice, sharing their expertise in various technologies, such as FACS analyses, fluorescence microscopy and mouse genetics. They also have links and collaborations with members of the other programmes within the CRG. Group leaders, postdocs and students actively participate in Work in Progress and Journal Club Seminars of the Programme. They also attend the internal seminars organized by the other programmes, in particular, the Gene Regulation Programme. Cristina López-Rodríguez moved to the Department of Life and Health Science of the UPF on May, although until the moving to the new building she is still working in the facilities of the CRG. Her report covers only the first five months.

DIFFERENTIATION AND CANCER



Hematopoietic Differentiation and Stem Cell Biology

This group is still split between the Albert Einstein College of Medicine (New York, USA) and the CRG until October 2006, when Thomas Graf moves to Barcelona.

Group Leader
Thomas Graf

At Albert Einstein:

Postdoctoral Fellows
Min Ye
Weimin Ci
Matthias Stadfeld

PhD Students
Huafeng Xie
Cathy Laiosa

Technicians
Jinghang Zhang

At the CRG:

Postdoctoral Fellows
Florencio Varas
Alexis Schubert

Technicians
Luisa Irene de Andrés

SUMMARY

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

RESEARCH PROJECTS

1. Reprogramming lymphoid cells into macrophages by enforced transcription factor expression

During blood cell formation, the earliest multipotent progenitors branch into a common lymphoid and a common myeloid precursor. However, it is largely unknown which transcription factors determine the branching between the two compartments. In attempts to answer this question, we have chosen the approach used in our earlier work with chicken cell lines, consisting in the perturbation of transcription gfactor networks by enforced transcription factor expression in differentiated ('committed') blood cells. We have concentrated on the dichotomy between B cells and macrophages on the one hand and T cells and macrophages on the other

We found that the bZip type transcription factor C/EBPalpha, which is expressed in macrophages but not B cells, effectively induces a switch of B cell precursors towards functional macrophages. The activation of myeloid genes requires the collaboration between C/EBPalpha and the transcription factor PU.1, which is expressed in B cell precursors and B cells. In contrast, the extinction by C/EBPalpha of the late B cell marker CD19 is PU.1 independent, and is caused by an inhibition of the CD19 regulator, Pax5. Recent results indicate that this interaction is mediated by direct protein protein interactions, and can be visualized by FRET in cell cultures.

More recently these studies were extended to T cell precursors. CD4/CD8 double negative T cell precursors show a dramatic restriction of plasticity at the boundary between pro-T cell (DN1 and DN2) and pre-T cells (DN3 and DN4). Thus, while pro-T cells exhibit T lineage as well as NK, macrophage and dendritic potentials, pre-T cells are fully committed. Exploring how plasticity in this lineage becomes restricted, we found that the myelomonocytic-dendritic transcription factors C/EBPalpha and PU.1 are expressed in pro-T but not in pre-T cells. Testing the effects of their enforced expression in pre-T cells (using myeloid culture conditions) C/EBPa was found to induce the formation of macrophages and PU.1 of dendritic cells. When cells were grown under T cell conditions (OP9-DL1 stroma cells) the conversion induced by C/EBP alpha was partially and the one induced by PU.1 completely inhibited. Cell reprogramming involves the coordinated upregulation of myelomonocytic genes and the down-regulation of T cell genes, in PU.1 dependent and independent processes. C/EBPalpha

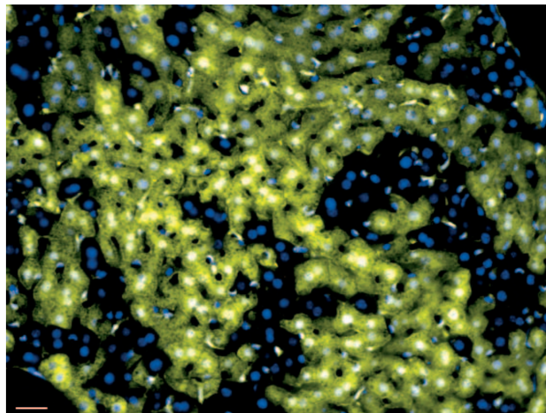


Figure 1. Using lineage tracing to explore developmental plasticity in vivo. The figure shows a section through the liver of a vavCre X Rosa26R-YFP mouse, with YFP positive cells in yellow and nuclei in blue. In this mouse all hematopoietc stem cells and their descendants are irreversibly labelled. The section (of a control mouse with a 'wide' expression pattern) shows a patch of labelled hepatocytes and in addition small YFP+ cells throughout the section that represent Kupffer cells. From: Stadtfeld and Graf, Development.

induced Thy-1 down-regulation does not require PU.1 and can be counteracted by both GATA-3 and activated Notch1, suggesting a cross antagonism between these factors. These results show that restoration of C/EBPalpha and of PU.1 expression in fully committed T cell precursors recapitulates the developmental plasticity in the earliest progenitors. They also suggest that during normal development the differentiation potential of pro-T cells becomes restricted by the selective inactivation of a subset of transcription factors, and that this process is modulated by Notch activity.

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

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

3. A CD41 EYFP knock-in mouse

CD41/GpIIb is one of the earliest markers detectable on definitive hematopoietic stem cells and is also a lineage specific marker of megakaryocytes/platelets. To study the development of this lineage from stem cells we have introduced the EYFP gene into the CD41 gene by homologous recombination. In this mouse, megakaryocytes and platelets are EYFP labeled, while no cells from other lineages express the reporter gene. In addition, a low percentage of hematoppoietic stem cells are EYFP+. For reasons that are unclear, only about 15-30% of the megakaryocytes and platelets in this mouse are labeled. Characterizing these cells revealed that the labeled platelets are more active than the unlabeled subset. The reason for this selectivity appears to be the age of the platelets, with newly made platlets being enriched compared to the

rest. These mice will be useful for the in vivo tracking of cells from the megakaryocyte lineage.

4 The role of the transcription factor PU.1 in B cell differentiation

The Ets family transcription factor PU.1 has been shown to be required for the development of B cells (as well as myelomonocytic cells and T cells). It has been proposed that its expression is required to up-regulate the transcription factor EBF and the IL-7 cytokine receptor, both of which are essential for B cell development (Singh, HR, PNAS 2005). We have now found that surprisingly PU.1-/- progenitors from fetal liver can develop into B cells, although at reduced efficiencies compared to wild type progenitors, suggesting that PU.1 is a facilitator of B cell development rather than strictly required. To address the role of PU.1 in vivo, we deleted the gene from the B cell lineage by Cre recombinase-mediated excision. We found that the excision of PU.1 induced a dramatic shift from B-2 cells (the major type of B cells, characterized by the expression of B220 antigen) to B-1 cells (characterized by the expression of the CD43 antigen and lack of B220 antigen). Interestingly, the cultured PU.1-/- B cells resemble chronic B cell leukemia cells by cell surface marker expression, and like the leukemic B cells inappropriately express the T cell markers lck and ZAP70. A novel research line in the lab explores the possibility that B-CLL is caused by the down-regulation or mutation of PU.1

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

Recent reports support the notion that the retroviral insertion sites into infected host cell DNA is less random than was previously assumed. Transcriptional active cellular genes are preferentially targeted and even cell type dependent hot spots for retroviral insertion have been described on specific chromosomes. In addition, it appears that in retrovirus infected stem cells transplanted into irradiated recipients a few clones become dominant. This may be due to the activation of a gene favoring the expansion of stem cells and may also constitute one of the contributing factors to the development of leukemias. To study whether specific retroviral insertions enhance the repopulation potential of hematopoietic stem cells a technique was developed to map to efficiently retroviral integration sites in the progeny of infected and transplanted stem cells. So far, more than 15 integration sites in transcribed sites have been mapped and the target genes identified. Current efforts aim at determining the biological significance of these genes for stem cell expansion.

PUBLICATIONS

Stadtfeld M, and Graf T
"Assessing the role of hematopoietic plasticity for endothelial and hepatocyte development by non-invasive lineage tracing."
Development, 132, 203-213 (2005)

Ye M, Ermakova O and Graf T
"PU.1 is not strictly required for B cell development and its absence induces a B-2 to B-1 cell switch"
J. Exp Med., 202, 1411-1422 (2005)

Stadtfeld M, Varas F, and Graf T
"Fluorescent protein-cell labeling and its application in time-lapse analysis of hematopoietic differentiation."
Methods Mol Med., 105, 395-412 (2005)

DIFFERENTIATION AND CANCER

Control of gene expression by transcription factors

Regulation of gene expression by NFAT5 during the immune response. Supported by a Leukemia & Lymphoma Society career development program. This group began its research activities at CRG in May 2003 and finished in May 2005.

Group Leader
Cristina López-Rodríguez

Students
Vanessa dos Reis Ferreira

Undergraduate students
Anaïs Estrada Gelonch

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-*kappa*B 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-*kappa*B) regulate specific gene expression in immune cells, we are interested in studying how NFAT5 regulates the development of the different immune-cell populations as well as their response to antigen. Beyond the analysis of NFAT5 in the immune system, we are also interested in characterizing the contribution of NFAT5 to major cellular processes.

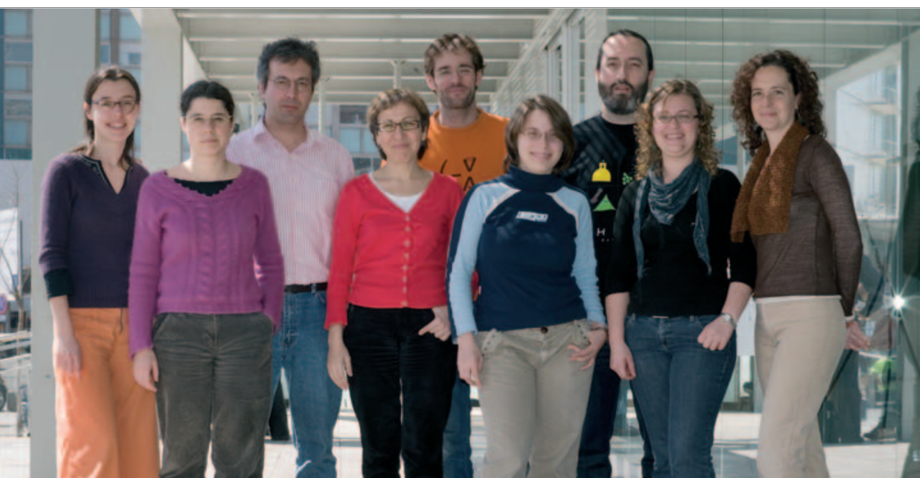
2. Gene expression pattern regulated by NFAT5

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

PUBLICATIONS

Esenten JH, Tsyttsykova AV, Lopez-Rodriguez C, Ligeiro FA, Rao A, Goldfeld AE
"NFAT5 binds to the TNF promoter distinctly from NFATp, c, 3 and 4, and activates TNF transcription during hypertonic stress alone."
Nucleic Acids Res., 33(12), 3845-54 (2005)

DIFFERENTIATION AND CANCER



Myogenesis

Group Leader
Pura Muñoz Cánoves

Postdoctoral Fellows
Mònica Suelves
Eusebio Perdiguero

PhD Students
Bernat Baeza-Raja
Berta Vidal
Vanessa Ruíz

Technicians
Mercè Jardí
Isabel-Cuartas
Gemma Cónsol

Ramón y Cajal Investigator
Antonio 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, Antonio Serrano (a Ramón y Cajal investigator in our laboratory) is analyzing the mechanisms involved in the regulation of muscle fiber type and size in vivo. In summary, our laboratory is pursuing three main lines of research:

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

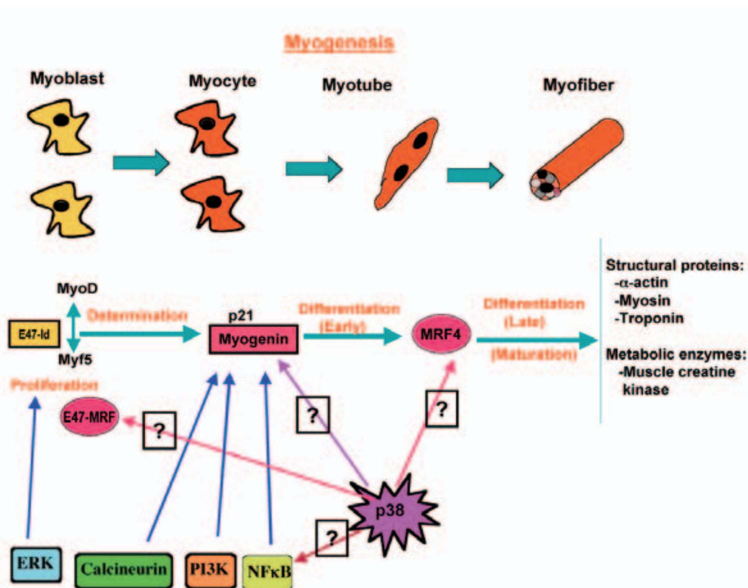


Figure 1. Possible phosphorylation targets of p38 MAPK that may account for the key role of this MAPK in myogenic differentiation (Lluis F, Perdiguero E, Nebreda AR, Muñoz-Cánoves P. Trends in Cell Biology 16: 36-44, 2006).

RESEARCH PROJECTS

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

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

1. p38 regulates formation of functional MRF-E47 dimers in myogenesis.

- Our recent results provide a novel mechanism demonstrating that p38 MAPK activity regulates MyoD/E47 association *in vitro* and *in vivo*. (*Lluis F, Ballestar E, Suelves M, Esteller M, Muñoz-Cánoves P; EMBO J. 24: 974-984, 2005*).

2. Differential role of p38 MAPK in early and late myogenesis: role of p38 on MRF4 activity.

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

3. Crosstalk between p38 and NF-kB signaling pathways in myogenesis.

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

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

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

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

- We have shown that uPA ameliorates dystrophy in mdx mice, an animal model of Duchenne Muscular Dystrphy (DMD), and injury-induced muscle regeneration via two mechanisms: fibrin degradation and mobilization of bone marrow cells during muscle repair. (In revision).
- We have demonstrated a role for uPA/plasmin-mediated pericellular fibrinolysis in myogenesis in vitro (Lopez-Alemany R, Suelves M, Muñoz-Canoves P. Thromb Haemost. 90: 724-733, 2003; Lopez-Alemany R, Suelves M, Vidal B, Muñoz-Canoves P. Frontiers in Bioscience, 10: 30-36, 2005).

2. Role of PAI-1 as a regulator of muscle growth. Preliminary results show that muscle size of PAI-1-deficient mice is larger than that of wild type mice, suggesting that PAI-1 may be negatively

regulating muscle growth (Suelves M, Vidal B, Ruiz V, Baeza-Raja B, Diaz-Ramos A, Cuartas I, Lluis F, Parra M, Jardi M, Lopez-Alemany R, Serrano AL, Munoz-Canoves P. Frontiers in Bioscience. 10: 2978-85, 2005). The mechanisms underlying PAI-1 function in muscle regeneration are being analyzed at present.

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

Mammalian skeletal muscle fibers comprise four major fiber types, including slow or type 1 and three subtypes of fast or type 2 fibers, type 2A, 2X and 2B. Each fiber type is defined by the presence of a specific isoform of myosin heavy chain and by a distinct program of gene expression. Skeletal muscle has also a remarkable capacity of self-regeneration due to the presence of a specialized cell type, the satellite cells. Neural activity controls muscle gene expression and regulates fiber size (Serrano AL, Murgia M, Pallafacchina G, Calabria E, Coniglio P, Lomo T, Schiaffino S. Proc Natl Acad Sci U S A. 98: 13108-13, 2001; McCullagh KJ, Calabria E, Pallafacchina G, Ciciliot S, Serrano AL, Argentini C, Kalhovde JM, Lomo T, Schiaffino S. Proc Natl Acad Sci U S A. 101:10590-5, 2004). Despite of the fact that changes in fiber types, reduction of fiber size (muscle atrophy) and a decrease of the muscle regenerative capacity are detected in many neuromuscular pathologies, the basic mechanisms underlying these processes have not been yet well characterised.

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

1. To investigate the signaling pathways, the transcription factors and their putative interactions, as well as the target genes involved in fiber type-specific gene expression and in the control of fiber size
2. To explore the relevance of the experimental manipulation of these networks for neuromuscular pathology in different animal models

PUBLICATIONS

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

Suelves M, Vidal B, Ruíz V, Baeza-Raja B, Díaz-Ramos MA, Cuartas I, Lluís F, Parra M, Jardí M, López-Alemany R, Serrano A, Muñoz-Cánoves P
"The plasminogen activation system in skeletal muscle regeneration: antagonistic roles of urokinase-typeplasminogen activator (uPA) and its inhibitor (PAI-1)"
Front. Biosci, 10, 2978-2985 (2005)

Nagamine Y, Medcalf R, Muñoz-Cánoves P
"Transcriptional and posttranscriptional regulation of the plasminogen activator system"
Thromb Haemost, 93, 661-675 (2005)

Lluís F, Ballestar E, Suelves M, Esteller M, Sartorelli V, Muñoz-Cánoves P.
"E47 phosphorylation by p38 MAPK promotes MyoD/E47 association and muscle-specific gene transcription."
EMBO J, 24, 974-984 (2005)

Lopez-Alemay R, Suelves M, Diaz-Ramos A, Vidal B, Muñoz-Canoves P
"Alpha-enolase plasminogen receptor in myogenesis."
Frontiers in Bioscience, 10, 30-36 (2005)

Vidal B, Parra M, Jardí M, Saito S, Paella E, Muñoz-Cánoves P
"The alkylating carcinogen N-methyl-N'-nitro-N-nitrosoguanidine activates the plasminogen activator inhibitor-1 gene throughsequential phosphorylation of p53 by ATM and ATR kinases"
Thromb Haemost, 93, 589- 591 (2005)

PATENTS

"Compound for the treatment of Duchenne muscular dystrophy", patent application No. PCT/ES2005/000619, filed on November 15, 2005.

DIFFERENTIATION AND CANCER

Epigenetics events in cancer

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

Group Leader
Luciano Di Croce

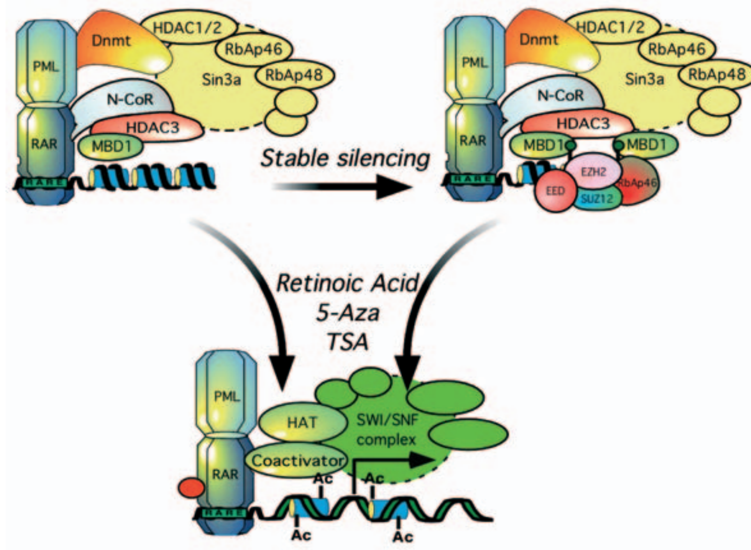
Postdoctoral
Marcus Buschbeck
Holger Richly
Ana Sofia Quina

PhD Students
Lluís Morey
Raffaella Villa
Iris Uribesalgo Micás

Technician
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-RARa are both required for fully silencing PML-RAR target genes. PML-RAR recruits MBD1 on its target promoter through an HDAC3-mediated mechanism. Furthermore, retroviral infected-hematopoietic precursors with MBD1 mutants (in either the MBD or the TRD domain) compromise the ability of PML-RAR to induce differentiation block, thus identifying MBD1 as a important player in PML-RARa promoter silencing subsequent to promoter hypermethylation, and as a potential candidate for cancer therapy. This research line thus has a strong potential impact on clinical aspects, as a point-mutated version of the MBD1 protein both prevents and reverts the PML-RARa hematopoietic differentiation block.

2. Role of epigenetic modification in cancer

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

3. Histone tail modification and heterochromatin

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

Di Croce L
"Chromatin modifying activity of leukemia associated fusion proteins"
Hum Mol Genet, 14, R77-84 (2005)

Brenner C, Deplus R, Didelot C, Danovi D, Pelicci PG, Amati B, Kouzarides T, de Launoit Y, Di Croce L, and Fuks F
"Myc represses transcription through recruitment of DNA methyltransferase corepressor".
EMBO J, 24, 336-346 (2005)

Buschbeck M, Hofbauer S, Di Croce L, Keri G and Ullrich A
"Abl-kinase-sensitive levels of ERK5 and its intrinsic basal activity contribute to leukaemia cell survival".
EMBO Report, 6 63-69 (2005)

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"The Polycomb group protein EZH2 directly controls DNA methylation"
Nature, 439, 871-874 (2005)

Villa R, De Santis F, Raker VA, Gutierrez A, Corsaro M, Minucci S, Pelicci PG and Di Croce L .
"MBD1 represses transcription and promotes leukaemia in cooperation with the oncogenic transcription factor PML-RAR".
Proc Natl Acad Sci (in press)

Carbone R, Di Croce L, Jenuwein T, Pelicci PG and Minucci S
"Histone methylation and establishment of a heterochromatin-like pattern at PML-RAR target genes".
Mol Cell Biol. (in press)

GENES AND DISEASE

Coordinator: Xavier Estivill

With the human genome sequenced, the sequences of the genomes of model organisms, and a massive amount of information on the genome variability in hand, we now have the opportunity to dissect the molecular components of common human disorders, such as cardiovascular disease, Alzheimer's disease, psychiatric disorders, diabetes and rheumatism. This basic understanding should help us to define major pathogenic pathways, and to develop strategies that could correct the biological defects that lead to disease. The Genes and Disease Programme of the CRG aims to study genomic variability related to disease, to investigate the function of genes with a potential role in common human disorders, and to design therapeutic approaches. The programme combines large-scale experiment approaches with forward genetic strategies to elucidate the biological basis of human disease.

Research Groups:

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

Efforts of Genes and Disease Programme investigators are focussed to the analysis of sequence and genomic variants of the human genome that could participate in the predisposition to human disorders, with particular emphasis in psychiatric disorders and mental retardation. These studies are enhanced by the use of neuropathological and behavioural characterization of models of disease. Specific collective work within the Genes and Disease Programme is focused on understanding the function of genes with potential implications in mental retardation and Down syndrome phenotypic traits. It is expected that dosage-sensitive genes will have critical consequences that can be explored by modifying the levels of their expression at the cellular level in vitro or in murine models in vivo. Investigators of the programme use murine models for the development of therapeutic approaches, that could correct features involved in mental retardation, anxiety disorders and cancer.

The Programme has set up facilities for high-throughput genotyping (Barcelona Genotyping Node of CeGen), neurobehavioral characterization of murine models and mice in vivo imaging. The Genes and Disease Programme has participated in several research networks supported by the "Instituto de Salud Carlos III – Fondo de Investigación Sanitaria" (ISCIII-FIS), including Clinical Genetics, Neuroscience, Hearing impairment, Psychiatric Genetics, and Cancer. In addition, Group Leaders of the Programme have participated in several research networks supported by the Department of Research Universities and Information Society (DURSI) of the "Generalitat de Catalunya", such as the Gene Therapy Network and Murine Models Network. The Programme participates in teaching activities in Human Genetics and Human Pathology of the Pompeu Fabra University. Finally, members of the Programme have strengthened links with several investigators of the PRBB and other investigators of the CRG.

During this year new PhD students and post-doctoral fellows have joined the programme. Data Meetings and Discussion Groups have become an important forum for of scientific life of all the members of the Programme. The programme has more than 20 ongoing research projects and 30 research fellows supported by national or international funding bodies.

Transgenics Unit:
Technician: Luís Sánchez Palazón (CRG)

During 2005 the microinjection lab has been set up and has produced transgenic mice by pronuclear injection for the CRG and Pompeu Fabra University groups. *Transgenesis by DNA injection has led to* different constructs with a total of 21 founders generated.

GENES AND DISEASE

Causes of Disease

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SUMMARY

The group focuses on the analysis of the variability of the human genome at the nucleotide and genomic levels and their relation with disease predisposition. This research has led to the identification of SNPs in BDNF and NTRK2 that protect or predispose to eating disorders and to detect gene variants involved in hearing loss. The group is also interested in studying the contribution of non-coding RNAs and copy number variants (CNVs) to human disease. Through the Genotyping Unit the group is searching for genes involved in the modification of non-syndromic hearing loss, the analysis of clinical variability in the response to methadone and to nicotine treatments, and the study of several psychiatric disorders (eating disorders, depression, anxiety and obsessive compulsive disorders, among others). The group is also exploring the contribution of non-coding RNAs, specially snoRNAs and miRNAs in the susceptibility to complex diseases.

RESEARCH PROJECTS

Segmental duplications, variability and human disease

Large-scale segmental duplications have played an important role in hominoid evolution and can be hotspots for non-allelic homologous recombination leading to deletion, duplication, inversion or translocation. Many of these segmental duplications coincide with structural variations or copy number variants (CNVs) of the human genome. We have performed a systematic analysis of the role of segmental duplications in disease. We have identified over 3,000 segmental duplications, of which about 50% are intrachromosomal. The group has designed an array for the complete characterization of CNVs and segmental duplications in relation with disease predisposition and subtle phenotype differences between subjects. The array contains about 6,000 BAC clones that cover the total length of each human chromosome, with a specific coverage for sequences located between and within segmental duplications.

The group has characterized is specifically working on two genomic regions that contain a complex organization of segmental duplications, one on chromosome 15q11-q13, and the other on chromosome 8p23.1. The 15q11-q13 region is involved in several genomic disorders. Three clusters of small nucleolar RNAs (snoRNAs), expressed in the central nervous system, are being studied for their potential implication in disease. The analysis of one of these snoRNA clusters in subjects of the general population, showing a high number of nucleotide changes indicative of gene conversion events at some of these snoRNAs. Since these hotspots for nucleotide changes in snoRNAs fall in regions that show complementation with the serotonin receptor type 2C, these sequences are being analysed for potential changes in eating disorders, for which we have detected association with the 5HT2C receptor. Another interesting region is a five-Mb segment of human chromosome 8p23.1, which is inverted in a significant proportion (about 25%) of individuals in the general population. The architecture of this region is being studied in humans and in other primates. Genes located at segmental duplications that flank this inversion, vary in copy number in different individuals.

We have compared the human, mouse and rat genome sequences and have demonstrated that recent segmental duplications correlate with breaks of synteny between these three species. Our data suggest that segmental duplications have participated in the recent evolution of these genomes. Furthermore, the boundaries of breaks of synteny are enriched in genes that have a role in adaptation to environment changes, immunity and response to external stimuli.

Role of neurotrophins and neurotransmiters in anorexia and bulimia

Eating disorders (ED) are complex genetic diseases that are classified into three subgroups: 1/ Anorexia Nervosa (AN); 2/ Bulimia Nervosa (BN); and 3/ intermediate disorders, known as Eating Disorders Not Otherwise Specified (EDNOS). These traits are known to be heritable and may be partially responsible for the genetically driven phenotypical variability within the different ED categories and could influence susceptibility to AN and BN. The brain derived neurotrophic factor gene (BDNF) plays a role in synaptic efficiency and neuronal plasticity. We have previously reported an associa-

tion between the Met66 allele of the Val66Met BDNF variant and restricting AN (ANR) and low minimum body mass index in Spanish patients. This association for BDNF has been confirmed in a large collection of trios from different European countries (France, Germany, Italy, Spain and United Kingdom). We have found that the Val66Met variant and another SNP located in the promoter region of BDNF (–270C>T) are associated with ANR, binge-eating/purging AN (ANBP), and BN. We have analyzed SNPs with a minor allele frequency higher than 0.10, covering the entire BDNF gene using the SNPlex technology in 174 ED patients and 174 sex-matched unrelated controls. We have also screened the NTRK2 gene and found association of a specific NTRK2 haplotype with binge-eating/purging AN, and a reduced frequency of another haplotype in BN patients. Finally, we have assessed BDNF plasma levels in discordant sib pairs with ED and found that BDNF levels were significantly higher in ED patients than in their unaffected sibs.

We have examined the involvement of the 5HT2C and SLC6A4 genes in the psychopathological symptomatology of ED. We have genotyped four SNPs within the 5HT2C gene and two sequence variants within the SLC6A4 gene to evaluate their involvement in the psychopathological symptomatology. Significant evidence of association between the a specific haplotype of the 5HT2C gene and different anxious and depressive subscales, that included Somatization, Obsessive-Compulsiveness, Depression, Anxiety, Hostility, Phobic Anxiety and Paranoid Ideation, was observed in BN patients. We also detected a strong association between the SLC6A4 genotype and Anxiety in the same group of BN patients. The analysis of epistatic effects between 5HT2C and SLC6A4, however, showed that the effect of the haplotype on the different anxious and depressive subscales was independent of the 5HTTLPR genotype.

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

Genetic factors that predispose to hearing impairment

Genes encoding for beta-connexin proteins are involved in hearing impairment. The group has shown that most cases of congenital deafness are due to mutations in GJB2. The group has found that a mutation in the mitochondrial genome (A1555G) is the commonest genetic cause of familial progressive hearing loss and is currently searching for genes and environmental factors that modify hearing impairment in subjects that carry mutation A1555G. The group has made progress in the characterization of a region of human chromosome 8p23 associated with the A1555G deafness. Several clusters of genes encoding defensins are located in this region. The number of repeat units varies between control individuals, and the potential clinical implications have to be

defined. Several other genetic factors are being studied, including the presence of heteroplasmia for mutation A1555G in samples of subjects that do not have the hearing loss phenotype.

Functional Genomics of Neurological Disorders
The main scientific interest of this emergent subgroup is to define the functional bases that contribute to the specific pathological outcome of human neurological diseases with a genetic component. Special interest is currently focused on neurodegenerative related regulatory networks involving mRNA and small RNA altered expression in human brain samples and in model systems for neurodegenerative processes, including mouse models and primary neuronal cell cultures. Neurodegeneration-linked common processes, such as impaired metabolic pathways, oxidative stress, necrosis and apoptosis are being analyzed. The final purpose is to gain information about gene regulatory networks modulating neurodegenerative processes, which might be suitable for diagnosis and identification of putative therapeutic targets.

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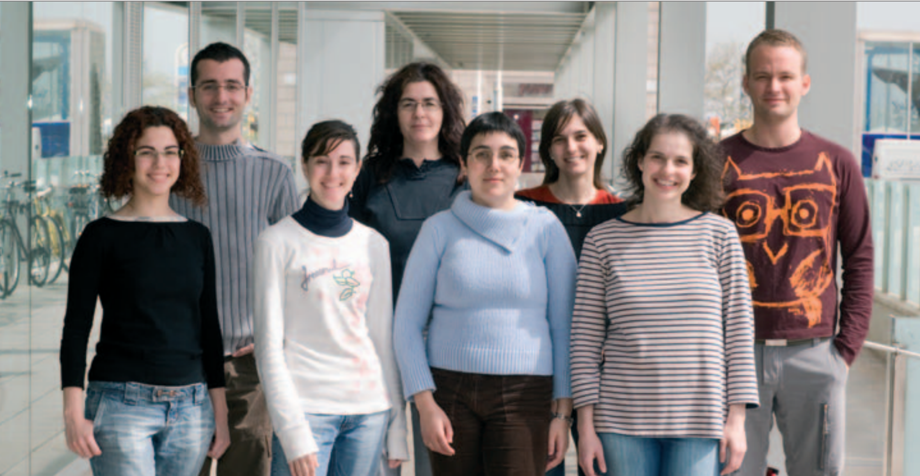
GENES AND DISEASE



Gene therapy

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Laura Garcia
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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. To be able to define gene transfer efficiency as well as the therapeutic response in living animals the use of molecular imaging techniques is fundamental. In that sense we have incorporated non-invasive bioluminescent systems that are becoming extremely helpful for gene transfer follow-up. The group is also working in the development of efficient gene delivery strategies for the local or systemic production of therapeutic proteins. These approaches allow us to study the role of hHGF as a renoprotective factor and to model the misuse of gene therapy for doping control.

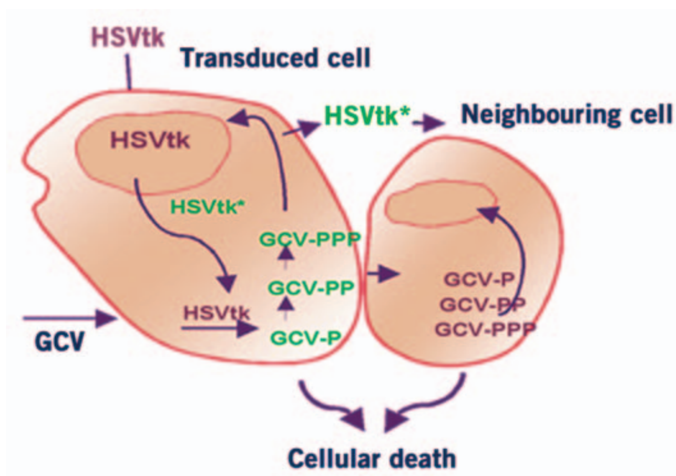

Figure 1. HSVtk/GCV suicide system

RESEARCH PROJECTS

1. Pancreatic cancer

One of the most devastating diseases that our society is presently facing is cancer. Particularly pancreatic cancer is the fifth cause of cancer deaths in industrialized countries. This neoplasia has a very bad prognosis mainly due to the late diagnosis together with the fact that current therapies are very inefficient. Gene therapy emerges as a candidate approach for their treatment The group has been involved in the past few years in exploring the feasibility of suicide gene therapy in pancreatic cancer, showing some but limited effects. Based on those observations we are presently interested in the development of more potent and selective agents. To be able to increase their potency we are working with different systems that may facilitate the spreading of the cytotoxic compound into the tumor mass. In that direction we have recently shown that an 8 aminoacid peptide of the Tat protein can provide transduction capabilities to the fused proteins, thus increasing their therapeutic outcome. 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. Alternatively the use of gene therapy approaches can be a very useful tool to validate potential targets for therapy. 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 mice 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

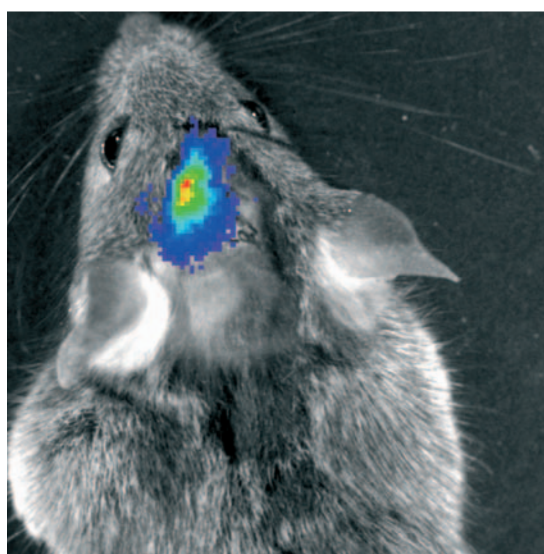

Figure 2. Bioluminescence imaging of striatum at 4 days post-viral infection.

Figure 3. WASP gene with 12 exons expanding 9 Kb.

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 aminoterminal 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. We have recently identified a novel WASP complex mutation that lead to the formation of two aberrant transcripts that result from the use of independent poly A signals. We are also working on the study of the functional consequences of WASP gene reintroduction in WAS patients lymphocytes.

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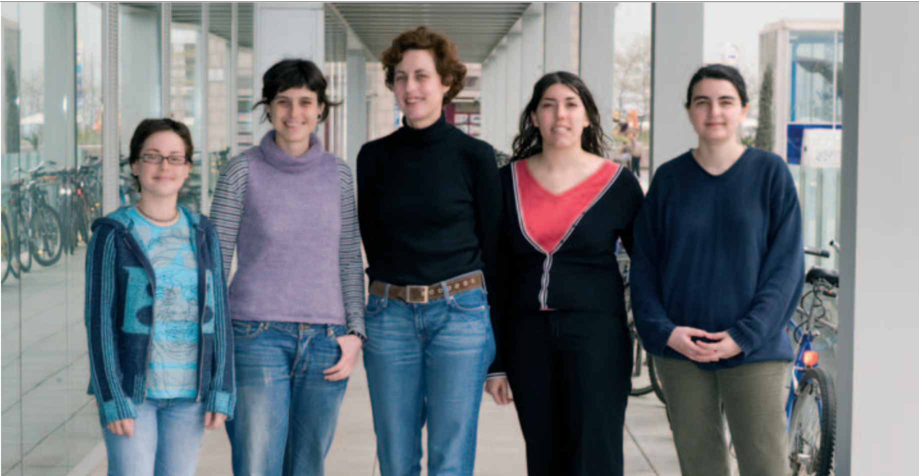
"Pitfalls and hopes in Down syndrome Therapeutic approaches: In the search for evidence-based treatments."
Behavioural Genetics (in press)

OTHER ACTIVITIES

Coordinator of the Gene Therapy Thematic Network (Xarxa Temàtica de Teràpia Gènica)

General Secretary of the Spanish Society of Gene Therapy

GENES AND DISEASE



Murine models of disease

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SUMMARY

Our interest is to study the in vivo function of particular genes that have potential implications in brain development and function. Our current research focuses on two chromosome 21 genes: *DYRK1A* encoding a dual-specificity protein kinase with several potential functions in brain development; and *DSCR1* encoding calcipressin1, a regulator of calcineurin mediated responses. Both, DYRK1A and CALP1 are highly expressed in central nervous system and are considered candidate genes for several phenotypic traits in Down syndrome. As a model system we use genetically modified mice.

RESEARCH PROJECTS

1. DYRK1A

Comprehension of DYRK1A function in the mammalian nervous system has raised an increased interest in the scientific community because transgenic mouse models overexpressing this gene recapitulate some of the neurological alterations in Down syndrome. We have previously shown that mice with only one copy of the gene ($Dyrk1A^{+/-}$ mice) present intrauterine growth retardation and microcephaly, supporting the notion that *DYRK1A* is a dosage sensitive gene. More recent studies have revealed that changes in the amounts of DYRK1A protein lead to altered numbers of particular neural cell types in mouse brain and retina. The phenotypes observed so far in both, gain and loss of function transgenic mice, indicate that DYRK1A might play a role in proliferation and/or cell-fate decision. Our present interest is to define how this protein kinase is regulating these processes. To this aim, we are mainly using the retina as a model system to study neurogenesis.

2. DSCR1

Calcipressin1 (CALP1) is a functional inhibitor of calcineurin, an ubiquitous and multifunctional calcium-activated protein phosphatase. Calcineurin is the most abundant protein in brain where regulates, among other neuronal functions, neurotransmitter release, neurite outgrowth and neuronal cell death. The only calcineurin inhibitor which expression is regulated by calcium-calcineurin signalling is CALP1, indicating that this protein can function in a feedback inhibition loop to suppress sustained calcineurin activity. CALP1 is widely express in brain, both during development and in the adult. Our present interest is to analyze the potential role of this regulator in central nervous system development and its possible implication in processes related to learning and memory. To address this question we generated a Dscr1 knockout mouse model and we are characterizing it at the molecular and histological levels. Specific behavioural tests have been performed to reveal any possible neurological and cognitive alterations in these mice.

PUBLICATIONS

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GENES AND DISEASE

Neurobehavioral phenotyping of mouse models of disease

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Technicians
Carla Obradors

SUMMARY

The overall goal of our research is the understanding of the role of putative candidate genes for human complex genetic diseases impairing the structural elements connecting the neurons with consequences on brain circuits that underly cognitive systems. This will lead to a better knowledge of the genetic substrates regulating the expression of complex behavioral traits. Using a wide variety of relevant behavioral paradigms, our laboratory is investigating specific links between cognitive impairments and memory disorders in patients with Down syndrome and behavioral deficits in mouse models of this disease. We are also currently working on candidate genes involved in dendrites/spine dysmorphology and altered neural plasticity in learning and memory brain circuits. Our second research line in directed to the study of panic disorder. We are interested in candidate genes that participate in the dysfunction of brain circuits involved in fear-related memories and in mouse behavioral traits relevant to panic and to anxiety. Most of our work employs the use of transgenic and knockout mice for genes expressed in the brain, but we also obtain important information about the genetic basis of behavior performing inbred strain surveys and studying recombinant inbred strains.

Our experimental approaches include behavioral analysis with multiple assessment tools that will detect basic alterations in nervous system function. Our assessment targets are basic neurological function, brainstem-spinal cord reflex, motor function/control centers, exploratory activity, anxiety-related responses, depression, sensorimotor gating, social interactions, and learning and memory. We also use neurohistological and morphometric approaches to determine a structural correlation for the detected phenotypic traits and cellular/molecular biology techniques to get insight into the underlying mechanism.

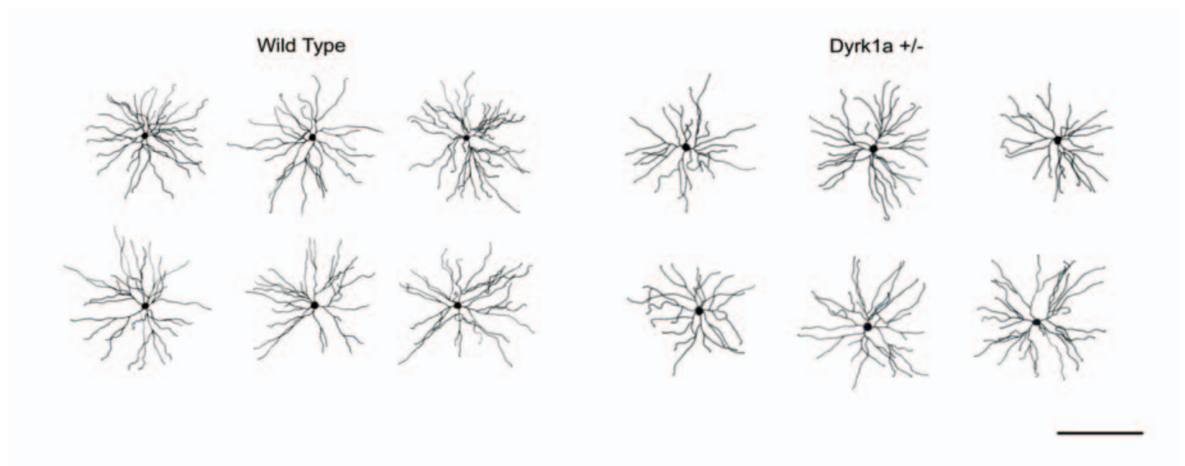

Figure 1. Down syndrome phenotypes in pyramidal neurons of the Cerebral Cortex

RESEARCH PROJECTS

1. Down syndrome

M. Martínez de Lagrán, G. Arqué

The neuropathological processes underlying Down syndrome (DS) mental retardation and their genetic dependence remain an open question. The crucial point is to define how does an excess of normal gene products in interaction with the environment direct and constrain neural maturation, and how does this abnormal development translate into cognition and behaviour. The Genes and Disease Program has generated murine models with different levels of expression of selected candidate genes for DS neuropathology. Our objective is to identify the physiological role of gene products and the dosage-dependent effects on neurodevelopment, learning and memory, and neurodegenerative processes. Our goal is to identify cellular and molecular substrates that regulate the emergence of different forms of learning and memory. We use a global model of the disease the trisomy 16 mouse model Ts65Dn (Ts65Dn) to study the pathogenetic mechanism of mental retardation, and to assay pharmacological and non-pharmacological interventions.

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

Our central hypothesis is that the cognitive impairment in DS is due to abnormalities in the structure and function of dendrites and synapses with specific consequences in neuronal network formation or in properties of brain plasticity. Thus, we study different forms of learning and memory and other cognitive domains, and the microstructural characteristics of the brain regions involved, basically cerebral cortex and hippocampus, and the hippocampal–cortical networks since the hippocampus is crucial in integrating information from distributed cortical modules including the stereological and microarchitectural analysis of specific cell types including the analysis of the architecture of the dendritic trees and the morphology of the dendritic spines. We are also involved in the experimental study of cellular mechanisms affecting cortical structure in animal models of DS. Based on evidence from people with DS and studies in mouse models, we hypothesize that cognitive dysfunction is in part due to abnormalities in the structure and function of dendrites and synapses with specific consequences in neurotrasmission. To address the question if pyramidal cells in postnatal development do not achieve the structural complexity, or if they undergo greater dendritic retraction of those in euploid brains during maturation, we use primary cultures of dissociated neurons of WT and DS model mice transfected with GFP-actin, and recorded and analyzed quantitatively to study growth cone morphology, dynamics of neurite outgrowth and branching and the actin cytoskeleton by confocal microscopy of fluorescent labelled neurons followed by quantitative image analysis and video-

time-lapse microscopy and confocal bleaching to study the dynamics of actin. Finally the decreased dendritic branching and reduced spine density in Ts65Dn and TgDyrk1A mice may be determined by intrinsic genetic programms but also by extracellular factors. To determine the extracellular factors that may affect cortical function in DS, we will generate mice with chimeric cortices in which neuronal precursor cells of trisomic or transgenic (DS) genotypes are in contact and under the influence of non-transgenic/trisomic host neurons and viceversa.

The second phase of this project is oriented to determine how the interaction between these gene products and the environment contribute to the expression of learned behaviours in in vivo neuroplasticity models. Findings from these studies should provide insight into some of the underlying neurobiological dysfunctions associated with human disorders resulting in impaired learning and memory such as mental retardation. We have been able to recreate certain conditions that result in abnormal learning capabilities in DS and to demonstrate that experience-dependent plasticity is impaired in shaping the three-dimensional architecture of neurons in the frontal cortex of Ts65Dn mice using environmental manipulations as a paradigm. Our research in the last years has bring to light some interesting and unsuspected culprits that will give us entry points into novel biological pathways. The phenotyping screens have identified a major dosage dependent effect for some of these genes in neurobehavioral development and in the formation of new memories. Moreover, we have determined synaptic transmission alterations are underlying features in some of these models as demonstrated by means of ultrastructural, pharmacological and microdyalisis experiments. We also explore the formation and maintenance of memories, the formation of activity-dependent neural connections and the molecular changes underlying activity-dependent neural plasticity at the structural and molecular levels, in primary cerebral cortex cultures.

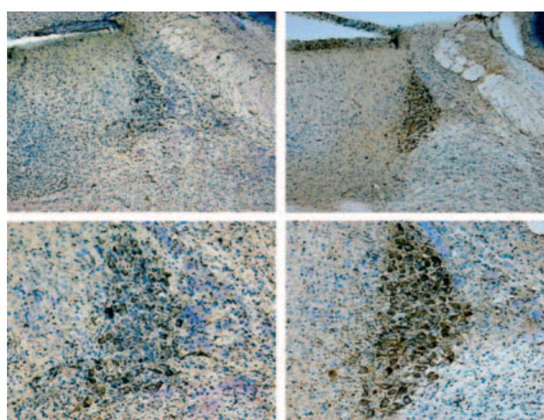

Figure 2. Neurotrophic Effect on Locus Coeruleus noradrenergic neurons after overexpression of NTRK3

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

knockout models for Dyrk1A, DSCR1 and BACE2.

2. Panic/Anxiety
I. Sahún, A. Amador, X. Gallego

Patients with neuropsychiatric disorders, such as panic/anxiety disorders, have altered sensorimotor gating responses, a differential emotionality profile and abnormal social behaviors. A second research line in our group is aimed at identifying genetic causative and vulnerability factors underlying anxiety-related behaviour and that could predict the onset of panic disorder. To this aim we use genetically modified animal models that help to elucidate mechanisms that may be acting in humans. Our current project is focused on the biochemical changes that occur in the CNS during development that are determinant of emotionality-related behaviors and to the development of therapeutic strategies that may overcome and/or prevent the brain alterations leading to panic attacks. Our interest is also aimed at elucidate the deficits of specific neurotransmitter systems that possibly underlie the inability of persons with anxiety disorders to correctly identify the fear-related information and the possible common neurobiological pathways responsible for co-morbid processes. We have raised the possibility that NTRK3 (TrkC) can exert a major role in anxiety disorders based on the observation that its overdosage leads to an increased anxiety-like behavior and panic reaction, possibly due to the trophic effect attained on the catecholaminergic nuclei. Now we evaluate the mechanism of this effect studying if noradrenergic neuron neurogenesis, survival or plasticity can be affected. Our initial studies on mice with overexpression of TrkC, are using pharmacological and behavioral strategies to better understand the nature of the behavioral abnormalities in these mice. Our hypothesis is that TrkC ligands regulate the development of LC NA neurons in vivo and developmental alterations in LC may facilitate an anxiety prone phenotype and predispose to the development of panic disorder. We have demonstrated that overexpression of TrkC in mouse leads to an increase in number of catecholaminergic neurons in the LC and SN and show elevated anxiety and panic reaction. The open questions are now i/ if this effect is achieved by specifically promoting TH-positive neurons or by affecting survival, and ii/ if it affects other brain regions involved in fear circuits. To explore these aspects we are studying the development and function of the fear system in these mice, using behavioral, electrophysiological, and cellular experimental approaches.

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

To this end we will evaluate the functional and structural modulations that occur upon application of specific neuromodulators or non-pharmacological stimuli that may regulate overall structure and plastic features of neurons and synapses. Finally we explore specific factors affecting the course of the disease in particular those that are associated with the regulatory capacity of the stress system. We

have also initiated the study of the role of other candidate genes (nicotinic receptors) for which murine models have already been generated in our Program.

In collaboration with Dr. R. Maldonado (Pompeu Fabra University) we have initiate a project for studying the implication of NTRK3 in processes comorbid to panic disorder, such as predisposition to substance abuse. Also we will analyze the predisposition to stress (collaboration with Dr. A. Armario, Autonomous University of Barcelona).

3. Technical development

We have set up new techniques: a/ Neurobehavioral: radial arm maze, fear conditioning, startle response, pre-pulse inhibition, Y-maze b/ Activity-dependent neuritogenesis and synaptic connectivity in primary cultures. We have also implemente the Neurolucida software to analyze the topological characteristics of neuronal populations.

PUBLICATIONS

Ferrer I, Barrachina M, Puig B, Martínez de Lagrán M, Martí E, Gómez-Isla T, Avila J, Dierssen M
"Constitutive Dyrk1A is abnormally expressed in Alzheimer disease, Down syndrome, Dementia with Lewy bodies, Pick disease and related transgenic models."
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Benavides-Piccione R, Elston GN, Arbones ML, Fotaki V, Estivill X, DeFelipe J, Dierssen M.
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Lumbreras M, Baamonde C, Martínez-Cué C, Lubec G, Cairns N, Sallés J, Dierssen M, Flórez J
"Brain G protein-dependent signaling pathways in Downsyndrome and Alzheimer's disease."
Amino Acids (in press)

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

Dierssen M, Ramakers G
"Dendritic Pathology in Mental Retardation: from Molecular Genetics to Neurobiology."
Genes, Brain and Behavior (in press)

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

OTHER ACTIVITIES

Organization of the Brain Awareness Week 2005

Organization of the 7th Annual Meeting IBANGS

Meeting of the Disease Models Thematic Network (Xarxa Temàtica Models Enfermetat SNC)

Co-Organization of the Course of Statistics

Member of the Executive Committee of the Spanish Society Neurosciences

Member of the Working Group on Pharmacology of the Catalan Institute of Neuroscience Research (Institut Català d'Investigació en Neurociència)

GENES AND DISEASE

Gene function

Group Leader
Susana de la Luna

Postdoctoral Fellows
Lali Genescà
Mónica Alvarez

PhD Students
Sergi Aranda
Eulàlia Salichs
Krisztina Arató *(since October)*

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 several HSA21 genes.

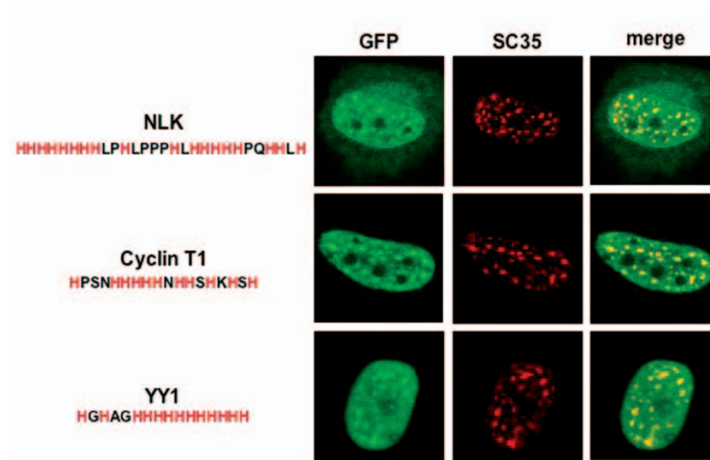

Figure 1. The ability of accumulating in nuclear speckles through histidine-rich regions is shared by several proteins in the human proteome. Co-localization of GFP fusions of the indicated full-length proteins (their histidine-rich segments are included) with SC-35 marker is shown.

RESEARCH PROJECTS

1. DYRK1A: a crossroads for signal transduction pathways

Mónica Alvarez, Sergi Aranda, Eulàlia Salichs

DYRK1A is one of the HSA21 genes for which changes in gene doses result in neuropathological alterations as it become evident from the analysis of the phenotypes shown by mouse transgenic mice in which the gene is either overexpressed or has been deleted. However, a clear role for the encoded kinase in different cellular processes and signal transduction pathways has not been defined yet. We intend to study DYRK1A from the molecular and cellular points of view. There are several reasons for choosing DYRK1A as one of our target molecules. First, we regard as pretty exciting the growth-related phenotypes shown by the Dyrk1A +/- mice, and second, we believe that DYRK1A might act as a crossroads for different signalling pathways since its substrates list consists of a variety of both cytosolic and nuclear proteins, transcription factors included. Finally, a great advantage to be considered is that the group can work in close collaboration with other groups in the Program interested in other aspects of the DYRK1A biology.

DYRK kinases (DYRK and HIPK subfamilies) constitute one of the families that belong to the CMGC group of protein kinases, formed by the CDKs, MAPKs, GSKs, CLKs and SRPKs. In the DYRK subfamily, the lower eukaryotic members, such as Yak1p and Pom1p in yeast and YakA in *Dyctiostelium* have been associated with pathways controlling growth and development. Studies with null alleles for the *C. elegans* DYRK genes, *mbk-1 and mbk-2* have shown no apparent phenotypes for *mbk-1* and lack of viability for *mbk-2* mutants. Mbk-2 has been postulated as a candidate master regulator of maternal-protein degradation during the oocyte-to-embryo transition. In flies, three DYRK members are found: *minibrain*, involved in postembryonic neurogenesis, dDYRK2, the putative product of the *smell-impaired* gene and dDYRK3. Finally, five members of the DYRK subfamily exist in mammals, DYRK1A, DYRK1B, DYRK2, DYRK3 and DYRK4 that share a high degree of conservation in the catalytic domain, but are very divergent in their N- and C-terminal domains. Although rather limited information is available for all these kinases, in general terms, all DYRKs might perform functional roles related to cell proliferation and/or differentiation.

We have previously reported that DYRK1A manages to accumulate in the splicing factor compartment (SFC) or nuclear speckles by using a histidine-rich region at its C-terminus. We have found out that there are a short number of human proteins with histidine-rich tracts in their primary sequence and we are testing them for their ability to accumulate in the SFC subnuclear compartment. The results obtained (see a few examples in Fig. 1) suggest that these regions can act as general nuclear speckle-targeting signals and allow us to establish a functional connection for these homopolymeric tracts.

Although DYRKs phosphorylate their substrates on serine and threonine, they autophosphory-

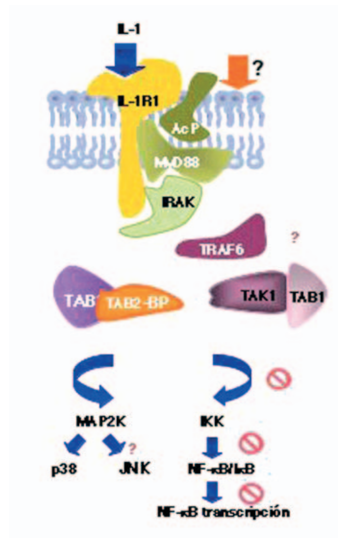

Figure 2. Schematic representation of the interaction of *C21orf7* ORFs with the IL-1 signalling pathway.

late their activation loop on an essential tyrosine. This event is the result of an intramolecular phosphorylation reaction coupled to DYRKs translation that renders fully active enzymes. For that reason, and since an activating kinase appears not to be necessary unlike it happens in most of the kinase-dependent signalling cascades, one might think that there is no room for activity regulation. However, DYRK1A seems to be extremely sensitive to gene dosage, and thus it is sensible to think that minimal changes in its activity would give rise to profound effects on the pathways it might control. With this in mind, we have become interested in finding mechanisms that could possibly regulate the activity of DYRK1A. In this sense, we have found 14-3-3 isoform ß in a yeast two-hybrid screening using DYRK1A as the bait, and concentrated part of our efforts in establishing a mechanism for the regulation of the DYRK1A kinase activity by this molecule.
Finally, other candidates found in the screening are being explored to confirm the detected binding and to assign a functional role to the intractions.

2. C21orf7
Lali Genescà

C21orf7 is one of the HSA21 genes with no function associated yet. Based on the similarity of its encoded products with the C-terminus of the protein kinase TAK1, we have focused our analysis in the signalling pathways controlled by this kinase.

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

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

GENES AND DISEASE

Associated Technological Unit: Genotyping Facility

Group Leader
Xavier Estivill

Unit Responsible
Mònica Bayés

Postdoctoral Fellows
Rafael de Cid
Mònica Gratacòs

Technicians
Carles Arribas
Cecília García
Magda Montfort
Anna Puig

SUMMARY

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in the human genome. A small fraction of this genetic variation is likely to explain the majority of the differences between individuals, including their predisposition to develop common human disorders, such as cardiovascular disease, hypertension, diabetes, asthma and cancer. SNP genotyping can be useful for genetic mapping, disease association studies, population genetics, and in other types of research, including research in model organisms and trait selection for agricultural, cattle farming or aquaculture applications. Some of the genotyping technologies also enable customers to identify visualize copy number variants (CNVs) and to accurately characterize loss of heterozygosity (LOH).

SERVICES

The Genotyping Unit, supported by Genoma España, through the National Genotyping Center (CeGen) provides support to scientists for genotyping projects in every aspect of research, from planning, DNA extraction, genotyping, data interpretation, through statistical analysis. The Unit offers custom, cost effective and flexible solutions for projects of any scale to both internal and external users, from public or private institutions. Investigators should obtain their own funds for genotyping, while the Unit covers the costs of equipment and personnel.

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

- Genotyping by **SNPlex** (Applied Biosystems): genotyping of 24-400 SNPs selected by customer
- Genotyping by **Pyrosequencing** (Biotage): genotyping of 1-20 SNPs selected by the customer
- Automated **DNA extraction** from blood or other tissues (Chemagen)
- **DNA quantification** using Picogreen (Molecular Probes)
- **Whole Genome Amplification** using GenomiPhi (Amersham)

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

- Mutation screening or SNP discovery through **dHPLC**
- Custom Genotyping by **Illumina**: genotyping of 90-1500 SNPs selected by the customer
- **Illumina Linkage IV Panel**: genotyping of 5.861 human SNPs genome-wide distributed
- **Illumina Medium-Density Mouse Linkage Panel**: 1536 mouse SNPs genome-wide distributed
- **Illumina Low-Density Mouse Linkage Panel**: 384 mouse SNPs genome-wide distributed
- **Illumina Whole Genome Genotyping with Human-1 BeadChip**: 109,000 human SNPs, 70% of which are located in exons or within 10 kb of transcripts
- **Illumina Whole Genome Genotyping** with HumanHap300 BeadChip: 317,000 tagSNP markers derived from the International HapMap Project

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

Table 1. Summary of services provided during the period 1/04/05 to 31/12/05

SERVICE	Total produced
SNPlex (nº genotypes)	2,822,489
PYROSEQUENCING (nº genotypes)	3,272
DNA EXTRACTION (nº samples)	1,850
WHOLE GENOME AMPLIFICATION (nº samples)	5,618
DNA QUANTIFICATION (nº samples)	10,294
dHPLC (nº injections)	15,829

RESEARCH PROJECTS

We are part of the Genotyping and Psychiatric Genetics Network (http://davinci.crg.es/rgpg/), which explores the genetic contribution to the susceptibility to psychiatric disorders, including eating disorders, anxiety, postpartum depression, autism and attention deficit and hyperactivity disorder. Other topics under investigation within the unit include the variability in the response to methadone and nicotine treatments, and asthma. Association studies using either case-control or family-based designs are currently been performed for these traits with state-of-the-art statistical tools.

PUBLICATIONS

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"Frailtypack: a computer program for the analysis of correlated failure time data using penalized likelihood estimation."
Comput Methods Programs Biomed., 80(2):154-64 (2005) [Epub 2005 Sep 6]

BIOINFORMATICS AND GENOMICS

Coordinator: Roderic Guigó

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

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

Research Groups:

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

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

BIOINFORMATICS AND GENOMICS

Bioinformatics and Genomics (join group with GRIB, IMIM-UPF)

Group Leader
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Research Associate
Eduardo Eyras (UPF)

Postdoctoral Fellows
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France Denoeud (IMIM)
Tyler Alioto (IMIM)
Nuria Lopez-Bigas (CRG)
Sylvain Foissac (CRG)

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Josep F. Abril (UPF)
Charles Chapple, (UPF)
Hagen Tilgner (CRG)

Technicians
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Julien Lagarde (IMIM)
Francisco Camara (IMIM)
Arnadu Kerhornou (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

B

Selenoprotein	Chromosomal location (number of exons)	Sec location in protein (length of protein)	Selenoprotein structure
15kDa	1p22.3 (5)	93 (162)	
DI1	1p32.3 (4)	126 (249)	
DI2	14q31.1 (2)	133 (265)	
DI3	14q32	144 (278)	
GPx1	3p21.31 (2)	47 (201)	
GPx2	14q23.3 (2)	40 (190)	
GPx3	5q33.1 (5)	73 (226)	
GPx4	19p13.3 (7)	73 (197)	
GPx6	**6p22.1 (5)**	**73 (221)**	
H	**11q12.1 (4)**	**44 (122)**	
I	**2p23.3 (10)**	**387 (397)**	
K	**3p21.31 (5)**	**92 (94)**	
M	22q12.2 (5)	48 (145)	
N	1p36.11 (12)	428 (556)	
O	**22q13.33 (9)**	**667 (669)**	
P	5p12 (4)	59, 300,318,330, 345, 352, 367, 369, 376, 378 (381)	
R	16p13.3 (4)	95 (116)	
S	**15q26.3 (6)**	**188 (189)**	
SPS2	-	60 (448)	
T	3q24 (6)	36 (182)	
TR1	12q23.3 (15)	498 (499)	
TR2	3q21.2 (16)	655 (656)	
TR3	22q11.21 (18)	522 (523)	
V	**19q13.13 (6)**	**273 (346)**	
W	19q13.32 (6)	13 (87)	

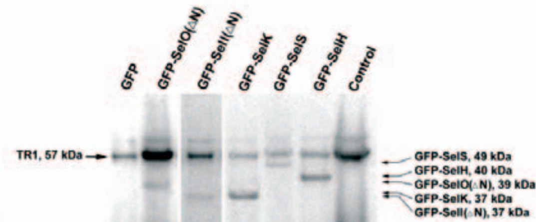

Figure 1. Identification of novel mammalian selenoproteins (in orange). Computational prediction followed by experimental verification. Taken from Kryukov et al. (2003)

RESEARCH PROJECTS

1. Gene Prediction

We are working in the development of geneid, an “ab initio” gene prediction program. Recently geneid has been used in the annotation pipeline of *Tetraodon nigroviridis* (Jaillon et al., 2004). We are in the process of implementing versions of geneid for a number of eukariotic genomes according to the priorities set at the National Human Genome Research 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, and with Genoscope in the analysis of the Paramecium genome.

2. Prediction of Selenoproteins

Particularly difficult in eukaryotic genomes is the prediction of selenoprotein genes, because selenocysteine is specified by the UGA codon, normally an stop codon. Since year 2000 we have been developing computational methods for selenoprotein prediction. During the last year we have successfully used this methods to characterize mammalian selenoproteins (Kryukov et al., 2003, figure 1). Recently, using comparative genomics methods we have discovered a novel selenoprotein family whose phylogenetic distribution is challenging long standing assumptions about the taxonomic distribution of eukaryotic selenoproteins (Castellano et al., 2004). The analylsis of the *T. nigroviridis* genome discovery further challenged these assumptions (work in progress).

3. Splicing

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

Table 3 **Sensivity of gene prediction**

Feature	Ensembl	Twinscan	SGP-2
Exact exon (%)	61	53	60
80% coverage exon (%)	85	77	85
Total exons	179,084	195,665	203,834

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

4. Comparative Genomics

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

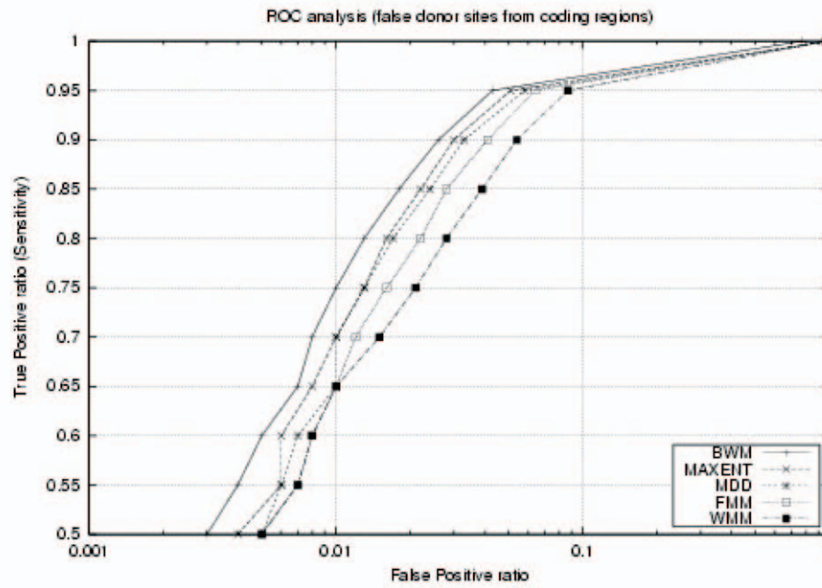

Figure 2. Comparative ROC of different methods to predict donor sites. The methods are ordered according to accuracy: BWM, MAXENT (Yeo and Burge, 2003), MDD (Burge and Karlin, 1997), FMM (First Order Markov Model) and WMM (Weigth Matrix Model)

Sequencing Consortium, 2004). In the table below the comparative accuracy of SGP-2 in the chicken genome.

5. Recognition of Promoter Regions

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

6. ENCODE Project

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

PUBLICATIONS

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Proc Natl Acad Sci USA, 102(45), 16188-93 (2005)

BIOINFORMATICS AND GENOMICS

Genomic analysis of development and disease

Associated Technological Unit: Microarray Laboratory

Group Leader
Lauro Sumoy

Postdoctoral Fellow
Franc Llorens

Microarray Core Facility:

Bioinformatician
Juanjo Lozano

Senior Technician
Eva Gonzalez

Senior Technician
Belen Miñana

Technician
Ana Mosquera

Guest or associated members:

Senior Technician
Mònica Grau (PRBB, P. Real – IMIM)

Technician
Manuel García (CRG, X. Estivill)

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

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

SUMMARY

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

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

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

MICROARRAY UNIT

SUMMARY

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

RESEARCH PROJECTS

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

LRRN6A is a novel gene with a hypothesized function in axon path-finding during development and in neuronal plasticity in the adult. This gene was found in the context of the study of the 15q24-q26 region of the human genome (DUP25) associated to panic and anxiety syndrome and joint laxity that lead to exhaustive characterization of cDNAs and construction of a transcript map of the region. We have undertaken functional studies centered on genes from 15q24-q26 and chose the LRRN6A gene for further study. With relevance to the disease, we have found that LRRN6A is expressed in the adult limbic system and shown it maps within the duplication. Recent findings involve LRRN6A, also known ans LINGO-1, in a pathway which inhibits axon regeneration in damaged nerves in reponse to myelin. 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.

2. Microarray meta-analysis

One of the main current challenges in microarray research is the comparison between different data sets. We intend to advance in the development of methods that allow inference of conserved patterns of gene co-regulation using meta-analysis methodologies. We plan to apply these to understand the signaling pathways affected by LRRN6A/LINGO1 during neuronal development and differentiation, and in the adult brain in response to pathological situations.

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

PUBLICATIONS

Camps J, Armengol G, Del Rey J, Lozano J, Vauhkonen H, Prat E, Egozcue J, Sumoy L, Knuutila S, Miro R
"Genome-wide differences between microsatellite stable and unstable colorectal tumors".
Carcinogenesis, Nov 4 (2005) [Epub ahead of print]

MICROARRAY UNIT

TECHNOLOGY DEVELOPMENT PROJECTS

1. Microarray technology

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

We are involved in projects focusing on breast cancer (collaboration with Dr. Miguel Beato, CRG; Dr. Francesc Solé, Hospital del Mar-IMAS), bladder and prostate cancer (collaboration with Drs. Antonio Alcaraz, Fundació Puigvert), genome structure variation in control population and genomic disorders mediated by homologous recombination events between segmented duplications (collaboration with Drs. Xavier Estivill, CRG, and Luis Perez Jurado, UPF), and variation in splicing (collaboration with Drs. Roderic Guigó and Juan Valcarcel, CRG). There is an ongoing

project to develop standardized procedures for amplification of RNA from small sample amounts, and to validate and compare different labelling methodologies (collaboration with Dr. Paco Real, IMIM). We are testing procedures for CHIP on chip to detect differences in binding of transcription factors and chromatin components to DNA (collaborations with Dr. Jorge Ferrer, IDIBAPS; Dr. Anna Bigas, IRO). Currently planned applications include diagnostics of breast, prostate and bladder cancer, and of genomic mutations mediated by segmental duplications through participation in collaborative projects. We have also developed tiling path BAC arrays for human chromosomes X, 15 and 22, and a 5,300 BAC array with 0.5 Mb density coverage of the human genome.

2. Microarray bioinformatics

We have set up automated image data acquisition, pre-processing, filtering, normalization and quality control Web based software for analysis of microarray experiments. We are implementing an Oracle based database for direct data browsing by service users. In the future, web tools for advanced data set classification, clustering, grouping, discriminant, factor analysis and data mining purposes, already under development (Sanchez-Corbayo et al, 2003). This also includes capabilities for anañysis of other types of microarrays data such as Affymetrix.

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

SERVICES

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

Microarray-related publications from laboratory members

Galy B, Ferring D, Minana B, Bell O, Janser HG, Muckenthaler M, Schumann K, Hentze MW.
"Altered body iron distribution and microcytosis in mice deficient in iron regulatory protein 2 (IRP2)"
Blood, 106(7), 2580-9 (2005)

Lozano JJ, Soler M, Bermudo R, Abia D, Fernandez PL, Thomson TM, Ortiz AR.
"Dual activation of pathways regulated by steroid receptors and peptide growth factors in primary prostate cancer revealed by Factor Analysis of microarray data"
BMC Genomics, 17;6, 109 (2005)

Grau M, Sole X, Obrador A, Tarafa G, Vendrell E, Valls J, Moreno V, Peinado MA, Capella G.
"Validation of RNA arbitrarily primed PCR probes hybridized to glass cDNA microarrays: application to the analysis of limited samples"
Clin Chem, 51(1), 93-101 (2005)

Publications by core facility users

Del Bas JM, Fernandez-Larrea J, Blay M, Ardevol A, Salvado MJ, Arola L, Blade C. "Grape seed procyanidins improve atherosclerotic risk index and induce liver CYP7A1 and SHP expression in healthy rats"
FASEB J., 19(3), 479-81 (2005)

Khymenets O, Ortuno J, Fito M, Covas MA, Farre M, de la Torre R.
"Evaluation of RNA isolation procedures from human blood and its application for gene expression studies (Sod-1, Sod-2)"
Anal Biochem, 1;347(1), 156-158 (2005)

CELL AND DEVELOPMENTAL BIOLOGY

Advisor: Alfonso Martinez-Arias

The programme of Cell and Developmental Biology is a new programme in the CRG. It has been designed to take into consideration the importance that findings in the area of animal development might have in human health. With this idea in mind, the focus of the programme is the analysis of developmental events from a cellular perspective.

The last decade of the XX century saw a big emphasis on the genetic analysis of developmental processes culminating with the sequencing of the genomes of several model organisms, which opened enormous possibilities for functional analysis. However, it is very clear that the functional unit of an organism is the Cell rather than the gene and the genome is only one way to begin to unravel the organization and functioning or organisms and by and large, organisms. It is this premise that leads the strong focus of the programme on events at the cellular level.

The bulk of the programme will be operational in 2006, when the new building opens. In the meantime 2005 saw the appointment of two group leaders, the incorporation of one of them and a small number of developments, which will provide important infrastructures for the CRG. The two groups are Isabelle Vernos (Senior group leader) and Hernan Lopez Schier (Junior group leader). Isabelle Vernos joined the CRG in August with an ICREA position. She came from the EMBL in Heidelberg (Germany) with a strong background and a wealth of experience in Cell Biology. Her main interest in the analysis of the activity of the cytoskeleton with a special focus on cell division and uses Xenopus as her model system. Hernan Lopez Schier comes from the Rockefeller University in New York (USA) and has an interest in the organization and activity of the cytoskeleton in the assembly of the auditory organ of the zebra fish. He will move to the CRG by the middle of next year. Both have complementary interests, skills and knowledge. At the end of the year a new round of selection of group leaders was made and five candidates were interviewed. Decision pending at the beginning of the year,

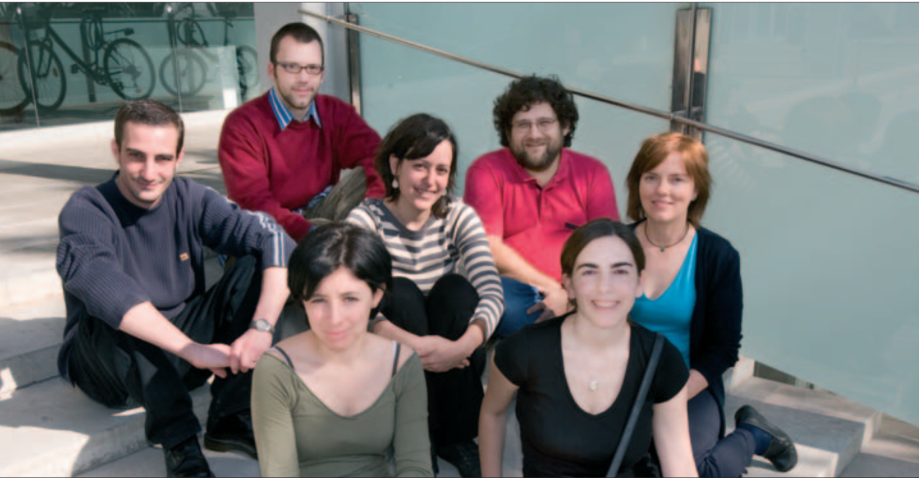
One of the most important developments for the programme is the establishment of an Advance Light Microscopy Unit, which will serve as an important resource for the programme, and the CRG. The Unit, modelled on that of the EMBL, is being developed by Isabelle Vernos. The CRG has set aside a substantial budget for this important project. Under her stewardship at the end of the year interviews took place and an offer was made to one of the candidates. In addition the programme will try to promote the zebra fish as an organism of choice in the analysis of vertebrate questions at the cellular level.

The Programme will have very string links with the Systems Biology Programme and, in addition, by nature with the programme of Cancer and Cell differentiation. However it is also obvious that there are good links with Gene Regulation and Bioinformatics and that there is little question that relationships are being forged with Genes and Disease, which has shown some interest in the use of the fish as a model system for the analysis of specific pathologies.

The programme is seeking an interaction with the Institute of Photonics that will be of interest as an interdisciplinary development, which will promote interactions between different institutes in Barcelona and also open the possibility of technological development. To this effect a meeting was organized by Luis Serrano, Isabelle Vernos, Lluis Torner and Alfonso Martinez Arias in December, hosted at ICFO on the issue of "Frontiers of Biological Imaging". The meeting was sponsored by the Department of Univerisities, Research and Information Society of the *Generalitat de Catalunya*. It was deemed a success and some joined projects emerged between ICFO and the CRG.

The perspectives for 2006 are good. The new appointees in the new building and the Advanced Optical Microscopy Unit in place will see research gathering place.

CELL AND DEVELOPMENTAL BIOLOGY



Microtubule function and cell division

Group Leader
Isabelle Vernos

Postdoctoral Fellows
Laurent-Herve Perez
Teresa Sardon (at EMBL in 2005)

Students
Vanessa Dos Reis Ferreira
(since August 2005)
Isabel Peset
David Vanneste (since October 2005)

Technician
Luis Bejarano

SUMMARY

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

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

RESEARCH PROJECTS

1. Mechanism of spindle assembly and chromosome segregation

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

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

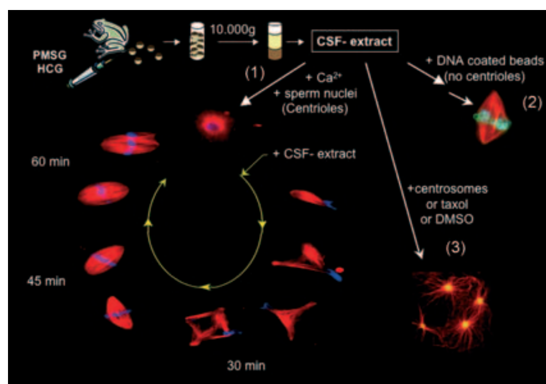


Figure 1. The Xenopus egg extract system. Eggs laid by the female are collected and crushed by low speed centrifugation to collect a concentrated cytoplasm locked in M-phase. This cytoplasm can then be used to follow various cell cycle related events in the test tube like (1) spindle assembly in the presence of chromosomes and centrosomes, (2) spindle assembly around chromatin without kinetochores and centrosomes (3) microtubule dynamics in the absence of chromatin.

spindle. We are currently investigating these issues and trying to determine the role of Aurora A activation in spindle assembly and cell cycle progression.

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

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

ments to understand their respective role in this pathway.

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

The Xenopus protein Maskin, previously identified and characterized in the context of its role in translational control during oocyte maturation, belongs to the TACC protein family. In other systems, members of this family have been shown to localize to centrosomes during mitosis and play a role in microtubule stabilization. We have examined the putative role of Maskin in spindle assembly and centrosome aster formation in the Xenopus egg extract system. We found that Maskin plays an essential role for microtubule assembly during M-phase and that both its localization and function are regulated by phosphorylation by the Xenopus Aurora A kinase. We also obtained some evidence indicating that Maskin works in concert with XMAP215 to oppose the destabilizing activity of XKCM1, therefore promoting microtubule growth from the centrosome (Peset I, Seiler J, Sardon T, Bejarano LA, Rybina S, Vernos I. J. Cell Biol. 26;170(7):1057-66, 2005).

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

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

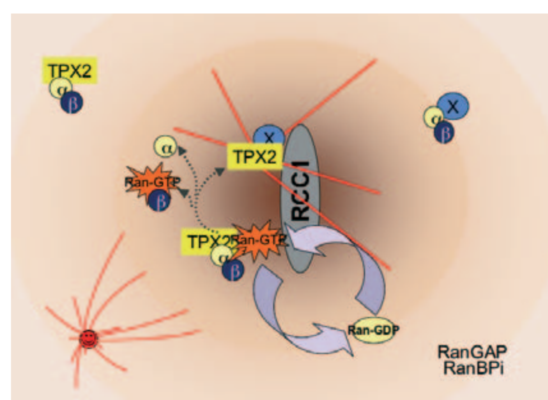


Figure 2. Model for the role of chromatin in spindle assembly. A gradient of RanGTP forms around the mitotic chromatin due to the enrichment of the exchange factor RCC1 on the chromatin and the presence of various factors that stimulate Ran GTPase activity (RanGAP and RanBPi) in the cytoplasm. RanGTP promotes the dissociation of NLS-proteins (like TPX2 and factor X) from importins. The released proteins trigger de novo microtubule nucleation and stabilization. In addition, some RanGTP regulated proteins increase microtubule nucleation and stabilization at the centrosome.

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

excess of Xklp1 in the egg extract resulted in the formation of spindles with reduced microtubule density. Similar results were obtained on centrosome nucleated microtubule asters *(Castoldi M, Vernos I. Mol Biol Cell. In Press)*. These results and the study of the Xklp1 motor domain in vitro (Bringmann et al, 2004) indicate that Xklp1 has unique properties and plays an important role for spindle assembly and function in the Xenopus egg extract system.

We found previously that Xkid has a very different function. It is required for chromosome alignment on the metaphase plate, (Antonio et al., 2000). In addition Xkid has a role in cell cycle progression during Xenopus oocyte meiotic maturation (Perez et al, 2002). We are currently trying to understand how a molecular motor may be required for meiotic cell cycle progression.

3. Screening assays for the identification of new inhibitors for mitotic motors

In collaboration with the groups of Thomas Surrey at EMBL and Prof. A. Giannis at the University of Leipzig we have examined the mitotic phenotypes induced by different collections of compounds with potential inhibitory activity on mitotic motors. We identified derivatives from Monastrol that are significantly more potent than Monastrol itself for inhibition of the kinesin-like protein Eg5 (*Gartner M, Sunder-Plassmann N, Seiler J, Utz M, Vernos I, Surrey T, Giannis T. ChemBioChem. 6(7):1173-7, 2005).*

2. Role of Kinesin II in membrane traffic

In interphase, microtubules originating from the centrally located centrosome irradiate towards the cell periphery. They provide polarized tracks used by motors to move and position different intracellular components. These transport events define membrane traffic inside the cell. Kinesin II is a heterotrimer consisting of two closely related kinesin-like proteins that heterodimerize through a coiled coil and interact with a third non-motor subunit named KAP (Kinesin Associated Protein). Kinesin II appears to be involved in multiple transport processes (Betley et al, 2004). Some time ago we identified Xklp3, a Xenopus kinesin-like protein that is one of the two kinesin-like proteins forming the Xenopus heterotrimeric kinesin II complex. We showed that Xklp3 activity is required for traffic between the ER to the Golgi apparatus (Le Bot et al, 1998) but we could not define its role more precisely. In order to get a further understanding on the role played by Kinesin II in membrane traffic we have used an RNAi approach in HeLa cells to knock down independently the expression of the three subunits of the complex.

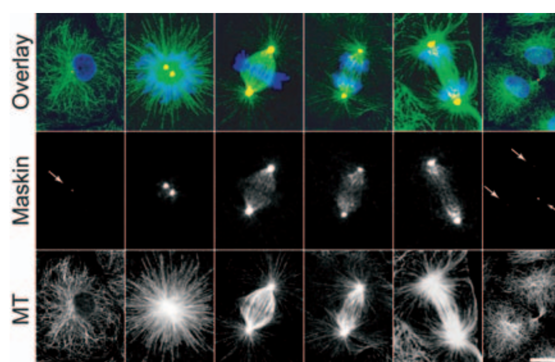


Figure 3. Maskin localization in XL177 cells. Maskin localizes to the centrosome of some interphase cells. During mitosis it becomes strongly enriched at the centrosome and localizes faintly on some spindle microtubules. In the overlay, microtubules are green, Maskin is red and DNA is blue. Scale bar, 20mm except in interphase and telophase cells 10mm.

We first tested whether Kinesin-2 could serve as a motor protein in rab6 directed Golgi to ER transport by knocking down KAP expression in a GalNAc-T2^{GFP} stable expressing HeLa cell line. We found that Kinesin II is not an effector in the rab6-induced redistribution event *(Young J, Stauber T, del Nery E, Vernos I, Pepperkok R, Nilsson T. Mol Biol Cell. 16(1):162-77, 2005).* KAP knockdown cells show an altered distribution of late endosomes/lysosomes that become clustered in the perinuclear area instead of being scattered throughout the cytoplasm as in control cells. This data and other complementary experiments performed in T.Schroer's lab indicate that one of the function of kinesin-2 is to participate in the movement and positioning of this class of organelles in the cell *(Brown CL, Maier K.C, Stauber T, Ginkel LM, Wordeman L, Vernos I, Schroer TA. Traffic 6(12):1114-24, 2005).*

PUBLICATIONS

Young J, Stauber T, del Nery E, Vernos I, Pepperkok R and Nilsson T
"Regulation of microtubule-dependent recycling at the TGN by rab6A and rab6A'".
Mol Biol Cell., 16(1), 162-77 (2005)

Gartner M, Sunder-Plassmann N, Seiler J, Utz M, Vernos I, Surrey T, Giannis T
"Development and Biological Evaluation of Potent and Specific Monastrol Analogues as Inhibitors of Mitotic Kinesin Eg5."
ChemBioChem., 6(7), 1173-7 (2005)

Peset I, Seiler J, Sardon T, Bejarano LA, Rybina S and Vernos I
"Function and regulation of Maskin, a TACC family protein, in microtubule growth during mitosis."
J. Cell Biol., 26;170(7), 1057-66 (2005)

Brown CL, Maier KC, Stauber T, Ginkel LM, Wordeman L, Vernos I and Schroer TA
"Kinesin II is a motor for late endosomes and lysosomes in vivo"
Traffic, 6(12), 1114-24 (2005)

Castoldi M and Vernos I
"Chromokinesin Xklp1 contributes to the regulation of microtubule density and organization during spindle assembly"
Mol Biol Cell. (in press)

Christodoulou A, Lederer CW, Surrey T, Vernos I, and Santama N
"Motor protein KIFC5A interacts with Nubp1 and Nubp2 and is implicated in the regulation of centrosome duplication"
J. Cell Science (in press)

Book chapters

Antonio C, Heald R and Vernos I
"In vitro assays for mitotic spindle assembly and function." In *Cell Biology: A Laboratory Handbook*. 3rd Edition. Celis, ed. Academic Press. ISBN: 0-12-164730-7 (2005)

OTHER ACTIVITIES

Organizer of the International Jacques Monod Conference (CNRS, France) "Molecular Machines in Cell Division", Roscoff (France), 11-14 September 2005.

SYSTEMS BIOLOGY

Coordinator: Luis Serrano

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

At the CRG we have a strong Biocomputing programme that could cover the data gathering and the comparative genomics, thus in our programme we will put more emphasis on data integration, computer modeling and experimental validations as well as on the design aspect. In these aspects we will establish strong links with the Gene Regulation, Cell Differentiation and Cancer and Cell Biology and Development programmes. We expect that a systematic analysis of biological systems will allow new insights in human diseases and for this strong ties should be made with the Gene and Diseases programme.

In 2005 we recruited our first two group leaders: James Sharpe and Mark Isalan. Together they cover modelization and experimental analysis of developmental systems and the engineering and design of new gene networks (see below). Our aim for 2006-2007 will be to hire more theoretical groups that will complement the expertise of the coordinator's group in simulation.

Research Groups

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

The goal of **James Sharpe**'s group is to bring together an interdisciplinary team of scientists to focus on the research of a particular complex system – development of the vertebrate limb. The group aims at understanding this process both at the level of gene regulatory networks, and at the level of the physical interactions between cells and tissues. To achieve this the group (currently in Edinburgh) includes embryologists, computer scientists, imaging specialists and engineers. They plan to develop a global strategy of understanding, from novel approaches for data-capture (live time-lapse OPT imaging) to finite-element simulations of the growing 3D structure and computer models of the gene networks responsible

for pattern formation across the organ. This combination of approaches allows them to address the following questions: What kinds of cellular movements are responsible for creating to correct 3D shape of the limb? How are these behaviours coordinated? How is the correct spatial pattern of gene expression controlled? What topology of gene regulatory network may be responsible for this complex phenomenon?

Mark Isalan' s group is interested in engineering synthetic gene networks to control gene expression in cells and to construct self-organising patterns, analogous to those used by organisms in morphogenesis and development. By transfecting cell populations with various gene networks, they hope to find the underlying 'design principles' of how certain networks form particular structures or functions.

APPENDIX

IV Annual Symposium of the Centre for Genomic Regulation

Appendix 1

“New challenges in the mechanistics of human disorders. Connecting the Genome with Disease”

The IV CRG Symposium was held on 2 and 3 December 2005, in the premises of the CRG in Barcelona. This edition was titled “NEW CHALLENGES IN THE MECHANISTICS OF HUMAN DISORDERS. CONNECTING THE GENOME WITH DISEASE”, because its purpose was to highlight different and very innovative topics within the world of the mechanistics of human molecular genetics, connecting them with psychiatric and neurological diseases.

Studies on monogenic diseases have produced knowledge about the molecular basis of most of severe hereditary defects affecting humans. Despite these great advances, the biological bases of common diseases affecting the general population are largely unsolved. This symposium tried to go deeply into the knowledge of new molecular mechanisms potentially involved in human disorders. The symposium gathered the main experts in the areas of small RNAs, epigenetics, genomic variation and gene expression variation. All attendants agreed that the speakers selected for the 5 sessions of the program were the best worldwide in their respective areas, taking into account the novelty of the topics presented. For this reason, the symposium was considered of great interest not only for the scientists at the CRG, but for the entire Catalan, Spanish and international scientific community, who had the chance to participate in high level discussions about leading research topics. The symposium worked also as a catalyser of potential international collaborations with groups of recognized prestige.

The symposium included excellent presentations that encouraged discussions and the exchange of experiences. The first day presentations dealt with the relation between genomic variations and some neurological diseases. The session gathered renowned scientists, as Dr. A La Spada (University of Washington) or Dr. J. Lupski (Baylor College of Medicine), both internationally prestigious for their studies on Huntington disease and Charcot-Marie-Tooth syndrome (CMT), respectively. The second day started with one session about small RNAs and their relevance in the genetics of different organisms as *C. Elegans*, some mammals and plants. To this session, followed a second one about the relation between epigenetic mechanisms and diseases like cancer, and the syndromes of Prader-Willi, Angelman and Bardet-Biedl. The symposium closed with a session about the relation between variability in gene expression and diseases, mainly neurological,

by Dr. V. Cheung (Children's Hospital of Philadelphia) and Dr. E. Dermitzakis, amongst other speakers.

The symposium was announced at lots of websites of institutes and genetics society all around the world apart from published several press releases in different media. Thanks to that, we got considerable international participation, both attendants and speakers, which gave to the symposium, the CRG and Barcelona a great visibility in the scientific world of genomics related to human diseases, apart from bringing science closer to society.

We do believe the final result of this symposium was really interesting for those attending, due to the relevance and prestige of experts in this area who, therefore, were able to offer a panoramic and up-to-date view of the mechanistics of different alterations.

The symposium gathered around 200 participants who, apart from the strictly scientific contents, were able to establish professional links and share experiences and results. In this sense, the activity contributed to the internationalisation of research in Catalonia and Spain and to a high level training of your scientists. The contents and format of the symposium worked as a forum, clearly suitable for the consecution of these objectives and for this reason, we consider it a great success.

CRG Seminars 2005

Appendix 2

CRG SEMINARS

20/12/05
Timo Zimmermann
EMBL, Heidelberg, Germany
"FRET and spectral imaging approaches for confocal and widefield microscopy"

20/12/05
Olaf Selchow
Central Imaging Facility, Microscopy & Image Analysis, Institute of Cell Biology & Immunology, University of Stuttgart, Germany
"Imaging topology and dynamics of signalling processes from the sub-cellular to the organ and organism level: Covering a large range of applications a

19/12/05
Robert Terry
Independent Biotech Consultant, Frederiksberg C., Denmark
"BioImaging - The Application of Quantitave Fluorescence Methods to Biomedical Research and Discovery"

19/12/05
Susanna Castel
Confocal Microscopy & Cellular Micromanipulation Facility, SCT - University of Barcelona, Barcelona Science Park, Barcelona, Spain
"Advanced imaging in a multi-user facility"

19/12/05
Graziano Pesole
Lab. of Bioinformatics & Comparative Genomics, Dept. of Biomolecular Sciences & Biotechnologies, Università degli Studi di Milano, Milan, Italy
"Computational approaches for the Identification of novel genes and gene isoforms"

16/12/05
Sergi Puig
Dept. de Bioquímica i Biologia Molecular, Universitat de València, Spain
"Mechanisms of response to iron deficiency in Saccharomyces cerevisiae"

01/12/05
Antonio Giráldez
Skirball Institute, New York University School of Medicine
"The roles of miRNAs during vertebrate development"

25/11/05
Patrick Varga-Weisz
Babraham Institute, Babraham, Cambridge, UK
"ATP-dependent chromatin remodelling factors in chromatin replication"

18/11/05
Eduard Batlle
Biomedical Research Institute, Parc Científic de Barcelona, Barcelona, Spain
"The role of Wnt signaling and EphB/ephrinB receptors in colorectal cancer"

18/11/05
Manolis Kellis
Broad Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA
"Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals."

14/11/05
Eyal Bengal
Dept. of Biochemistry, Rappaport Institute for Research in the Medical Sciences, Technion-Israel Institute of Technology, Haifa, Israel
"P38 MAPK signaling and the regulation of myogenesis in Xenopus development"

07/11/05
Isabel Palacios
Dept. of Zoology, University of Cambridge, Cambridge, UK
"A novel mechanism of Kinesin mediated transport"

04/11/05
Tony Kouzarides
Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, UK
"Chromatin modifications and their function"

28/10/05
Elizabeth R. Gavis
Associate Professor, Dept. Molecular Biology, Princeton University, USA
"Localized RNAs, localized translation, and developmental asymmetry"

21/10/05
Jean-Christophe Marine
Lab. of Molecular Cancer Biology, Fladers Interuniversity, Institut for Biotechnology, Ghent, Belgium
"Studies on the regulation of the p53 tumor suppressor pathway in vivo"

18/10/05
Bruno Amati
Dept. of Experimental Oncology, European Institute of Oncology, Milan, Italy
"Myc, chromatin and cancer"

07/10/05
Peter Becker
Molekularbiologie, Adolf-Butenandt-Institut, München, Germany
"Dosage compensation in Drosophila: Mechanism, Models, Mystery"

07/10/05
Angus I. Lamond
Wellcome Trust Biocentre, University of Dundee, Dundee, Scotland, UK
"Nuclear Dynamics: a quantitative view of the protein flux through subnuclear organelles"

03/10/05
Fabio Rossi
Assistant Professor, The Biomedical Research Centre, Medical Genetics Medicine, The University of British Columbia, Vancouver, Canada
"Molecular mechanisms modulating the migration of hematopoietic progenitors"

03/10/05
Ramin Shiekhattar
Associate Professor, The Wistar Institute, Philadelphia, USA
"Genomic repression and its link to cancer"

30/09/05
Susanna Chiocca
Dept. of Experimental Oncology, European Institute of Oncology, Milan, Italy
"Gam1: a model to study viral interference with cellular pathways"

22/09/05
Mikhail S Gelfand
Institute for Information Transmission Problems, Russian Academy of Sciences, Moscow, Russia
"Riboswitches: possibly the oldest regulatory system"

09/09/05
Wahle, Elmar
Universitaet Halle, Institut fuer Biochemie, Halle, Germany
"Deadenylation of mRNA - an important step in the regulation of gene expression"

22/07/05
Auboeuf, Didier
Group Leader AVENIR 2002, INSERM U685, Centre G. Hayem, Hôpital Saint-Louis, Paris, France
"A Subset Of Nuclear Receptor Coregulators Act As "Coupling" Proteins During Synthesis And Maturation Of RNA Transcripts"

21/07/05
Verdin, Eric
Gladstone Institute of Virology & Immunology, University of California, San Francisco, USA
"Transcriptional regulation of HIV: role of chromatin"

15/07/05
Goodwin, Elisabeth
Associate Professor, Genetics Dept., University of Wisconsin, Madison, USA
"A novel ncRNA that controls development in C. elegans"

08/07/05
Martín-Bermudo, Lola
Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide, Sevilla, Spain
"Interactions between cells and the extracellular matrix: a key regulator of embryonic development"

07/07/05
González, Acaimo
Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide, Sevilla, Spain
"A genetic approach to the study of stem cells and their surroundings in Drosophila"

17/06/05
Serfling, Edgar
Dept. of Molecular Pathology, Institute of Pathology, Würzburg, Germany
"NFAT Transcription Factors in Lymphocyte Activation, Apoptosis and Carcinogenesis"

13/06/05
Sardet, Christian
BioMarCell UMR 7009 CNRS/UPMC, Station Zoologique, Villefranche sur Mer, France
"Establishment of embryonic axes: ascidians compared"

09/06/05
Heintz, Nathaniel
Professor, Howard Hughes Medical Institute, The Rockefeller University, New York, USA
"Genetic Dissection of the Mammalian Brain"

09/06/05
Ioshikhes, Ilya
Assistant Professor, Dept. of Biomedical Informatics, Ohio State University, Columbus, USA
"Influence of variant histone H2A.Z on local chromatin dynamics"

03/06/05
Rodríguez de Córdoba, Santiago
Dpto. Inmunología, Centre Investigaciones Biológicas, CSIC, Madrid, Spain
"Integrating genomic methods and functional analyses to characterize predisposition to Hemolytic Uremic Syndrome"

23/05/05
Shiekhattar, Ramin
Associate Professor, The Wistar Institute, Philadelphia, USA
"Regulation of genomic repression and its link to cancer"

20/05/05
King, Ross D.
Dept. of Computer Science, The University of Wales, Aberystwyth, UK
"The Robot Scientist Project"

19/05/05
Corbí López, Ángel L.
Profesor de Investigación, Centro de Investigaciones Biológicas, CSIC, Madrid, Spain
"DC-SIGN: pathogen recognition vs. escape from immunosurveillance"

06/05/05
Warner, Jonathan R.
Dept. of Cell Biology, Albert Einstein College of Medicine, New York, USA
"The Central Role of Ribosome Biosynthesis"

04/05/05
Stratmann, Thomas
Departamento de Fisiología, Universidad de Barcelona, Barcelona, Spain
"I-Ag7 - A TOP PLAYER IN TYPE I DIABETES"

03/05/05
Isalan, Mark
Structures and Biocomputing (Luis Serrano group), EMBL, Heidelberg, Germany
"Engineering gene networks on magnetic beads"

02/05/05
Sharpe, James
Programme Leader, MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK
"Building a 4D computer model of mouse limb development"

29/04/05
Nebreda, Ángel R.
CNIO, Spanish National Cancer Center, Madrid, Spain
"Signal integration in the regulation of the meiotic cell cycle and oncogenesis"

18/04/05
Baranov, Pavel
Human Genetics Dept., University of Utah, Salt Lake City, USA
"Recoding: A conspiracy against the genetic code"

15/04/05
Nekrutenko, Anton
Dept. Biochemistry & Molecular Biology, Center Comparative Genomics & Bioinformatics, The Huck Institutes for Life Sciences, Penn State Univ., USA
"Xlas-ALEX: Forced compensatory evolution of essential signaling proteins encoded by overlapping reading frames"

15/04/05
Walter, Peter
Howard Hughes Medical Institute/University of California in San Francisco, Biochemistry & Biophysics Dept., San Francisco, USA
"Mechanism of intracellular signaling from the endoplasmic reticulum to the nucleus"

14/04/05
Makova, Kateryna
Dept. of Biology, Center for Comparative Genomics & Bioinformatics, Penn State University, USA
"Strong and weak male mutation bias at different sites in the primate genomes"

01/04/05
Query, Charles C.
Department of Cell Biology, Albert Einstein College of Medicine, New York, USA
"How does the spliceosome select and position pre-mRNA for catalysis?"

21/03/05
Esteller, Manel
Director, Cancer Epigenetics Lab, Molecular Pathology Program, Spanish National Cancer Centre (CNIO), Madrid, Spain
"Cancer Epigenetics: Breaking the DNA Methylation and Histone Codes"

14/03/05
Orozco, Modesto
Reconeixement molecular, Institut de Recerca Biomèdica de Barcelona (IRBB-PCB), Barcelona, Spain
"Convergent views on the physics of nucleic acids"

11/03/05
Hall, Michael N.
Professor of Biochemistry, Biozentrum, University of Basel, Switzerland
"TOR signalling and control of cell growth in yeast and mammals"

25/02/05
Koenig, Michel
Institute de Génétique et de Biologie Moleculaire et Cellulaire (IGBMC), Illkirch, France
"Genetics of recessive ataxias: from genes identification to mouse models"

23/02/05
Soreq, Hermona
Prof. Molecular Biology, Vice Dean R&D, Fac. Science, The Hebrew Univ. Jerusalem, Dept. Biol.Chem., Inst. Life Sciences, Jerusalem, Israel
"Maladjusted alternative splicing accelerates dopaminergic-cholinergic imbalances and parkinsonism"

15/02/05
Moreira do Carmo, Alexandra
Group Leader of the "Cell Activation & Gene Expression" Lab, Instituto de Biologia Molecular e Celular (IBMC), Porto, Portugal
"Decoding the non-coding region: the 3' UTR of the cell cycle kinase polo"

11/02/05
Misteli, Tom
National Cancer Institute, NIH, Bethesda, USA
"Nuclear architecture in health and disease"

14/01/05
Bachs Valldeneu, Oriol
Catedràtic de Biologia Cel·lular, Dept. Biologia Cel·lular i Anatomia Patològica, Facultat de Medicina, Universitat de Barcelona, Spain
"A new regulatory pathway for the cell cycle inhibitor p27Kip1"

PROGRAMME SEMINARS 2005

GENE REGULATION

17/11/05
Oscar Puig
Institute of Biotechnology, University of Helsinki, Viikinkaari 9, Finland
"Role of FOXO in the regulation of stress, growth and metabolism through the insulin signaling pathway"

10/10/05
Stefan Fritz
Dept. Molecular Biology, University Aarhus, Aarhus, Denmark
"Dynamics of organelles and retroviral RNA"

28/07/05
Begoña Aguado
Centro Nacional de Biotecnologia, Universidad Autonoma de Madrid, Madrid, Spain
"The MHC class III region: a model for comporative and functional genomic and proteomic studies"

14/07/05
Miguel Lopez de Heredia
Gene Center and Institute for Biochemistry, University of Munich, Germany
" Composition of cellular RNPs. A Biochemical Approach"

19/04/05
Korbel Jan O.
Department Biocomputing, EMBL, Heidelberg, Germany
"Protein function prediction: utilising the genomic context"

15/03/05
Josefine Lundgren
Dpt. Molecular Biology and Functional Genomics, Stockholm University, Sweden
"Studies of Proteasome subunit function and regulation in Drosophila cell lines using different RNAi approaches"

31/01/05
Victoria Lunyak
Department of Medicine, University of California, San Diego, La Jolla, California, USA
"Epigenetic language" - when and ehere for cell specific transcriptional programs

28/01/05
Rosa Maria Marion
Department Biochemistry and Biophysics, University of California, San Francisco, USA
"High throughput screen for stress-responsive transcription factors: Identification of Sfp1 as a stress-and nutrient-sensitive regulator of ribosomal"

14/01/05
Valeria De Turris
"La Sapienza", Università degli studi di Roma, Roma, Italy
" RNA factory of TOP genes: analysis of the elements regulating the biosynthesis of intron-encoded snoRNAs"

DIFFERENTIATION & CANCER

13/10/05
Miranda Grounds
Professor, School of Anatomy & Human Biology, University of Western Australia, Australia
"Factors controlling the breakdown and repair of skeletal muscle: focus on TNFa and IGF-1"

02/09/05
Félix Prado Velasco
Dpto. de Genética, Facultad de Biología, Universidad de Sevilla, Sevilla, Spain
"Genetic instability during DNA replication in yeast"

25/08/05
Estanislao Bachrach
Genetics Department, Harvard Medical School, Boston, USA
"Muscle Engraftment of Myogenic Progenitor Cells Following Intra-Arterial Transplantation"

21/04/05
Aurelie Rossin
PhD student, H Gronenemeyer's lab, IGBMC, Strasbourg, France
"Regulation of cell differentiation and death by Rexinoids"

10/03/05
Holger Richly
Department of Molecular Cell Biology, Max Planck Insitute of Biochemistry, Germany
"An escort pathway to the proteasome."

GENES & DISEASE

22/12/05
Mario Cáceres
Dept. of Human Genetics, Emory University School of Medicine, Atlanta, USA
"Increased expression in human cerebral cortex of two thrombospondin genes involved in synapse formation"

28/06/05
Antoni Matilla-Dueñas
Director, EUROSCA Proteomics Project. Institute of Child Health. University College London, UK
"The EUROSCA Proteomics Project: Aplicacions proteòmiques a l'estudi de les Ataxies Espinocerebel.loses (SCA)"

08/02/05
Alexandra De Lille
Xenogen Corporation, Paris, France
"Biophotonic Imaging and its Uses for Monitoring Gene Therapy, Oncogenic events and Tracking Stem Cells in Live Animals"

BIOINFORMATICS & GENOMICS

21/11/05
Chaysavanh Manichanh
Unite d'Ecologie et de Physiologie du Système Digestif, Institut National de Recherche Agronomique, Jouy-en-Josas, Paris, France
"Metagenomic approach to analyze human intestinal microbiota".

03/11/05
Darrell Conklin
Department of Computing School of Informatics City University London, London, UK
"Gene finding for the helical cytokines"

24/10/05
Steffen Mueller
qPCR Field Application Scientist
"Applications of Real Time Quantitative PCR: Assay Validation, Optimization and Troubleshooting"

05/10/05
Tom Gingeras
AFFIMETRIX, Santa Clara, California, USA
"Functional RNAs : Lessons Learned and Forgotten"

04/10/05
Jan Korbel
Yale University, New Haven, USA
"Tiling Arrays in the Analysis of the Transcriptome"

26/09/05
Christoforos Nikolaou
Computational Genomic G.Inst.Biology National C. Research in Physical Sciences"Demokritos", Athens, Greece
"Addressing non-random aspects of genomic sequences in different length scales"

19/09/05
Francois Enault
PhD student IGS, CNRS UPR 2589 Marselle, France
"Inference of gene function using genomic context analysis"

12/09/05
Fazel Famili
IIT/ITI, National Research Council Canada, Ottawa, Canada
"Knowledge Discovery in Genomics and BioIntelligence".

10/06/05
Hagen Tilgner
Postdoc, Wellcome Trust Sanger Institute, Cambridge, UK
"Using Mass spectrometry to confirm (parts of) gene structures"

CELL & DEVELOPMENTAL BIOLOGY

19/12/05
Sylvain Gaudan
EBI, EMBL, UK Outstation, Cambridge, UK
"EbiMed: A web application that combines information retrieval and extraction from Medline. A step beyond PubMed"

30/11/05
Stefan Jäger
Evotec Technologies GmbH, Hamburg, Germany
"Spreading the Power of Single Molecule Analysis into Cells (Insight3D Cell)

Grants

Appendix 3

The grants that the CRG obtained from 1st January to 31st December 2005 are the following:

Organism	Amount (euros)
MINISTERIO DE EDUCACION Y CIENCIA	1.822.905,43
MINISTERIO DE SANIDAD Y CONSUMO-FIS	904.945,39
FUNDACION DESARROLLO INVEST. GENOMICA Y PROTEOMICA	521.709,31
EUROPEAN COMMISSION	520.328,37
FUNDACIO LA CAIXA	118.001,00
MUSCULAR DYSTROPHY ASSOCIATION	115.690,70
AGAUR - AGENCIA GESTIO D'AJUTS UNIVERSITARIS	105.547,66
FUNDACION CIENTIFICA DE LA ASOC ESP CONTRA EL CANCER	99.879,30
GLAXOSMITHKLINE	98.950,30
FUNDACIO MARATO TV3	83.626,00
CENTRE D'INNOVACIÓ I DESENVOLUPAMENT EMPRESARIAL	30.000,00
FUNDACIO IMIM	25.536,80
INTERNATIONAL BEHAVIOURAL AND NEURAL GENETICS SOCIETY	23.829,19
BUNDESMINISTERIUM FOR BILDUNG UND FORSCHUNG	20.000,00
NOVARTIS FARMACEUTICA S.A.	18.000,00
GRANTS (MISCELLANEOUS)	15.473,61
FONDATION JEROME LEJEUNE	14.000,00
MINISTÉRIO DA CIÉNCIA E DO ENSINO SUPERIOR	12.500,00
FUNDACIÓ AGRUPACIÓ MUTUA	9.000,00
DURSI	8.145,75
BUNDESMINISTERIUM FOR BILDUNG UND FORSCHUNG	7.000,00
CAIXA CATALUNYA	6.000,00
CONTRATAS Y OBRAS EMPRESA CONSTRUCTORA, SA	6.000,00
GRUPO FERRER INTERNACIONAL, SA	6.000,00
LABORATORIOS DR. ESTEVE, SA	6.000,00
PFIZER	6.000,00
PROUS SCIENCE	6.000,00
PANLAB S.L.	4.000,00
TOTAL AMOUNT	**4.615.068,81**

Honors and prizes of CRG Scientists during 2005

Appendix 4

Miguel Beato was appointed Dr. Honoris Causa of the Univeristy Pablo Olavide, Seville, Spain

Miguel Beato was elected member of the Scientific Advisory Board of the CENIEH (National Centre for Research on Human Evolution), Burgos, Spain

Miguel Beato was elected member of the Scientific Advisory Board of the Fundación Genoma España, Madrid, Spain

Miguel Beato's group was recognized as a "Consolidated Research Group" in 2005 by the Department of Universities, Research and Information Society (DURSI), of the Catalan Government, Spain

Luciano Di Croce's group was recognized as a "Emergent Research Group" in 2005 by the Department of Universities, Research and Information Society (DURSI), of the Catalan Government, Spain

Xavier Estivill's group was recognized as a "Consolidated Research Group" in 2005 by the Department of Universities, Research and Information Society (DURSI), of the Catalan Government, Spain

Fatima Gebauer's group was recognized as a "Singular Research Group" in 2005 by the Department of Universities, Research and Information Society (DURSI), of the Catalan Government, Spain

Raúl Méndez's group was recognized as a "Singular Research Group" in 2005 by the Department of Universities, Research and Information Society (DURSI), of the Catalan Government, Spain

Pura Muñoz received the 2005 Research Award by Fundacio Agrupacio Mutua, Spain

Pura Muñoz's group was recognized as a "Consolidated Research Group" in 2005 by the Department of Universities, Research and Information Society (DURSI), of the Catalan Government, Spain

Lauro Sumoy's group was recognized as an "Singular Research Group" in 2005 by the Department of Universities, Research and Information Society (DURSI), of the Catalan Government, Spain

Juan Valcárcel's group was recognized as a "Consolidated Research Group" in 2005 by the Department of Universities, Research and Information Society (DURSI), of the Catalan Government, Spain

Isabelle Vernos was elected EMBO member in 2005, Germany

Josep Vilardell's group was recognized as a "Singular Research Group" in 2005 by the Department of Universities, Research and Information Society (DURSI), of the Catalan Government, Spain

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