Heptahelical protein PQLC2 is a lysosomal cationic amino acid exporter underlying the action of cysteamine in cystinosis therapy

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AUTHOR SUMMARY

Transport of solute across membranes is crucial to eukaryotic cell physiology, as illustrated by diverse diseases associated with defective transport and the presence of ~400 solute transporter genes in humans. However, the function of many putative transporters remains unknown, such as the proteins responsible for lysosomal export of metabolites. Cystinosin, the lysosomal cystine exporter defective in cystinosis (1), is characterized by a duplicated motif termed the PQ loop. PQ-loop proteins are more frequent in eukaryotes than in prokaryotes, and, except for cystinosin, their molecular function remains unknown. The substrate-coupled proton-binding site is nested in the second PQ loop, suggesting that these motifs have functional significance (2). Here, we showed that another PQ-loop protein, PQLC2, is a lysosomal amino acid transporter that is relevant for the treatment of cystinosis.

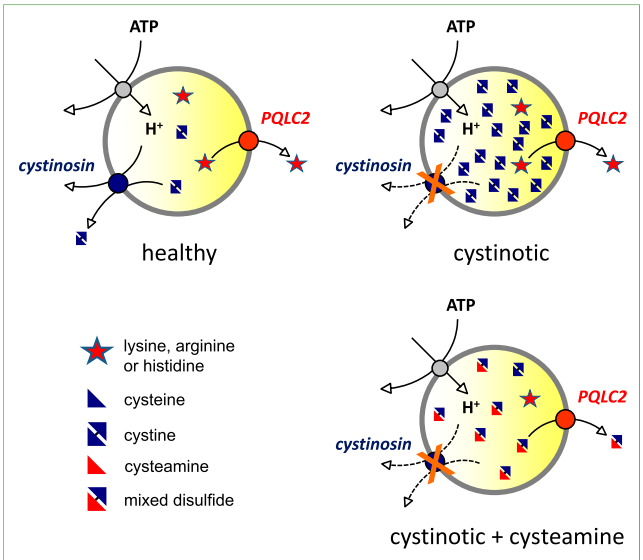

Fig. P1. PQLC2 is a lysosomal cationic amino acid exporter that plays a key role in cysteamine therapy of cystinosin. (*Upper Left*) In healthy subjects, cystinosin and PQLC2 export cystine (the oxidized form of cysteine) and cationic amino acids, respectively, from the lysosomal lumen. Their activity is stimulated by the acidity of the lumen. (*Upper Right*) In patients with cystinosis, lysosomes accumulate cystine because cystinosin is impaired. (*Lower Right*) The drug cysteamine can reverse this accumulation by entering lysosomes and reacting with cystine to form a cysteamine-cysteine mixed disulfide, which resembles lysine. The mixed disulfide is then exported by PQLC2, thus depleting cystine from lysosomes and alleviating symptoms.

We first showed that three yeast PQ-loop proteins of unknown function, Ypq1, Ypq2, and Ypq3, localize to the vacuolar membrane and are involved in homeostasis of cationic amino acids. Genetic inactivation of Ypq1 and Ypq2 decreases the sensitivity of yeast cells to canavanine, a natural toxic analog of arginine. This resistance phenotype requires prior accumulation of cationic amino acids in the vacuole. Moreover, transcription of the *YPQ3* gene is activated by lysine starvation. We thus hypothesized that Ypq1–3 proteins export cationic amino acids from the yeast vacuole.

We next identified PQLC2, a mammalian PQ-loop protein closely related to the yeast Ypq proteins, in purified lysosomal membranes. Because of the strong homology between PQLC2 and Ypq1–3, we reasoned that cationic amino acids are likely substrates. Indeed, frog oocytes expressing PQLC2 at their plasma membrane displayed robust transport activity that was strongly activated in acidic extracellular medium (mimicking the lysosomal lumen) and exhibited narrow selectivity for cationic amino acids, including arginine, histidine, and lysine. Moreover, heterologous expression of PQLC2 at the vacuole of the yeast *ypq2* mutant restored canavanine sensitivity, and PQLC2 efficiently transported canavanine, suggesting that the increased canavanine sensitivity provided by PQLC2 results from increased vacuolar export. We concluded that PQLC2 and Ypq1–3 are evolutionarily conserved lysosomal/vacuolar exporters of cationic amino acids.

We next showed that PQLC2 exports from lysosomes a key chemical intermediate (cysteamine-cysteine mixed disulfide) underlying the current drug therapy of cystinosis, a rare inherited disease caused by mutations in the cystinosin gene. In this condition, large amounts of cystine accumulate in the patient's lysosomes (Fig. P1) and progressively impair the function of multiple organs, including the kidney, endocrine glands, muscles, and CNS (1). The drug cysteamine (Cystagon) depletes cystine from cystinotic lysosomes and, with lifelong treatment, alleviates symptoms.

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According to an early biochemical model (1), cysteamine reacts with lysosomal cystine and forms a lysine-like mixed disulfide that exits lysosomes through an unknown lysosomal transporter of cationic amino acids (Fig. P1). The elucidation of PQLC2 function prompted us to examine whether it corresponded to this mixed disulfide transporter. Using our frog oocyte assay, we found that PQLC2 efficiently transports the mixed disulfide. Moreover, silencing of the *PQLC2* human gene in cultured cells of patients trapped this intermediate when cells were exposed to cysteamine. We concluded that PQLC2 plays a key role in the therapeutic action of cysteamine.

Except for cystinosin, the molecular activity of other PQ-loop proteins remains unknown. The elucidation of PQLC2 function suggests that small-molecule transport is a conserved feature of the PQ-loop protein family, in agreement with the recent identification of SWEET sugar transporters (3) and of the mitochondrial pyruvate carrier (4, 5) in related protein families. The characterization of PQLC2 also has clinical implications. Its role in cysteamine therapy of cystinosis should form the basis of rationales to improve this treatment and alleviate its constraints and side effects. For instance, allosteric or transcriptional activators of PQLC2 might potentiate cysteamine and help reduce the doses. The study of PQLC2 may also help clarify the origin of cationic amino acid abnormalities in Batten disease, another lysosomal disease characterized by early-onset neurodegeneration and the accumulation of "aging pigment" (lipofuscin) in lysosomes.

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