Ups and downs of lysosomal pH: conflicting roles of LAMP proteins?

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Abstract

The acidic pH of lysosomes is critical for catabolism in eukaryotic cells and is altered in neurodegenerative disease including Alzheimer and Parkinson. Recent reports using Drosophila and mammalian cell culture systems have identified novel and, at first sight, conflicting roles for the lysosomal associated membrane proteins (LAMPs) in the regulation of the endolysosomal system.

Main text

Macromolecules including proteins, nucleic acids, and complex sugars are degraded in lysosomes by >60 acid hydrolases, which function optimally at a pH of 4.5-4.9 [1]. Lysosomal pH is established by a dynamic equilibrium between proton influx and efflux across the lysosomal membrane, balanced with changing concentrations in other cations and anions [2,3]. Regulation of lysosomal pH is imperative to ensure that acid hydrolase activity remains optimal in lysosomes. The endocytic pathway is critical for lysosomal function as it traffics lysosomal membrane proteins and acid hydrolases that are key for the lysosome's degradative capacity [3]. Endosomes mature from early endosomes to late endosomes through a tightly regulated maturation process guided by RAB GTPases, SNARE complexes, and phospholipids [4]. Their maturation is accompanied by a progressive decrease in pH from 6.8-6.1 in early endosomes, to 6.0-5.0 in late endosomes, and finally to a pH of 4.9-4.5 in endo-lysosomes [4] .

The pH gradient is essential for endosomal trafficking and cargo sorting as it provides receptors with different conditions in which they bind and release ligands and restricts hydrolase activity to the terminal compartment. Endo-lysosomal acidification is mainly regulated by an ATP-dependent proton pump, the vacuolar-type H^+-translocating ATPase (V-ATPase). The pH adjustment of each endosomal compartment is accomplished based on V-ATPase concentration, selection of V-ATPase isoforms, and association and dissociation of the V_0 and V_1 domains [4,5]. Acid hydrolase trafficking and activation represents one of the most critical pH-dependent processes, as highly acidic pH ranges like those seen in endo-lysosomes and lysosomes cause hydrolase release from M6P transport-receptors, making them available for hydrolysis [6].

Consequently, aberrant endosomal pH regulation is detrimental. For example, dysregulation of lysosomal acidification is a significant risk factor for Alzheimer disease (AD) and Parkinson disease (PD) [2,7-10]. A decrease in autolysosomal acidification precedes amyloid-β accumulation, a common pathology of AD, in mouse cortical neurons [7]. In contrast, a lower lysosomal pH is associated with mutations in GBA1/β-glucocerebrosidase (glucosylceramidase

beta 1) [8-13] which increase the lifetime risk of PD by as much as 30% [14], and cause faster decline with more severe symptoms in affected patients [15].

Lysosomal associated membrane proteins (LAMPs) contribute to the glycocalyx through their highly N-glycosylated lumenal domain to protect the lysosomal membrane from the harsh pH conditions in the lumen [16]. The most abundant LAMPs found on lysosomal membranes are the partially functionally redundant LAMP1 and LAMP2, making up ~50% of proteins found on the lysosomal membrane [17,18]. Whereas *lamp1* mutant mice are grossly normal [19], *lamp2* and particularly *lamp1 lamp2* double-mutant cells show a block in macroautophagy (MA) with undegraded material and cholesterol accumulating in late autophagic vacuoles [18,20-22], as well as an absence of chaperone-mediated autophagy [23,24]. LAMP2 deficiency in humans causes Danon disease, in which patients show similar cellular phenotypes including altered autophagic flux [25].

Recently, two publications described unanticipated functions of LAMP proteins in endo-lysosomal pH regulation. First, our labs showed that *Drosophila melanogaster* deficient for *Lamp1*, the *bona fide* ortholog of mammalian *LAMP1* and *LAMP2*, show a highly increased number of LysoTracker (LTR)-positive, acidic vesicles in the fat bodies (functionally similar to mammalian adipose tissue and liver) of 3rd instar larvae and adult flies (Fig. 1) [26]. These LTR-positive vesicles equally colocalize with Rab5, Rab7, and Rab11 thus indicating dysregulation in the acidification of the endo-lysosomal compartment. It is currently unknown whether the endo-lysosomal acidification of *Lamp1* mutant fat body is caused directly by the absence of Lamp1 on lysosomes, or indirectly by the lipid transport defects that these mutants show as well [26]. Nevertheless, the acidification phenotype of fly *Lamp1* mutants is reminiscent of *Gba1b*-deficient flies [12,13,27], and indeed mutation of *Lamp1* or its overexpression can enhance and suppress *Drosophila* PD models [28], respectively, suggesting that an accumulation of acidic vesicles indeed poses a significant risk for PD.

The second recent study elegantly showed that both human LAMP1 and LAMP2 are capable of complexing with TMEM175, another prominent PD risk factor and a lysosomal proton exporter that counterbalances the proton-influx of V-ATPase (Fig. 1) [29]. Using cell culture and purified liposome systems, Zhang *et al.* showed that binding of LAMP1 or LAMP2 to TMEM175 inhibits its proton efflux function, thus lowering the lysosomal pH [29]. Making use of the fact that overexpressed LAMP proteins and TMEM175 localize to the plasma membrane, they were able to utilize patch-clamp electrophysiology to investigate how LAMP1 and LAMP2 affect TMEM175 proton conductance. LAMP1 inhibits proton transport as long as it contains its membrane proximal LAMP domain and is able to bind TMEM175 via its TM domain. Critically, *TMEM175* mutants

unable to bind to LAMP1 and LAMP2 are inert to inhibition by LAMP proteins, and consistently, the LAMP1 TM-domain and C terminus (sufficient to bind to, but not inhibit TMEM175) act as a dominant negative, ultimately increasing proton export and thus the lysosomal pH. Conversely, *TMEM175* knockout HAP1 cells show significantly higher LTR fluorescence and lower lysosomal pH compared to WT cells, which is consistent with their model of LAMP1 and LAMP2 inhibiting TMEM175 proton efflux function.

Thus, clearly, LAMP proteins regulate endolysosomal acidification in flies as well as in human cells, although apparently in possibly opposite ways: Lamp1 in flies is required to prevent excessive endolysosomal acidification (EELA) [26], but in mammalian cells LAMP1 and LAMP2 can inhibit the lysosomal proton export function of TMEM175 [29] to maintain a low pH, suggesting opposite net effects. These conflicting findings call into question what the basic function(s) of LAMPs are in relation to endo-lysosomal acidification, how those are altered by interacting proteins, and whether and to what extent such functions are conserved or diverged during evolution. At least in flies, Lamp1 somehow must antagonize net proton influx, a function that also can be exerted by human LAMP2A in rescue experiments [26]. In contrast, results from Zhang *et al.* would suggest that the lysosomes of cells deficient in *LAMP1* and *LAMP2* would be more basic. While they have not addressed this in their system, no changes were found in LTR puncta of *lamp1 lamp2* double-knockout MEFs [18,24] or the pH of various autophagic and endosomal compartments of *lamp2* or *lamp1 lamp2* double-mutant tissues when assessed by N-(3-[{2,4-dinitrophenyl}amino] propyl)-N-(3-aminopropyl) methylamine/DAMP [17].

One plausible explanation for this discrepancy could be that LAMPs interact with positive and negative regulators of lysosomal pH (Fig. 1). In flies, the net effect would be to prevent hyper-acidification of lysosomes, a function that could be overshadowed in the presence of an active proton exporter in human cells. Along these lines it is interesting to note that, first, large scale interaction approaches have found LAMP1 and LAMP2 proteins associated with V-ATPase subunits, suggesting that LAMPs may modulate its activity [30]. Second, *D. melanogaster* encodes no direct homolog of TMEM175 easily identifiable in database searches and no proton efflux channel has been identified, which could explain why the acidification phenotype of *Lamp1* mutants is unmasked. If so, *TMEM175 LAMP1 LAMP2* triple-mutant cells would be expected to show an even lower lysosomal pH. Likewise, overexpression of LAMPs should suppress the *TMEM175* KO acidification phenotype. Of course, many additional explanations are possible, including evolutionary divergence, or indirect mechanisms that lead to EELA in *Lamp1* mutants. Clearly though, LAMP proteins have a role in the regulation of the acidification of the endolysosomal system and shedding light on the underlying mechanisms will provide much

needed insight into endo-lysosomal acidification and has the potential to inform the pathology of neurodegenerative diseases.

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Declaration of interest statement

The authors declare no conflict of interest.

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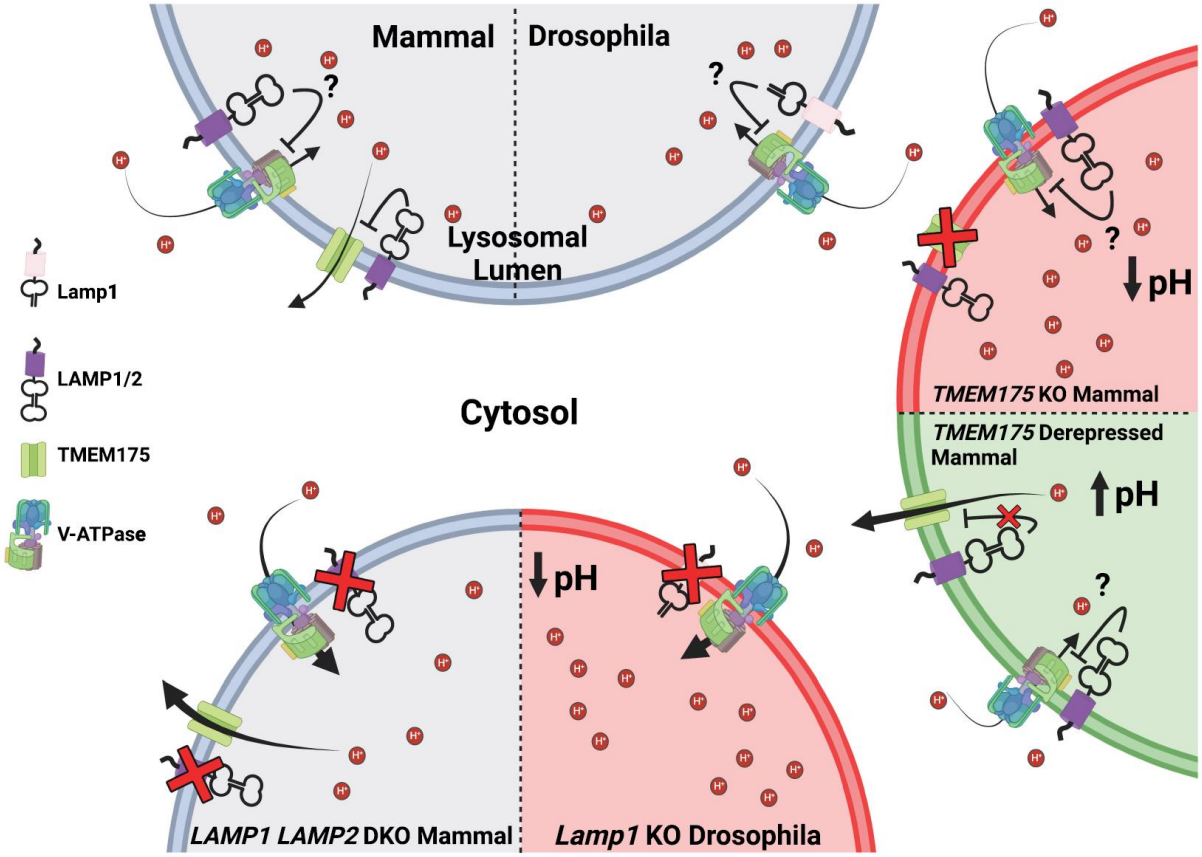

Figure 1. Models of lysosomal pH regulation by LAMP proteins. Top: In mammalian cells (left), the acidic pH is maintained through a balance of proton influx (V-ATPase) and efflux (TMEM175), the latter being inhibited by LAMP1 and LAMP2. Drosophila (top right) lack a TMEM175 homolog. Additionally, it is plausible that LAMP proteins in either species directly or indirectly regulate V-ATPase to control lysosomal pH. Bottom: When *LAMP1* and *LAMP2* are knocked out in mammalian cells, both TMEM175 and V-ATPase are no longer inhibited, but lysosomal pH is maintained, potentially due to a mutual increase of both proton influx and efflux (left). In *Drosophila Lamp1* mutants, lysosomal pH decreases possibly due to overactivation of V-ATPase (and no efflux compensation). Upon lack of TMEM175 in mammalian cells, lysosomal pH also decreases due to a loss of proton efflux (right). Conversely, TMEM175 mutants unable to be inhibited by LAMP proteins cause excessive protein efflux and thus higher lysosomal pH. See text for details. Biorender.