Unusual properties of the lysosomal amino acid transporter PQLC2

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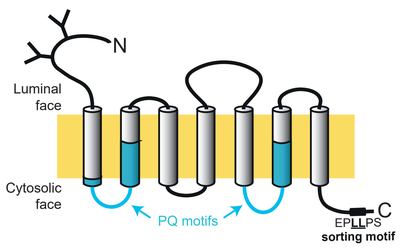
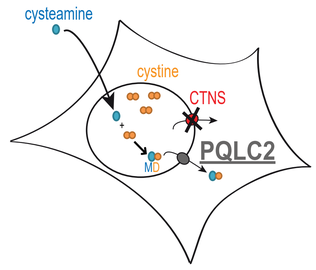


PQLC2

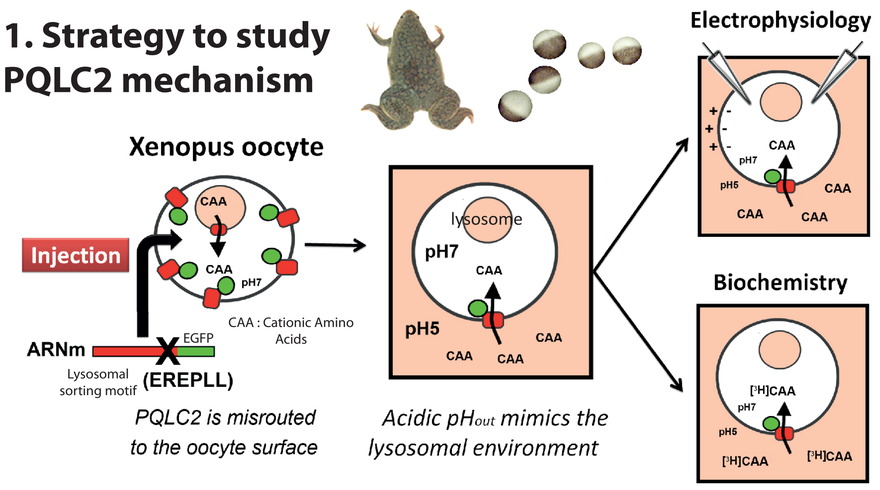
The PQ-Loop Containing type-2 (PQLC2) protein is a lysosomal cationic amino acid transporter required for cysteamine therapy of cystinosis. Indeed, cysteamine induces formation of a mixed cysteine/cysteamine disulfide (MD) resembling lysine, which is exported from cystinotic lysosomes by PQLC2 (Jézégou et al., PNAS 2012; Liu et al., Science 2012).

We previously showed that PQLC2 mediated efflux of MD is a rate-limiting step in lysosomal cystine clearance in human cystinotic fibroblasts treated with cysteamine (unpublished data, see point 2.). Here, we studied the functional properties of PQLC2 using two-electrode voltage clamp of Xenopus oocytes expressing PQLC2 at their surface.

Taken together, our results sugest that both modulation of the electrical potential of the lysosomal membrane and increasing cytosolic Arg levels might accelerate efflux of the mixed disulfide from cystinotic lysosomes, thus improving cysteamine therapy.

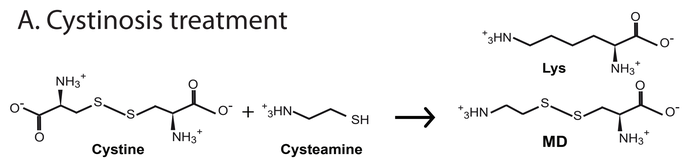


1. Strategy to study PQLC2 mechanism

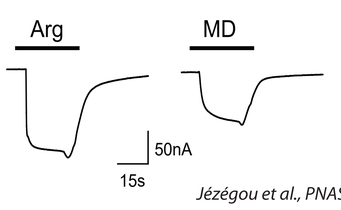


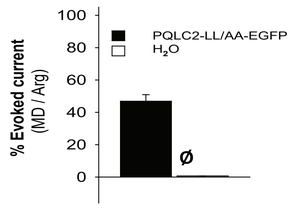
2. PQLC2 mediates the rate-limiting step in cysteamine therapy

A. Cystinosis treatment



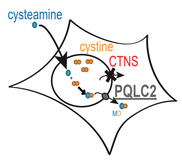
B. MD transport current carried by PQLC2





Jézégou et al., PNAS 2012

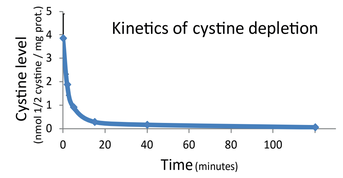
B. MD clearance is substantially slower than its synthesis

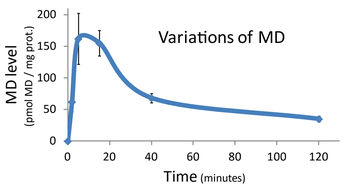


Human cystinotic fibroblasts

± cysteamine
100µM, 37°C
variable time

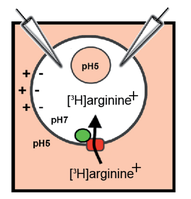
LC-MS/MS

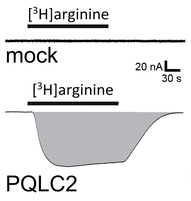


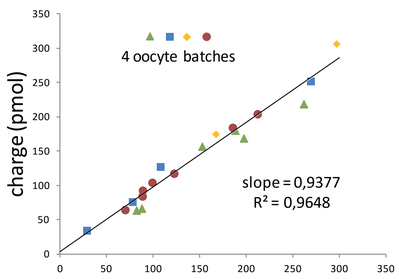


3. PQLC2 is a uniporter strongly modulated by membrane voltage

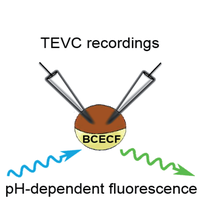
A. PQLC2 transports one charge per arginine

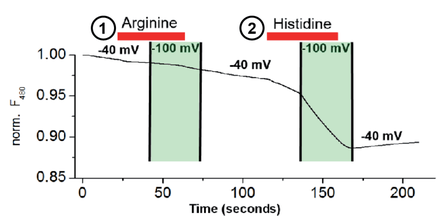




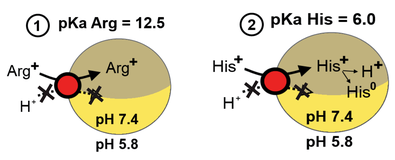


B. PQLC2 does not cotransport protons

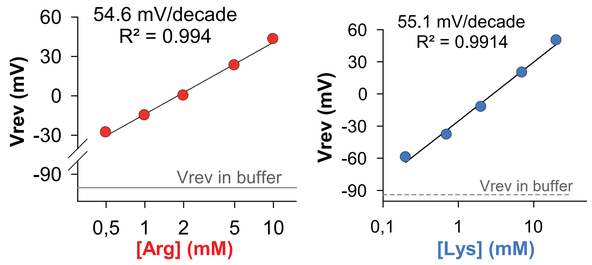




Interpretation :

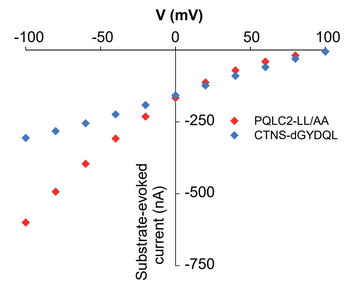


C. Measure of stochiometry confirms the uniport mecanism



Slope values for Arg (54.6) and Lys (55.1) stochiometry curves are consistant with the expected value for a uniporter mode (58.9) according to Nernst's equation.

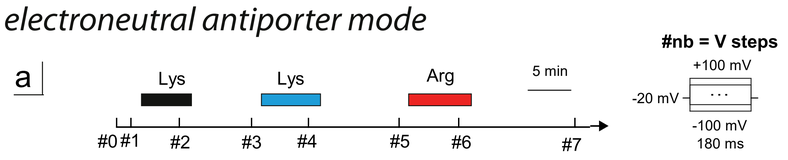
D. Current-voltage curves

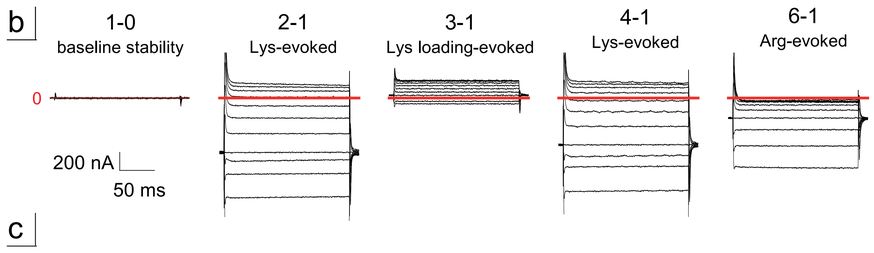


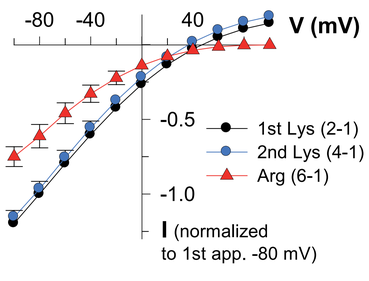
In agreement with the uniport mechanism, PQLC2 activity showed strong voltage dependence.

4. Arg (but not Lys) shifts PQLC2 from uniport mode to electroneutral antiport mode that may accelerate PQLC2 lysosomal substrate efflux

A. External Arg, but not Lys, shifts PQLC2 from uniporter mode to electroneutral antiporter mode





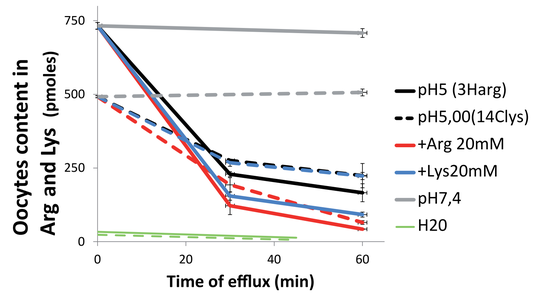


First perfusion of Lys (a. black) loads the oocyte with Lys, generating an efflux when returned to the buffer (3-1). Second application of Lys (a. blue) didn't silence the efflux (see b. positive current over «0 current» on trace 4-1, and c. blue trace).
Interestingly, Arg (third application, a. red) silenced the efflux observed at positive potential (see b. 6-1 and c. red trace). In this configuration, PQLC2 realizes an electroneutral echange of one effluxed molecule with one extracellular molecule of arginine.

B. External Arg accelerates Arg and Lys efflux (preliminary data)

Oocytes are first loaded with 3H Arg and 14C Lys. Efflux is started by incubating oocytes into pH 5.0 buffer containing no CAA (control condition, black traces), 20mM Arg (red traces) or 20mM Lys (blue traces).

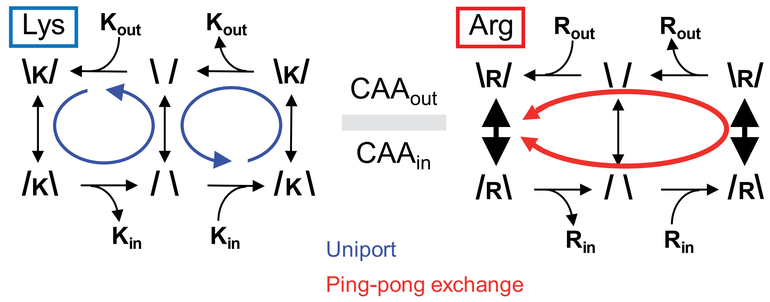
After 0 (control), 30 or 60min of incubation, oocytes are lysed and their contents in radioactivity are counted (dotted lines for 14C Lys and solid lines for 3H Arg).



Efflux is only mediated by PQLC2 (no efflux at pH7.4). Extracellular Arg, but not Lys, accelerates both Arg and Lys transport by PQLC2.

5. Conclusion and perspectives

Proposed model for transport mode



Arg would bypass reorientation of empty transporter, thus accelerating the overall transport cycle.

Perspectives

- Test whether modulation of Arg concentration accelerate cystine efflux in human cystinotic fibroblasts treated with cysteamine.

- Test whether Arg supplementation of CTNS knock out mice treated with cysteamine lowers cystine accumulation level in tissues.

- Test whether modulation of lysosomal membrane voltage improves cysteamine therapy.

- Try to find key residues in PQLC2 sequence that may contribute to these properties.

Xavier Leray has a PhD fellowship from the Cystinosis Research Foundation.