Neurophotonics Laboratory

2017 ESGLD Graduate Course

Lysosomal channels and transporters

Bruno Gasnier, PhD

Paris Descartes University & CNRS

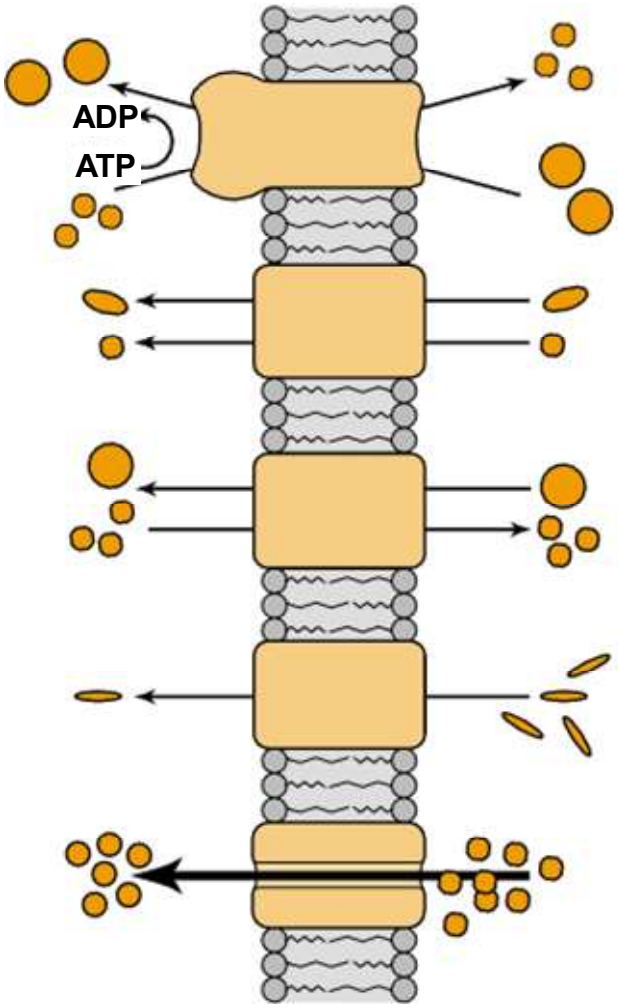
Outline

- The weird world of membrane transport
- How to study lysosomal channels and transporters
- The v-ATPase
- Ion channels and transporters
- Catabolite exporters

The weird world of membrane transport

- The membrane transport ecosystem
- Critical differences between channels and transporters
- Key role of membrane potential

Diversity of membrane transport proteins



Primary transporters
ion pumps,
ABC transporters

build up…

Secondary transporters
uni-,
anti-,
sym-porters

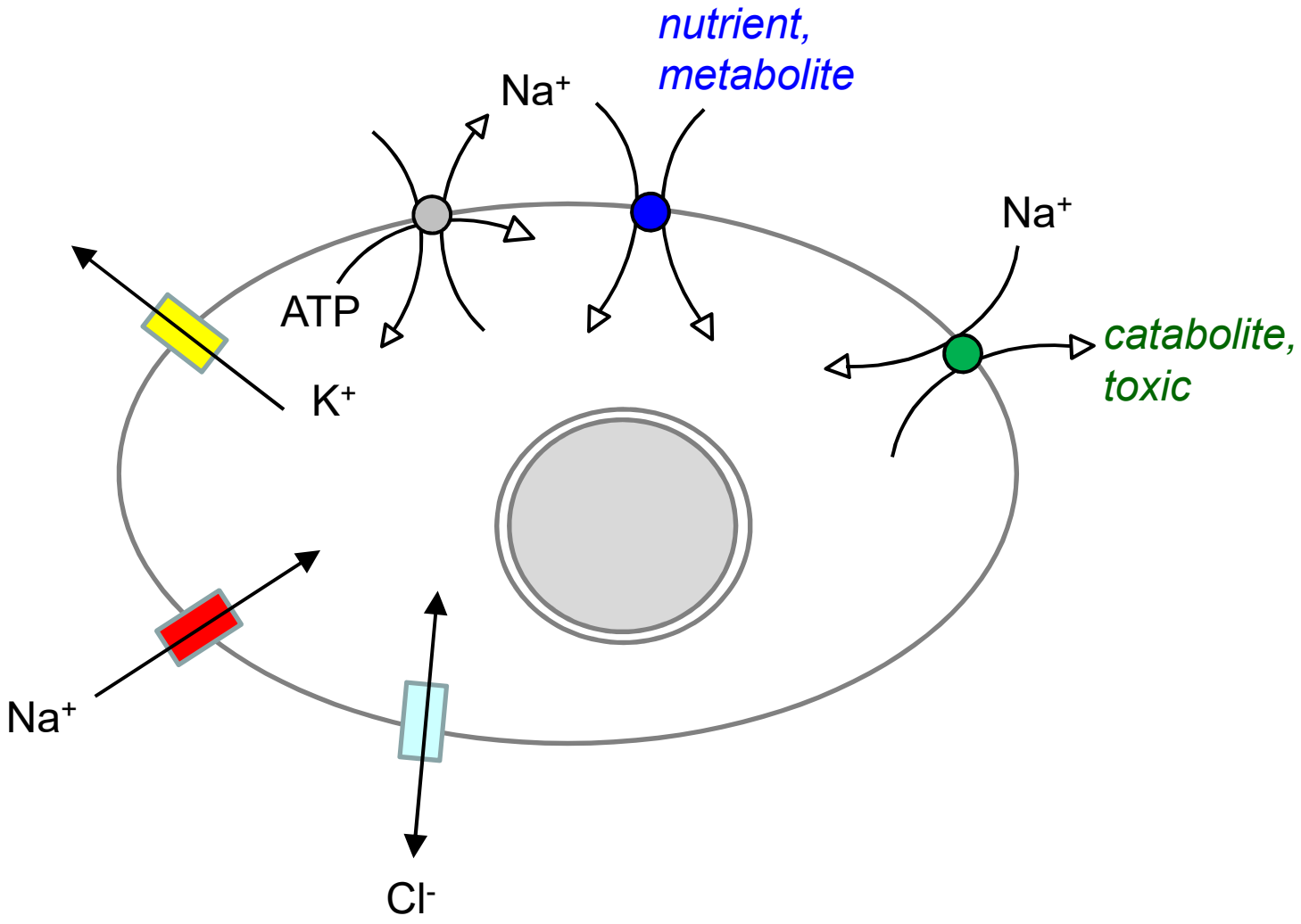
convert…

… concentration gradients

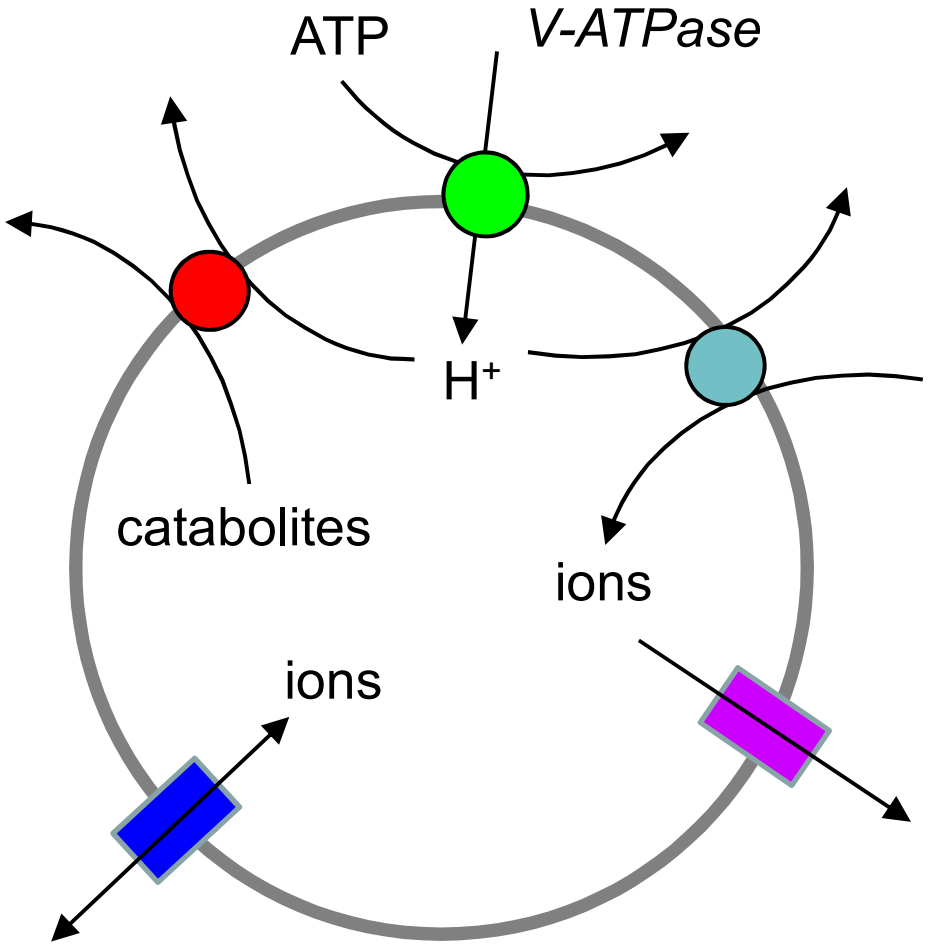
Channels
ion channels,
porins

dissipate…

Interdependence of transport proteins at the plasma membrane

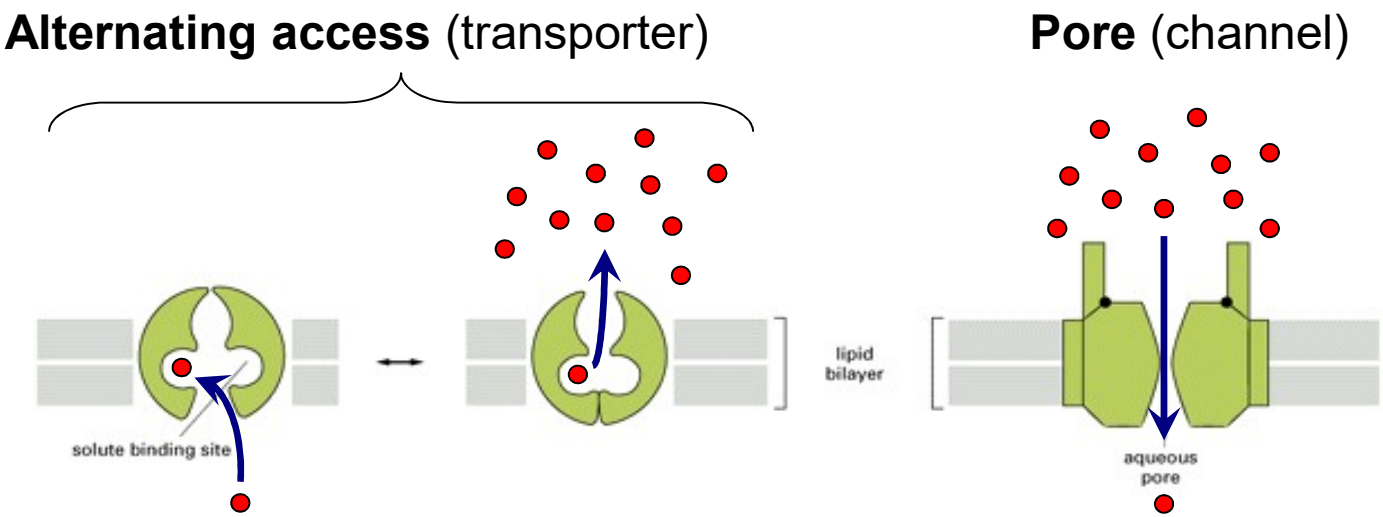


The lysosomal transport ecosystem



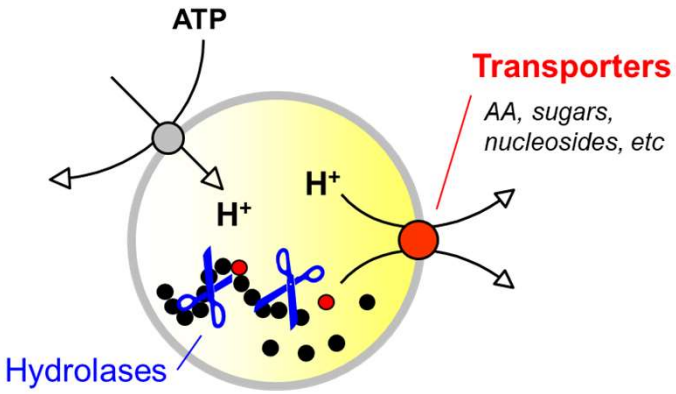
Channels and transporters operate through distinct mechanisms

Channels open a pore through the membrane while transporters shuttle between outward-facing and inward-facing conformations

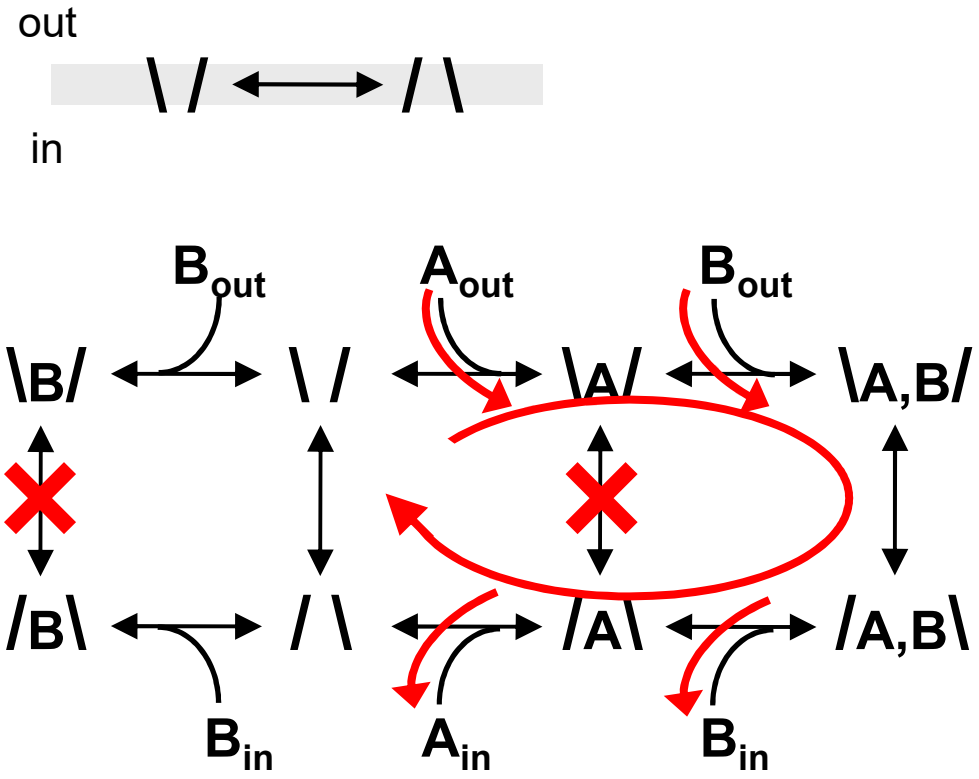


'Uphill' transport requires alternating access and energy

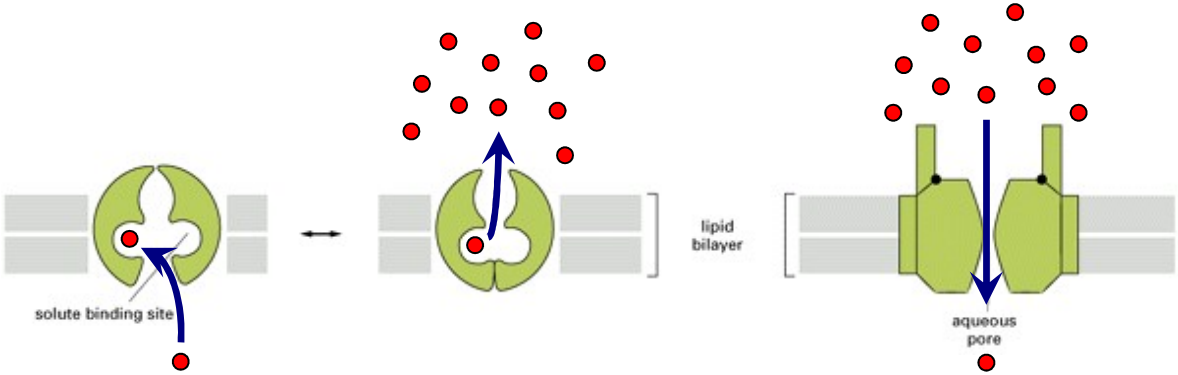
How alternating access harnesses energy for uphill transport



Symport mechanism



Uphill transport has a high kinetic cost



	Transporter	Channel
Turnover:	10 to 10^3 s^{-1}	~10^8 s^{-1}

Transporters are painfully slow!

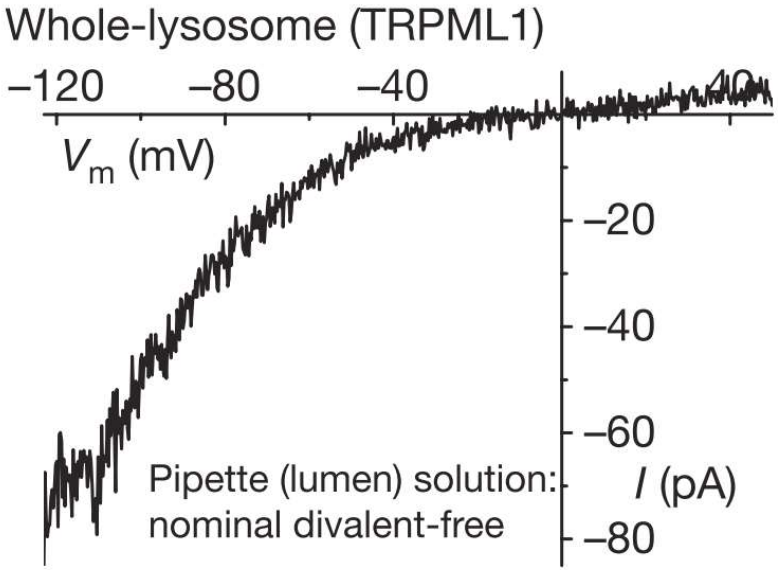
→ Stronger and faster contribution of channels to ion concentrations and membrane potential

The weird world of channels and transporters

- The membrane transport ecosystem
- Critical differences between channels and transporters
- **Key role of membrane potential**

Many channels and transporters are highly sensitive to the membrane electrical potential

Lysosomal cation channel
TRPML1 (= mucolipin 1)



$V_m = V_{cytosol} - V_{lumen}$

XP Dong… H Xu (2008) Nature

Conversely, membrane potential is highly sensitive to ion fluxes

Values for a 700-nm lysosome

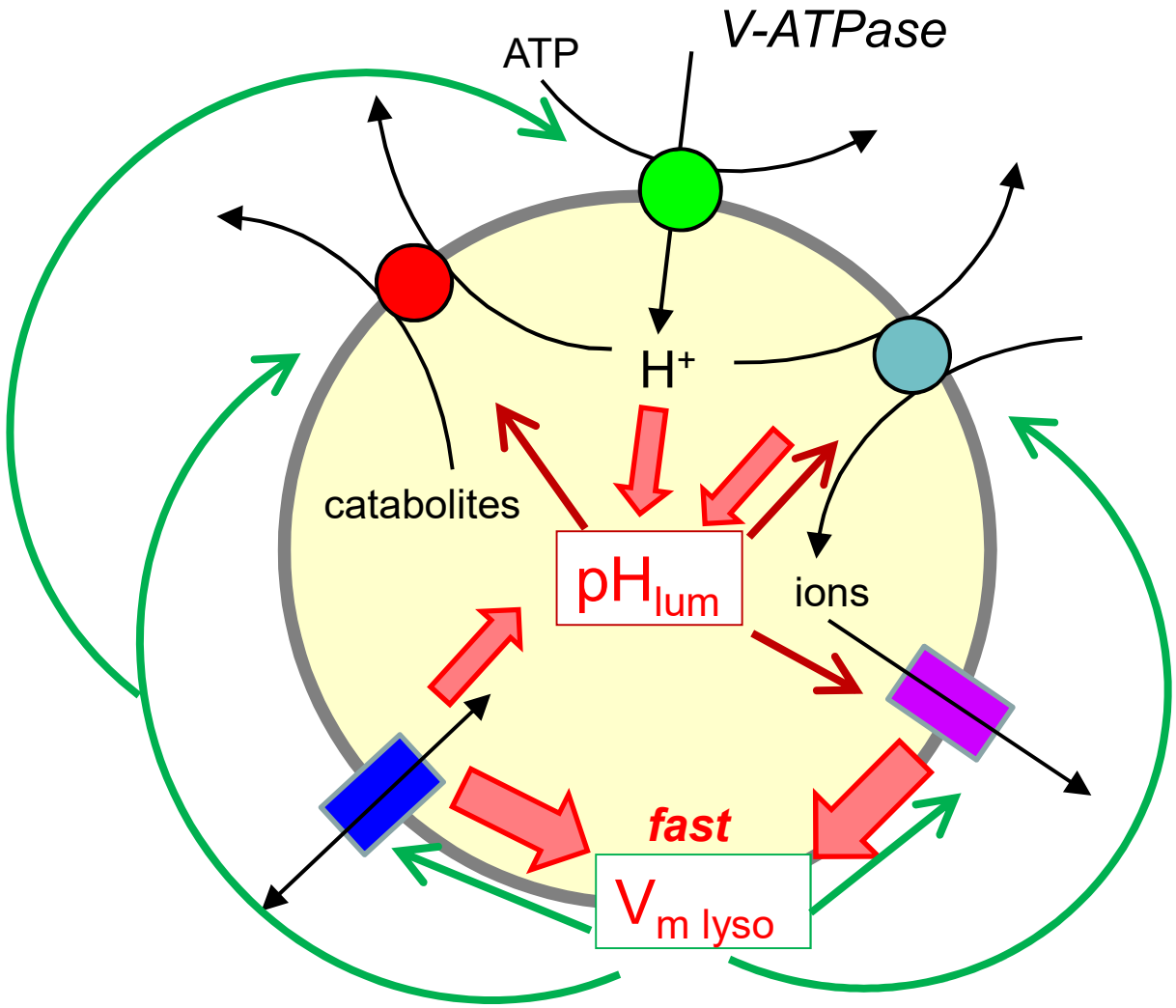
- Volume = 1.7×10^{-16} L
- Membrane area = 1.5×10^{-8} cm^2
- Bilayer capacitance = 1 µF/cm^2
- Buffering capacity* = 60 mM/pH at pH_{lumen} 4.5-5.0

- Number of H^+ (or monovalent ions) needed to shift $\mathbf{V_m}$ by 60 mV **≈ 5 500**

- Number of H^+ to acidify $\mathbf{pH_{lumen}}$ by 1 Unit from 5.5
  - without buffering: 1.7×10^{11}
  - with buffering **≈ 2×10^{38}**

** Value based on BE Steinberg… S Grinstein (2010) J Cell Biol*

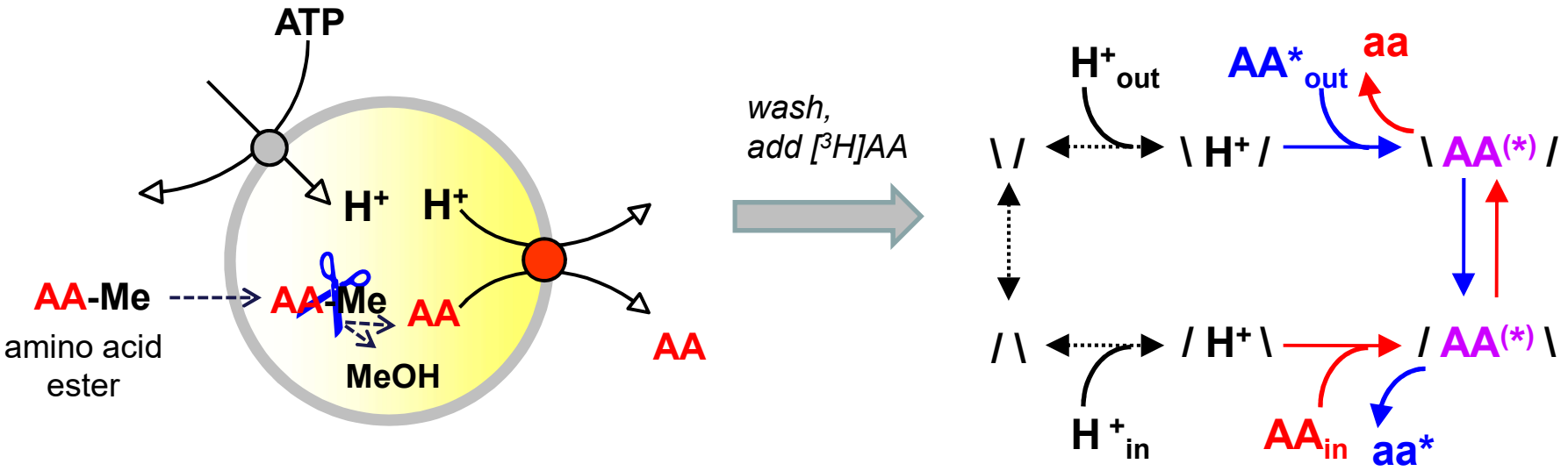
The lysosomal transport ecosystem



- The weird world of membrane transport
- **How to study lysosomal channels and transporters**
- The v-ATPase
- Ion channels or transporters
- Catabolite exporters

1) Good old techniques with lysosome preps

- Usually demanding: high amounts needed; low affinity; purity
- For AA transport: ‘counter-transport’ of artificially loaded lysosomes



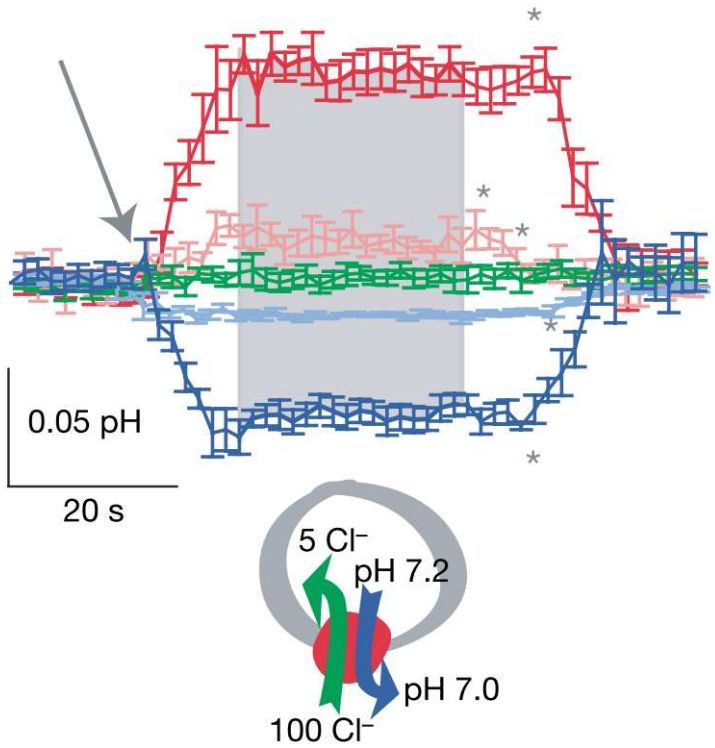
Purity is not an issue with this assay: low ester cleavage in contaminating organelles

1bis) Fluorescent techniques with lysosome preps

- Fluorescent assays can be used for major electrogenic pathways

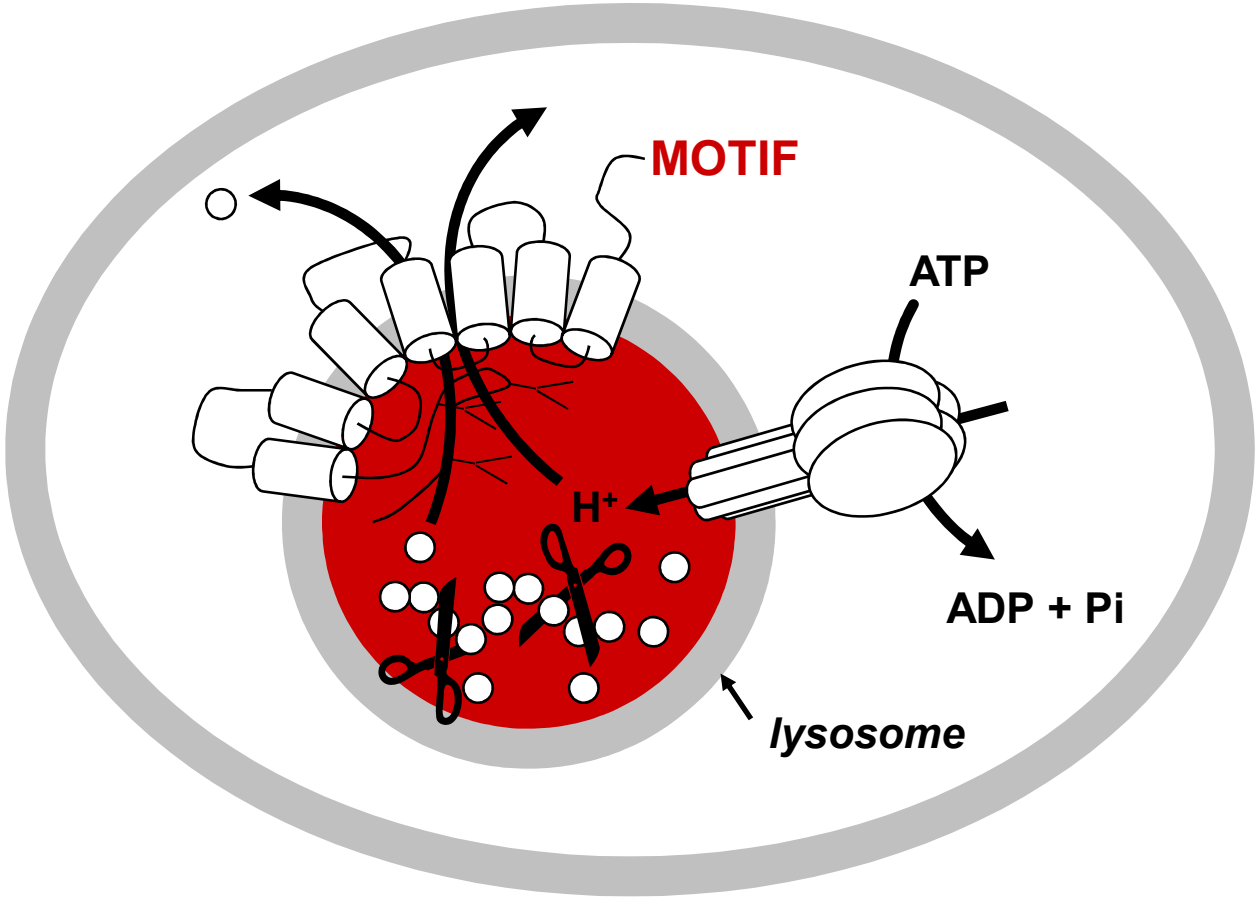
Study of Cl-/H+ exchange by ClC-7:

- *Lysosome membranes resealed on varying media*
- *pH monitored with BCECF*
- *Valinomycin (↓) starts reaction by clamping V to K+ diffusion potential*

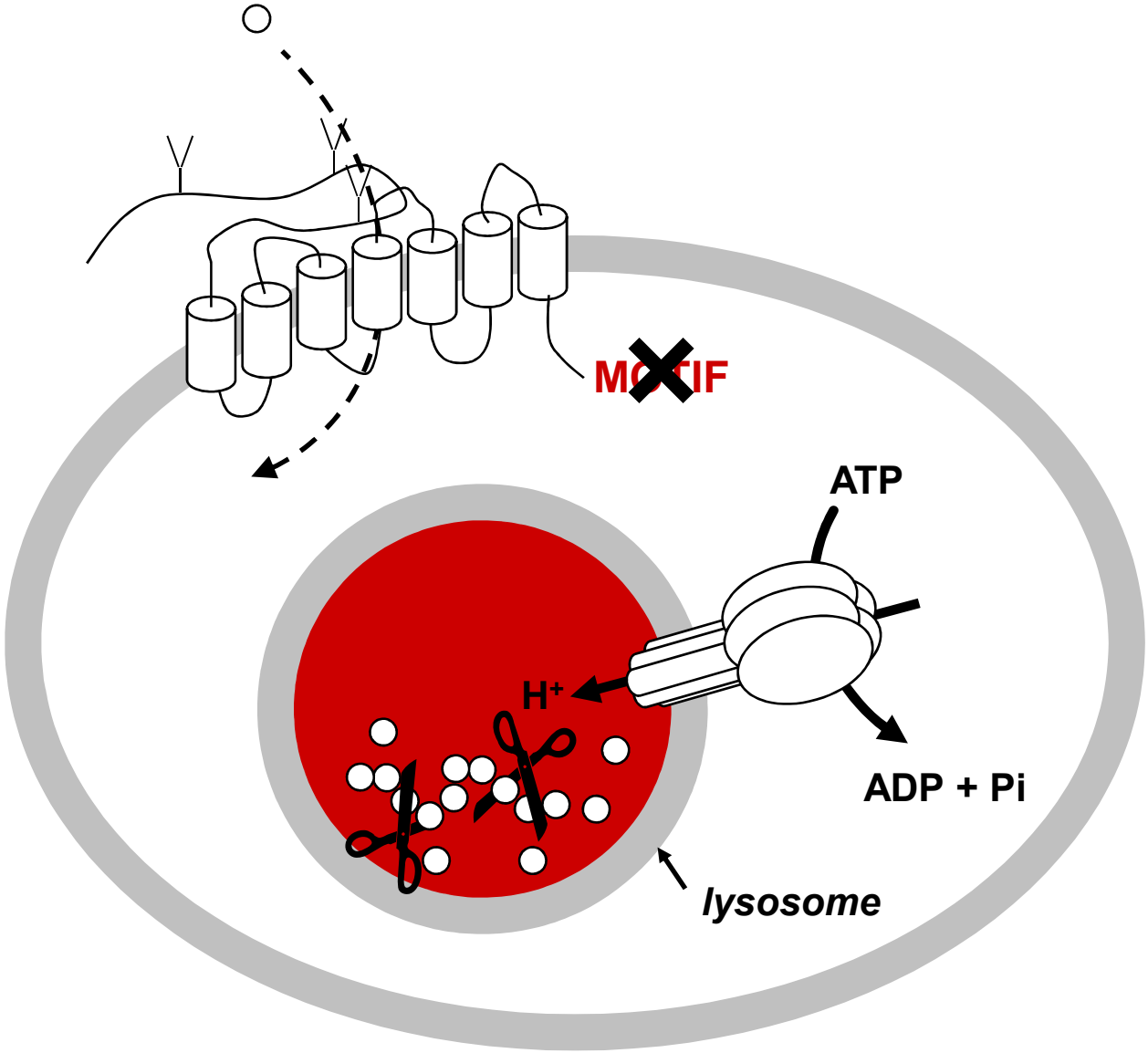


A Graves…. J Mindell (2008) Nature

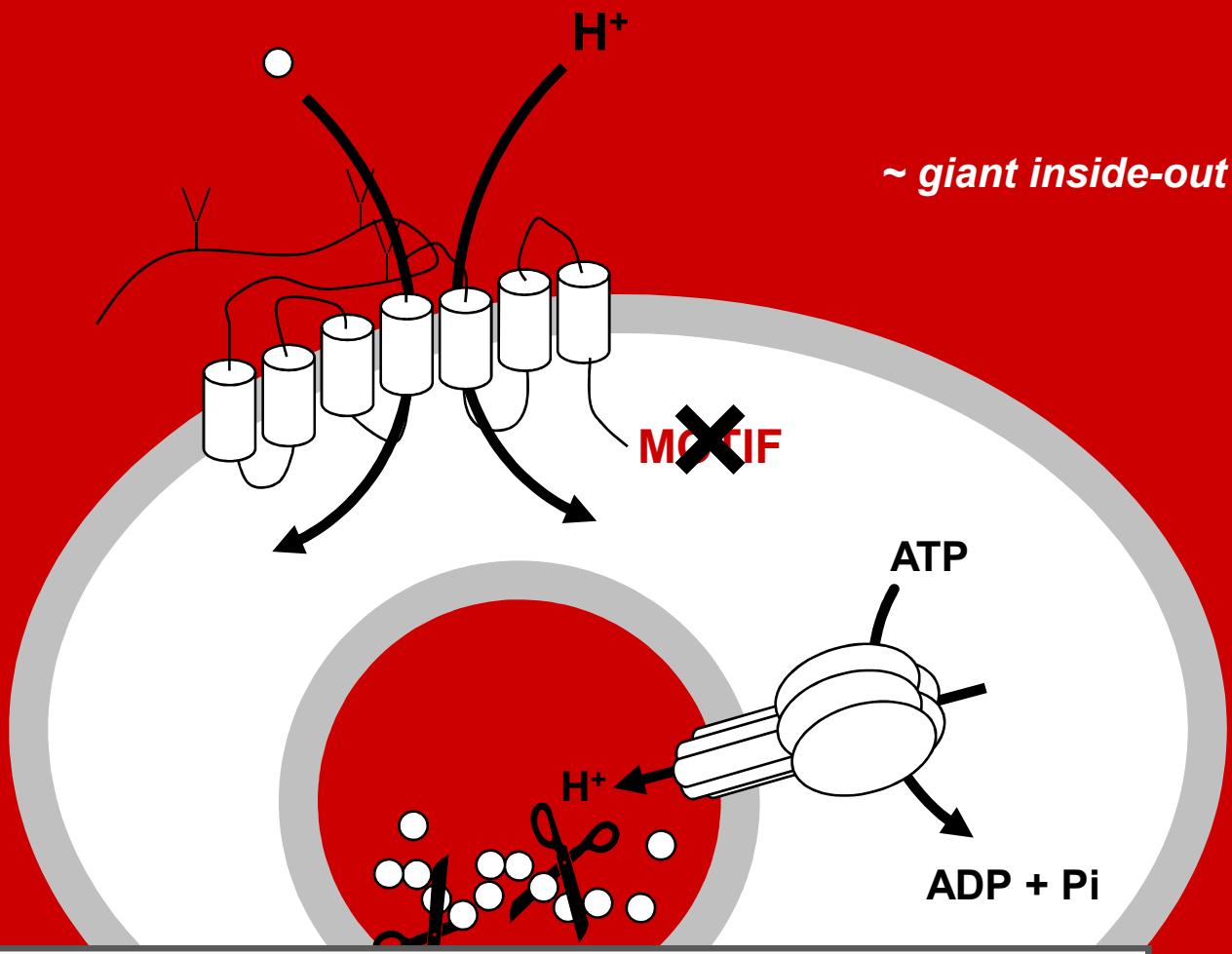
2) Whole-cell approach to study lysosomal transporters



Kalatzis et al (2001) EMBO J



Kalatzis et al (2001) EMBO J

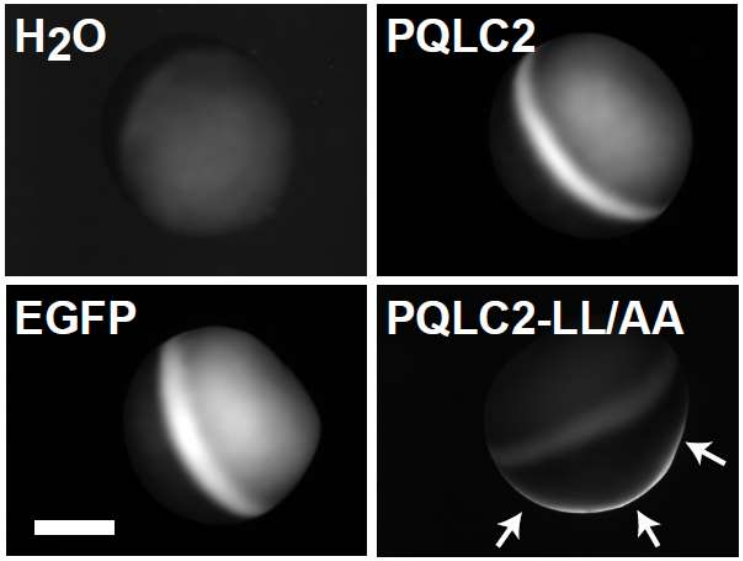


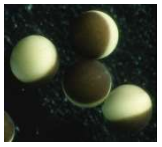
- topologically equivalent to lysosomal efflux
- faster and easier than lysosomes
- amenable to diverse techniques, including voltage clamp

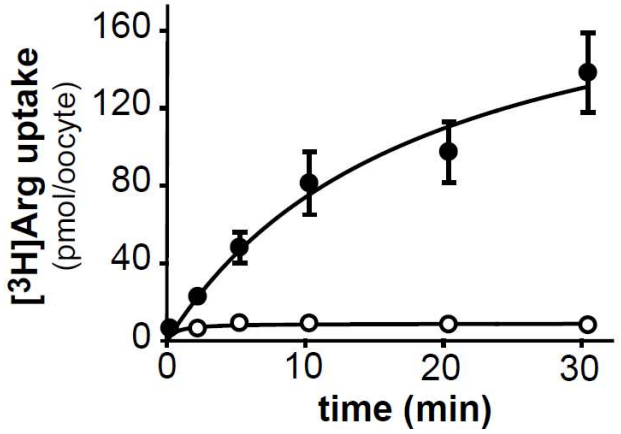
Kalatzis et al (2001) EMBO J

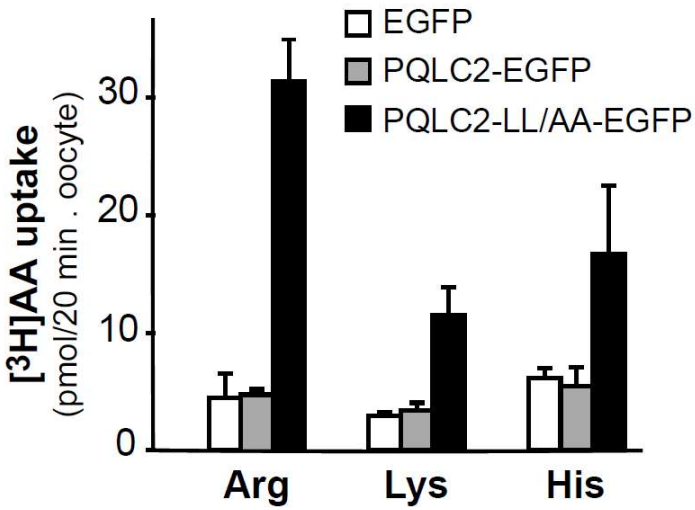
Application to PQLC2: radiotracer flux

PQ Loop repeat Containing protein 2
'Picklock 2'



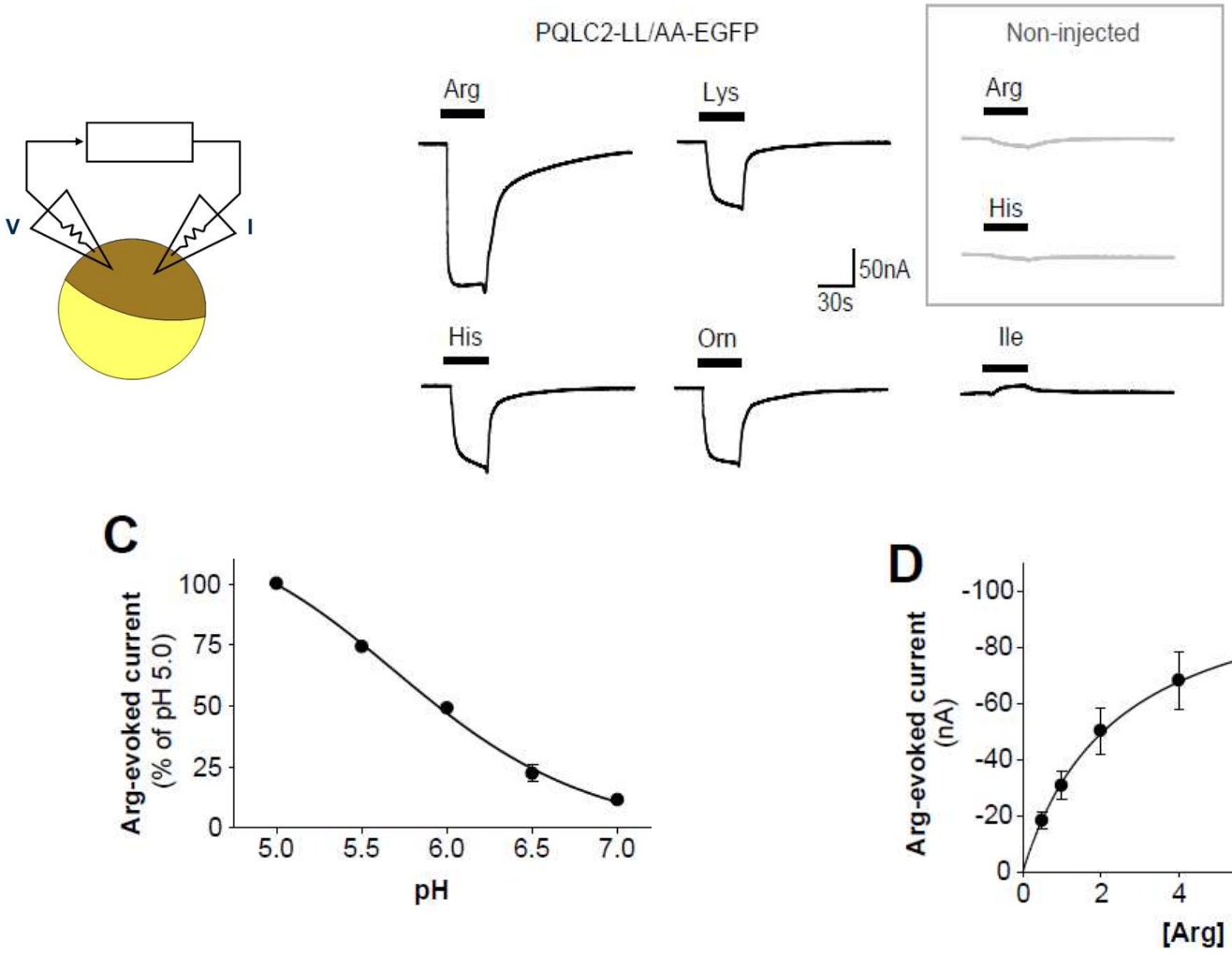






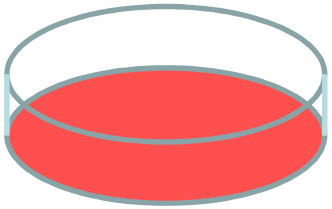
Jézégou et al (2012) PNAS

Application to PQLC2: voltage-clamp measurements



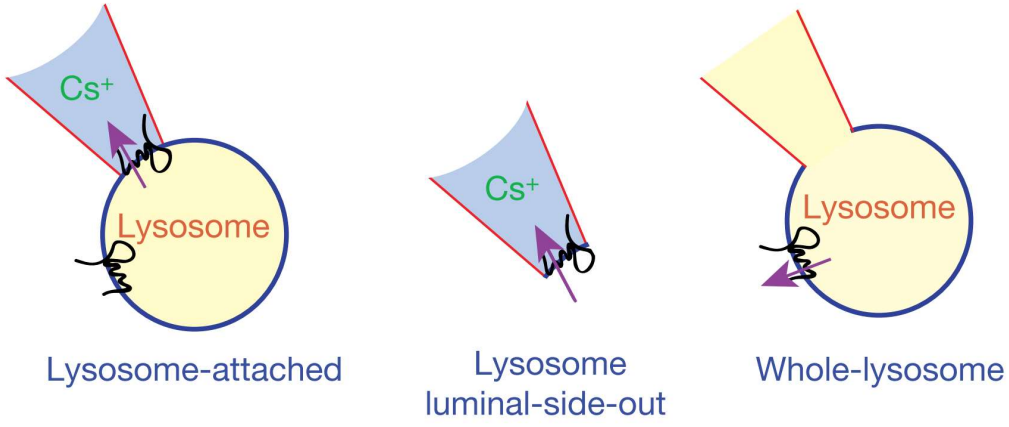
Jézégou et al (2012) PNAS

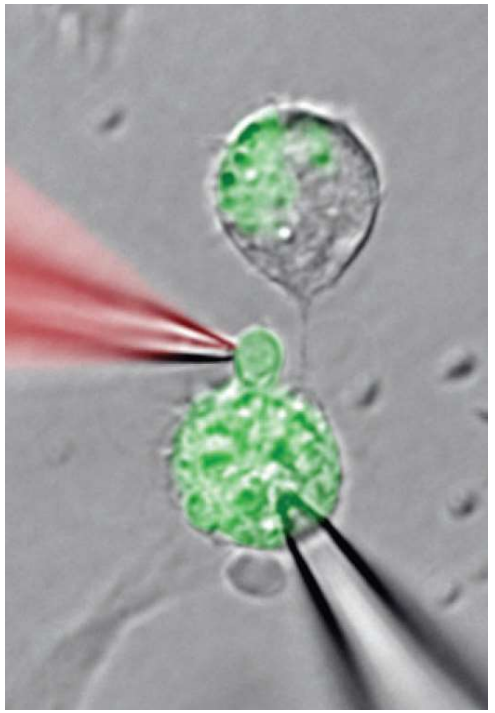
3) Lysosome patch-clamp



Lysosome enlargement

Vacuolin 1
Rab5 mutant
etc





Courtesy of Xianping Dong and Haoxing Xu, University of Michigan.

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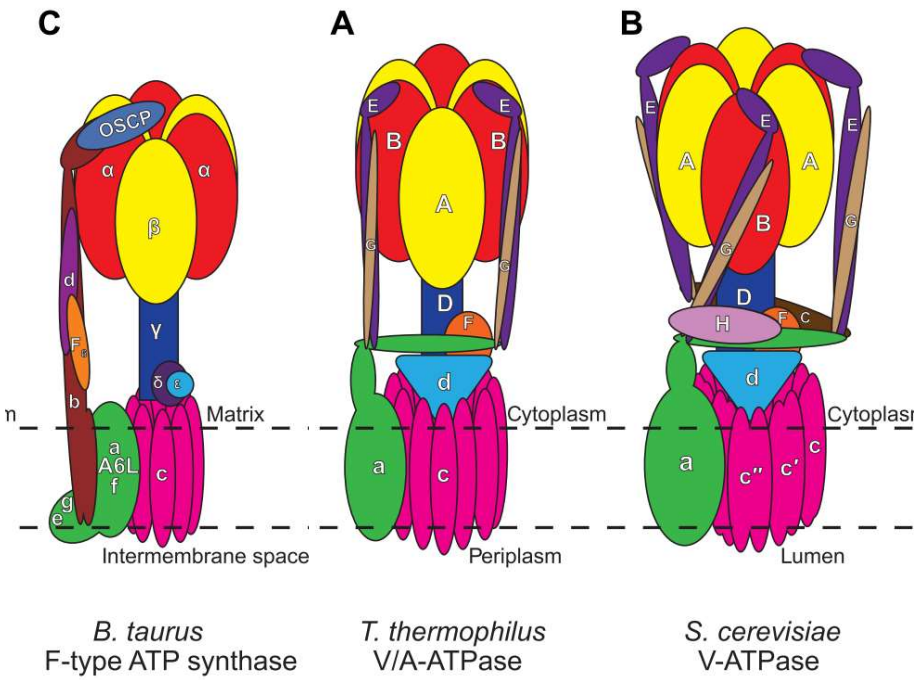
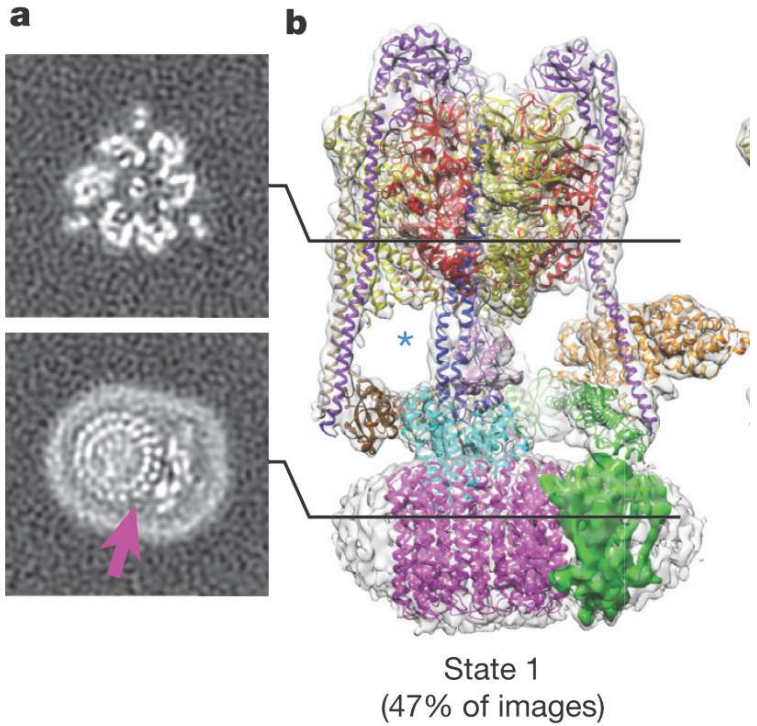
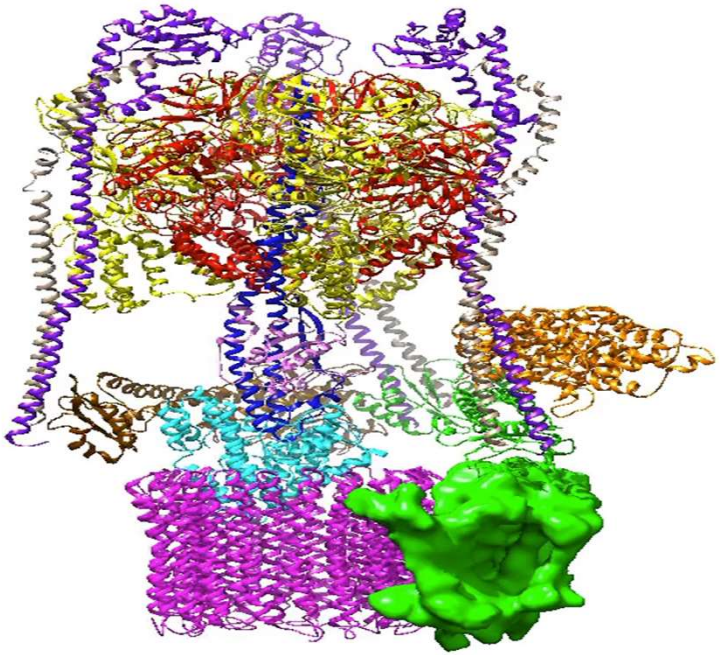
The V-type H+ ATPase

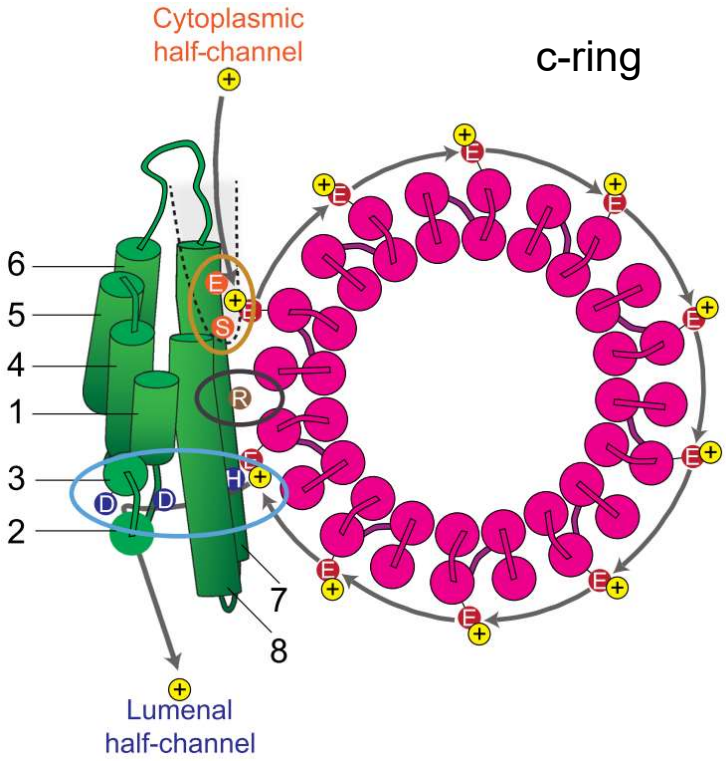




J. Zhao, S. Benlekbir & JL. Rubinstein, Nature 2015

Rotary mechanism of H+ pumping by V-ATPase





→ Sustained H+ pumping for organelle acidification requires an electrical shunt (counter-ions) to prevent build up of opposing membrane potential

J. Zhao, S. Benlekbir & JL. Rubinstein, Nature 2015
MT Mazhab-Jafari…. & JL. Rubinstein, Nature 2016

- The weird world of membrane transport
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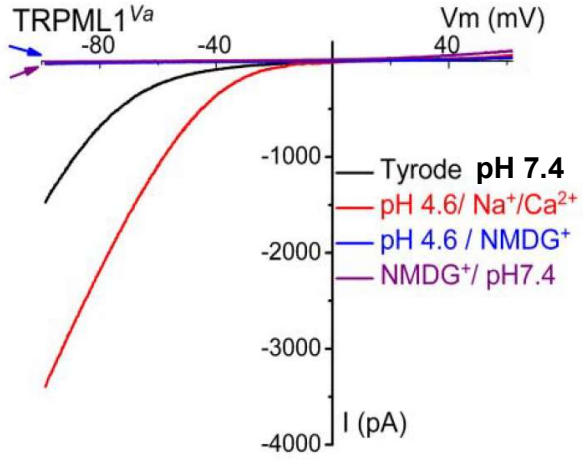
TRPML1 (mucolipin 1): properties

- Defective in Mucolipidosis type IV
- Belongs to the Transient Receptor Potential superfamily
- 6 TM + pore loop
- Lysosomes and late endosomes (TRPML2 and 3 as well)
- Cation selectivity: Ca^{2+}, Fe^{2+}, Zn^{2+} etc; Na^{+}, K^{+}
- Inward rectification (= lysosomal cation export)

- Regulated by:
  - luminal pH
  - $PI3,5P_2$



H Xu… D Clapham (2007) PNAS

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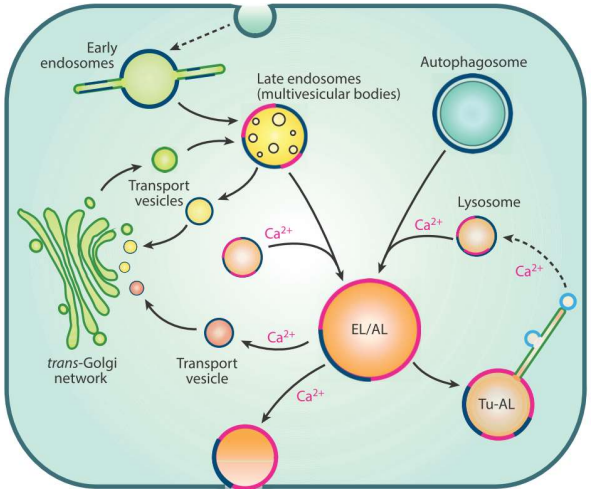
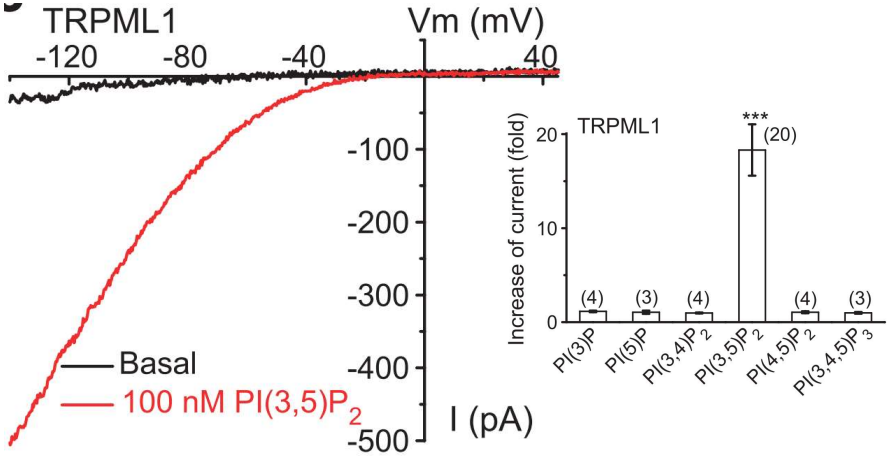
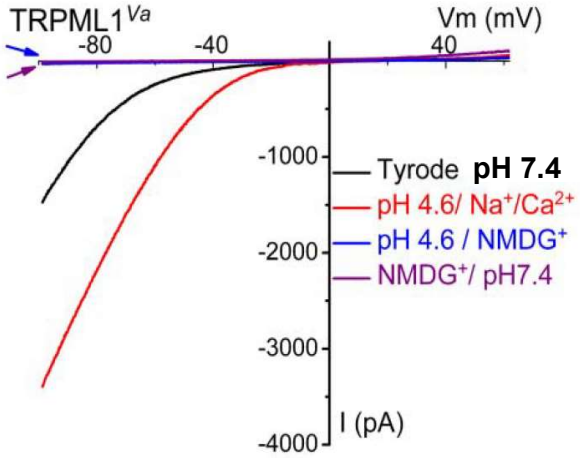




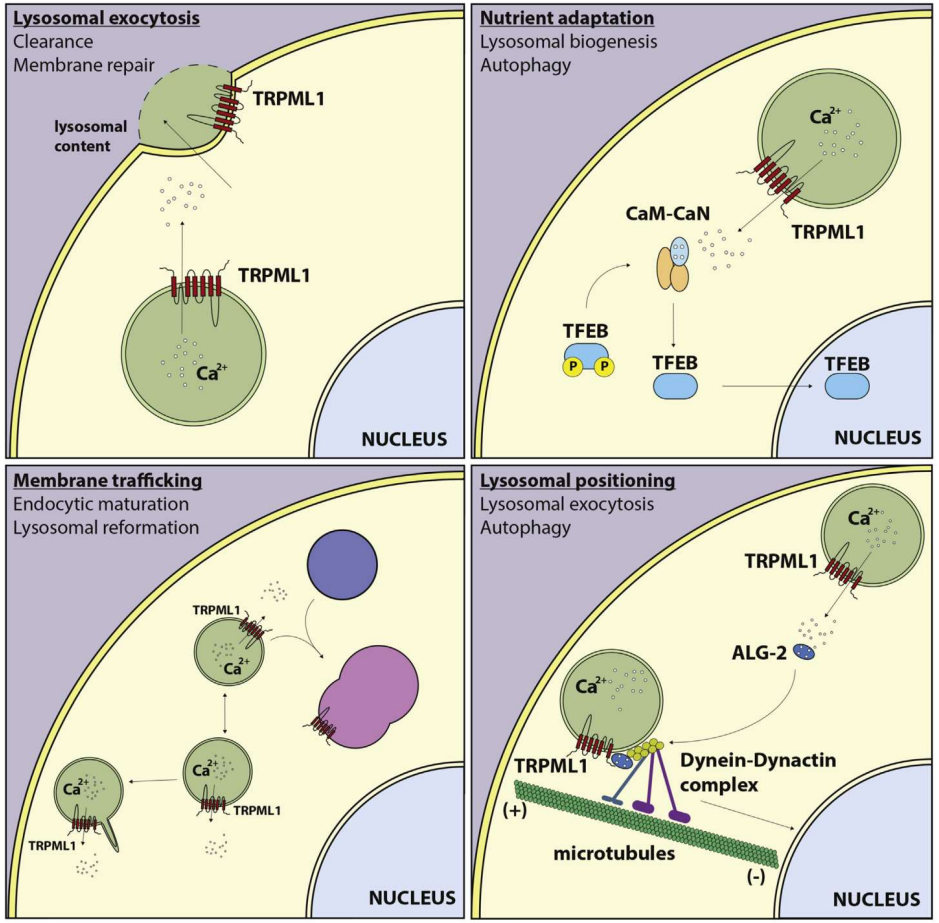
XP Dong…. H Xu… (2010) Nat Comm



H Xu… D Clapham (2007) PNAS

TRPML1 (mucolipin 1): cellular roles

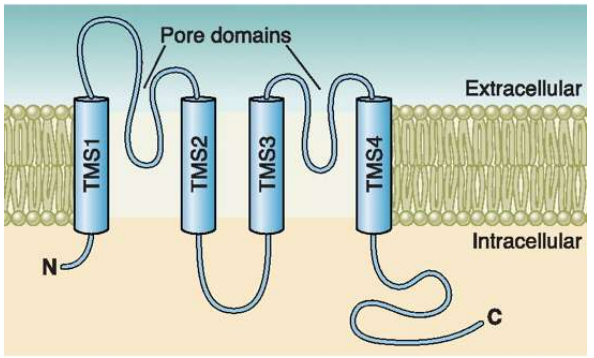
- Local Ca^{2+} release for
  - Lysosome / LE or autophagosome fusion
  - Lysosome exocytosis
  - Lysosome positioning
  - Lysosome adaptation by TFEB
- Export of divalent metals (Fe^{2+}, Zn^{2+}…) released by metalloprotein degradation



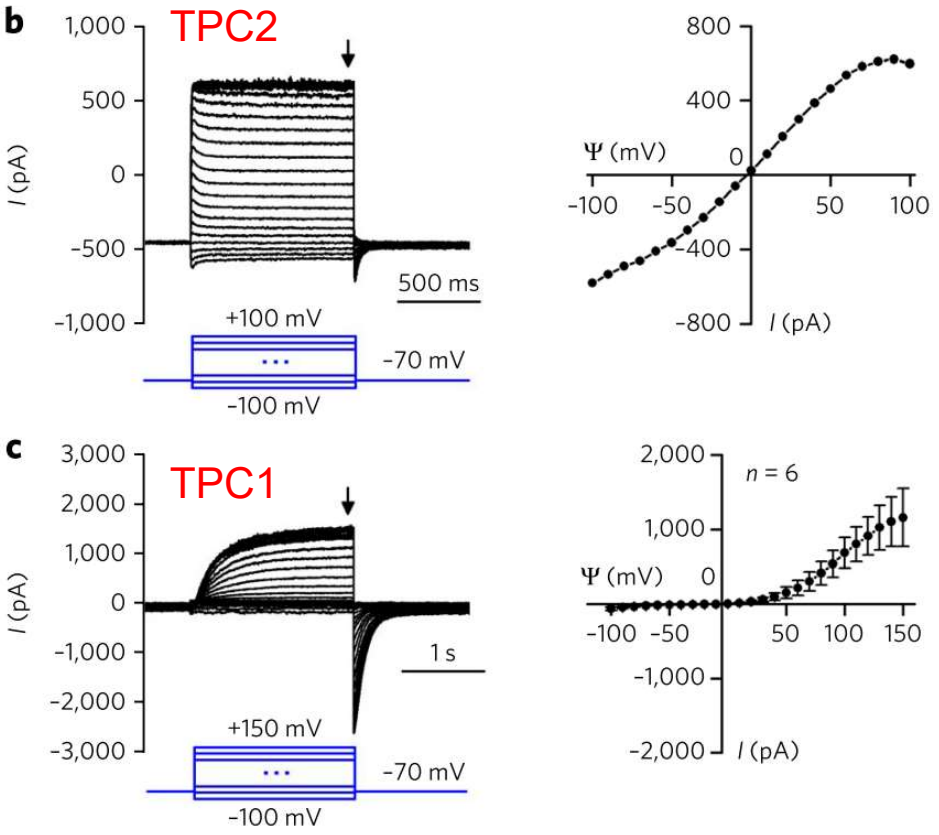
Reviewed in S Di Paola… DL Medina (2017) Cell Calcium

TPC1 and 2

- Belong to the Two-Pore domain channel superfamily
- 4 TM + 2 pore loops
- Ion selectivity: $Na^+ >> K^+$ (P_{Na}/P_K = 80 for TPC1)



- Regulated by:
  - Membrane potential (TPC1)
  - Luminal pH (TPC1)



C Cang…. D Ren… (2014) Nat Chem Biol

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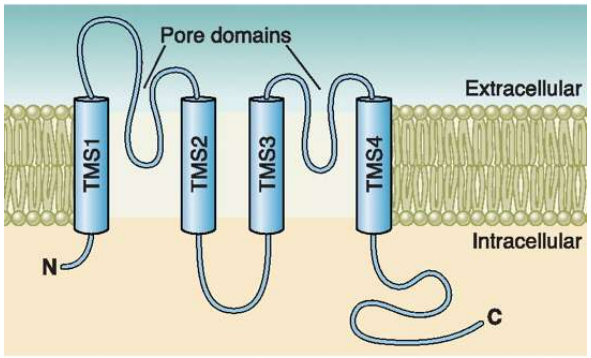
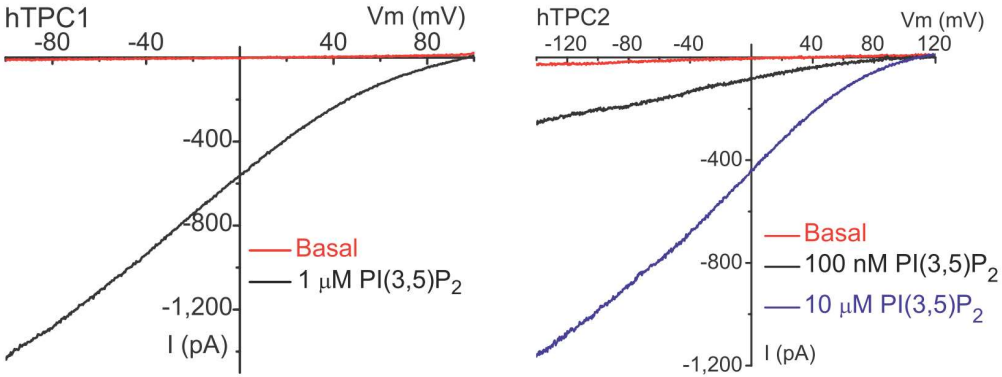


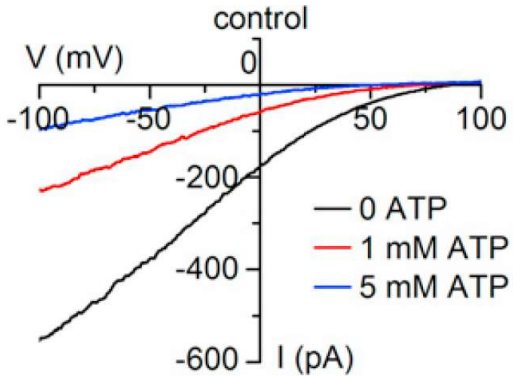
- Regulated by:
  - Membrane potential (TPC1)
  - Luminal pH (TPC1)
  - $PI3,5P_2$ (both)
  - **mTORC1**

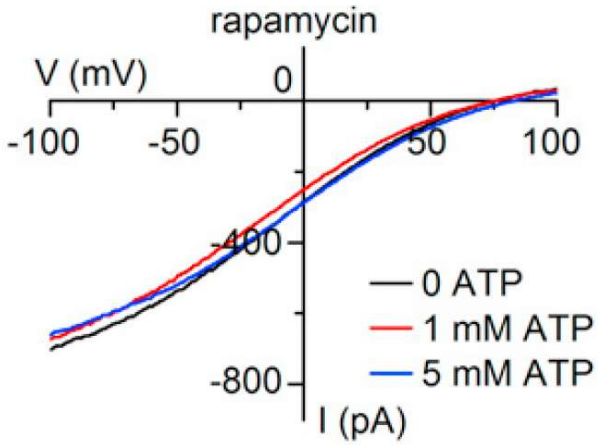


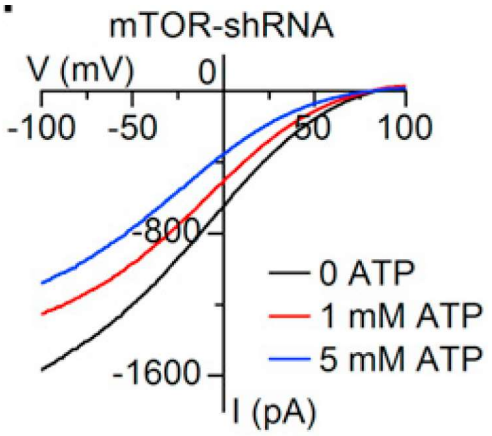
- Conflicting reports on regulation by NAADP

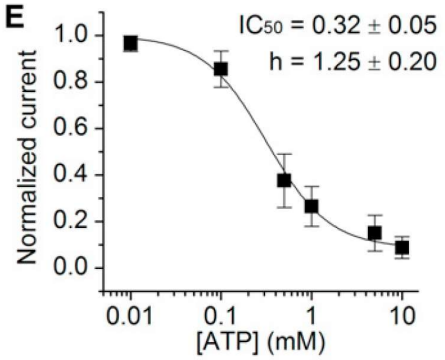
X Wang…. H Xu… (2012) Cell

Regulation of TPC1 and 2 by mTORC1





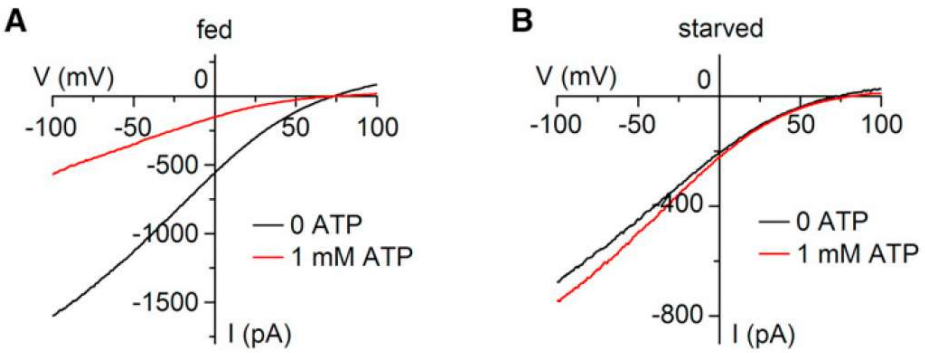




Mediated by kinase activity of mTORC1 (not mTORC2) through unidentified phosphorylated target

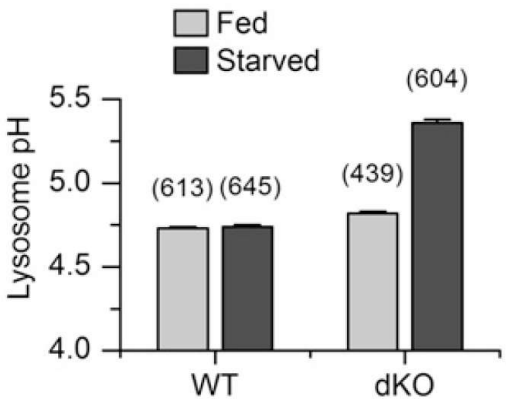
C Cang…. D Ren… (2013) Cell

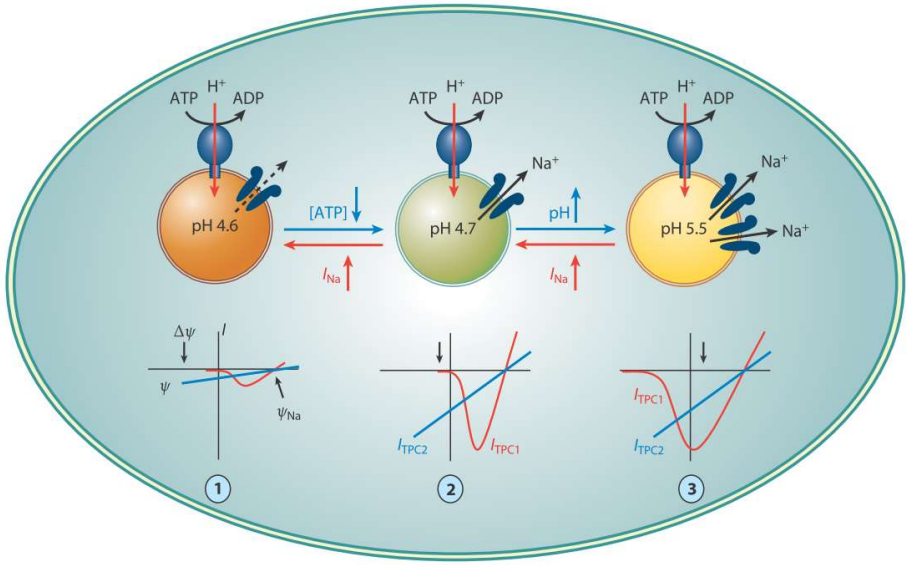
Regulation of TPC1 and 2 by mTORC1



- down regulated by AAs (not glucose)
- involves mTOR recruitment to lysosomes

Suggested cellular role: keep luminal pH acidic under starvation

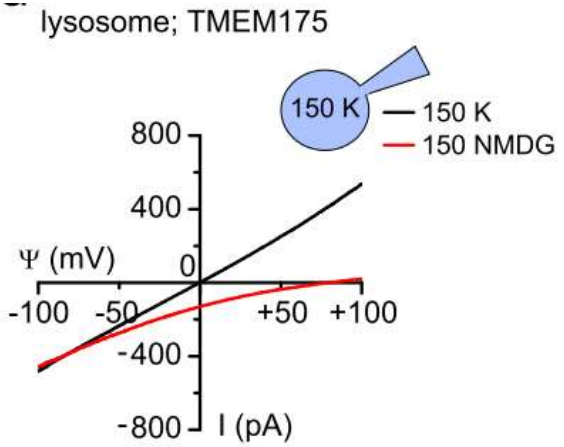




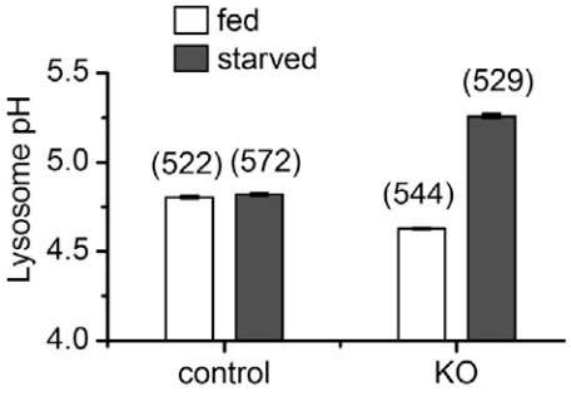
C Cang…. D Ren… (2013) Cell
H Xu & D Ren (2015) Annu Rev Physiol

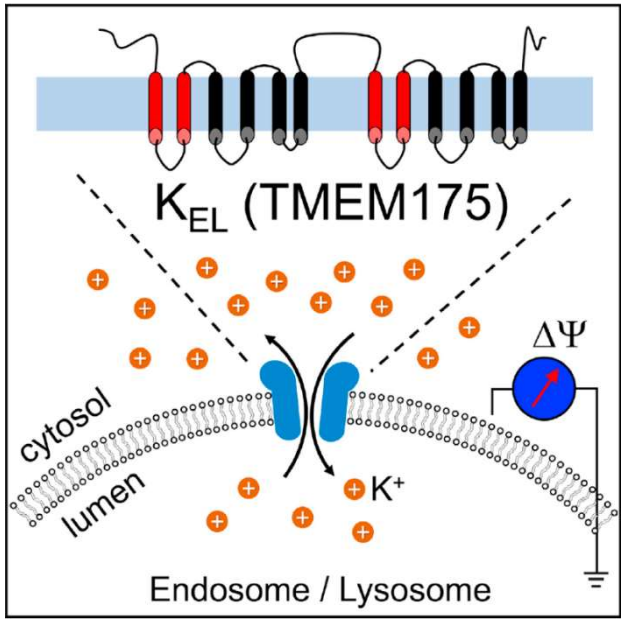
TMEM175

- Identified by gain-of-function patch-clamp screen
- Unrelated to know K+ channels
- Selective for K^+ (P_{Na}/P_K = 36; P_{Na}/P_{Ca} = 140)
- = major K+ permeation pathway of lysosomes



- Suggested cellular role: contribution to lysosomal acidification (K^+ as counter-ion to sustain v-ATPase activity; *specific for autolysosomes?*)





C Cang…. D Ren… (2015) Cell

ClC-7 transporter

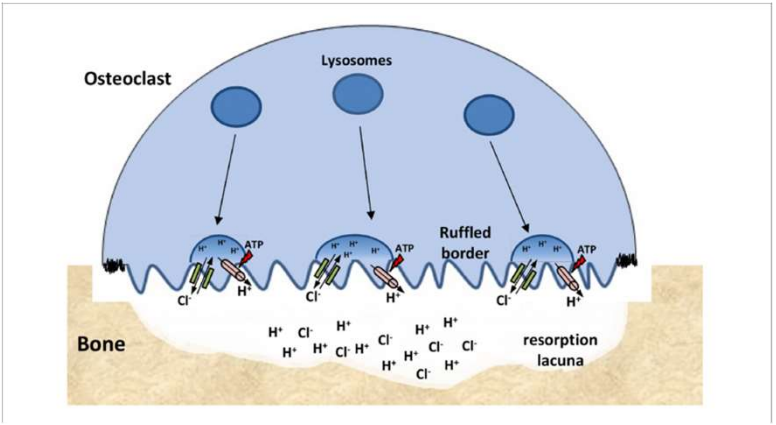
- Member of the ClC chloride channel/transporter family
- Exchanges 2 Cl- for 1 H+
- Requires beta subunit Ostm1 for stability and activity
- Defective in infantile malignant osteopetrosis

Suggested cellular roles:

- Acidification of bone resorption lacuna

(electrical shunt for V-ATPase at osteoclast ruffled membrane)

- Controversed role in lysosomal acidification
- Accumulation of chloride into lysosomal lumen



L Leisle… T Stauber (2011) EMBO J
TJ Jentsch (2015) J Physiol
JA Mindell (2012) Annu Rev Physiol.

- The weird world of membrane transport
- How to study lysosomal channels and transporters
- The v-ATPase
- Ion channels and transporters
- **Catabolite exporters**
- ABC transporters

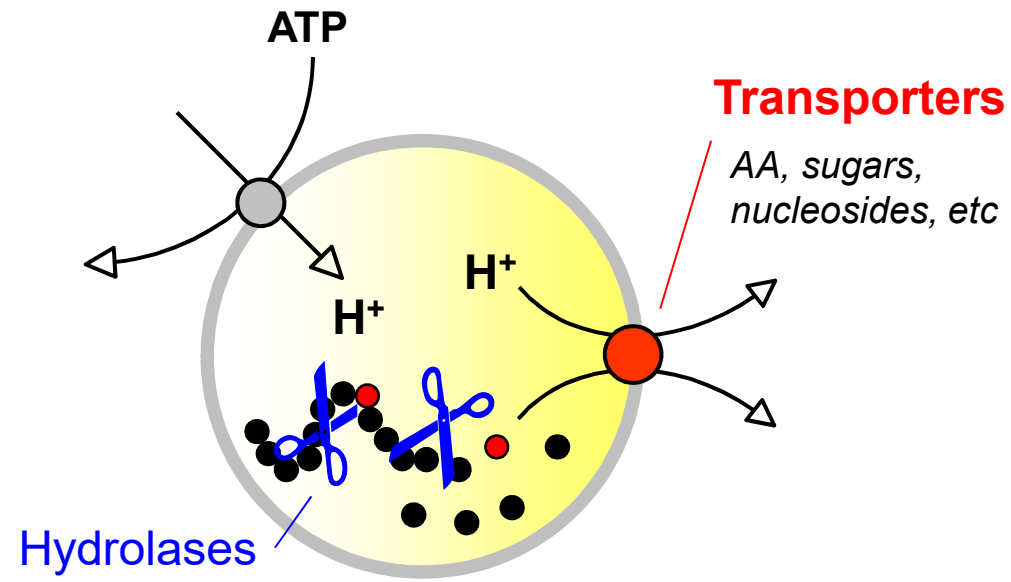
Lysosomal catabolite transporters

• Main function: export amino acids, sugars, nucleosides, lipids, Pi etc released by luminal hydrolysis of macromolecules

• Generally coupled to proton cotransport

• Thus the huge (2.5-Unit) proton gradient drives them in export direction (even when facing high cytosolic substrate concentration)

• Exception: cysteine import (unknown protein)



Lysosomal amino acid transporters

- Many transporters are still missing

Table 1 Lysosomal transport activities and proteins from the lysosomal membrane with demonstrated or putative transport function

Substrates		Transport protein (*human gene*)	Mechanism	Associated inherited disorder (OMIM no.)	References
Protein catabolites	Lys, Arg (system c)	PQLC2*	?	Treatment of cystinosis	* Pisoni et al (1985), Pisoni et al (1987b)
	Glu, Asp (system d)	?			Collarini et al (1989)
	Ala, Ser, Thr (system e)	?			Pisoni et al (1987a)
	Pro, Ala, Ser, Thr (system f)	?			Pisoni et al (1987a)
	Pro (system p)	?			Pisoni et al (1987a)
	Pro, Ala, Gly	LYAAT1 (*SLC36A1*)	H^+symport		Sagné et al (2001)
	Leu, Phe, Tyr (system t)	?			Stewart et al (1989)
	Ile, Leu, Phe, Trp, Tyr (system h)	?			Bernar et al (1986)
	Leu, Ile, Val, Met, Phe (system l)	?			Stewart et al (1989)
	Cystine	Cystinosin (*CTNS*)	H^+symport	Cystinosis (219800)	Town et al (1998), Kalatzis et al (2001)
	Cysteine*, cysteamine*	?			Pisoni et al (1990)
	Di-and tripeptides	?			Thamotharan et al (1997)
	His, dipeptides	PHT2 (*SLC15A3*)	H^+symport		Sakata et al (2001)
	Gln, Asn	SNAT7** (SLC38A7)	H+ symport?	Nutrition of cancer cells	
	Arg	SNAT9$ (SCL38A9)	Nutrient sensor		

Updated from Sagné & Gasnier (2008) J Inherit Metab Dis 31:258

** Liu, et al Science 2012; Jézégou et al PNAS 2012*

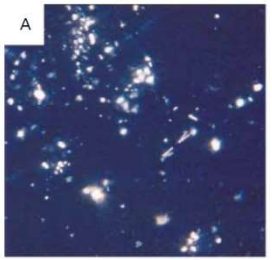
** * Verdon et al PNAS 2017*

Lysosomal transporters for other metabolites

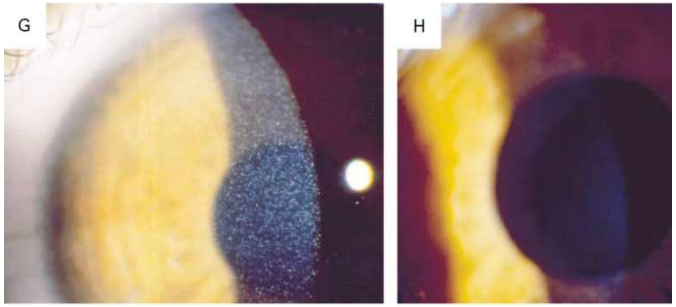
Degraded macromolecule	Substrates	Transporter	Disease
Carbohydrates	Sialic acids, acidic hexoses	Sialin	Salla disease, ISSD
	Neutral hexoses	GLUT8	
Lipids	cholesterol	NPC1	Niemann-Pick C
Nucleic acids	nucleosides	ENT3	histiocytosis, H syndrome, PHID syndrome
Internalized transcobalamin	cobalamin	ABCD4, LMBD1	cobalamin F and J diseases

Cystinosis

- rare: ~1/100,000 live births
- autosomal recessive; *CTNS* gene, 17p13
- hallmark: lysosomal storage of cystine
- *CTNS* gene encodes the lysosomal cystine transporter, cystinosin
- prominent kidney dysfunction, then multisystemic
- current treatment: cysteamine



cystine crystals



+ cysteamine eyedrops

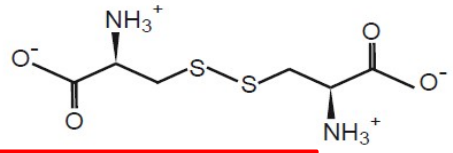
TABLE 1. AGE-RELATED CLINICAL CHARACTERISTICS OF UNTREATED NEPHROPATHIC CYSTINOSIS.

AGE	SYMPTOM OR SIGN	PREVALENCE IN AFFECTED PATIENTS
		%
6–12 mo	Renal Fanconi's syndrome (polyuria, polydipsia, electrolyte imbalance, dehydration, rickets, growth failure)	95
5–10 yr	Hypothyroidism	50
8–12 yr	Photophobia	50
8–12 yr	Chronic renal failure	95
12–40 yr	Myopathy, difficulty swallowing	20
13–40 yr	Retinal blindness	10–15
18–40 yr	Diabetes mellitus	5
18–40 yr	Male hypogonadism	70
21–40 yr	Pulmonary dysfunction	100
21–40 yr	Central nervous system calcifications	15
21–40 yr	Central nervous system symptomatic deterioration	2

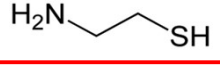
Gahl, Thoene, Scheider (2002) N Engl J Med, 347:111

The cationic AA transporter PQLC2 underlies cysteamine therapy of cystinosis

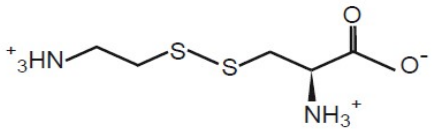
cystine



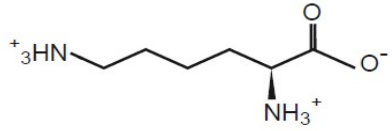
cysteamine

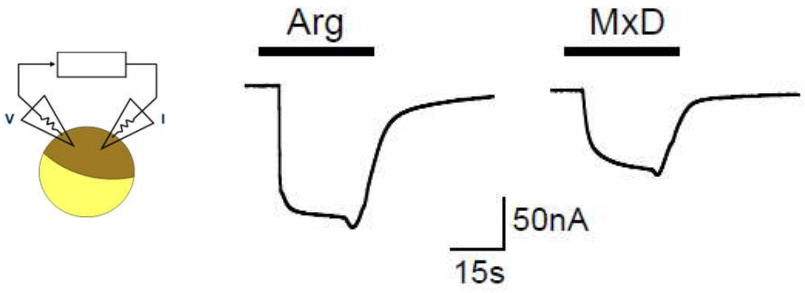


mixed disulfide



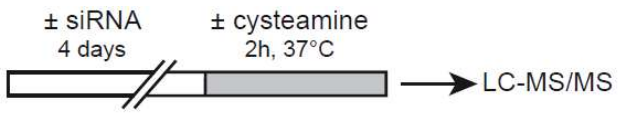
lysine



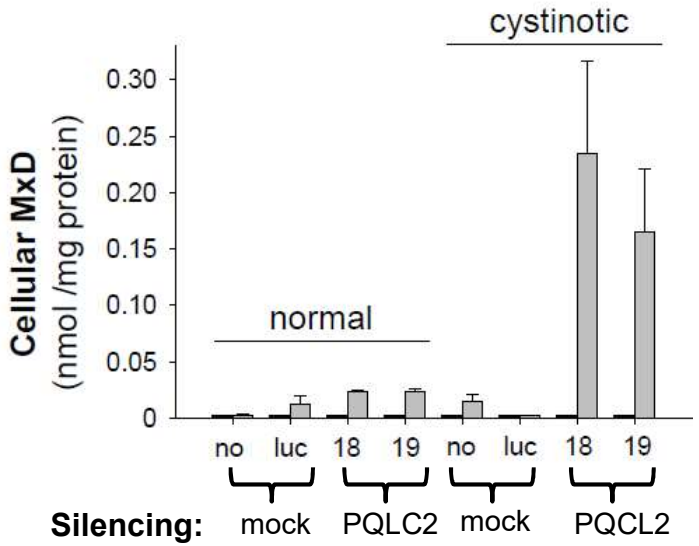


PQLC2-LL/AA oocyte

Patient skin fibroblasts



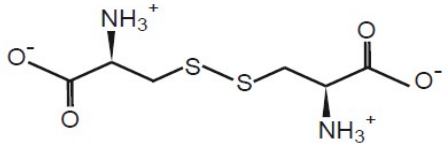
■ - cysteamine
□ + cysteamine



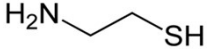
Jézégou et al PNAS 2012

The cationic AA transporter PQLC2 underlies cysteamine therapy of cystinosis

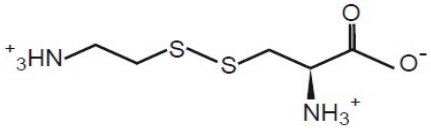
cystine



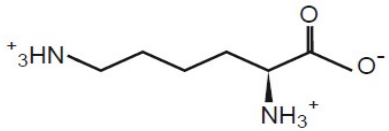
cysteamine

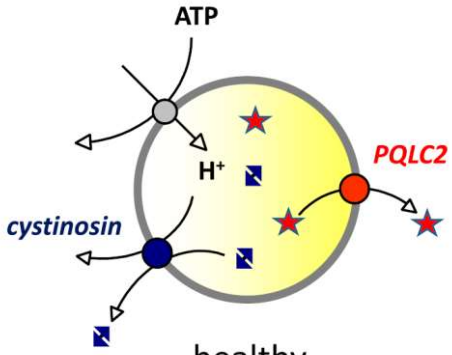


mixed disulfide

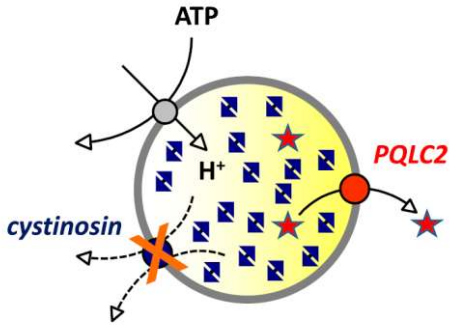


lysine

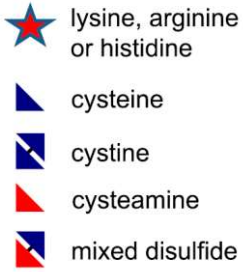


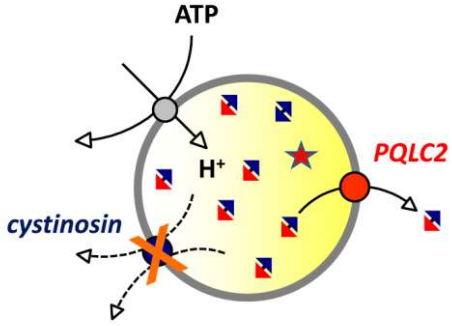


healthy



cystinotic

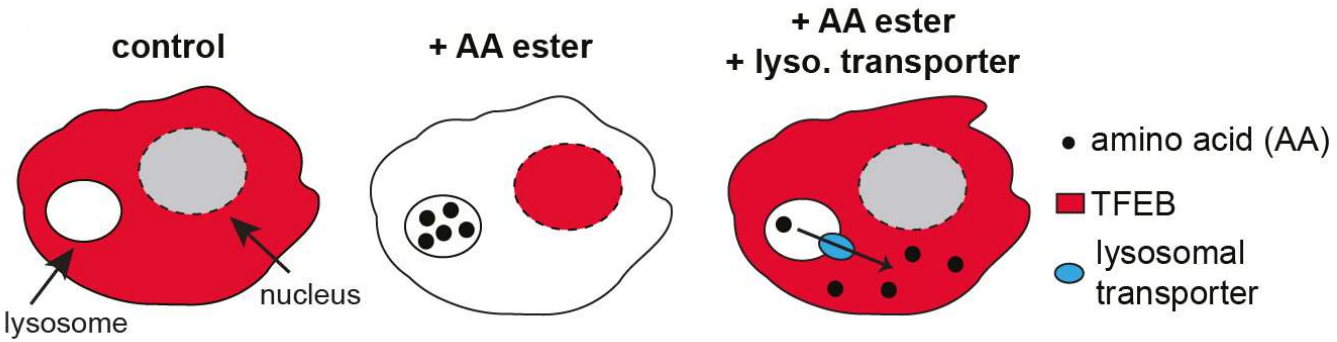


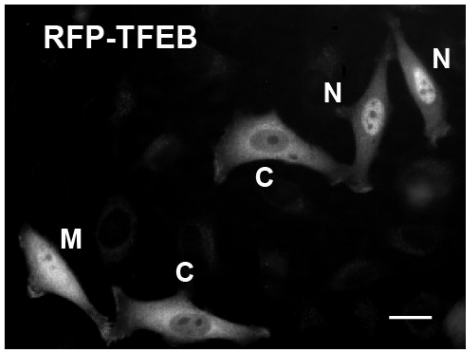


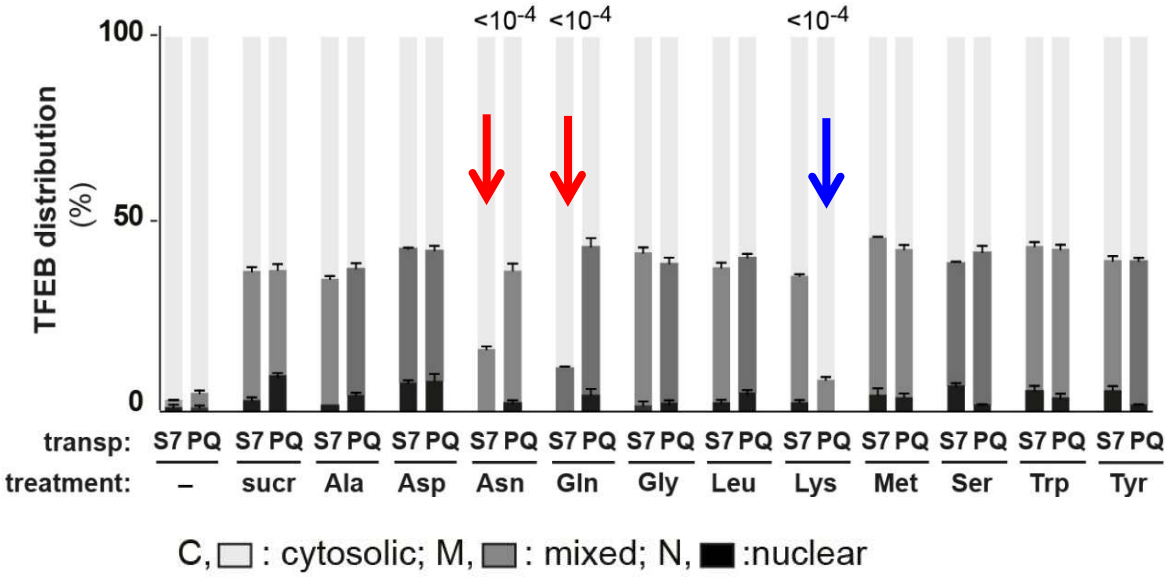
cystinotic + cysteamine

Jézégou et al PNAS 2012
see also B. Liu… X. Wang Science 2012

Identification of Gln/Asn transporter SNAT7 based on a novel in-cell TFEB-based assay

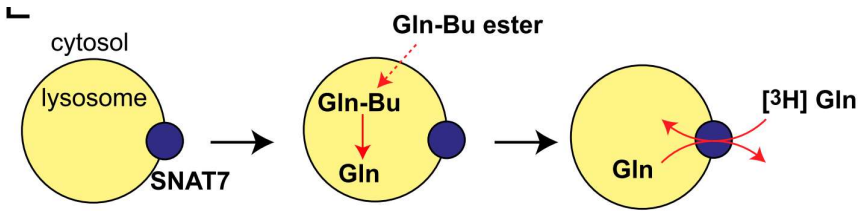


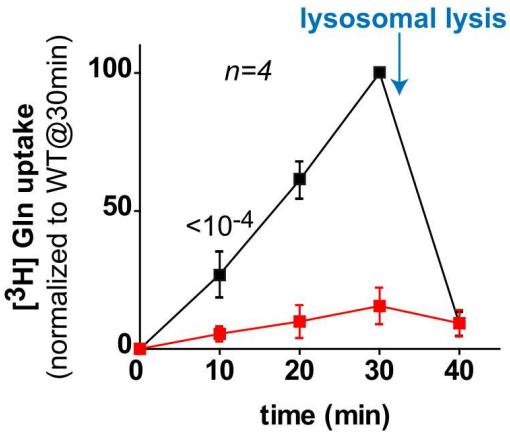


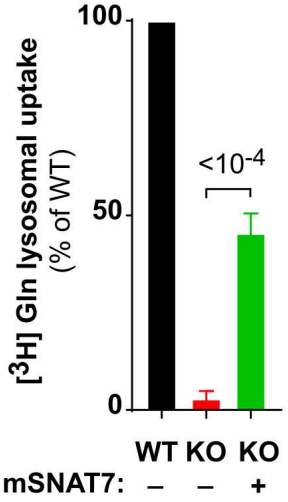


Verdon et al (2017) PNAS 2017

Identification of Gln/Asn transporter SNAT7 based on a novel in-cell TFEB-based assay

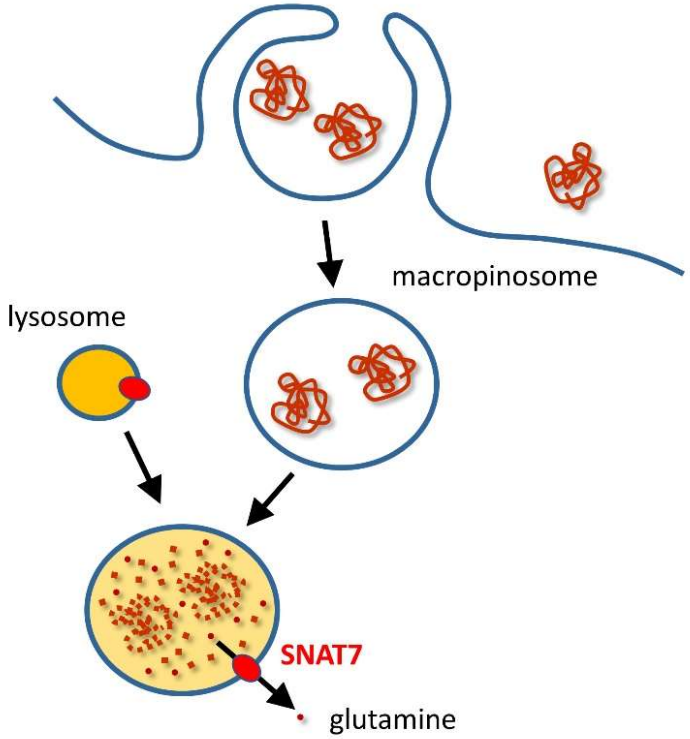






Verdon et al (2017) PNAS 2017

Cellular role:
micropinocytosis-dependent growth of cancer cells ‘addicted’ to glutamine



SNAT9 *(SLC38A9)* is involved in Arg-sensing and cholesterol-sensing at the lysosomal membrane to regulate mTORC1

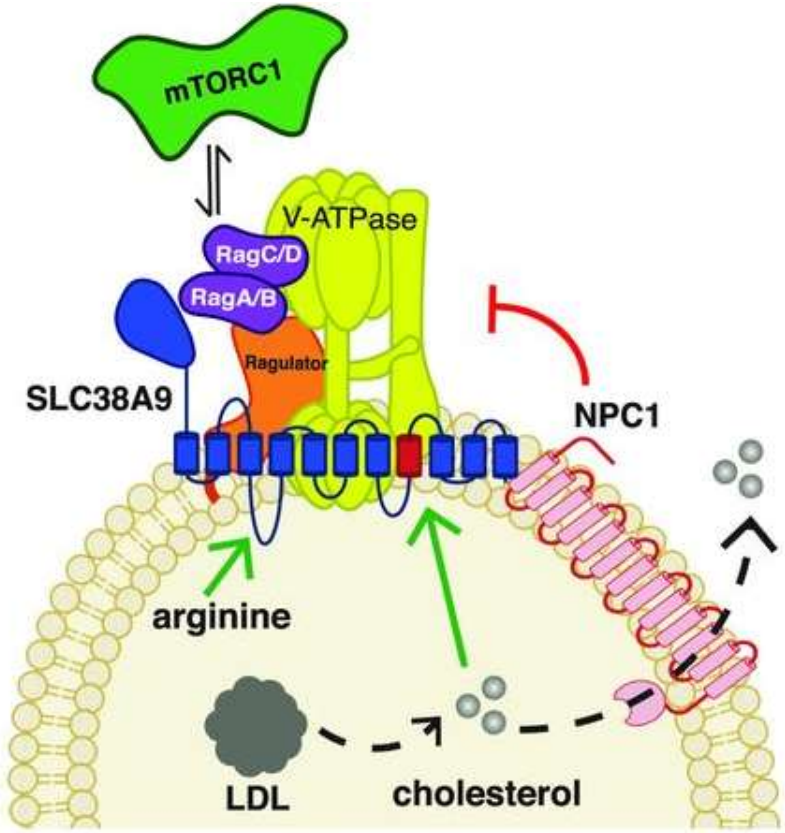
SNAT9 = 'transceptor' rather than transporter?

Arg-sensing

Wang S, …. Sabatini DM Science 2015
Rebsamen M, …. Superti-Furga G. Nature. 2015
Jung, Genau & Behrends C. Mol Cell Biol. 2015

Cholesterol sensing

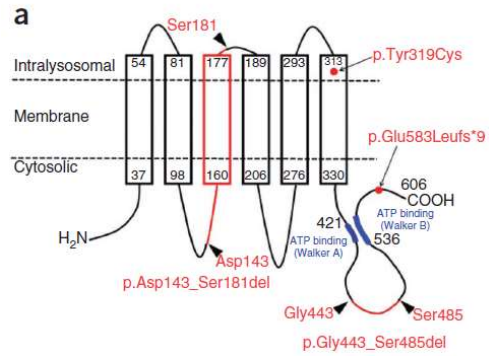
BM Castellano…R Zoncu Science 2017



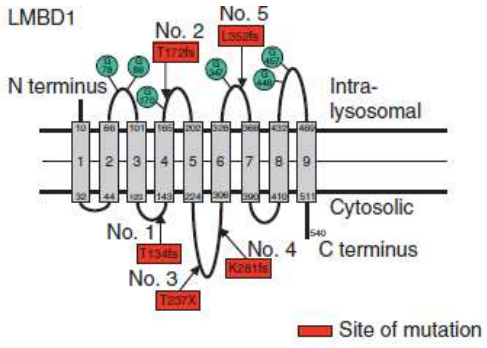
Cell entry of cobalamin (= B12) through lysosomal ABC transporter and LMBD1

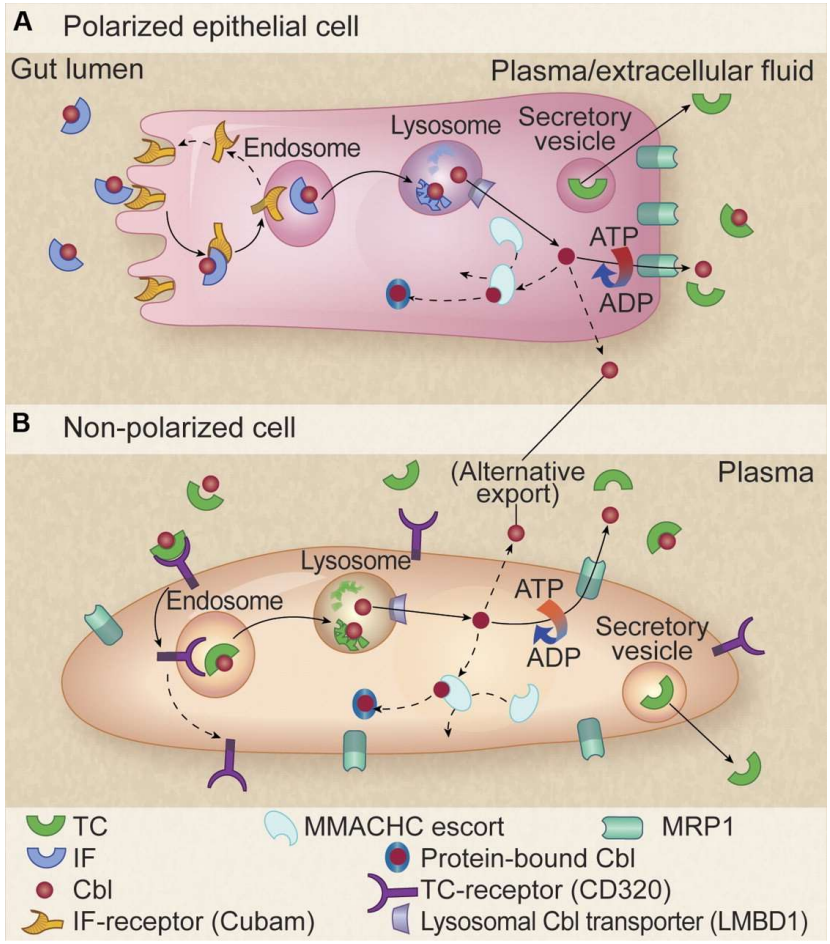
Vitamin B12 (cobalamin) metabolism diseases:
cblF and *cblJ* complementation groups

ABCD4(*cblJ*)



LMBD1 (*cblF*)





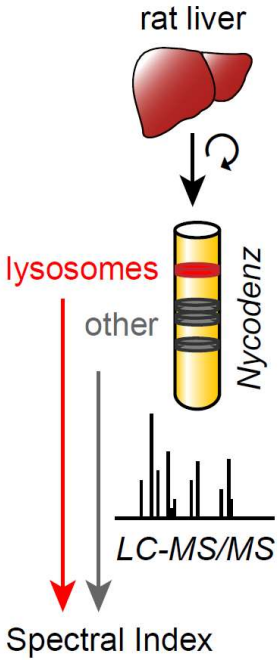
Rutsch et al (2009) Nat Genet
Coelho et al (2012) Nat Genet

Membrane proteomics provide a good starting point to mine new lysosomal transport activities

An Extended Proteome Map of the Lysosomal Membrane Reveals Novel Potential Transporters*

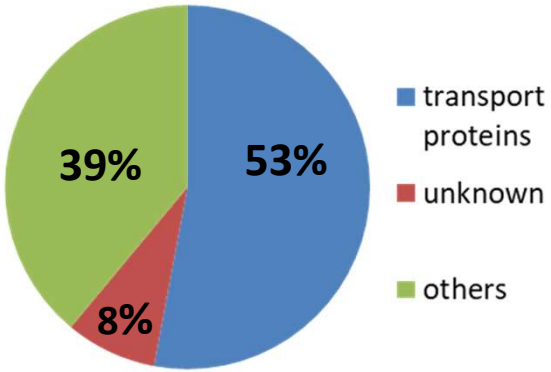
Agnès Chapel‡§¶, Sylvie Kieffer-Jaquinod‡§¶, Corinne Sagné‖, Quentin Verdon‖§§, Corinne Ivaldi‡§¶, Mourad Mellal‡§¶, Jaqueline Thirion**, Michel Jadot**, Christophe Bruley‡§¶, Jérôme Garin‡§¶, Bruno Gasnier‖, and Agnès Journet‡§¶‡‡

Mol Cell Proteomics (2013)



734 proteins

263 integral mb proteins (TM ≥ 1)
136 polytopic proteins (TM ≥ 2)



Among polytopic proteins

Take home messages

• Channels and transporters have distinct mechanisms and kinetics

• Stronger and broader impact of ion channels, including through membrane potential

• The v-ATPase provides the energy for most processes

• Ion channels and transporters regulate ion homeostasis

• TRPML1 releases Ca2+ in the vicinity of the lysosome for fusion, trafficking and signalling processes

• Secondary transporters (+ some ABC transporters) export lysosomal catabolites or micronutrients for reuse in metabolism

• Most transporters remain to be discovered