TRPML1: An Ion Channel in the Lysosome

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Contents

Abstract

The first member of the mammalian mucolipin TRP channel subfamily (TRPML1) is a cation-permeable channel that is predominantly localized on the membranes of late endosomes and lysosomes (LELs) in all mammalian cell types. In response to the regulatory changes of LEL-specific phosphoinositides or other cellular cues, TRPML1 may mediate the release of Ca^{2+} and heavy metal Fe^{2+}/Zn^{2+}ions into the cytosol from the LEL lumen, which in turn may

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regulate membrane trafficking events (fission and fusion), signal transduction, and ionic homeostasis in LELs. Human mutations in *TRPML1* result in type IV mucolipidosis (ML-IV), a childhood neurodegenerative lysosome storage disease. At the cellular level, loss-of-function mutations of mammalian *TRPML1* or its *C. elegans* or *Drosophila* homolog gene results in lysosomal trafficking defects and lysosome storage. In this chapter, we summarize recent advances in our understandings of the cell biological and channel functions of TRPML1. Studies on TRPML1's channel properties and its regulation by cellular activities may provide clues for developing new therapeutic strategies to delay neurodegeneration in ML-IV and other lysosome-related pediatric diseases.

Keywords
Lysosome • Endosome • TRP channel • Membrane trafficking • Ca^{2+} • Phosphoinositide

1 Gene

Human TRPML1 (or mucolipin-1/MCOLN1), the founding member of the TRPML subfamily, is encoded by the *MCOLN1* gene localized on chromosome 19 (19p13.2–13.3; base pair positions 7,587,496–7,598,895) (Bargal et al. 2000; Bassi et al. 2000; Slaugenhaupt et al. 1999; Sun et al. 2000). No splicing variant has been reported for the human *TRPML1* gene. In contrast, the almost identical mouse *Trpml1* gene contains two alternatively spliced isoforms (Slaugenhaupt 2002). Although there are two other *TRPML1*-related genes, i.e., *TRPML2* and *TRPML3*, in human and mouse genomes (Cheng et al. 2010), only one single gene in *C. elegans* and *Drosophila*, *cup*-5 and *trpml* (CG8743), respectively, encodes the TRPML protein, which shares 30–40 % sequence identity with human TRPML1 (Fares and Greenwald 2001). Genetic studies on model organisms suggest that human *TRPML1* plays an evolutionarily conserved role in the cell biology of the lysosome.

2 Expression and Subcellular Localization

TRPML1 is ubiquitously expressed in every mouse tissue, with the highest levels of mRNA expression in the brain, kidney, spleen, liver, and heart (Falardeau et al. 2002; Samie et al. 2009). Consistent with this expression pattern, the loss of TRPML1 results in enlarged late endosomes and lysosomes (LELs) and the accumulation of lysosomal storage materials in most cell types of ML-IV patients and *Trpml1* knockout mice (Slaugenhaupt 2002; Venugopal et al. 2007).

Cellular phenotypes of ML-IV and its mouse model indicate that TRPML1 is predominately localized on the membranes of LELs, but heterologously expressed

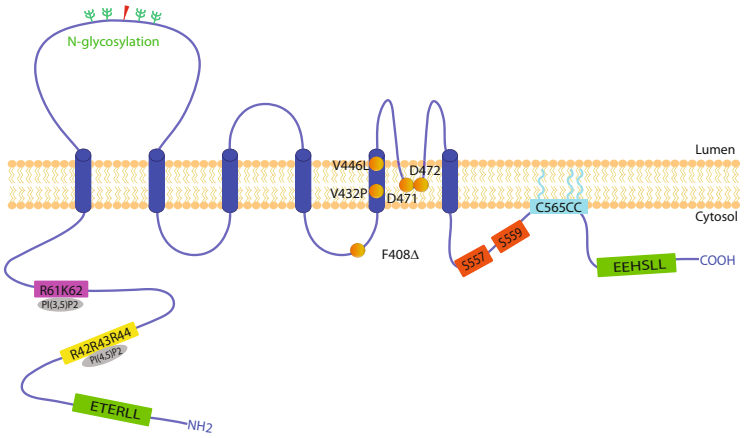

Fig. 1 Structural aspects of TRPML1. TRPML1 consists of six transmembrane (6TM) domains with the amino-terminal (NH_2) and carboxyl-terminal (COOH) tails facing the cytosol. The first luminal loop is uniquely large and contains four N-glycosylation sites and a cleavage site. Two di-leucine motifs ETERL^{15}L and EEHSL^{577}L are located separately at each tail to mediate the localization of TRPML1 to late endosomes and lysosomes (LELs). At the N-terminus of TRPML1, several positively charged amino acid residues are predicted to interact with phosphoinositides with Arg61 and Lys 62 for PI(3,5)P_2 and Arg42/Arg43/Arg44 for PI(4,5)P_2, respectively. At the C-terminus, there are two potential PKA sites (S^{557} and S^{559}) and three potential palmitoylation sites (C$^{565–567}$). Two negatively charged amino acid residues (D^{471} and D^{472}) are potential pore-forming determinants. Gain-of-function mutations are found in the lower part of the S5 (e.g., V^{432}P). Loss-of-function mutations that cause ML-IV patients are throughout the protein (e.g., F408Δ)

GFP or mCherry-tagged TRPML1 proteins are also detected in the early endosomes and plasma membrane (Thompson et al. 2007; Vergarajauregui and Puertollano 2006). Immunostaining and gradient fractionation studies have confirmed the LEL localization of TRPML1 (Kim et al. 2009; Zeevi et al. 2009). GFP fusion proteins of TRPML1 are co-localized nicely (>80 %) with the lysosomal-associated membrane proteins1, 2, and 3 (Lamp1–3) (Manzoni et al. 2004). Moreover, in the gradient fractionation analysis, TRPML1 proteins were found primarily in the Lamp1-positive fractionations (Dong et al. 2010a; Kim et al. 2009).

The LEL localization of TRPML1 is instructed by two di-leucine motifs located separately in the N-terminal and the C-terminal cytosolic tails (see Fig. 1). The N-terminal motif (L^{15}L) interacting with clathrin adaptor protein 1 and 3 (AP1 and AP3) mediates a direct transport of TRPML1 proteins from trans-Golgi network (TGN) to LELs, whereas the C-terminal motif (L^{577}L) directs AP2-dependent internalization from the plasma membrane, which is followed by endocytic trafficking to LELs (Abe and Puertollano 2011; Pryor et al. 2006; Vergarajauregui and Puertollano 2006) (Fig. 1). When both di-leucine motifs are mutated (TRPML1-L^{15}L/AA-L^{577}L/AA), whole-cell TRPML1 currents become detectable (Zhang et al. 2012a).

3 The Channel Structure

Human TRPML1 is a 580-amino acid protein with a molecular mass of 65 kDa (Slaugenhaupt 2002). Due to the lack of a crystal structure for TRPML1, our knowledge about the topology of TRPML1 is mainly gained from bioinformatic analysis and biochemical and structural studies on other TRP channels (Cheng et al. 2010; Dong et al. 2010b). Similar to other TRP channels, TRPML1 consists of six putative transmembrane-spanning domains (TMs, S1–S6) with the amino-terminal (NH_2 or N) and carboxyl-terminal (COOH or C) tails facing the cytosol (Fig. 1). Strikingly, TRPML1 possesses a large highly N-glycosylated luminal loop separating the first two TMs, in which a proteolytic cleavage site with uncharacterized function is located (see Fig. 1) (Kiselyov et al. 2005; Miedel et al. 2006; Puertollano and Kiselyov 2009). The channel pore of TRPML1 is predicted to be formed by the linker or the so-called "pore-loop" region between S5 and S6 (Cheng et al. 2010). Consistently, the pore mutations of TRPML1 are known to affect the conductance and selectivity of TRPML1 channels (Dong et al. 2010a; Pryor et al. 2006; Vergarajauregui and Puertollano 2006). S5 and S6 are presumed to form the channel gate, and the gain-of-function gating mutations are found in the lower part of the S5 domain (Dong et al. 2009; Grimm et al. 2012; Xu et al. 2007) (Fig. 1). The COOH-terminus of TRPML1 contains two potential PKA sites: ($Ser^{557}$and Ser^{559}; see Fig. 1) (Vergarajauregui et al. 2008b) and three cysteine residues ($C^{565}CC$) for potential palmitoylation to ensure association with LEL membranes (Vergarajauregui and Puertollano 2006). In addition, there are multiple positively charged amino acids (Arg and Lys, Fig. 1) in a polybasic domain of the N-terminus of TRPML1(Dong et al. 2010a). Phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$, an LEL-localized phosphoinositide (Zhang et al. 2012b; Zolov et al. 2012), may directly bind to these sites to activate or increase the channel activity of TRPML1 (Dong et al. 2010a). The plasma membrane-localized $PI(4,5)P_2$, however, inhibits the activity of TRPML1 through distinct sites within the same polybasic domain (Zhang et al. 2012a).

4 Interacting Proteins

To gain a better understanding of how TRPML1 regulates multiple lysosomal functions (Cheng et al. 2010; Grimm et al. 2012), it is important to define the molecular context for TRPML1's channel function. One potential effector of TRPML1 channel is penta-EF-hand apoptosis-linked gene 2 protein (ALG-2). ALG-2, a Ca^{2+} binding protein, is found to directly bind to a stretch of amino acid residues (positions 37–49) on the N-terminus of TRPML1 in a Ca^{2+}-dependent manner (Vergarajauregui et al. 2009). Notably, the aberrant accumulation of enlarged endolysosomes induced by TRPML1 overexpression was dramatically attenuated when the potential ALG-2-interacting sites are mutated (Vergarajauregui et al. 2009). Hence ALG-2 may serve as a downstream Ca^{2+} sensor that couples the TRPML1-mediated lysosomal Ca^{2+} release to cellular

functions (Cheng et al. 2010; Vergarajauregui et al. 2009). Alternatively, ALG-2 may directly regulate TRPML1' channel activity (Cheng et al. 2010; Grimm et al. 2012; Vergarajauregui et al. 2009).

Another TRPML1 interaction partner is lysosome-associated protein transmembrane member proteins (LAPTMs), as demonstrated by a yeast two-hybrid screen and co-immunoprecipitation experiments (Vergarajauregui et al. 2011). Interestingly, knockdown of endogenous LAPTMs by specific siRNA induced the accumulation of endolysosomes with electron dense and multi-laminar structures, reminiscent of storage materials in ML-IV cells (Chen et al. 1998; Slaugenhaupt 2002; Zeevi et al. 2009).

Venugopul et al. identified an interaction between TRPML1 and a molecular chaperone complex including heat shock cognate protein of 70 kDa (Hsc70) and heat shock protein of 40 kDa (Hsp40) (Venugopal et al. 2009). The interaction appears to be through the large intraluminal loop between S1 and S2 of TRPML1 (Venugopal et al. 2009). Hsc70 and Hsp40 are required for recognizing the target cytosolic proteins during chaperone-mediated autophagy (CMA), which is defective in ML-IV fibroblasts (Venugopal et al. 2009). Interestingly, an increase in intracellular Ca^{2+} concentration enhances the co-immunoprecipitation and co-localization between Hsc70 and TRPML1 (Venugopal et al. 2009). TRPML1 is also found to interact with two-pore TPC proteins (Yamaguchi et al. 2011), but whether TRPML1 forms heteromeric channels with TPCs is unknown. Finally, a comprehensive and systematic screen for TRPML1 interactors has been recently performed, which resulted in the discovery of a large set of TRPML1-interacting proteins (Spooner et al. 2013).

5 The Channel Biophysical Properties and Function

5.1 Permeation Properties

The LEL localization of TRPML1 has made it difficult to analyze the permeation and gating properties of the channel. However, the recent development of the whole-endolysosome patch-clamp technique has allowed a direct study of TRPML1 on artificially enlarged lysosomes, which are induced by vacuolin-1, a small-molecule chemical compound that selectively enlarges lysosomes (Dong et al. 2010a; Wang et al. 2012). By using the whole-lysosome recordings, it was shown that TRPML1-mediated currents exhibit strong inward rectification (inward indicates cations moving out of the lysosomal lumen). TRPML1 is permeable to Ca^{2+}, Fe^{2+}, Zn^{2+}, Na^{+}, and K^{+} (Table 1) (Dong et al. 2008, 2009; Xu et al. 2007). TRPML1 has been shown to be impermeable to protons, although TRPML1-mediated currents are potentiated by low luminal pH (pH_L) (Dong et al. 2008; Xu et al. 2007) and loss of TRPML1 reportedly affected the lysosomal luminal pH (Miedel et al. 2008; Venkatachalam et al. 2008).

The single-channel conductance (see below) is 76 pS (from −140 mV to −100 mV) and 11 pS (from −80 mV to −40 mV) (Table 1; Xu et al. 2007) for

Table 1 Summary of the channel properties of TRPML1

Properties	TRPML1
Tissue distribution	Ubiquitously expressed with the highest expression levels in the brain, kidney, spleen, liver and heart
Subcellular localization	Late endosomes and lysosomes (LELs)
Ion selectivity	Non-selective: Ca^{2+}, Fe^{2+}, Zn^{2+}, Na^{+}, K^{+}, etc.
I–V plot	Strong inwardly rectifying (inward indicating cations flowing out of the lumen to the cytoplasm)
Single channel conductance (pS)	45 pS (−140 mV to −40 mV)
Activation mechanisms	Voltage; low luminal pH; $PI(3,5)P_2$
Activators	$PI(3,5)P_2$; ML-SA1; SF-22; SF-51
Inhibitors	Sphingomyelins; $PI(4,5)P_2$; Verapamil, Gd^{3+}, and La^{3+}
Interacting proteins	TRPML2, TRPML3; TPC2; ALG-2; LAPTMs; Hsc70; and Hsp40
Cellular functions	Lysosomal membrane trafficking; lysosomal exocytosis; autophagy; lysosomal ion homeostasis
Human diseases	Mucolipidosis IV and Niemann-Pick diseases
Genetic models	Human MLIV patients; Mouse TRPML1 knockout; *Drosophila trpml* mutant; *C elegans* CUP-5 mutant

$TRPML1^{Va}$ and 45 pS (−140 mV to −40 mV) for wild-type TRPML1 (Zhang et al. 2012a). Lysosome transmembrane potentials are presumed to be positive in the luminal side (> + 30 mV), which may provide a driving force for cation efflux from the LEL lumen into the cytosol (Dong et al. 2010b; Morgan et al. 2011).

The putative pore of TRPML1 is formed by the linker region of S5 and S6 (Fig. 1), which constitutes the pore loop and the selectivity filter of the channel (Cheng et al. 2010). Consistent with this prediction, replacing two negatively charged amino acid residues in the pore loop with positively charged ones ($D^{471}D^{472}$-KK) results in a pore-dead non-conducting channel (Dong et al. 2010a; Grimm et al. 2012; Xu et al. 2007).

5.2 Gating

The putative channel gate of TRPML1 is formed by S5 and S6. A proline substitution at Vla^{432} (Vla^{432} P or *Va*, a mutation at the homologous position in TRPML3 causing the varitint-waddler (*Va*) phenotype with pigmentation and vestibular defects in mice (Di Palma et al. 2002; Xu et al. 2007; Fig. 1) in the lower part of S5 in TRPML1 results in gain-of-function (GOF) constitutively active TRPML1 channels at both the plasma membrane and endolysosomal membranes (Xu et al. 2007). The constitutive channel activity caused by Pro substitutions is proposed to be related to locking or facilitating channel conformation at the open state (Dong et al. 2010a; Grimm et al. 2012; Xu et al. 2007). Furthermore, unlike the wild-type TRPML1 channel, $TRPML1^{Va}$ showed a dramatically increased plasma membrane localization, suggesting that the constitutive release of luminal cations

(most likely Ca^{2+}) promotes the delivery of TRPML1 to plasma membrane, likely via lysosome exocytosis (Dong et al. 2009).

Using whole-endolysosome and whole-cell recordings, endogenous activators and inhibitors have been identified for TRPML1. First, phosphoinositides are shown to regulate TRPML1 in a compartment-specific manner. $PI(3,5)P_2$, a phosphoinositide that is mainly localized in the LEL, potently activates TRPML1 with an $EC_{50} = 48$ nM, potentially through a direct binding mechanism (Dong et al. 2010a; Zhang et al. 2012a). Neutralizing the potential $PI(3,5)P_2$ binding sites in the N-terminus ($R^{42}R^{43}R^{44}K^{55}R^{57}R^{61}R^{62}$, 7Q; Fig. 1) completely abolished the activation effect (Zhang et al. 2012a). On the other hand, $PI(4,5)P_2$, a plasma membrane-specific phosphoinositide, inhibits TRPML1 in the inside-out patches (Zhang et al. 2012a). Interestingly, the inhibitory effect of $PI(4,5)P_2$ is also largely abolished in TRPML1-7Q, suggesting that the positively charged amino acid residues in the N-terminus of TRPML1 are required for the isoform-specific regulation of TRPML1's channel activity.

To further investigate the activation mechanisms of TRPML1, several synthetic small-molecule compounds have been recently identified as TRPML agonists (Grimm et al. 2010; Shen et al. 2012). Of them, Mucolipin Synthetic Agonist 1 (ML-SA1) robustly activates TRPML1 at low micromolar concentrations with a response comparable to $PI(3,5)P_2$ (Shen et al. 2012). These agonists may be helpful not only in investigating the gating mechanisms of TRPML1 but also in probing the cell biological functions of the channel (Grimm et al. 2012).

5.3 Channel Function

The permeation and gating properties of TRPML1 suggest that the channel functions of TRPML1 are to release $Ca^{2+}/Fe^{2+}/Zn^{2+}$from the LEL lumen in response to various cellular cues (Cheng et al. 2010; Shen et al. 2011), such as an alteration of lysosomal concentration of $PI(3,5)P_2$. Ca^{2+} efflux from endosomes and lysosomes is thought to be important for signal transduction, organelle homeostasis, and endosomal acidification (Dong et al. 2010b; Luzio et al. 2007a, 2007b; Morgan et al. 2011). The luminal concentration of Ca^{2+} is ~0.5 mM, which is 5,000-fold higher than the cytosolic Ca^{2+} at ~100 nM (Dong et al. 2010b; Morgan et al. 2011). TRPML1 is a natural candidate for lysosomal Ca^{2+} release, and the released Ca^{2+} may drive organelles fusion or fission within the endocytic pathway (Cheng et al. 2010). Consistently, lysosomal trafficking defects are observed in ML-IV and *TRPML1*$^{-/-}$ cells, suggesting that a primary role of TRPML1 is to mediate Ca^{2+} release from LEL upon physiological stimulations.

As TRPML1 is also permeable to Fe^{2+} and Zn^{2+}, TRPML1 may also participate in the regulation of the cellular homeostasis of these heavy metals (Dong et al. 2008). Indeed, cells that lack TRPML1 exhibit a cytosolic Fe^{2+} deficiency and an overload of lysosomal Fe^{2+}, suggesting that TRPML1 contributes to iron transport out of the lysosomes (Dong et al. 2008). Similarly, the permeability of TRPML1 to Zn^{2+} and elevated Zn^{2+} levels in *TRPML1*$^{-/-}$ cells are suggestive of an

essential role of TRPML1-mediated lysosomal Zn^{2+} transport in maintaining Zn^{2+} homeostasis (Eichelsdoerfer et al. 2010; Kukic et al. 2013).

Lysosomes contain a variety of acid hydrolytic enzymes that mediate the breakdown of waste materials and cellular debris (Luzio et al. 2007a). The activities of these enzymes require the luminal pH (pH_L) to be maintained at 4.5 – 5.0, which is mainly established by a vacuolar (V)-type H^+-ATPase (Luzio et al. 2007a). Despite being impermeable to protons, TRPML1 is potentiated by low pH_L (Dong et al. 2008; Xu et al. 2007), and *TRPML1*$^{-/-}$ cells exhibit hyperacidification (Miedel et al. 2008), suggesting that TRPML1 plays a role in regulating pH_L homeostasis. It is possible that elevated juxtaorganellar $[Ca^{2+}]_{cyt}$ caused by lysosomal Ca^{2+} release via TRPML1 induce H^+ efflux through a unidentified Ca^{2+}-H^+ exchanger (Cheng et al. 2010).

6 Physiological Functions

As a lysosomal Ca^{2+} release channel, in addition to regulating lysosome ion homeostasis (see above), TRPML1 has also been proposed to regulate lysosomal membrane trafficking and signal transduction. Lysosomal membrane trafficking refers to all the membrane fusion and fission steps related to the LEL, including endosome maturation, autophagosome–lysosome fusion, LEL-to-TGN retrograde trafficking, and lysosomal exocytosis. Many of these trafficking steps are known to be Ca^{2+} dependent and are defective in *TRPML1*$^{-/-}$cells, suggesting that TRPML1 functions are important in these processes (LaPlante et al. 2002; Shen et al. 2012; Thompson et al. 2007).

6.1 Endosome Maturation

In the endocytic pathway, early endosomes mature into late endosomes and subsequently lysosomes by undergoing lumen acidification, alterations in featured lipids, and dissociation and recruitment of compartment-associated proteins. The process of late endosomal maturation may be regulated by TRPML1 (Fig. 2), as the lysosomal delivery and subsequent degradation of endocytosed proteins, such as BSA and platelet-derived growth factor receptors (PDGF-R), are delayed in ML-IV fibroblasts and macrophage cells stably expressing shRNA against *TRPML1* (Thompson et al. 2007; Vergarajauregui et al. 2008a). In contrast, such a delay is not observed in HeLa cells with transient knockdown of TRPML1 (Miedel et al. 2008), suggesting that the regulatory role of TRPML1 on endosome maturation is cell type specific and subtle. Alternatively, the accumulation of endocytosed proteins caused by TRPML1 deficiency might be secondary to chronic lysosomal storage or other primary defects associated with TRPML1 deficiency (Miedel et al. 2008).

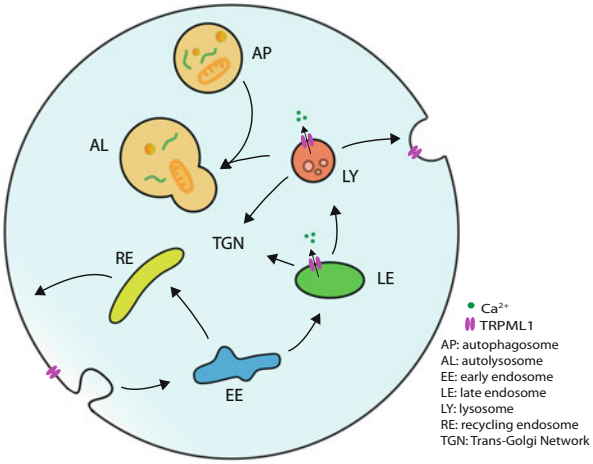

Fig. 2 TRPML1 in lysosomal membrane trafficking. Early endosomes (EEs) are formed upon endocytosis. EEs then undergo endosome maturation into LEs, which become LYs upon further acidification. TRPML1 is predominantly localized in the late endosomes (LEs) and lysosomes (LYs). TRPML1 may mediate Ca^{2+} release from LEs and LYs, which are Ca^{2+} stores with luminal Ca^{2+} concentration approximately 0.5 mM. Transport vesicles derived from LEs and LYs can mediate LEL-to-TGN retrograde trafficking. The autolysosomes (ALs) are formed from fusion of LYs with autophagosomes (APs). LYs can also undergo membrane fusion with plasma membrane (lysosomal exocytosis). Ca^{2+} release from TRPML1 may regulate the above-mentioned trafficking steps

6.2 LEL-to-TGN Retrograde Trafficking

Retrograde trafficking from the LEL to *trans*-Golgi-network (TGN) allows the reutilization of the digested products of lysosomes and the recycling of shuttle proteins that facilitate the transport of lysosomal proteins from TGN to LEL after the biosynthetic processes. For example, the mannose-6-P receptor is required for the lysosomal delivery of hydrolytic enzymes (Luzio et al. 2007b) (Fig. 2). The LEL-to-TGN retrograde trafficking is defective in ML-IV cells, as fluorophore-conjugated lactosylceramide, a lipid that is normally located in the Golgi-like compartment, is accumulated in LEL-like vesicles (Chen et al. 1998). Notably, the delayed or blocked retrograde trafficking in ML-IV and Niemann–Pick C (see below) cells is rescued by expression of TRPML1 or by synthetic TRPML1 agonist ML-SA1 (Shen et al. 2012), suggesting that the channel activity of TRPML1 is required for this specific trafficking step.

6.3 Autophagosome–Lysosome Fusion

The fusion of autophagosomes with lysosomes is essential for the degradation of damaged organelles and aged proteins during autophagy (Fig. 2), thus affecting cell

survival especially under starvation or stress conditions. Loss of TRPML1 function significantly enhances constitutive autophagy but not the starvation-induced autophagy (Vergarajauregui et al. 2008a). In ML-IV fibroblasts, elevated staining of microtubule-associated protein 1A/1B-light chain 3 (LC3, a protein marker for autophagosomes)-positive puncta was observed under complete medium, suggestive of an increase in the basal levels of autophagic flux (Curcio-Morelli et al. 2010; Vergarajauregui et al. 2008a). The constitutive activation of autophagy observed in ML-IV cells and *TRPML1*$^{-/-}$ neurons seems to be caused by both increased autophagosome formation and delayed fusion of autophagosomes with lysosomes (Curcio-Morelli et al. 2010; Vergarajauregui et al. 2008a). Consequently, autophagosomes undergo inefficient digestion and accumulate in TRPML-deficient cells. Interestingly, overexpression of TRPML1 in NRK cells also increases constitutive autophagy, which is also seen in TRPML1-deficient cells. However, because aberrant autophagy is not only found in TRPML1-related diseases but also observed in a wide spectrum of lysosome storage diseases (LSDs) (Lieberman et al. 2012), it remains to be elucidated whether TRPML1 plays a direct role in autophagosome–lysosome fusion.

6.4 Lysosomal Exocytosis

HEK293 cells transfected with TRPML1^{Va} (a gain-of-function mutation) exhibit enhanced lysosomal exocytosis (Dong et al. 2009). On the other hand, lysosome exocytosis induced by lysosome biogenesis transcription factor TFEB requires TRPML1 (Medina et al. 2011), and fibroblasts from ML-IV patients exhibit impaired ionomycin-induced lysosomal exocytosis (LaPlante et al. 2006; Medina et al. 2011). Likewise, shRNA-mediated TRPML1 knockdown in mouse macrophages results in a delay in the plasma membrane transport of the major histocompatibility complex II (MHCII) from LEL compartments in response to the immune stimulation (Thompson et al. 2007). Taken together, these results suggest that activation of TRPML1 may positively regulate lysosomal exocytosis.

6.5 Signal Transduction

Ca^{2+} release from LELs is believed to play an essential role in the transduction of extracellular signals such as glucose-induced insulin secretion in clonal pancreatic beta cells, arterial smooth muscle contraction, T-lymphocyte Ca^{2+} signaling, and neurotransmitter release (Galione et al. 2009). Nicotinic acid adenine dinucleotide phosphate (NAADP) is a Ca^{2+}-mobilizing second messenger produced in response to extracellular stimuli and believed to act on lysosome Ca^{2+} stores (Morgan et al. 2011). TRPML1, as well as two-pore TPC channels, is proposed to be the NAADP receptor (Grimm et al. 2012; Morgan et al. 2011; Zhang and Li 2007). However, while TPCs are shown to be $PI(3,5)P_2$ (but not NAADP)-activated Na^+-selective ion channels in endolysosomes (Wang et al. 2012), NAADP-induced

lysosomal Ca^{2+} release is still intact in TRPML1 KO cells (Yamaguchi et al. 2011). Hence it remains to be established whether TRPML1 plays a role in lysosomal signal transduction.

7 Lessons from Knockouts

Using genetic knockout (KO) approaches, animal models of ML-IV have been well established in mice, *C. elegans*, and *Drosophila*, providing opportunities to better understand the underlying pathogenic mechanisms at the organism and cellular levels and develop potential therapeutic strategies for ML-IV. The first murine model of TRPML1 KO generated by Slaugenhaupt's group (Venugopal et al. 2007) displays neurological, gastric, and opthalmological abnormalities that are reminiscent of the clinic features of ML-IV patients, which include mental retardation, retinal degeneration, constitutive achlorhydria, and iron deficiency. The progressive neurodegeneration phenotypes are prominent in TRPML1 KO mice, which exhibit gait changes at an age of 3 months and gradually develop hind-limb paralysis and typically die at the age of 8–9 months (Venugopal et al. 2007). At the cellular level, ML-IV-like dense granulomembranous storage bodies are observed in TRPML1$^{-/-}$ neurons and glial cells. Evident vacuolization was seen in parietal cells with elevated serum gastrin (Venugopal et al. 2007).

ML-IV-like phenotypes are also observed in the knockout models of *C. elegans* and *Drosophila*. The *cup-5* mutant worms exhibit decreased degradation of endocytosed proteins and accumulation of large vacuoles labeled with LEL markers, indicative of the defective endocytic trafficking (Fares and Greenwald 2001). Studies on the *Drosophila* model of ML-IV demonstrate that progressive neuronal death in *TRPML*-null flies is likely due to impaired autophagy, which results in the accumulation of lysosomal lipofuscin and damaged mitochondria and hence high levels of apoptosis (Venkatachalam et al. 2008). Meanwhile, the inefficient clearance of apoptotic neurons by *trpml*-null glial cells may aggravate cell death in neurons.

8 MI-IV and Other TRPML1 Related Diseases

ML-IV is an autosomal recessive lysosomal storage disorder first described as a new variant of the mucolipidoses characterized by prominent accumulation of lipids and cholesterols inside the cells (Berman et al. 1974). The most common clinical features of ML-IV patients include severe psychomotor retardation, retinal degeneration, and constitutive achlorhydria (Slaugenhaupt 2002). ML-IV-causing *MCOLN1* mutations have been identified predominantly in Ashkenazi Jews (Bargal et al. 2001). To date, there are more than 20 known mutations identified, most of which are severe loss-of-function mutations. Mutations with milder phenotypes, such as ΔF408 (Fig. 1), have only partial loss of the channel function (Dong et al. 2008). No effective treatment has been identified for ML-IV.

TRPML1's role may also be extended to other LSDs including Niemann–Pick A and C diseases (Shen et al. 2012). NPC exhibits lysosomal accumulation of sphingomyelin (SM), cholesterol, and glycolipids and insufficient activity of acid sphingomyelinase (aSMase). TRPML1-mediated lysosomal Ca^{2+} release is dramatically reduced in NPC cells, suggestive of a potential block of channel activity in NPC lysosomes (Shen et al. 2012). Interestingly, TRPML1 channel activity is inhibited by SM, but potentiated by aSMase. In the cellular assays, increasing TRPML1's expression or activity was shown to reduce lysosome storage and cholesterol accumulation in NPC cells (Shen et al. 2012). Collectively, TRPML1 channel deregulation in the lysosome may be a primary pathogenic mechanism that causes secondary lysosome storage, presumably by blocking TRPML1-dependent lysosomal trafficking in NPC.

9 Concluding Remarks

TRPML1 is a lysosomal Ca^{2+} release channel that is important for the regulation of lysosomal membrane trafficking and lysosome ion homeostasis. However, it is still difficult to know whether any of the cellular defects are directly caused by TRPML1 deficiency or indirectly caused by the chronic storage of lysosomal materials. Employing approaches to acutely activate and inhibit TRPML1's channel function may prove helpful in distinguishing these possibilities.

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