Trends in Biochemical Sciences

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Review

Lysosomal Ion Channels as Decoders of Cellular Signals

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Lysosomes, the degradation center of the cell, are filled with acidic hydrolases. Lysosomes generate nutrient-sensitive signals to regulate the import of H^+, hydrolases, and endocytic and autophagic cargos, as well as the export of their degradation products (catabolites). In response to environmental and cellular signals, lysosomes change their positioning, number, morphology, size, composition, and activity within minutes to hours to meet the changing cellular needs. Ion channels in the lysosome are essential transducers that mediate signal-initiated $Ca^{2+}/Fe^{2+}/Zn^{2+}$ release and $H^+/Na^+/K^+$-dependent changes of membrane potential across the perimeter membrane. Dysregulation of lysosomal ion flux impairs lysosome movement, membrane trafficking, nutrient sensing, membrane repair, organelle membrane contact, and lysosome biogenesis and adaptation. Hence, activation and inhibition of lysosomal channels by synthetic modulators may tune lysosome function to maintain cellular health and promote cellular clearance in lysosome storage disorders.

Highlights

Lysosomes generate nutrient-sensitive signals to regulate the import of H^+, hydrolases, and endocytic and autophagic cargos, as well as the export of the degradation products.

In response to environmental and cellular signals, lysosomes change their positioning, number, morphology, size, composition, and activity within minutes to hours to meet the changing cellular needs.

Selective Na^+, K^+, and Ca^{2+} channels are present in the lysosome to maintain the concentration gradients of H^+, Na^+, K^+, and Ca^{2+} across the lysosomal membrane.

Ion channels in the lysosome are essential transducers of cellular signals and are required for cellular homeostasis.

Signal-initiated lysosomal Ca^{2+} release and membrane potential changes regulate lysosome movement, membrane trafficking, nutrient sensing, membrane repair, organelle membrane contact, and lysosome biogenesis.

Activation and inhibition of lysosomal channels may tune lysosome function to promote cellular clearance in lysosome storage disorders.

Lysosome Function

Lysosomes are traditionally viewed as macromolecule degradation center of the cell, and express more than 60 types of acidic hydrolases [9]. Recent studies, however, have clearly demonstrated that lysosomes are also a signaling hub that hosts the major nutrient sensors in the cell [2–4]. The 'degradation' and 'signaling' arms of lysosome function regulate each other reciprocally, such that degradation products are often the nutrient-sensitive signals that in turn control the duration and rate of degradation [5]. Instructed by these signals, lysosomes undergo constant movement, membrane fusion and fission, exocytosis, proliferation (biogenesis and reformation), and self-repair [5,6]. At resting conditions, each mammalian cell contains hundreds of primary and secondary (i.e., endolysosomes, autolysosomes, and phagolysosomes) lysosomes that are heterogeneous in protein and lipid composition, size (diameter = 100–500 nm), and positioning (e.g., peripheral vs perinuclear) [5,7,8]. Upon nutrient starvation and refeeding, these lysosome parameters change rapidly and reversibly to meet changing cellular needs. In this review we discuss recent advances in our understanding of the signals and signal transducers that regulate lysosomal membrane trafficking, the formation of lysosome–organelle membrane contact sites, nutrient-sensing, biogenesis, and adaptation (Figure 1).

Degradation

Lysosomes are the primary sites of cellular recycling in which proteins, complex lipids, polysaccharides, and nucleotides are broken down by proteases, lipases, glycosidases, and nucleases into their respective monogenetic building-block molecules (amino acids, monosaccharides, free fatty acids, and nucleosides) for reuse in biosynthetic pathways [9] (Figure 1). Mutations in the hydrolases result in accumulation of the cargo substrates, which in turn causes

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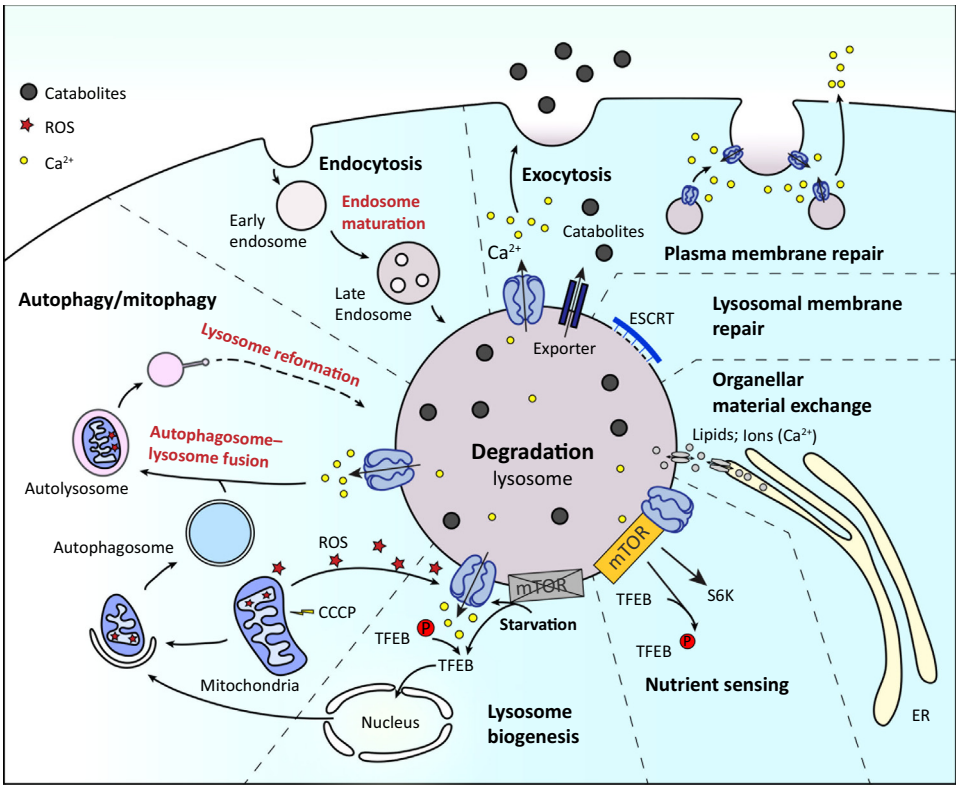

Figure 1. Function and Regulation of Lysosomes. Receiving inputs from both endocytic and autophagic pathways, lysosomes are the degradation center in the cell under both normal and starvation conditions. The degradation products (i.e., catabolites) are released through exporters, vesicular trafficking, lysosomal exocytosis, or interorganelle material exchange at membrane contact sites. Lysosomes are formed *de novo* through endosome maturation, or are reformed from autolysosomes or endolysosome hybrids. Lysosomes form membrane contact sites with the ER and other organelles, at which export of lipids and ions takes place. Lysosomal membrane proteins bridge the information of cytoplasmic signaling and luminal degradation. Lysosomal ion channels, by mediating signal-dependent lysosomal ion flux, participate in various lysosomal functions, including lysosomal membrane trafficking, catabolite export, nutrient sensing, and mTOR signaling. Triggered by various cellular cues, lysosomal Ca^{2+} release regulates lysosomal trafficking, lysosomal exocytosis, autophagic clearance of damaged mitochondria, plasma membrane repair, lysosomal membrane repair, TFEB nuclear translocation, and lysosome biogenesis. Abbreviations: ER, endoplasmic reticulum; P, phosphorylation; ROS, reactive oxygen species.

lysosomal insufficiency at the cellular level, and more than 50 types of lysosome storage diseases (LSDs) at the organismal level [9].

Membrane Trafficking: Movement, Fusion, and Fission

Lysosomes are mobile vesicles that undergo frequent membrane trafficking, in other words fusion and fission, with other intracellular compartments [10]. Lysosomes receive cargo materials delivered by endocytosis and autophagy (Figure 1). Exogenous endocytic cargos are fed into lysosomes through the endosome maturation process (Figure 1), which also includes lysosomal acidification and hydrolase delivery via mannose-6-phosphate receptors [7]. Conversely, intracellular autophagic cargos such as damaged mitochondria are packed into autophagosomes and delivered to lysosomes through autophagosome–lysosome fusion [11]. During these processes, lysosomes, with the aid of motor proteins, move constantly in both anterograde (nucleus to plasma membrane) and retrograde directions [12,13]. Degradation

products are exported into the cytoplasm or extracellular space through multiple efflux mechanisms: permeases and transporters, vesicular trafficking, exchange at lysosome–organelle membrane contact sites, and lysosomal exocytosis [8] (Figure 1). Whereas insoluble lipids and empty mannose-6-phosphate receptors are retrogradely transported to the *trans*-Golgi network (TGN) for reuse [7,8,14], catabolite-containing lysosomes are anterogradely transported and release their contents into the extracellular space through lysosomal exocytosis (fusion with the plasma membrane) [15] (Figure 1).

Lysosome–Organelle Membrane Contact

Catabolite export may also employ the non-vesicular, interorganelle material exchange pathways at lysosome–organelle membrane contact sites [14,16] (Figure 1). Lysosomes form membrane contact sites with all types of organelles, including endoplasmic reticulum (ER), mitochondria, and peroxisomes [16,14]. Free cholesterol can be exported from lysosomes to the ER and peroxisomes through lysosome–ER and lysosome–peroxisome membrane contact sites [17–19]. At ER–lysosome membrane contact sites, ER may sequester lysosomal Ca^{2+} [20], and ER Ca^{2+} can refill lysosomal Ca^{2+} stores [21]. In addition, membrane contact sites may also regulate the fission and positioning of endosomes and lysosomes [22].

Nutrient Sensing

Lysosomes provide a mobile physical platform that monitors nutrient levels in the cell [3]. Whereas the yeast vacuole (a lysosome equivalent) is the major storage site for nutrients, in mammalian cells the majority of the building-block molecules used in biosynthesis are supplied from lysosomes [9]. The mammalian target of rapamycin complex 1 (mTORC1) kinase that integrates the information of cellular nutrients and growth signals is primarily localized on the lysosomal membrane [23]. By forming a protein complex with lysosomal GTPases (e.g., Rags and Rheb), V-ATPase, and amino acid exporters (e.g., SLC38A9), mTORC1 'senses' both the luminal and cytosolic pools of amino acids [5]. Upon amino acid starvation, mTORC1 dissociates from the lysosomal membrane and its substrates (Figure 1), resulting in halted protein synthesis but elevated autophagy [3]. Lysosomes also host AMP-activated protein kinase (AMPK), the primary glucose sensor in the cell [5,24]. Glucose starvation activates AMPK by increasing the AMP/ATP ratio or the production of fructose 1, 6-bisphosphatase, a glycolytic metabolite [24]. Moreover, SLC38A9, as well as the cholesterol transporter NPC1, are involved in the sensing of lysosomal sterol lipids [25]. Hence, lysosomes are focal sensing sites of cellular nutrients.

Lysosome Adaptation and Heterogeneity

Nutrient-derived signals regulate lysosome function, trafficking, and adaptation [3,5]. Lysosomal degradation is likely regulated by multiple feedback mechanisms: an increase in cargo vesicles such as autophagosomes can stimulate degradation, and the end-products of degradation can inhibit degradation [5,3]. Indeed, removing metabolites from the cell culture medium (amino acid starvation) serves as the most commonly used experimental paradigm to stimulate autophagic degradation [11]. In addition to promoting autophagosome formation, amino acid starvation also boosts lysosome function, as manifested by increases in the delivery of V-ATPase, membrane fusion and fission, and directional movement [26]. Lysosomal signals may recruit multiple adaptation mechanisms to sustain lysosome consumption (Figure 1). First, nutrient-dependent changes in mTOR activity and phosphoinositide levels generate new lysosomes from tubulated autolysosomes (autophagic lysosome reformation) [13,27]. Second, an ESCRT-dependent membrane self-repair process increases the pool of 'healthy' lysosomes [6]. Third, a transcriptional factor EB (TFEB)-dependent, lysosome-to-nucleus transcriptional program is triggered to stimulate the expression of genes for lysosome biogenesis [28]. Hence,

catabolite-sensitive signals, through their effectors mTOR, AMPK, Rags, and various lipid kinases [5,29], play essential roles in lysosome regulation. Non-nutritional signals, for example, other lysosomal stressors such as oxidants and pathogen invasion, may also activate lysosome adaptation (Figure 1), often through the shared signal transducers and effectors such as mTOR and TFEB [3,5,30].

Although lysosomes can be synchronized in their fusion/reformation cycle upon stimulation, under resting and basal states lysosomes are largely unsynchronized and heterogeneous in individual cells: primary versus secondary lysosomes, endolysosomes versus autolysosomes, activated (acidified) versus resting (neutral) lysosomes, and perinuclear versus peripheral lysosomes [31,32]. Conceivably, the same set of nutrient-sensitive signals, transducers, and effectors, at their ambient levels, determine the basal rate of degradation. Because increasing cargo load and experimentally changing the catabolite levels have profound effects on lysosomal functions and signaling, we propose that the heterogeneity of individual lysosomes reflects differences in their cargo and catabolite contents [3,5], and subsequent differences in the recruitment of transducers and effectors.

Lysosome Ion Homeostasis and Membrane Potential

Lysosomal ion channels and transporters establish the concentration gradients for H^+, Na^+, K^+, Ca^{2+}, and Cl^-, as well as the lysosomal membrane potential ($\Delta\Psi$) across the lysosomal membranes (Figures 1 and 2).

H^+

A hallmark feature of lysosomes is an acidic luminal pH [9,33]. During endosome maturation, lysosomes are gradually acidified by the V-ATPase to reach a plateau pH_{Lumen} (pH_L) of 4.6 [32] (Figure 2). pH_L for individual lysosomes, however, is heterogeneous depending on location and nutrient status in the cell [31,32], which regulates the activities of both V-ATPase and an unidentified H^+ 'leak' conductance [34].

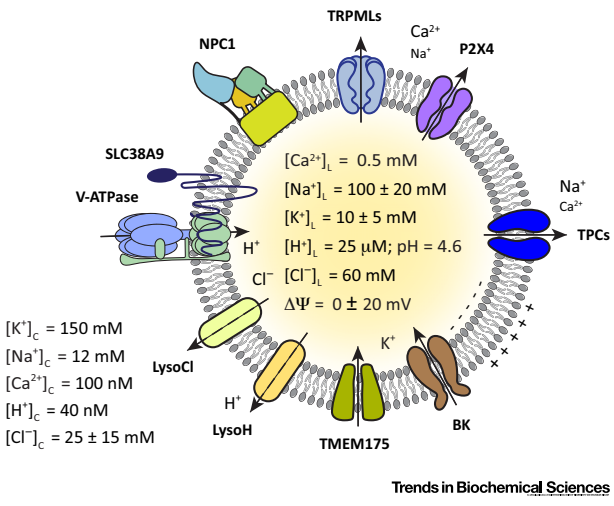

Figure 2. Lysosomal Ion Channels and Transporters. Compared to the cytoplasm, the lysosome lumen contains high concentrations of H^+, Ca^{2+}, and Na^+, but low K^+. At resting conditions, lysosomal membrane potential $\Delta\Psi$ is close to 0 mV. Lysosomal channels that have been characterized using lysosomal patch-clamp include Na^+/Ca^{2+}-permeable TRPMLs, P2X4, and TPCs, and K^+-selective channels BK/Lysok$_{VCa}$ and TMEM175. V-ATPase is the proton pump that acidifies lysosomes. The molecular identities of the lysosomal Cl^- and H^+ conductances are not yet known. SLC38A9 is the lysosomal arginine sensor and transporter; NPC1 is the lysosomal cholesterol transporter. Abbreviations: $[ion]_C$, cytosolic ion concentration; $[ion]_L$, lumenal ion concentration.

Ca^{2+}

Lysosomes serve as small-volume, mobile intracellular Ca^{2+} stores, with a $[Ca^{2+}]_L$ of ~0.5 mM, 5000-fold higher than $[Ca^{2+}]_{Cytosol}$ [35]. A putative Ca^{2+}/H^+ exchanger was proposed to drive lysosomal Ca^{2+} uptake [36,37], but recent studies suggest that ER Ca^{2+} may refill lysosome stores independently of pH [38]. In the latter model, ER Ca^{2+} release-channel inositol trisphosphate (IP_3) receptors, together with a putative lysosomal Ca^{2+} uptake channel/transporter, mediate Ca^{2+} exchange at ER–lysosome membrane contact sites [21,35]. Lysosomal Ca^{2+} release regulates most lysosomal functions [8,13,16,39], including lysosome movement, membrane trafficking, and membrane contact site formation.

Na^+/K^+

The lysosome lumen was thought to be high in K^+ and low in Na^+, suggesting that, like the ER, there are no substantial concentration gradients of Na^+ or K^+ across lysosomal membranes [37,40]. This view has been challenged by several recent whole-endolysosome patch-clamp studies showing the presence of multiple Na^+- and K^+-selective channels in the lysosome [21,41–43]. In addition, isolated lysosomes are reported to contain high $[Na^+]_L$ and low $[K^+]_L$ [43] (Figure 2). The ion transporters that establish the lysosomal Na^+ and K^+ gradients are not yet known, but disruption of the gradients using Na^+/H^+ and K^+/H^+ ionophores is known to impair various lysosomal functions, including lysosomal acidification and catabolite export [1,21].

Cl^-

$[Cl^-]_L$ was estimated to be 60–80 mM using the pH-insensitive probe Clensor [44]. Lysosome Cl^-, maintained by the CLC family of Cl^-/H^+ exchangers, may provide counterions to support acidification [45–47].

$\Delta\Psi$

Given the steep concentration gradients of Na^+, K^+, and H^+, lysosomal $\Delta\Psi$ ($= \Psi_{cytosol} - \Psi_{lumen}$) is determined by the relative permeabilities of these ions [21]. For a lysosome with a diameter of 0.3 μm, there are only 9×10^5 (in molecules) of Na^+, 9×10^4 K^+, and 200 H^+ in the lumen (assuming $[Na^+]_L$ = 100 mM, $[K^+]_L$ = 10 mM, and $[H^+]_L$ = 25 μM; Figure 2). Theoretically, if this lysosome has 10 channels with a single-channel conductance of 10 pS and 0.1 open probability, at a driving force of 10 mV, 600 ions would pass across the lysosomal membrane per millisecond. However, upon channel opening, luminal ion composition would be altered quickly (in milliseconds) to reduce the electrochemical gradients (Figure 3). Hence, luminal ion depletion and large or lasting changes in $\Delta\Psi$ would not occur *in situ* (Figure 3). The reported lysosomal $\Delta\Psi$ values are scattered but cluster at small negative values [37], for example −19 mV in macrophage phagolysosomes [48]. In the current-clamped vacuolin-enlarged lysosomes at assumed high $[Na^+]_L$, $\Delta\Psi$ is found to be slightly positive (e.g., +20 mV) [21,49]. We propose that, under ambient levels of cellular signals, 'resting' lysosomal $\Delta\Psi$ is near to 0 mV (±20 mV; Figures 2 and 3). Upon stimulation, lysosome-associated cellular cues activate various resident Na^+, H^+, and K^+ channels to rapidly but transiently change the $\Delta\Psi$ of individual lysosomes to regulate various $\Delta\Psi$-dependent lysosomal functions such as catabolite export (Figure 3).

Lysosomal Ca^{2+} and Na^+ Channels

There are at least three types of lysosomal Ca^{2+} (LysoCa) channels in the mammalian cells: TRPML1–3, TPC1–2, and P2X4 (Figure 2), all of which have been directly measured and verified by direct recordings on native lysosomes. Because many lysosomal functions are Ca^{2+}-dependent, cellular cues may regulate them by activating LysoCa channels [35]. Unlike

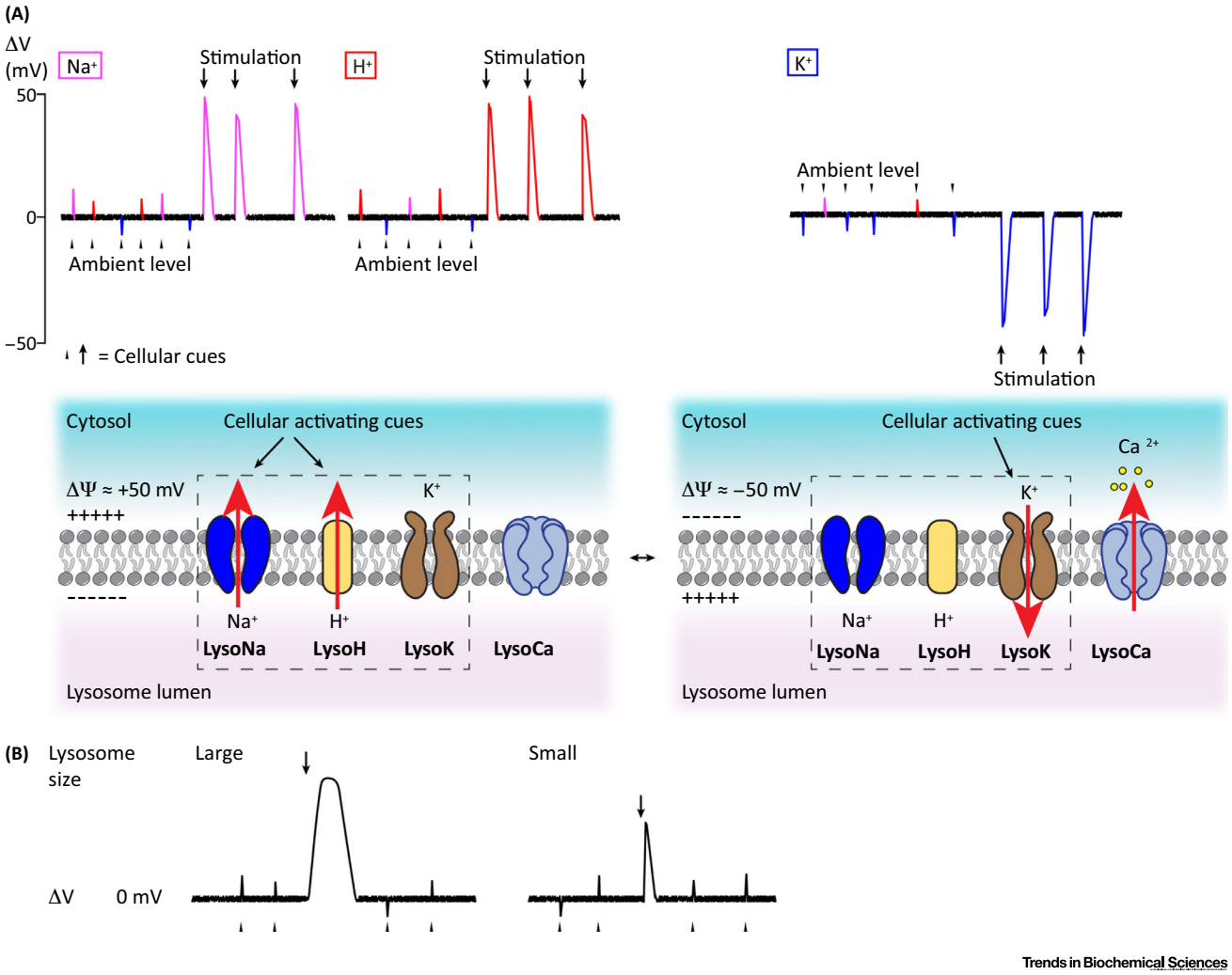

Figure 3. Regulation of Lysosomal Membrane Potential. (A) Lysosomal membrane potential ($\Delta\Psi$) is determined by the relative permeability of the lysosomal membranes to H^+, Na^+, and K^+ through an unidentified H^+-leak conductance (LysoH), LysoNa channels (e.g., TPCs), and LysoK channels (e.g., BK/LsyoK$_{VCa}$ and TMEM175). TPCs are Na^+ channels activated by phosphatidylinositol 3,5-bisphosphate [PI(3,5)P_2]; TMEM175 is a K^+ leak channel; BK channels are activated by both voltage and Ca^{2+}. Lysosomal $\Delta\Psi$ is depolarized when LysoH and LysoNa channels are open (left panels), and hyperpolarized when LysoK channels are open (right panels). In resting conditions, lysosomal $\Delta\Psi$ fluctuates around 0 mV (±20 mV) owing to minimal activation of lysosomal ion channels by ambient levels of cellular signals. Large $\Delta\Psi$ changes (±50 mV) occur when the levels of lysosomal ion channel-activating signals are high. Activation of LysoK reduces or reverses lysosomal $\Delta\Psi$, providing a larger driving force for LysoCa-mediated lysosomal Ca^{2+} release. (B) Lysosome size may influence the duration and amplitude of lysosome $\Delta\Psi$ changes. Luminal ion composition changes more dramatically in small-sized lysosomes. Hence, larger lysosomes exhibit bigger and longer-lasting $\Delta\Psi$ changes upon identical (in strength and duration) stimulation.

TRPMLs and P2X4, TPCs, although Ca^{2+}-permeable, are Na^+-selective [43,49,50], and are hence more likely to be involved in direct regulation of lysosomal $\Delta\Psi$- and Na^+-dependent functions. The focus of the current review is to discuss how cellular cues regulate lysosomal functions through modulation of channels in the lysosomal membranes. Hence, additional candidate LysoCa and LysoNa channels that are only inferred by pharmacology or antibodies are not discussed here.

Table 1. Lysosome Ion Channels and Their Functions[a]

Lysosomal ion channel	Permeability	Subcellular localization	Lysosome targeting motif[b]	Acidic pH_L dependence	Endogenous agonists	Endogenous Inhibitors	Synthetic agonists	Synthetic inhibitors	Lysosome function	Human disease and mouse knockout phenotype
TRPML1	Cation, non-selective $Ca^{2+} > K^+ = Na^+ > Cs^+$	LEL	$L^{577}L^{578}$ $L^{15}L^{16}$	↑	$PI(3,5)P_2$, ROS	$PI(4,5)P_2$, sphingomyelin, adenosine	ML-SAs, SF-51, MK6-83	ML-SIs	Lysosomal exocytosis; retrograde transport; plasma membrane repair; TFEB activation; ROS sensing	ML-IV; NPC; AD
TRPML2	Cation, non-selective $Na^+ \sim K^+ \sim Cs^+$, $Ca^{2+} \sim Fe^{2+}$	RE, LEL, PM			$PI(3,5)P_2$		ML-SAs	ML-SIs	Lysosome trafficking regulation	Impaired immune response
TRPML3	Cation, non-selective Ca^{2+} $> Na^+ > K^+ >> Cs^+$	EE, LEL, PM		↓	$PI(3,5)P_2$	$PI(4,5)P_2$	ML-SAs, SFs	ML-SIs	Exosome release	
TPC1	Na^+/Ca^{2+}	EE, LEL	$L^{83}I^{84}$	↑	$PI(3,5)P_2$, voltage sphingosine (?), NAADP (?),	Cytoplasmic ATP, mTOR		Tetrandrine (?), Ned19 (?)	Amino acid export; endolysosomal excitability; pH_L homeostasis; cellular ATP sensor; ER–endosome membrane contact site formation	Reduced virus entry; impaired exercise endurance after fasting
TPC2	Na^+/Ca^{2+}, $P_{Na}/P_{Ca} > 10$, $P_{Na}/P_K > 60$	LEL	$L^{11}L^{12}$		$PI(3,5)P_2$, NAADP (?)	Cytoplasmic ATP, mTOR, Mg^{2+}		Tetrandrine (?), Ned19 (?)	Amino acid export; pH_L homeostasis autophagy regulation; cellular ATP sensor; lysosome trafficking regulation	Hypercholesterolemia; impaired exercise endurance after fasting; PD; NAFLD; reduced virus entry
P2X4	Ca^{2+}, Na^+	LEL, PM	$L^{22}I^{23}$ $Y^{372}xxV$ $Y^{378}xxGL$	↓	Luminal ATP				Lysosome fusion	
TMEM175	K^+, $P_K/P_{Na} > 30$	EE, LEL	$L^{499}L^{500}$ (?)	↓				4-AP, Zn^{2+}	pH_L homeostasis; autophagosome–lysosome fusion; lysosome resting $\Delta\Psi$ maintenance	PD
SLO1 (BK)	K^+	LEL, MT, PM	$L^{488}I^{489}$, $L^{734}I^{735}$ (?)	↓	Ca^{2+}		NS1619	Paxillin, IBTX	Lysosomal Ca^{2+} release and refilling; lysosome $\Delta\Psi$ control	Various neurological symptoms; impaired glucose homeostasis

[a]Abbreviations: AD, Alzheimer's disease; 4-AP, 4-aminopyridine; EE, early endosome; ER, endoplasmic reticulum; IBTX, iberiotoxin; LEL, late endosome and lysosome; ML-IV, type IV mucolipidosis; ML-SAs, mucolipin synthetic agonists (TRPML1 synthetic agonists); ML-SIs, TRPML1 synthetic inhibitors; MT, mitochondria; mTOR, mammalian target of rapamycin; NAADP, nicotinic acid adenine dinucleotide phosphate; NAFLD, non-alcoholic fatty liver disease; NPC, Niemann–Pick type C disease; PD, Parkinson's disease; pH_L, lysosome luminal pH; PM, plasma membrane; RE, recycling endosome; ROS; reactive oxygen species; TFEB, transcription factor EB.
[b]Based on the human protein.

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Mucolipin TRP Channels (TRPMLs)

The mucolipin subfamily of transient receptor potential (TRP) channels, TRPML1–3, are tetrameric six-transmembrane (TM) channels that are localized exclusively on endosomes and lysosomes [51] (Table 1). Whereas TRPML1 is ubiquitously expressed, TRPML2 and TRPML3 are more restricted in their expression [8,51]. Although TRPML1 has been most extensively studied, genetic and cell-biological studies of mammalian TRPML2 and TRPML3, as well as non-mammalian (e.g., *Drosophila* and *C. elegans*) TRPMLs, suggest conserved roles of mucolipin channels in regulating lysosomal functions [52,101]. Loss-of-function mutations of human TRPML1 cause type IV mucolipidosis (ML-IV), a LSD with symptoms of neurodegeneration and muscular dystrophy [53]. Di-leucine motifs at the intracellular N- and C-termini are responsible for the lysosomal localization of TRPML1 [8]. Whole-endolysosome patch-clamp studies reveal that mammalian TRPML1–3 channels are permeable to both Ca^{2+} and Na^{+}, as well as to K^{+} and heavy metal ions such as Fe^{2+} and Zn^{2+} [54]. Recent high-resolution structural studies have confirmed that the cationic selectivity is determined by negatively charged amino acid residues in the pore loop, and the activation gate is made of the segment S6 TM helices [55,56].

Endogenous Agonists: $PI(3,5)P_2$ and ROS

Phosphatidylinositol 3,5-bisphosphate [$PI(3,5)P_2$] is a late endosome and lysosome (LEL)-specific phosphoinositide that regulates several trafficking steps of lysosomes [29]. Inhibiting $PI(3,5)P_2$ synthesis genetically or pharmacologically causes lysosomal trafficking defects mimicking ML-IV (TRPML1 null mutant) cells [29]. $PI(3,5)P_2$ potently activates whole-lysosomal TRPML1–3 and *Drosophila* TRPML channels by binding to positively charged amino acid residues in the cytosolic N-terminal domain, as modeled in high-resolution structures [55,57,58] (Figure 4). $PI(3,5)P_2$ binding may lead to conformational changes in the S2–S3 linker to open the S6 gate (Figure 4) [55,58]. Whereas cellular $PI(3,5)P_2$ levels change before lysosomal trafficking events, in $PI(3,5)P_2$-deficient cells many lysosomal functions are defective, including retrograde movement, exocytosis, and reformation [8,29,59]. Hence, TRPMLs may serve as an essential signal transducer for lysosomal $PI(3,5)P_2$. Consistently, mutations in the $PI(3,5)P_2$ binding sites of TRPML1 (Figure 4) affect $PI(3,5)P_2$-dependent lysosomal functions [13]. However, other $PI(3,5)P_2$ effectors may also contribute to the regulation of these lysosomal functions [29].

Reactive oxygen species (ROS) are environmental stress signals that regulate multiple cellular functions, including autophagosome and lysosome biogenesis [30,60]. ROS levels are elevated upon mitochondrial damage, and this triggers mitophagy to remove damaged mitochondria and excessive ROS, working as a negative feedback mechanism to maintain cellular health [30] (Figure 1). ROS induce nuclear translocation of TFEB, a transcriptional regulator of autophagosome and lysosome biogenesis [28], in a TRPML1- and lysosomal Ca^{2+}-dependent manner [30]. ROS directly and robustly activate lysosomal TRPML1 channels, suggesting that TRPML1 may function as a signal transducer for ROS to regulate lysosome function [30,60]. Consistent with this hypothesis, ROS sensitivity of TRPML1 is required for ROS-induced TFEB activation and mitophagy [30]. Generally speaking, because TRPML1 is activated by more than one cellular cue, it will be necessary to introduce knock-in mutations at agonist-specific binding sites to test which activation mechanism is key to a specific function.

Endogenous Inhibitors: mTOR, $PI(4,5)P_2$, Sphingomyelin, Adenosine

mTOR was recently shown to phosphorylate and inhibit TRPML1 [61–63]. Because TRPML1 is activated during amino acid starvation [64], it is possible that starvation-induced mTOR inhibition serves as a nutrient-derived signal to modulate TRPML1-mediated Ca^{2+} release.

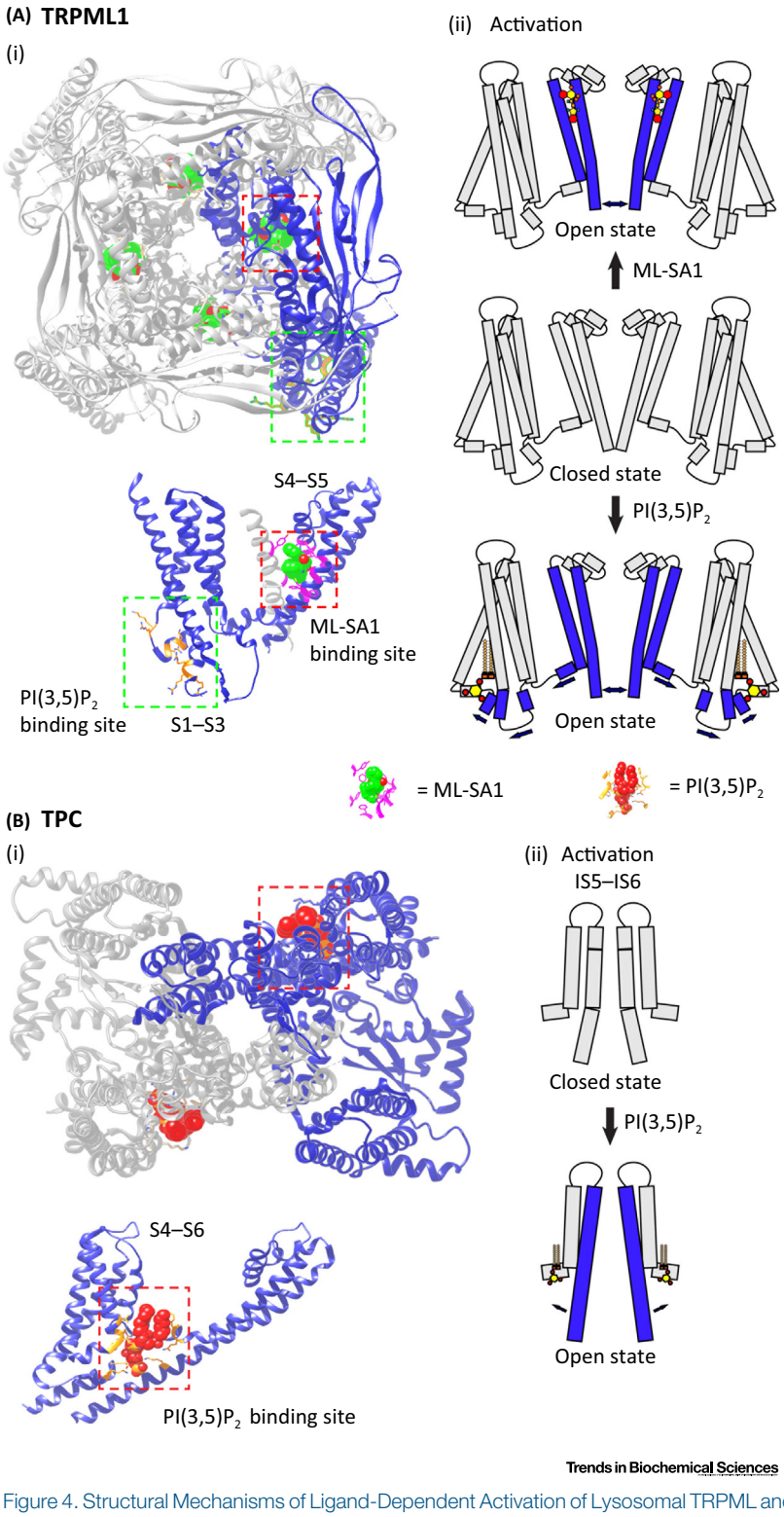

Figure 4. Structural Mechanisms of Ligand-Dependent Activation of Lysosomal TRPML and TPC Channels.
(A) High-resolution structures of TRPML1. (i) The upper panel shows the top view of TRPML1 in the tetrameric assembly. Red

(Figure legend continued on the bottom of the next page.)

Two cell-surface lipids reportedly inhibit TRPML1: phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2] and sphingomyelin, and this inhibition is proposed to prevent TRPML1 from being active in non-lysosomal compartments [65,66]. However, PI(4,5)P_2 was recently reported to be generated on the lysosomal membrane to regulate mTOR-dependent lysosome reformation [27]. Because lysosome reformation is a Ca^{2+}-dependent process [13], it is possible that PI(4,5)P_2 inhibition of TRPML1 plays an important role in lysosome reformation. Lysosomal PI(4,5)P_2 and sphingomyelin levels are aberrantly elevated in some LSD cells [65,67]. Likewise, in adenosine deaminase-deficient cells, luminal adenosine accumulation may inhibit TRPML1 to cause lysosomal dysfunction [68]. Hence, pathogenic TRPML1 inhibition may underlie the trafficking defects in many LSDs.

Other Modulators: Luminal Acidic pH and cAMP/PKA

Luminal pH modulates the channel activities of TRPML1 and TRPML3 [69] (Table 1). Hence, cellular cues affecting lysosome acidification may regulate lysosome functions via TRPML-dependent mechanisms. In uroepithelial cells, pathogen invasion induces lysosome alkalization to trigger TRPML3- and Ca^{2+}-dependent exosome release [69]. cAMP signaling is known to regulate lysosome acidification and function, but the underlying mechanisms are not clear [70]. In parietal cells, PKA increases the activity of TRPML1 that is localized in the tubulovesicles [71,72]. Future studies may reveal whether cAMP/PKA signaling regulates lysosome function through TRPML1.

TRPML-Specific Synthetic Agonists and Inhibitors

Small-molecule synthetic modulators have been used to probe TRPML-dependent lysosomal functions [65,73]. ML-SA (mucolipin synthetic agonist 1) compounds, by binding to a hydrophobic pocket above the S5–S6 gate (Figure 4) [56], specifically activate whole-lysosome TRPMLs, but not other lysosomal ion channels. Binding mutations were reported to selectively abolish ML-SA1 activation without interfering with PI(3,5)P_2 activation [56]. Hence, synthetic agonists may provide a powerful tool to acutely activate and inhibit TRPML1, linking channel activity with specific lysosomal functions. For example, ML-SA activation of TRPML1 is sufficient to trigger lysosomal exocytosis, TFEB activation, and retrograde transport, and the effects were abolished in TRPML1 knockout cells [8,13,30]. Moreover, many lysosomal functions mediated by endogenous activation of TRPML1 are blocked by ML-SIs (synthetic inhibitors) [13,30].

Two-Pore TPC Channels

TPC1 and TPC2 are dimeric 12-TM cation channels that are localized exclusively on endosomes and lysosomes via di-leucine motifs [8,74]. TPCs, ubiquitously expressed in mammalian tissues [8,52,75], have been shown to be K^+-impermeable Na^+-selective cation channels in lysosomal electrophysiological analyses [43,52]. In cell studies, TPCs are believed to be lysosomal Ca^{2+}-release channels [52,43].

NAADP, PI(3,5)P_2, and Sphingosine

TPCs were first suggested to mediate nicotinic acid adenine dinucleotide phosphate (NAADP)-dependent Ca^{2+} release from endolysosomes [74,76]. Small NAADP-activated Ca^{2+} currents

and green boxes indicate the ML-SA1 and PI(3,5)P_2 binding sites, respectively. The lower panels illustrate the ML-SA1 and PI(3,5)P_2 binding sites in one single TRPML1 subunit. (ii) Cartoon illustrations of ligand-induced channel opening. (B) High-resolution structures of TPC2. (i) The upper panel shows the top view of TPC2 in the dimeric assembly. The PI(3,5)P_2 binding sites are enclosed in the red box. The lower panel illustrates the PI(3,5)P_2 binding sites in one single TPC2 subunit. (ii) Cartoon illustrations of PI(3,5)P_2-induced channel opening. Abbreviations: IS5–6, pore-lining inner helices 5–6; ML-SA1, mucolipin synthetic agonist 1; PI(3,5)P_2, phosphatidylinositol 3,5-bisphosphate; S1–S6, transmembrane segments 1–6.

were reported in TPC2-overexpressing cells in some but not other studies [52]. By contrast, PI(3,5)P_2 robustly activates TPC currents in all lysosomal electrophysiological studies [43,49,77]. Hence, TPCs are unlikely to be the direct target of NAADP, as suggested by photolabeling studies using TPC knockouts [78]. NAADP-mediated Ca^{2+} release and TPCs reportedly facilitate formation of endosome–ER membrane contact sites [79] and Ebola virus entry [52,79,80]. However, highly potent inhibitors of the NAADP receptor (e.g., Ned-19), which are widely used in NAADP/TPC cell studies to draw firm conclusions, barely block whole-lysosome TPC currents ([43]; X. Zhang and H. Xu, unpublished). Sphingosine is also shown to trigger TPC1-dependent Ca^{2+} release and subsequent TFEB activation [81]. However, sphingosine failed to directly activate TPCs in the lysosomal recordings (X. Zhang and H. Xu, unpublished).

Several recent studies demonstrated that mammalian TPCs are PI(3,5)P_2-activated Na^+-selective channels ($P_{Na}/P_{Ca} > 10$ and $P_{Na}/P_K > 60$) [43,50]. By contrast, plant TPC1 was found to be Ca^{2+}-selective. Structure-guided mutational analyses have identified the determinants in the pore loop that are responsible for Ca^{2+} versus Na^+ selectivity [50]. High-resolution structural studies have demonstrated that PI(3,5)P_2 opens the channel gate by binding to positively charged amino acid residues in the S4–S5 link and S6 [77] (Figure 4). In the absence of PI(3,5)P_2, the background Na^+-permeability is minimal and lysosomal $\Delta\Psi$ is independent of TPCs (X. Zhang and H. Xu, unpublished); in the presence of PI(3,5)P_2, lysosomal $\Delta\Psi$ is depolarized via TPCs [21] (Figure 3). Hence, TPCs are essential signal transducers for PI(3,5)P_2 to regulate lysosomal $\Delta\Psi$ [49].

ATP, mTOR, pH, and Voltage

TPCs are also modulated by several cytosolic and luminal factors. TPC2 is modulated by intracellular ATP through mTORC1 kinase [49]. In pulmonary arterial smooth muscle cells, pharmacological inhibition of mTOR leads to TPC-dependent Ca^{2+} release [82]. Hence, TPC2 may couple the metabolic status of the cell with lysosome function. TPC knockout cells exhibit defects in autophagy regulation, lipid metabolism, Ebola virus infection, lysosome pH stability, and amino acid export [52,83]. It is not clear whether these TPC functions are mediated by mTOR inhibition or PI(3,5)P_2 activation. It was presumed that mTOR inhibition plays an important role during amino acid starvation [49]; however, PI(3,5)P_2 levels are known to decrease dramatically upon amino acid starvation [29,59]. Therefore, separating these effects may require knock-in mutations that selectively abolish PI(3,5)P_2 activation or mTOR inhibition.

Lysosome pH and voltage may specifically regulate TPC1 but not TPC2 [49]. The voltage-dependence of TPC1 is mediated by arginine residues in the S4 segment of the second repeat [77,84]. The role of such regulation is unclear because TPC1 and TPC2 are believed to play similar or redundant roles in most TPC-dependent lysosomal functions [52]. It was recently reported that NAADP activation of TPC1 required arginine residues that are present in TPC1 but not in TPC2 [84]. However, NAADP is believed by many investigators to activate both TPC1 and TPC2 [52].

P2X4

P2X4 is a trimeric two-TM channel that is permeable to both Na^+ and Ca^{2+} when activated by ATP [85,86]. P2X4 is dually localized on both lysosomal and plasma membranes, and lysosomal targeting requires both di-leucine and tyrosine motifs [85,87]. Lysosomal P2X4 is activated by luminal ATP and inhibited by acidic lysosomal pH [85,88]. Activation of P2X4-dependent Ca^{2+} release may drive homotypic lysosome fusion in a calmodulin-dependent manner [89].

Lysosome K^+ channels

Consistent with the presence of a large K^+ concentration gradient across the lysosomal membranes, there are multiple K^+-selective channels in the lysosome. Furthermore, K^+ ionophores and changes in cytosolic K^+ are known to affect lysosomal $\Delta\Psi$ [21,49].

TMEM175

Human TMEM175 is a dimeric 12-TM K^+- and Cs^+-selective channel that is expressed in both early endosomes and LELs [41] (Table 1). Unlike canonic K^+ channels that use the GYG motif as the selectivity filter, TMEM175 harbors a FSD motif on both TM1 and TM7 to achieve a high K^+ over Na^+ selectivity [90]. Whereas overexpression of TMEM175 decreased lysosomal $\Delta\Psi$, knockout of TMEM175 in macrophages slightly increased lysosomal $\Delta\Psi$ [41]. These results are consistent with the early observations showing that lysosomes isolated from liver cells are permeable to both Cs^+ and K^+ [91]. Hence, TMEM175 may mediate a constitutively active background K^+ conductance in the lysosome [41] (Figure 3). However, TMEM175 currents are relatively small and are not detectable in most isolated lysosomes [21], suggesting that TMEM175 might be further potentiated by particular cellular cues to reach more negative $\Delta\Psi$ under physiological conditions. One potential candidate is luminal pH, and pH regulation of TMEM175 is required for maintaining lysosomal pH homeostasis during starvation [41] (Table 1). TMEM175 may also function as a negative regulator of autophagosome–lysosome fusion during starvation [41,92]. Hence, TMEM175 may be required for nutrient- and pH-dependent regulation of lysosome $\Delta\Psi$.

BK/LysoK$_{VCa}$

BK channels are tetrameric large-conductance Ca^{2+}-activated Kv channels that are expressed at the plasma membrane of excitable cells where they act as negative regulators of membrane excitability. Two recent studies suggest that BK-like currents are also present in the lysosomes of both excitable and non-excitable cells (lysosome voltage-dependent Ca^{2+}-activated K^+ channels; LysoK$_{VCa}$) [21,42]. Upon peri-lysosomal Ca^{2+} increase, lysosomal $\Delta\Psi$ changes rapidly in a LysoK$_{VCa}$ dependent-manner [21,42]. Given the large conductance, opening of a single LysoK$_{VCa}$ channel is sufficient to produce significant changes of lysosomal $\Delta\Psi$ [21]. A negative $\Delta\Psi$ may increase the driving force for lysosomal Ca^{2+} release [21,42] (Figure 3), allowing LysoK$_{VCa}$ to regulate Ca^{2+}-dependent lysosomal functions. In addition, LysoK$_{VCa}$ and its Ca^{2+} sensitivity are required for refilling of lysosomal Ca^{2+} stores [21,35]. Hence, LysoK$_{VCa}$ is a positive regulator of lysosomal Ca^{2+} signaling. Because ER–lysosome membrane contact sites may be required for Ca^{2+} refilling, it is possible that BK/LysoK$_{VCa}$ regulate ER–lysosome membrane contact site formation through a $\Delta\Psi$-dependent mechanism [35].

Lysosomal Ion Channels in Lysosomal Diseases

LSDs

Mutations of TRPML1 cause ML-IV, and ML-IV patients cannot walk or talk [53]. Owing to the lysosomal trafficking defects, undigested biomolecules and lipofuscin accumulate in ML-IV lysosomes [53]. LSDs may also be caused by human mutations that affect the production of cellular cues that activate or inhibit lysosomal channels [57]. Mutations in NPC1 cause Niemann–Pick type C disease (NPC), a neurodegenerative LSD characterized by cholesterol/sphingolipid accumulation [25]. Excessive sphingomyelin accumulation in the lysosome inhibits TRPML1 [65]. Likewise, mutations in PIKfyve, the PI(3,5)P_2-synthesizing enzyme, and OCRL, a PI(4,5)P_2 phosphatase, also cause LSD-like symptoms [57,67]. In all these LSD cells, reduced lysosomal TRPML1 activity may underlie or contribute to trafficking defects and lysosome dysfunction.

Common Neurodegenerative Diseases

Alzheimer's (AD) and Parkinson's (PD) are common neurodegenerative diseases in which lysosome insufficiency is believed to be pathogenic [93]. Whereas impaired TRPML1 signaling may contribute to AD [94,95], genome-wide association studies (GWAS) have identified a link between TMEM175 and PD [96]. TMEM175-deficient neurons are susceptible to α-synuclein aggregation [92]. A major cause to inherited and sporadic PD is gain-of-function mutations in LRRK2 kinase [97,98], and LRRK2-dependent lysosomal defects are ameliorated by TPC2 inhibition [97]. Hence, lysosomal channels may have a general role in lysosome-related diseases.

Small-Molecule Lysosomal Channel Modulators in the Potential Treatment of Lysosomal Diseases

Synthetic modulators may mimic endogenous agonists to regulate lysosomal functions. Small-molecule TRPML1 agonists were sufficient to restore lysosome function in ML-IV patient fibroblasts carrying partial loss-of-function mutations [52]. In most LSDs, hydrolase deficiency causes primary lysosomal storage, which in turn affects lysosome function to cause secondary storage, resulting in a vicious cycle [8]. Because TRPML1 is a major regulator of lysosomal trafficking [65], activation of TRPML1 may facilitate lysosomal trafficking to promote cellular clearance and reduce lysosomal storage in many LSDs. Indeed, TRPML1 overexpression and ML-SAs can promote cholesterol clearance in NPC cells [65,99,100]. Likewise, BK agonists were reported to decrease cellular storage in several LSD fibroblasts [99]. Given the similarity between LSDs and common neurodegenerative diseases [93], lysosomal channel modulators may promote cellular clearance in lysosome-related diseases in general.

Future Directions

We have witnessed the discovery of many lysosomal ion channels, as well as the cellular cues that regulate them. However, the molecular determinants responsible for several lysosomal conductances have yet to be established. For instance, an unidentified H^+ 'leak' channel seems to be regulated by nutrient-sensitive cellular cues to affect lysosomal pH [34,49]. Likewise, there are several unidentified Cl^- conductances in the lysosome (H. Xu *et al.*, unpublished) which are not mediated by the known lysosomal CLC transporters [46]. Although lysosomal degradation is known to generate large amount of osmolytes, it remains unclear whether there are water channels and osmo-sensitive channels. To study how cellular cues regulate lysosomal ion flux and $\Delta\Psi$ changes in intact cells, it will be necessary to develop lysosome-targeted voltage sensors and pH-insensitive luminal $Ca^{2+}/Na^+/K^+$ sensors. Finally, super-resolution live imaging of lysosomes may help to reveal how lysosomal Ca^{2+} release and $\Delta\Psi$ changes are correlated with lysosomal membrane dynamics and membrane contact site formation.

Outstanding Questions

What are the molecular determinants responsible for several lysosomal conductances? For instance, an unidentified H^+ 'leak' channel is regulated by nutrient-sensitive cellular cues to affect lysosomal pH. Likewise, there are several unidentified Cl^- conductances in the lysosome, which are not mediated by the known lysosomal CLC transporters.

How are lysosomal Na^+, and K^+, and Ca^{2+} channels activated by endogenous cellular cues and nutrient signals?

Although lysosomal degradation is known to generate large amount of osmolytes, it remains unclear whether water channels and osmo-sensitive channels are also present.

To study how cellular cues regulate lysosomal ion flux and membrane potential changes in intact cells, it will be necessary to develop lysosome-targeted voltage sensors and pH-insensitive luminal $Ca^{2+}/Na^+/K^+$ sensors.

Super-resolution live imaging of lysosomes may help to reveal how lysosomal Ca^{2+} release and membrane potential changes are correlated with lysosomal membrane dynamics and lysosome–organelle membrane contact site formation.

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