

ANIMAL MODELS BY GENETIC MANIPULATION



Lluís Montoliu

Summary

We are interested in understanding how mammalian expression domains work and how they are organised within genomes.

We would like to know the required regulatory elements that identify a given expression domain and specify its expression pattern in space, time and level, to improve the design

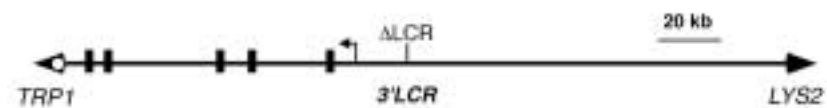
of gene transfer strategies, used for animal transgenesis and for gene therapy.

We use two experimental models: the mouse tyrosinase and whey acidic protein genes, two independent developmentally regulated and tissue-specific loci that have served us to identify a number of key regulatory elements, including boundaries and LCRs.

We address our experiments *in vivo*, using transgenic mice carrying large chromosomal-type constructs that we engineer by homologous recombination to investigate the functional role of specific sequences, and *in vitro*, using cells and chromatin DNA-protein analyses. Further, our laboratory generates and analyses new animal models of human diseases by genetic modification of mice.

We have used a number of tyrosinase transgenic mice to address the retinal deficits commonly associated with albinism, the normal mammalian retinal development and the genes involved in the process.

Finally, through collaborations, we have generated a number of additional animal models (transgenics and knockouts) for CNS-related diseases or conditions (Alzheimer, pain, psychosis).



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Figure 1. Albino and transgenic mouse (in front) generated by ICSI with a YAC carrying the tyrosinase gene with a deleted LCR prepared by homologous recombination in yeast cells. A severe decrease of tyrosinase expression is seen in the skin but not in the eye (see Moreira et al. 2004).
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PERSONNEL



Laboratory Picture (December 2004) From left to right: Lluís Montoliu, Alfonso Lavado, Victoria Tovar, Lucía Regales, Julio Pozueta, Rosa Roy (in front), Soledad Montalbán, Marta Cantero, Ángel García, Patricia Cozar.

Group Leader:

Dr. Lluís Montoliu José

Postdoctorals scientists :

Dr. Alfonso Lavado Júdez
(desde / from 01-04-2004)

Dra. Patricia Giraldo Carbajo
(hasta / up to 30-03-2003)

Dra. Victoria Tovar Herrador

Dra. Rosa Roy Barcelona

(desde / from 01-11-2003)

Dr. Francisco Javier Rodríguez Jiménez
(desde / from 01-10-2003 hasta / up to
28-02-2004)

Predocctoral Fellows:

Alfonso Lavado Júdez
(hasta / up to 31-03-2004)

Lucía Regales Álvarez

Ángel García Díaz

Julio Pozueta Larios

Julia Fernández Punzano (Titulado Superior,
desde / from 01-11-2004)

Visiting Graduate Students:

Rodolfo Moreno (desde / from 01-02-2003
hasta / up to 30-04-2003)

Visiting Undergraduate Students:

Elisa Jiménez (hasta / up to 30-06-2003)

Technical Assistants:

Patricia Cozar López

Marta Cantero González

Histology Facility at CNB:

Noemí Magán (desde / from 01-03-2004
hasta / up to 30-11-2004)

Soledad Montalbán Iglesias

(desde / from 01-12-2004)

Visiting Scientists:

Dra. Karoline Lassnig (IFA Tulln, Department
of Animal Production, Tulln, Austria) (March
2003)

Dra. M^a Carmen Muñoz (Trasngenic Unit,
Parc Científic de Barcelona, PCB) (July-
August 2004)

Dr. Glen Jeffery (University College London,
Institute of Ophthalmology, London, UK)
(January and December 2004)

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PUBLICATIONS

INTERNATIONAL SCIENTIFIC JOURNALS

Moreira, P.N., Giraldo, P., Cozar, P., Pozueta, J., Jiménez, A., Montoliu, L.* and Gutiérrez-Adan A*.(2004). Efficient generation of transgenic mice with intact yeast artificial chromosomes by intracytoplasmic sperm injection. *Biology of Reproduction* Dec;**71(6)**:1943-7.

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Giraldo, P., Martínez, A., Regales, L., Lavado, A., García-Díaz, A., Alonso, A., Busturia, A. and Montoliu, L. (2003). Functional dissection of the mouse tyrosinase locus control region identifies a new putative boundary activity. *Nucleic Acids Research* Nov **1;31(21)**:6290-305.

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Millot, B., Montoliu, L., Fontaine, M.L., Mata, T. and Devinoy, E. (2003). Hormone-induced modifications of the chromatin structure surrounding upstream regulatory regions conserved between the mouse and rabbit whey acidic protein genes. *Biochemical Journal* **15;372(Pt 1)**:41-52.

Montoliu, L. (2003). Simple databases to monitor the generation and organisation of transgenic mouse colonies. *Transgenic Research* **12(2)**:251-3.

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Montoliu, L. (2003). Large-scale preparation of agarose plugs of yeast DNA (pp. 326-328); Purification of YAC DNA with filtration units (pp- 329-331). Preparing injection buffer for BAC/YAC DNA (pp. 333). In: *Manipulating the Mouse Embryo. A Laboratory Manual (Third Edition)*. Andras Nagy, Marian Gertsenstein, Kristina Vintersten, Richard Behringer (Eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

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Montoliu, L. (2004). Clonación en mamíferos: aspectos científicos e implicaciones terapéuticas. En: Últimas investigaciones en biología: células madre y células embrionarias. José Fernández Piqueras (Ed.), Publicaciones de la Universidad Internacional Menéndez y Pelayo (UIMP), Ministerio de Educación y Ciencia. (pp. 55-88).

Montoliu, L. (2003). Traslado de genes (animales transgénicos, mutantes y clónicos). En: 50 años de ADN. Pedro García Barreno (Ed.), Editorial Espasa-Forum, capítulo 6, (pag. 183-227), Madrid.

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RESEARCH PROJECTS

Montoliu, Lluís

Functional and structural characterisation of genomic boundaries
Spanish Ministry of Science and Technology, National Plan R+D+i, Biotechnology Program, BIO2003-08196, 2004-2006.

Montoliu, Lluís (sub-project, Coordinator: Dr. Mara Dierssen, CRG, Barcelona)

Murine models of central nervous system (CNS) disease.
Autonomous Government of Catalunya, Generalitat de Catalunya, Thematic Network, 2003-2005, 2001-2003.

Montoliu, Lluís

Identification of genes associated with retinal development: analysis of visual deficits associated with hypopigmentary diseases (albinism).
Autonomous Government of Madrid, CAM, 08.5/0046.1/2003, 2003-2004.

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Artificial chromosomes in mammary gland transgenesis.
Joint Collaborative Project, Spain-Austria, HU2001-0025, 2002-2003.

Montoliu, Lluís

Genomic boundaries in gene transfer events.
Spanish Ministry of Science and Technology, National Plan R+D+i, Biotechnology Program, BIO2000-1653, 2001-2003.

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DOCTORAL THESES

Mata González, Teresa

Functional and structural characterisation of new mammary gland-specific expression vectors based in the mouse whey acidic protein gene.

Centro de Investigaciones y Estudios Avanzados (CINVESTAV), Instituto Politécnico Nacional, México DF (2004). Mark: Apto
Supervisors: Dr. Vianney Ortiz-Navarrete (CINVESTAV, México, DF) and Dr. Lluís Montoliu.

Lavado Júdez, Alfonso Javier

Animal models for the functional study of the mouse tyrosinase gene and the consequences associated with its mutation in the mammalian visual system.

Autonomous University of Madrid (2004). Mark: Excellent cum laude.

Supervisor: Dr. Lluís Montoliu

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CONTRACTS

Lluís Montoliu

Formation in techniques for the generation and the analysis of trasngenic and knockout mice.
Fundación Parc Científic de Barcelona (PCB), Barcelona Science Park Foundation (Barcelona), June-August 2004.

Lluís Montoliu

Generation of chimeras to obtain knockout mice for the LAT2 gene.
Fundación Institut de Recerca Oncològica (IRO), Institute of Oncology Research Foundation (Barcelona).
June-August 2004.

Lluís Montoliu

Production, rederivation and cryopreservation of knockout mice for the Sigma-I receptor gene.
Laboratorios del Dr. Esteve, S.A. (Barcelona).
July 2003- June 2006.

Lluís Montoliu

Analysis of genetic polymorphisms (microsatellite variants) in mice.
Bionostra, S. L. (Madrid).
May-July 2003.

Lluís Montoliu

Analysis of the phenotype of knockout mice for the Sigma-I receptor gene.
Laboratorios del Dr. Esteve, S.A. (Barcelona).
September 2001-August 2004.

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PATENTS

Moreira, P.N, Gutiérrez-Adán, A. and Montoliu, L.I.
A method for the stable introduction of large DNA sequences in the genome of non-human mammals
INIA and CSIC
Spain (OEP: P 200400857) 6 April 2004