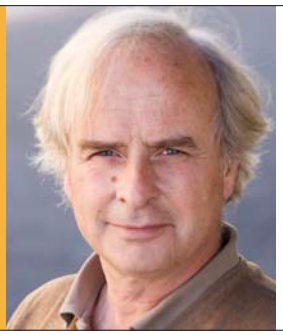
MANUEL PALACÍN



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 developing the three-dimensional (3D) 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 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. 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. 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 lysinuric protein intolerance (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

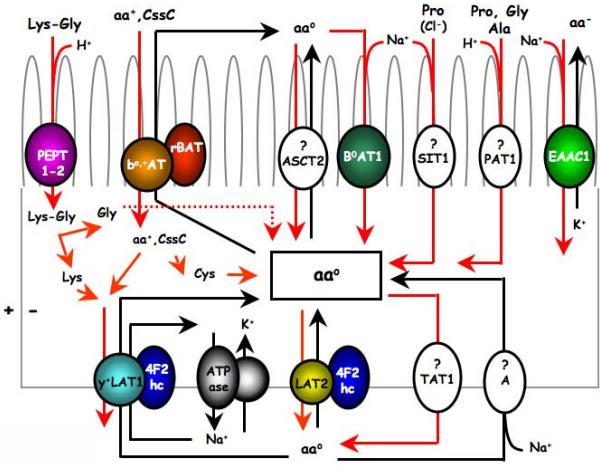


***Figure 1**. Proximal tubule model for amino acid transporters involved in renal and intestinal reabsorption of amino acids. Transporters with a proven role in renal reabsorption or intestinal absorption of amino acids are coloured, whereas those expressed in the plasma membrane of epithelial cells 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 et al, 2008.*

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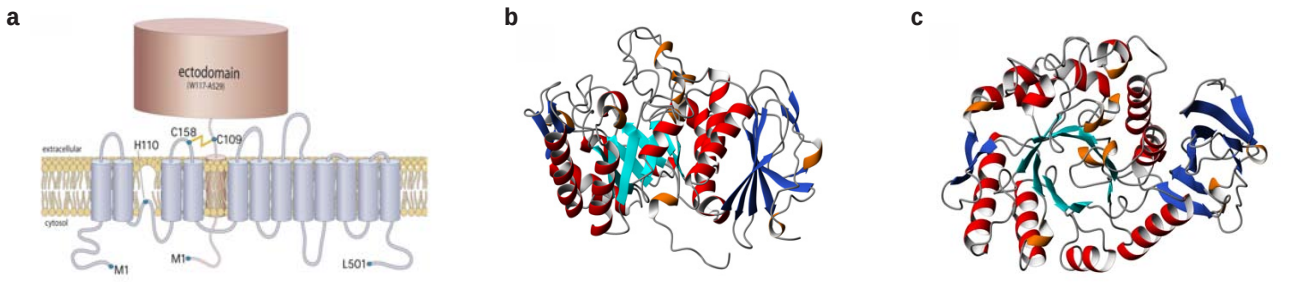

Figure 2. *Structure of 4F2hc-ED. A, HAT schematic representation. 4F2hc (pink) with a bulky N-glycosylated ectodomain (4F2hc-ED, covering residues Trp117 to the C-terminal Ala529) is linked by a conserved disulfide bridge (Cys109 in human 4F2hc) with a light subunit (blue), a 12 trans-membrane-spanning non-glycosylated protein. Lateral (B) and upper (C) views of 4F2hc-ED structure. The N-terminal position corresponds to Cys109. The structure is similar to that of* α*-glycosidases, including two domains: a TIM-barrel (/)8 and a C-terminal domain with eight antiparallel* β*-sheets. Adapted from Fort* et al, *J Biol Chem,* ***282****, 31444-52 (2007).*

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 the following: (i) the identification of molecular mechanisms to explain the distinct phenotypes in cystinuria, using animal and cell models; (ii) the identification of modulator genes of lithiasis in cystinuria, using animal models; (iii) the search for new drugs to treat lithiasis in cystinuria, using our murine cystinuria model *Stones*; and (iv) the identification of the mechanisms that lead to immunological disorders associated with LPI, using a newly generated floxed y^+LAT1 mouse line.

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 oligomeric state of HATs, the atomic structure of the ectodomain of 4F2hc (CD98hc; in collaboration with IRB Barcelona researcher Ignasi Fita; Figure 2), the light subunit as the catalytic component, the membrane topology of the light subunits, and the 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 at a subnanometer scale (6.5Å). This map revealed striking similarities with unrelated transporters with the so-called 'double inverted repeat' fold (Figure 3). At present, we are conducting 3D crystallisation screenings of several transporter homologues of the light subunits of HATs (APC superfamily) within the European Union project EDICT (European Drug Initiative on Channels and Transporters). Functional studies in parallel seek to identify key residues for amino acid transport function within HATs.

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 has allowed us to study the role of the extracellular domain of 4F2hc in its multiple functions, including interaction with β1 integrins. Moreover, the recombinant extracellular domain of 4F2hc is a powerful tool for the identification of potential ligands of 4F2hc.

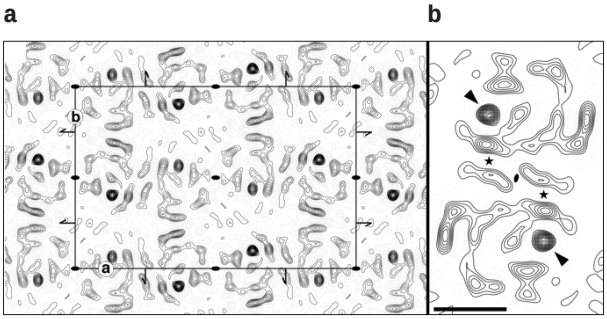

Figure 3. *Projection structure of AdiC-W293L. A, p22121-symmetrised projection map of AdiC-W293L at 6.5Å resolution calculated from five electron micrographs. The black rectangle marks the unit cell (lattice dimensions: a=184Å, b=119Å, γ=90°), which contains four AdiC-W293L dimers (two up- and two down-oriented dimers). B, improved projection map of AdiC-W293L after symmetrisation of one of the four identical dimers in the unit cell exploiting the internal, non-crystallographic 2-fold symmetry axis of the dimer. The only strong density peak in the projection structure of the AdiC-W293L monomers is marked by arrowheads. This projection map shows similarities to the amino acid transporter LeuT (Yamashita et al, Nature, 437, 215-23, 2005), one of the structural paradigms for transporters with the "double inverted repeat" fold. The putative intradimeric contact sites are indicated by stars. The 2-fold axes perpendicular to the membrane plane and the screw axes parallel to the membrane plane are indicated. Solid lines indicate density above the mean, whereas negative contours are shown as light grey lines. The scale bar represents 25Å. Figure adapted from Casagrande et al, 2008.*

SCIENTIFIC OUTPUT

Publications

Bartoccioni P, Rius M, Zorzano A, Palacín M and Chillarón J. Distinct classes of trafficking rBAT mutants cause the type I cystinuria phenotype. *Hum Mol Genet*, **17**(12), 1845-54 (2008)

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Research networks and grants

CIBER de enfermedades raras (CIBERER)
Instituto de Salud Carlos III (2007-2010)
Principal investigator Unit 731: Manuel Palacín

European Drug Initiative on Channels and Transporters (EDICT)
European Commission, 7th Framework Programme, 201924 (2008-2012)
Principal investigator: Manuel Palacín

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

Role of 4F2hc in tumorogenesis
'La MTV3' Foundation (2006-2009)
Principal investigator: Manuel Palacín

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

Other funding sources

Support to incentive research activity in the University of Barcelona (2007-2010)

Collaborations

Physiopathology of inherited aminoacidurias cystinuria and lysinuric protein intolerance (LPI)
Virginia Nunes, Bellvitge Institute for Biomedical Research and University of Barcelona (Barcelona, Spain), Gianfranco Sebastio, Federico II University (Naples, Italy) and Josep Chillarón, University of Barcelona (Barcelona, Spain)

Study of the multiple functions of heavy chains of HATs
María Antonia Lizarbe, Universidad Complutense de Madrid (Madrid, Spain), Joaquín Abian, CSIC-Autonomous University of Barcelona (Barcelona, Spain) and Mark H Ginsberg, University of California San Diego (La Jolla, USA)

Structure-function relationship in heteromeric amino acid transporters (HATs)
Dimitrios Fotiadis, University of Bern (Bern, Switzerland), Ignasi Fita, IRB Barcelona (Barcelona, Spain), Modesto Orozco, IRB Barcelona (Barcelona, Spain), Steve Baldwin, Astbury Centre for Structural Molecular Biology, Institute of Membrane and Systems Biology, University of Leeds (Leeds, UK) and Matthias Quick, Weill Medical College, Cornell University (New York, USA)

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