Manuel Palacín

Amino acid transporters: biochemistry, physiopathology, genetics and structural biology

Our research efforts focus on the molecular bases of renal reabsorption of amino acids, the physiopathology of the inherited aminoacidurias cystinuria and lysinuric protein intolerance (LPI), the structure-function relationship in heteromeric amino acid transporters (HATs), and the study of the multiple functions of heavy chains of HATs. With regards to the molecular bases of renal reabsorption of amino acids, we address the generation and characterisation of mutated mouse models of renal amino acid transporters. In the physiopathology of inherited aminoacidurias, our goals are the following: (i) to develop animal models to study the impact of several renal amino acid transporters on cystinuria; (ii) to identify mechanisms of pathology in this inherited disorder; (iii) to search for new drugs for the treatment of lithiasis in cystinuria; and (iv) to generate and characterise a mouse model for LPI. Finally, our group works towards elucidating the atomic structure of HATs, using both human transporters and prokaryotic homologues.

The molecular bases of renal reabsorption of amino acids

Our laboratory has identified and characterised three amino acid transporters involved in the renal reabsorption of amino acids: systems $b^{0,+}$ (heterodimer rBAT-$b^{0,+}$AT), y^+L (heterodimer 4F2hc-y^+LAT1) and exchanger L (heterodimer 4F2hc-LAT2; Figure 1). We have also demonstrated the role of systems $b^{0,+}$ and y^+L in cystinuria and lysinuric protein intolerance (LPI). This has allowed us to propose a mechanism of reabsorption in which these amino acid exchangers participate. This model requires basolateral transporters with a net flux of neutral amino acids. The search for these transporters is done mainly with functional studies of orphan transporters within the described amino acid transporter families. The characterisation of mutated mouse models of LAT2 and EEG1 might shed light on this issue. Moreover, in collaboration with Paolo Gasparini, we are studying whether there is an association between amino acid transporter polymorphisms and renal reabsorption of amino acids in genetically isolated human populations. In this regard, we have identified groups of amino acids with co-variation in urinary excretion (D'Adamo *et al*, in press). This activity was initiated within the European Union project EUGINDAT (European Union Genomic Initiative on Disorders of Amino acid Transporters).

Physiopathology of inherited aminoacidurias cystinuria and LPI

Our laboratory has identified the genes involved in cystinuria (system $b^{0,+}$; heterodimer rBAT-$b^{0,+}$AT) and LPI (system y^+L; heterodimer 4F2hc-y^+LAT1), and within the International Cystinuria Consortium, which we founded, we have identified most of the mutations causing these diseases. We have established a wide genotype-phenotype correlation in cystinuria that has allowed us to propose a new classification of the disease: type A, caused by SLC3A1 mutations, and type B, caused by SLC7A9 mutations. The objectives that we are currently pursuing are as follows: i) identification of molecular mechanisms to explain the distinct phenotypes in cystinuria, using animal and cell models; ii) identification of modulator genes of lithiasis in cystinuria, using animal models; iii) search for new drugs to treat lithiasis in cystinuria, using our murine cystinuria model Stones; and iv) identification of the mechanisms that lead to immunological disorders associated with LPI, using a newly generated floxed y^+LAT1 mouse line. Expression of CRE recombinase under the control of tamoxiphen in these animals has resulted in the first mouse model for LPI.

Structure-function relationship in heteromeric amino acid transporters (HATs)

Our laboratory has identified most of the members of the HATs. Moreover, we have approached the structure-function relationships of HATs by defining the transport mechanisms as obligatory exchangers, the oligomeric state, the atomic structure of the ectodomain of 4F2hc (CD98hc) (in collaboration with IRB Barcelona researcher Ignasi Fita), the light subunit as the catalytic component, the membrane topology of the light subunits, and key residues for transport. Recently, in collaboration with Dimitrios Fotiadis (EUGINDAT project), we obtained the first projection map of a prokaryotic homologue of the light subunits of HATs (LAT family of transporters) at a subnanometer scale (6.5 Å). This map revealed striking similarities with unrelated transporters with the so called '5 + 5 transmembrane repeat' fold (Casagrande *et al*, 2008). Recently, we offered evidence

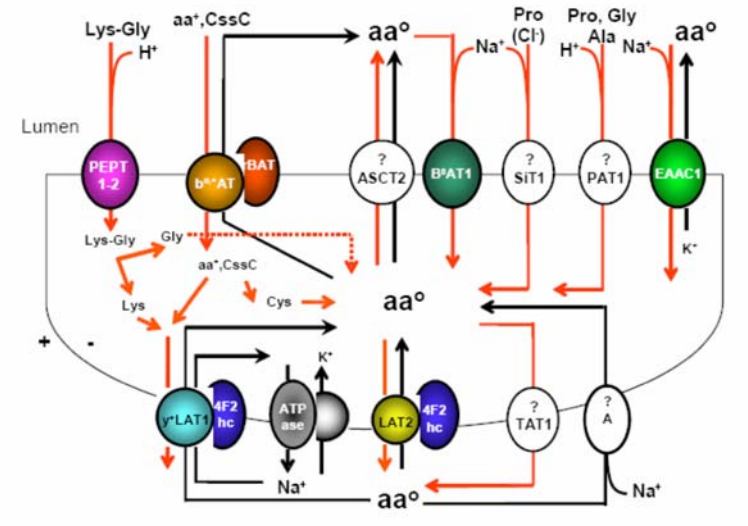
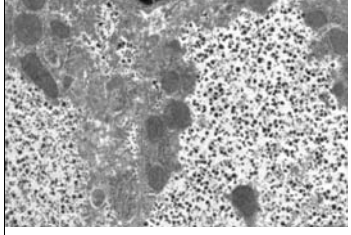


Figure 1. ***Proximal tubule model for amino acid transporters involved in renal and intestinal reabsorption of amino acids.*** *Transporters with a proved role in renal reabsorption or intestinal absorption of amino acids are coloured, whereas those expressed in the plasma membrane of epithelial cells of the proximal convoluted tubule (or of the small intestine) but with no direct experimental evidence supporting their role in reabsorption are shown in white. Amino acid fluxes in the reabsorption direction are in red. PEPT1 and PEPT2 are expressed in the small intestine and kidney respectively. Adapted from Moe, Wright and Palacín; Brenner & Rector's The Kidney, chapter 6, 214-47 (2008)*

by force spectroscopy that substrate binding increases the conformational flexibility of a LAT transporter (Bippes *et al*, 2009). At present, we are working on the atomic resolution of a prokaryotic homologue of the light subunits of HATs (Figure 2). Functional studies in parallel seek to identify key residues for amino acid transport function within HATs.



Research Group Members

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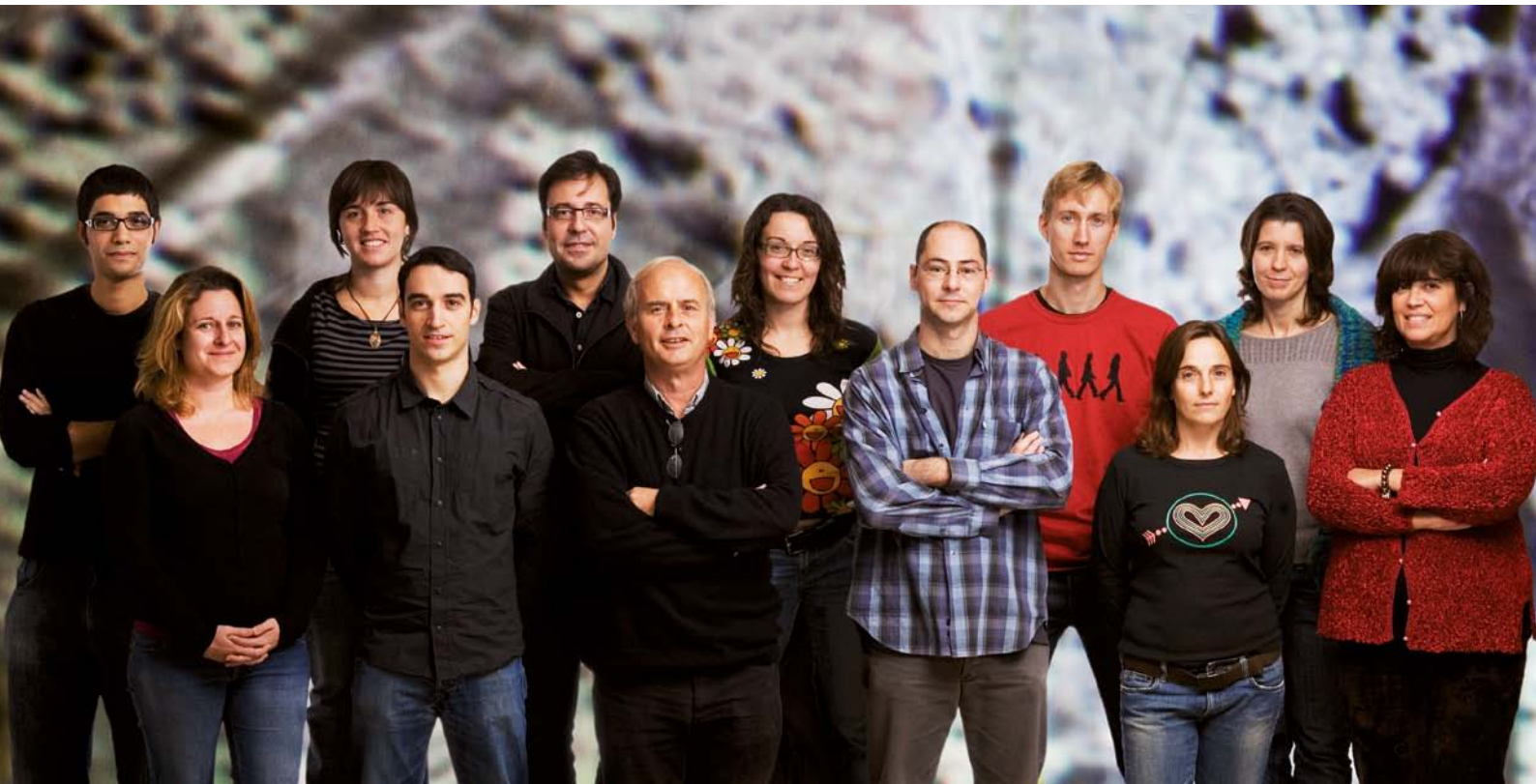

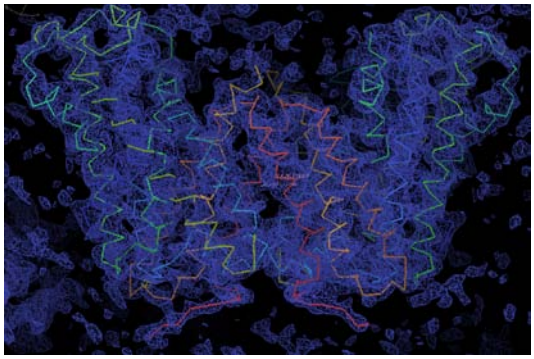

Figure 2. ***Electronic density of a prokaryotic homologue of the light subunits of HATs.*** *The atomic structure (Ca backbone; rainbow colour) has been solved at a resolution of 3.5 Å. Electronic density (1 σ) (violet).*

Study of the multiple functions of heavy chains of HATs

One of the heavy subunits of HATs identified, 4F2hc (CD98), is involved in many cellular functions, such as cellular transformation, adhesion and fusion. Very recently we have developed the 3D structure of the extracellular domain of 4F2hc (PDB 1Y4N and 1Y5Z). This allows us to study the role of the extracellular domain of 4F2hc in its multiple functions, including interaction with ß1 integrins. Moreover, the recombinant extracellular domain of 4F2hc is a powerful tool for the identification of potential ligands of 4F2hc.

Scientific output

Publications

Bippes CA, Zeltina A, Casagrande F, Ratera M, Palacin M, Muller DJ and Fotiadis D. Substrate binding tunes conformational flexibility and kinetic stability of an amino acid antiporter. *J Biol Chem*, **284**(28), 18651-63 (2009)

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Zorzano A, Sebastián D, Segalés J and Palacín M. The molecular machinery of mitochondrial fusion and fission: An opportunity for drug discovery? *Curr Opin Drug Discov Devel*, **12**(5), 597-606 (2009)

Research networks and grants

CIBER de enfermedades raras (CIBERER)
Carlos III Health Institute (since 2007)
Principal investigator: Manuel Palacín

European Drug Initiative on Channels and Transporters (EDICT)
European Commission, 201924 (2008-2012)
Principal Investigator: Manuel Palacín

Random approach to build a thermostable polytopic membrane protein for crystallization
Spanish Ministry of Science and Innovation, BFU2008-04637 (2008-2012)
Researcher: José Luís Vázquez-Ibar

Role of 4F2hc in tumorogenesis
'La Marató TV3' Foundation (2006-2009)
Principal investigator and coordinator: Manuel Palacín

Transportadores heteromericos de aminoácidos: estructura, genómica funcional y fisiopatoplogía (Cistinuria y Lisinuria con intolerancia a proteínas)
Spanish Ministry of Science and Innovation, BFU2006-14600-C02-01 (2006-2009)
Principal investigator: Manuel Palacín

Collaborations

Physiopathology of inherited aminoacidurias cystinuria and lysinuric protein intolerance (LPI)
Josep Chillarón, University of Barcelona (Barcelona, Spain); Virginia Nunes, IDIBELL (Barcelona, Spain); Gianfranco Sebastio, Università Federico II (Naples, Italy)

Structure-function relationship in heteromeric amino acid transporters (HATs)
Steve Baldwin, University of Leeds (Leeds, UK); Ignacio Fita, IRB Barcelona (Barcelona, Spain); Dimitrios Fotiadis, University of Bern (Bern, Switzerland); Eric Gouaux, Vollum Institute (Portland, USA); Modesto Orozco, IRB Barcelona (Barcelona, Spain); Matthias Quick, Cornell University (New York, USA)

Study of the multiple functions of heavy chains of HATs
Joaquín Abian, Autonomous University of Barcelona (Barcelona, Spain); Chloe Feral, Nice Sophia Antipolis University (Nice, France); Mark Ginsberg, University of California San Diego (La Jolla, USA); María Antonia Lizarbe, Universidad Complutense de Madrid (Madrid, Spain)

The molecular bases of renal reabsorption of amino acids
Paolo Gasparini, Institute for Maternal and Child Health IRCCS-Burlo Garofolo (Trieste, Italy); Virginia Nunes, IDIBELL (Barcelona, Spain)