CRF grant ¨Molecular anatomy and physiology of human cystinosin¨

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Topological study of human cystinosin

We have now tested 100 single-cysteine mutants to probe the membrane topology of cystinosin using scanning cysteine accessibility mutagenesis (SCAM). We included in this scan residues covering the lysosomal and cytosolic regions of transmembrane domains to determine their borders. In addition, we examined polar side chains embedded in the membrane as they might reveal the existence of an internal aqueous cavity hosting the cystine binding site.

Our results confirmed the seven-helix topology and they revealed the existence of clusters of water-accessible positions in transmembrane domains, especially the fifth and sixth ones. Interestingly, one cluster includes an essential aspartate that represents a proton-binding site responsible for the coupling between cystine binding and proton binding (see below). A manuscript is under preparation.

Molecular physiology of cystinosin

In a previous unpublished study (R. Ruivo's PhD thesis), we used voltage-clamp analysis of a human cystinosin construct expressed in *Xenopus* oocytes to analyse the mechanism of cystinosin transport. We had shown that cystine is translocated across the membrane with one proton, thus explaining how the lysosomal H^+-ATPase actively expels cystine out of the lysosome. We also showed that the voltage dependence of cystine transport suggests that cystine binding and proton binding are coupled and that the proton binding site is buried in the membrane. During the course of the CRF project, we pursued this study and identified a strong candidate for this proton binding site using a biophysical technique termed charge movement analysis. It consists in recording cystinosin-associated currents under voltage clamp and observing the transient currents elicited by sudden changes in membrane voltage ('voltage jumps'). We showed that: (i) cystine binding induces transient currents as expected if the proton bound to the cystine/cystinosin complex is expelled from, or attracted to, its buried binding site by the voltage jumps; (ii) that these transient currents absolutely require an aspartate located in the sixth transmembrane domain.

As charge movements are generally difficult to interpret, we recently went a step further and showed that they are attributable to proton movements, and not electrogenic conformational changes, since: (i) their voltage dependence is predictably shifted by pH changes in the bulk aqueous environment and (ii) that the transient currents are ~2-fold slower when water (H_2O) is replaced by heavy water (D_2O). A manuscript has been submitted for publication and we are now addressing the reviewers' concerns.

The fact that the proton-binding aspartate is buried in the electric field in our voltage-jump experiments, yet accessible to intracellular water-soluble reagents in our topology experiments suggests that the environment of this residue undergoes major conformational changes throughout the cystine transport cycle and that the identified aspartate is a relay site for translocation rather than, for instance, a regulatory site. Our topology map and functional study of human cystinosin should help understanding the mechanism of cystine transport, but also cast some light on the molecular effects of missense pathogenic mutations.