Research Summary

Lluís Montoliu

Animal models by genetic manipulation

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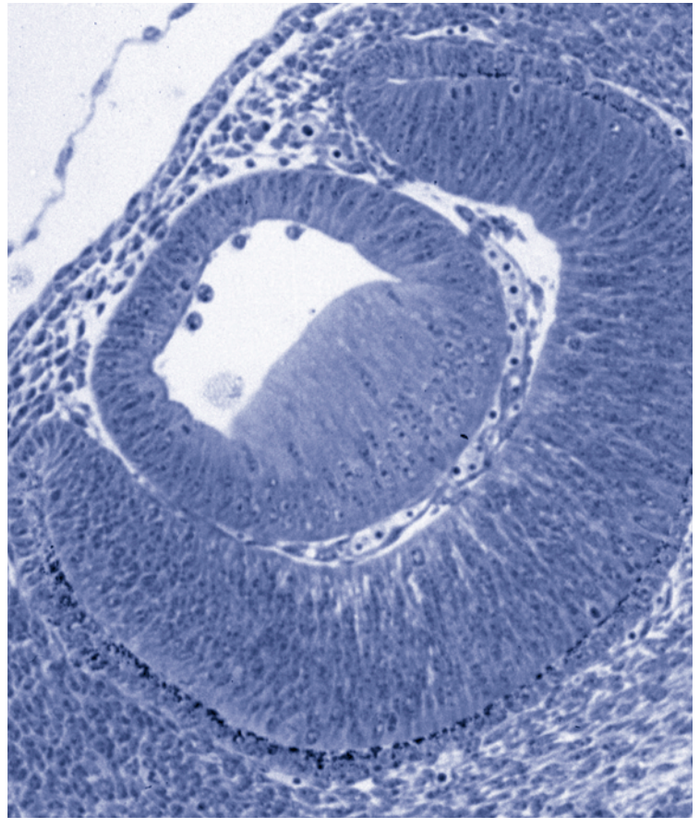
The experimentation with transgenic animals has shown temporal and spatial variability in transgene expression mainly due to position effects, related to the insertion of the transgene in the host genome. In this regard, the study of mechanisms regulating faithful transgene expression is essential for the development of animal models by genetic manipulation and, at the same time, allows increasing our knowledge on the organisation and structure of eukaryotic expression domains.

In our laboratory, we use two experimental models to analise regulatory elements from genomic expression domains: the tyrosinase gene, specific of melanocytes and of retinal pigment epithelium cells, and the whey acidic protein (WAP) gene, mammary gland-specific during the lactation period (in collaboration, the latter, with Dr. Eve Devinoy's laboratory at INRA in Jouy-en-josas, France, and Prof. M. Müller's laboratory at the Veterinary University of Vienna, Austria). We have used several natural mutants of the tyrosinase gene and made a number of transgenic animals with genomic fragment from both domains in order to uncover the most important regulatory elements, which restrict tissue-specific expression and prevent their ectopic activation. Among those identified elements, that are being characterised both at the structural and functional level, there is a locus control region (LCR), in the tyrosinase gene, and boundaries/insulators, in both experimental systems. The principal task of boundaries is to delimit the operative region of transcriptional regulators belonging to the domain and to prevent interferences from activators and repressors of neighbouring domains. Their existence can be uncovered with experiments in transgenic animals in which we evaluate the capacity of these sequences to protect a given heterologous transgene from position effects. In collaboration with Dr. Ana Busturia's laboratory (CBMSO, Madrid) we have generated and analised multiples *Drosophila* transgenic lines carrying different construct, with or without boundaries.

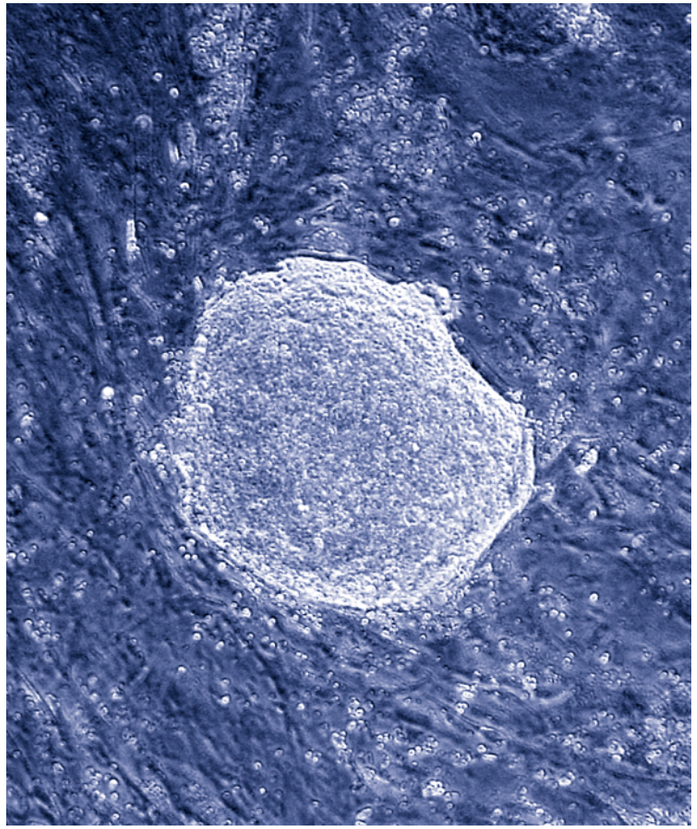
We are using also the tyrosinase experimental model to investigate the function of this protein, encoding the key enzyme in the melanin biosynthetic pathway, in mammalian retina development. Oculocutaneous albinism type I is caused by mutations in the tyrosinase gene. This genetic defect, affecting 1:10000 Europeans, is characterised by hypopigmentation and multiple abnormalities in the retina and the visual system. All these abnormalities disappear in transgenic mice obtained with tyrosinase functional constructs. In our laboratory, in collaboration with Dr. Glen Jeffery's laboratory (UCL, London, UK) we study how, directly or indirectly, tyrosinase influences the normal development of mammalian retina, using several transgenic mouse models.

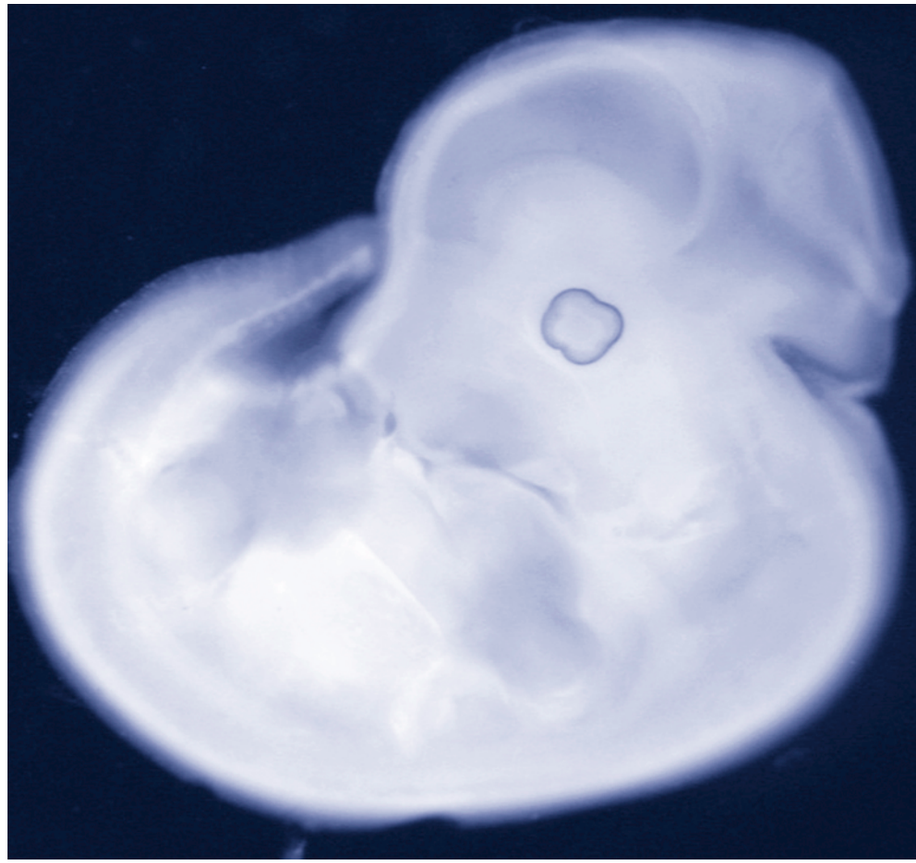
The study of the mechanisms, both functional and structural, implicated in obtaining an adequate pattern of transgene expression goes beyond the tyrosinase model being of capital relevance in projects of animal biotechnology and gene therapy, in which optimal expression must be guaranteed. In this sense, our laboratory has developed several transgenic constructs based in artificial chromosomes (BACs and YACs) aiming to improve heterologous expression in transgenic animals. In collaboration with Prof. F. Valdivieso's laboratory (CBMSO, Madrid) we are producing a new animal model for the Alzheimer's disease using a transgenic mouse prepared with a new artificial chromosome containing the APP gene.



Histological section of a mouse eye at embryonic day 11.5. Melanin can first be detected at the retinal pigment epithelium.

Finally, in collaboration with a national pharmaceutical company, we have generated and are analysing the phenotype of a mouse knock-out model carrying a mutation, generated by homologous recombination in ES cells, in the Sigma receptor Type I, involved in analgesia, schizophrenia and psychosis phenomena.





In situ hybridisation of a 12.5 mouse embryo with a tyrosinase specific probe. Specific signals with the expression of the gene can only be detected at the retinal pigment epithelium.

Microphotography of a mouse ES cell colony growing in culture over mouse embryonic fibroblasts. These cells are used to generate new knockont mice.

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Grant Support

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FEDER (FD97-2059), 2000-2001.

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Montoliu, L.
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Genetrix, S. L., 2002-2003.

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Bionostra, S. L., 2002.

Doctoral Thesis

Estela Giménez. (2002).
Estudios funcionales de la tirosinasa en el desarrollo de la retina de mamíferos.
Universidad Autónoma de Madrid, Sobresaliente *cum laude*.

Patricia Giraldo. (2002).
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Patents

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Courses and meetings

III Curso de Doctorado sobre "Transferencia Génica en Mamíferos" (Postgraduate course)
Dpto. Biología Molecular UAM (curso 2000-2001)
Montoliu, L. (coordinador).

Simposio sobre “Transgenic animal systems”
X Congreso Europeo de Biotecnología ECB10 (Madrid, Julio 2001)
Montoliu, L. (co-organizador).

II Reunión del Grupo de “Transgénesis en Mamíferos”
XXIV Congreso de la Sociedad Española de Bioquímica y de Biología Molecular, Valencia (2001)
Montoliu, L. (coordinador).

IV Curso de Doctorado sobre "Transferencia Génica en Mamíferos" (Postgraduate course)
Dpto. Biología Molecular UAM (curso 2001-2002)
Naranjo, J.R., Montoliu, L. (coordinadores).

Simposio sobre "Modelos Animales y Nuevas Estrategias Terapéuticas"
XXV Congreso de la Sociedad Española de Bioquímica y de Biología Molecular, León (2002)
Lucas, J.J., Montoliu, L. (coordinadores).

Staff

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Staff Scientists

Estela Giménez (until 28-02-2002)
Patricia Giraldo
Alfonso Lavado
Francina Langa (until 31-01-2001)
Lucía Regales
Ángel García Díaz (since 01-04-02)
Victoria Tovar (since 01-06-2002)
Patricia Cozar (since 16-02-2001)
Marta Cantero (since 01-12-2001)

Visitors

Miguel Angel Chinchetru (since 01-10-2001 until 31-09-2002)
Sabbatical, Universidad de León

Julio Pozueta (since 15-10-2001)
CBMSO-UAM/CSIC, Madrid

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CINVESTAV, Mexico DF

Glen Jeffery (Mayo 2001)
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