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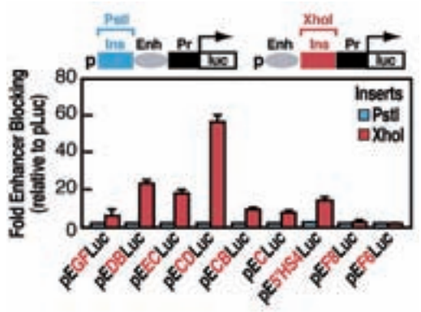
ANIMAL MODELS BY GENETIC MANIPULATION

In our laboratory, we are interested in understanding how mammalian expression domains work and how they are organised within genomes. In particular, we are focused in the identification and characterisation of genomic boundaries or insulators. These are the required regulatory elements that flank expression domains and counteract any interference from neighbouring loci, while allowing the internal enhancers and silencers to specify the expression pattern of the locus in space and time. The interest in studying insulator elements is double. First, it contributes to our understanding of the functional and structural organization of vertebrate genomes. And, second, they can be used effectively in biotechnological applications, as spacers, as boundaries, in any gene expression construct to be used in gene transfer experiments (i.e. transgenesis, gene therapy), preventing the inappropriate expression patterns of transgenes or gene therapy constructs and insulating them from neighbouring sequences surrounding the place of insertion in the host genomes.



Enhancer blocking assay serves to identify a new insulator (CD). From Lunyak et al. Science 2007; 317:248-251

We have explored several experimental models, including the mouse whey-acidic protein gene, the mouse growth hormone gene and, mainly, the mouse tyrosinase gene. These are three independent developmentally regulated and tissue-specific loci that have served us to identify three types of boundaries that appear to operate under different cellular mechanisms. In addition we have started to search for new insulator sequences found in vertebrate genomes. We address our experiments preliminary through bioinformatic analyses, *in silico*, that serve to identify potential insulator sequences. Subsequently, insulator candidates are functionally validated *in vitro*, using cells and the enhancer blocking assay and, eventually, *in vivo*, using transgenic animals (zebrafish, in collaboration with Jose Luis Gomez-Skarmeta, CABD, and mice, at CNB), carrying appropriate informative constructs.

In addition, our laboratory generates and analyses new animal models to study the neural alterations in vision, but also in hearing, associated with albinism, a rare disease, that substantiate our incorporation at the CIBERER. Using transgenic pigmented and albino mice, in collaboration with the laboratory of Isabel Varela-Nieto (IIB-CSIC/UAM) we have demonstrated that albino mice display premature severe hearing loss and that do not recover after a noise-induced hearing loss, as compared to their pigmented counterparts. Through the use of specific transgenic mice we have also identified the lack of intermediate reagents in the pigment biosynthetic pathway (such as L-DOPA) as the primary cause of the hearing abnormalities observed in hypopigmentary syndromes.

Finally, through collaborations, we have generated a number of additional animal models (transgenics) for CNS-related diseases or conditions (Alzheimer) exploiting our technology of yeast artificial chromosome (YAC)-type of transgenes.

The expertise of our laboratory and its implication with the Mouse Embryo Cryopreservation and Histology Facilities at CNB have been also instrumental for our effective participation in several FP7 European Projects within the field of mouse functional genomics, such as INFRAFRONTIER and EMMA, the European Mouse Mutant Archive, whose new Spanish node has been assigned to the CNB, under the coordination of Lluis Montoliu.

Using transgenic mice and tyrosinase-reporter transgenes to functionally validate new genomic insulators

Selected Publications

Moreira PN, Pozueta J, Pérez-Crespo M, Valdivieso F, Gutiérrez-Adán A, Montoliu L. *Improving the generation of genomic-type transgenic mice by ICSI.* **Transgenic Res.** 2007 Apr;16(2):163-8.

Moreira PN, Pérez-Crespo M, Ramírez MA, Pozueta J, Montoliu L, Gutiérrez-Adán. *Effect of Transgene Concentration, Flanking Matrix Attachment Regions, and RecA-Coating on the Efficiency of Mouse Transgenesis Mediated by Intracytoplasmic Sperm Injection.* **Biol Reprod.** 2007 Feb;76(2):336-43.

Lunyak VV, Prefontaine GG, Núñez E, Cramer T, Ju BG, Ohgi KA, Hutt K, Roy R, García-Díaz A, Zhu X, Yung Y, Montoliu L, Glass CK, Rosenfeld MG. Developmentally regulated activation of a SINE B2 repeat as a domain boundary in organogenesis. **Science.** 2007 Jul 13;317(5835):248-51.

Murray JD, Whitelaw B, Montoliu L. *Meeting report: UC Davis Transgenic Animal Research Conference VI.* **Transgenic Res.** 2007 Dec;16(6):835-7.

Montoliu L. *Gene targeting in mice awarded with the 2007 Nobel Prize in Physiology or Medicine.* **An. R. Acad. Nac. Farm.** 2008; 74(1):81-99.

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Lucia Morgado

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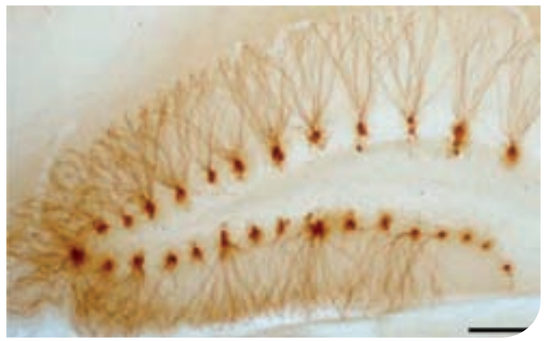
FUNCTIONAL ANALYSIS OF TRANSCRIPTIONAL REPRESSOR DREAM

We study the regulatory mechanisms that control activity-dependent gene expression in excitable cells. Our long-term goal is to develop a global model encompassing the different mechanisms that control gene expression and are regulated directly or indirectly by changes in the nuclear concentration of Ca^{2+}. Along these lines, we aim towards the complete identification of mediator complexes containing DREAM as well as other less well characterized Ca^{2+}-dependent nuclear cofactors. We also aim to understand changes at the chromatin structure that could be related to changes in nuclear Ca^{2+}, including modification in the activity of chromatin remodeling enzymes, DNA methylation and post-transcriptional changes at the level of the histones.

Specifically, work with the transcriptional repressor DREAM involves several research lines. One research line focus on the functional characterization of transcriptional repressor DREAM in vivo using several transgenic-based models with dominant active mutants of the DREAM protein. These mutants do not bind Ca^{2+} and do not interact with CREB or CREM proteins. Thus, these mutants will remain bound to DNA after membrane receptor activation and increase in Ca^{2+} and cAMP and, will continue to repress transcription even during membrane depolarizing. By the use of tissue-specific promoters we have targeted these mutants to the brain to obtain constitutive or conditional expression by the use of Tet-Off or Tet-On trans-activating systems.

A second research line tries to characterize putative functional domains within the DREAM protein, in particular those mediating the nuclear translocation, the tetramerization process and the DNA binding domain. Currently, there is strong evidence indicating that the domain responsible for binding to the DRE sequence is formed upon tetramerization. Sequence analysis of the DREAM protein does not reveal the presence of nuclear import or export sequences in DREAM, thus we are analyzing alternative mechanisms regulating the translocation of DREAM to and from the nucleus. We have identified several phospho-residues that modulate the repressor activity of DREAM either affecting its nuclear translocation, its tetramerization and/or its binding to DRE sites in the DNA. In this regard, we characterized the interaction between DREAM and GRK2 and we showed that DREAM is phosphorylated by GRK2.

Finally, we have used the two hybrid approach to characterize several proteins that are interacting with DREAM, both in Ca^{2+}-dependent and Ca^{2+}-independent manner. In this area our main interest is in those proteins that contribute to, or block the transcriptional function of DREAM, though several interactions without a direct relationship with the nuclear function of DREAM are also under investigation. In addition, we are developing the tools to use proteomic technology to decipher the composition of nuclear complexes containing DREAM as well as the changes in the composition of these complexes upon different experimental and/or physiological conditions.



DREAM regulates synaptic plasticity in hippocampal granular neurons.

Selected Publications

Ruiz-Gomez A, Mellstrom B, Tornero D, Morato E, Savignac M, Holguin H, Aurrekoetxea K, Gonzalez P, Gonzalez-Garcia C, Cena V, Mayor F Jr, Naranjo JR. *GRK2-mediated phosphorylation of DREAM regulates membrane trafficking of Kv4.2 potassium channel.* **J Biol Chem.** 282:1205-1215 (2007).

Naranjo JR, Mellstrom B. *Split personality of transcription factors inside and outside the nuclear border.* Sci STKE. 2007 Jan 30;2007(371):pe5. PMID: 17264316 [**PubMed - in process**]

Groves P, Kover KE, Andre S, Bandorowicz-Pikula J, Batta G, Bruix M, Buchet R, Canales A, Canada FJ, Gabius HJ, Laurents DV, Naranjo JR,

Palczewska M, Pikula S, Rial E, Strzelecka-Kiliszek A, Jimenez-Barbero J. T*emperature dependence of ligand-protein complex formation as reflected by saturation transfer difference NMR experiments.* **Magn Reson Chem.** 2007 Sep;45(9):745-8.

Marcos Rivas and José R. Naranjo. *Thyroid hormones, learning and memory.* **Genes, Brain and Behavior.** Jun;6 Suppl 1:40-4 (2007).

Britt Mellström, Magali Savignac, Rosa Gomez-Villafuertes and Jose R. Naranjo. *Ca^{2+}-operated transcriptional networks: molecular mechanisms and in vivo models.* **Physiol.** Rev 88:421-449 (2008).