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# Role of TRP Channels in the Regulation of the Endosomal Pathway

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Some members of the transient receptor potential (TRP) channel superfamily have proved to be essential in maintaining adequate ion homeostasis, signaling, and membrane trafficking in the endosomal pathway. The unique properties of the TRP channels confer cells the ability to integrate cytosolic and intraluminal stimuli and allow maintained and regulated release of $Ca^{2+}$ from endosomes and lysosomes.

## The Role of Ion Channels in the Endocytic Pathway

The endosomal pathway is a highly compartmentalized system comprising numerous membrane-bound organelles that provides precise spatial and temporal regulation of various physiological processes (34). Endosomes and lysosomes receive cargo from the cell surface via endocytosis, biosynthetic cargo from the late Golgi complex, and various molecules from the cytoplasm via autophagy (12). The endosomal pathway is recognized as a key regulator of endocytic membrane traffic, protein sorting, and signaling. It also plays an essential role in a multitude of other cellular functions, including cell adhesion and migration, pathogen entry, neurotransmission, antigen presentation, and cell polarity.

During the last two decades, the characterization of the sorting machinery that regulates the trafficking of proteins between different endosomal compartments has been at the forefront of the study of endosomal biology (13, 67). It has also become evident that the lipid composition of the endosomal membranes (i.e., rafts, phosphoinositides) plays a central role in both trafficking and signaling events. However, it is important to emphasize that the luminal composition of the endosomal organelles is also crucial for proper endosomal function. Luminal acidification is essential for the delivery and degradation of internalized ligands in lysosomes and regulates posttranslational modification and sorting of proteins along the endosomal pathway (56). Acidification must increase progressively from endocytic vesicles and early endosomes to late endosomes and lysosomes. Luminal $Ca^{2+}$ is also a key regulator of the endosomal pathway. Release of $Ca^{2+}$ from endosomes and lysosomes is required for several steps of intracellular trafficking, including fusion and fission events (54). $Ca^{2+}$ efflux is also important for signal transduction, organelle homeostasis, and endosomal acidification (31). Moreover, defects on endosomal calcium homeostasis have been directly linked to several pathologies including acute pancreatitis (75) and Niemann-Pick Type C disease (52).

The luminal composition of endosomal organelles is maintained by specific channels that function as conduits for ions to cross the intracellular membranes. Recently, several members of the transient receptor potential (TRP) superfamily of ion channels have been localized to the endosomal pathway, where they participate in the regulation of vesicular ion homeostasis (FIGURE 1). TRP channels are a large family of cation channels with diverse physiological functions and cellular distributions (60). Most TRP channels act as sensors of changes in the extracellular environment, such as temperature, light, osmolarity, mechanical stress, or chemicals. However, some TRP channels respond to variations in the intracellular environment like pH or intracellular concentration of specific molecules (23). The activation of TRP channels may be directly mediated by physical or chemical stimuli or indirectly by second messengers generated by G protein-coupled or tyrosine kinase receptors. The TRP superfamily is divided into seven subfamilies (TRPC, TRPV, TRPM, TRPN, TRPA, TRPML, and TRPP) based on their sequence and structural organization (88). TRPs exhibit a common six-membrane-spanning topology with both the amino- and carboxy-terminal tails oriented toward the cytosol and the pore located between *transmembrane segments 5* and *6*.

It was initially assumed that most TRPs function at the cell surface. However, when heterologously expressed, many TRP channels localize, at least partially, to intracellular vesicles. In many cases, it is uncertain whether this vesicular distribution is physiologically relevant. Intracellular TRPs might represent newly synthesized molecules in transit to the plasma membrane or internalized channels being delivered to lysosomes for degradation. In addition, some channels accumulate in large and stable recycling pools close to the plasma mem-

brane and are activated on translocation to the cell surface. Therefore, to be considered a bona fide endosomal TRP, we propose that the channel must follow at least one (or preferably all) of the following criteria: *1*) presence of targeting motifs that mediate the sorting of the protein to the endosomal pathway, *2*) channel activity recorded at endosome/lysosomes, and *3*) defects on the endosomal pathway caused by absence or mutation of the endogenous protein (FIGURE 1).

## TRPML1, a Prototypical Endo-Lysosomal Channel

TRPML1, also known as mucolipin-1 or MCOLN1, can be considered an example of an endosomal TRP channel as it complies with the three criteria mentioned above. First, several studies have established that both endogenous and heterologously expressed TRPML1 distribute to the late endocytic pathway (42, 68, 82, 86, 94). Trafficking of TRPML1 to late endosomes/lysosomes is mediated by two consensus acidic di-leucine motifs located at the $NH_2$- and COOH-terminal tails of the protein, respectively (68, 86, 94) (FIGURE 2). The di-leucine targeting motif at the $NH_2$ tail ($E^{11}$TERLL) directly interacts with clathrin adaptors AP1 and AP3. This interaction mediates direct sorting of TRPML1 from the trans-Golgi network (TGN) to the endosomal pathway. TRPML1 can also reach endosomes through an indirect pathway by traveling from TGN to the plasma membrane before being internalized and delivered to endosomes and subsequently to late endosomes/lysosomes. Endocytosis of TRPML1 from the cell surface is regulated by the di-leucine motif located at the COOH-terminal tail ($E^{573}$EHSLL) and is dependent on clathrin and the clathrin adaptor AP2 (94).

Second, TRPML1 activity has been recorded by direct patch-clamp of enlarged lysosomes isolated from vacuolin-treated cells (27). The intracellular distribution of TRPML1 made difficult the analysis of the selectivity and gating properties of this channel, leading to conflicting observations (reviewed in Refs. 69, 71). However, the above-mentioned recording of native endolysosomal membranes together with the use of a constitutive active mutant of TRPML1 (V432P) concluded that TRPML1 is an inwardly (from lumen to cytoplasm) rectifying channel permeable to $Ca^{2+}$, $Na^+$, $K^+$, and $Fe^{2+}$/$Mn^{2+}$ whose activity may be potentiated by low pH. Despite being a nonselective cation channel, it is thought that the main physiological function of TRPML1 is to facilitate $Ca^{2+}$ efflux from late endosomes/lysosomes.

Third, mutations in TRPML1 results in Mucolipidosis type IV (MLIV) (5, 6, 77, 81), a lysosomal storage disorder characterized by severe neurological and ophthalmological abnormalities (1, 2, 4). The first symptoms of the disease appear during the first year of life and include intellectual disabilities, psychomotor delay, weak muscle tone (hypotonia), corneal clouding, and progressive retinal degeneration (10, 70). MLIV patients also show impaired secretion of gastric acid (achlorhydria), leading to defective iron absorption and anemia (74). By their early teens, most affected individuals are unable to walk, present limited or no ability to speak, and have severe vision loss or blindness. Analysis of fibroblasts from MLIV patients by electron microscopy revealed the presence of enlarged vacuolar structures that accumulate mucopolysaccharides and lipids forming characteristic multiconcentric lamellae (10, 32, 70, 77). These enlarged vacuoles are present not only in fibroblasts but also in every tissue and organ of MLIV patients,

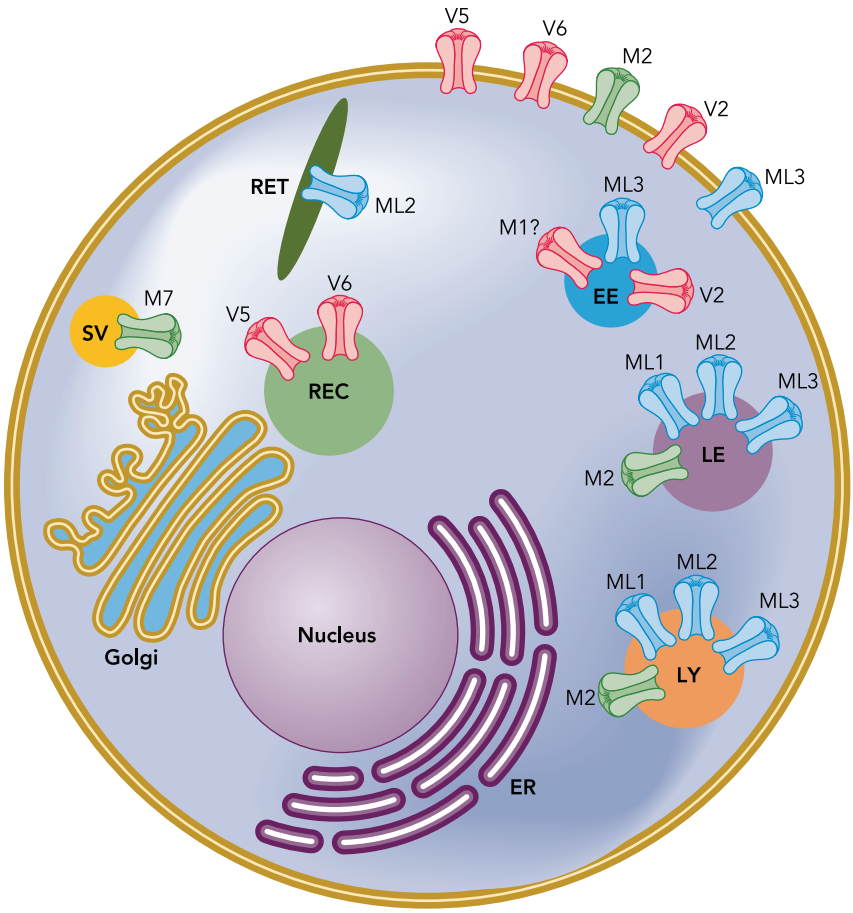


FIGURE 1. **Overview of TRP channels in endosomal trafficking pathways**
Members of some TRP subfamilies are widely distributed in various organelles along endolysosomal pathways. TRPML1 (ML1) is predominantly expressed in late endosomes (LE) and lysosomes (LY). Similarly, TRPML2 (ML2) is expressed in LE and LY but is also found on recycling endosomal tubules (RET). TRPML3 (ML3) is found not only in membranes of LE and LY but also in early endosomes (EE) and is distributed within the plasma membrane of the cell. TRPV5 (V5) and TRPV6 (V6) channels are found within the plasma membrane and recycling endosomal compartments (REC), but it is not yet known whether they are actively involved in this trafficking pathway. Recent reports indicate that TRPV2 (V2) is found to be expressed in both the plasma membrane and in early endosomes. TRPM2 (M2) is found in the plasma membrane, late endosomes, and lysosomes. TRPM1 (M1) is speculated to be present on endosomes, but conclusive evidence for its presence remains controversial. TRPM7 (M7) is thought to play a role in vesicular content release in neurons.

indicating an ubiquitous impairment of the lysosomal function. Absence of TRPML1 function leads to abnormal lysosomal pH (although different laboratories have reported conflicting observations with regard to the lysosomal pH in MLIV cells) (58, 79), delayed retrograde trafficking of LacCer from lysosomes to TGN (18, 58, 68, 79), lipofuscin accumulation (57, 87), decreased fusion of lysosomes with the plasma membrane (48), lysosomal $Fe^{2+}$ overload (27), and deficient autophagy (90). Indeed, MLIV fibroblasts as well as neurons from mouse and *Drosophila* TRPML1 knockouts show accumulation of autophagosomes, ubiquitinated aggregates, p62 protein inclusions, and abnormal mitochondria, all of which are indicative of decreased autophagosome degradation (21, 36, 57, 87, 89, 90). It has been suggested that the combination of all these factors ultimately generate oxidative stress, causing the neuronal death and neurodegeneration observed in MLIV (19).

It is important to note that altered lipid trafficking, lipofuscin accumulation, defective autophagy, and oxidative stress are common to many lysosomal storage disorders (95). Therefore, a full understanding of TRPML1 function is dependent on the clarification of its primary role as well as the initial events that trigger the pathological cascade in MLIV. Also of great importance will be elucidating the mechanisms that regulate activation of this channel under physiological conditions (FIGURE 3). A key observation was recently reported by Dong et al. showing that TRPML1 activity is modulated by phosphoinositides (PIs) (28). By performing direct patch-clamping of endolysosomal membranes, Dong et al. reported that PI(3,5)P2 strongly increases TRPML1 activity with high specificity.

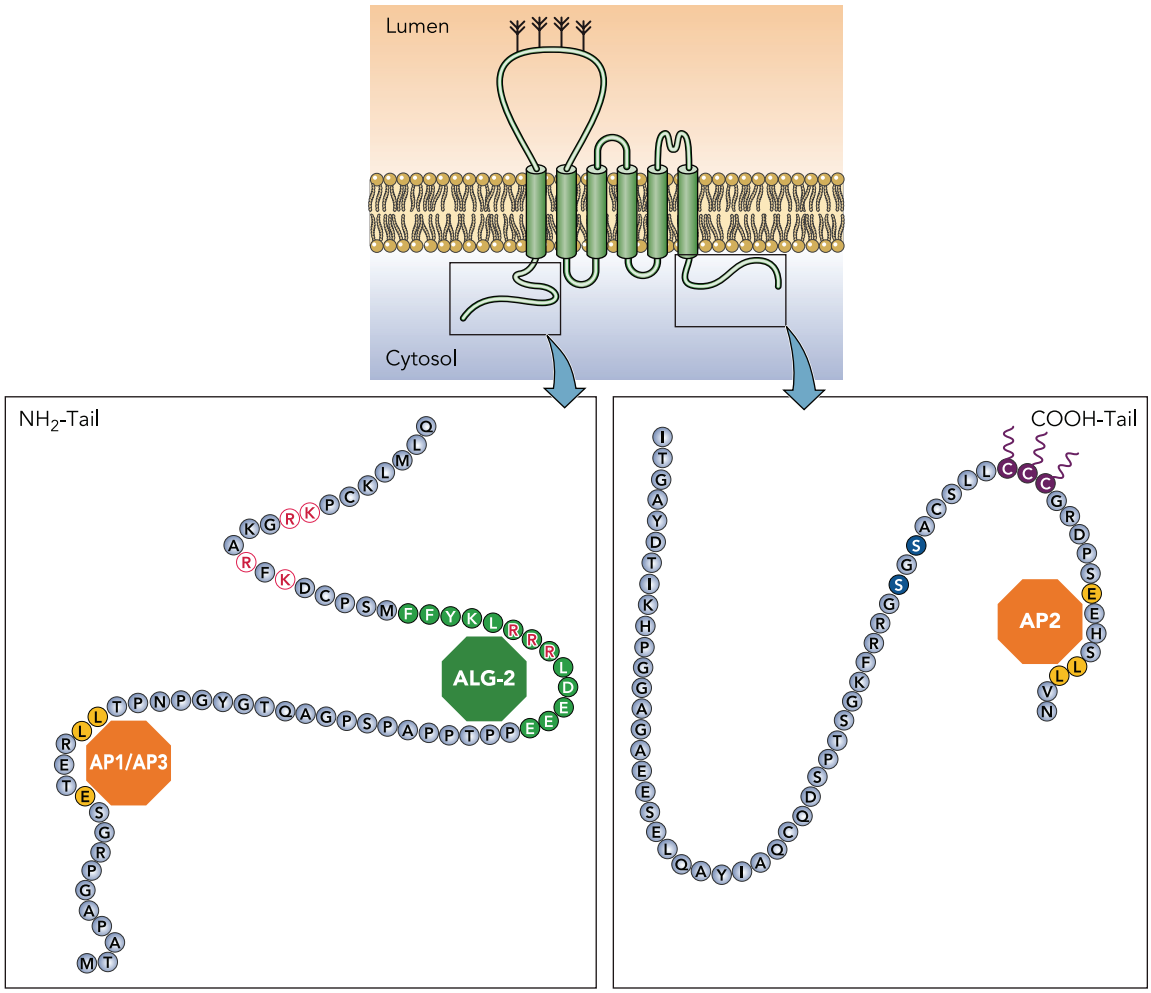


FIGURE 2. **Sorting and regulatory motifs in TRPML1**
TRPML1 is predicted to have six transmembrane domains with the $NH_2$- and COOH-terminus tails oriented toward the cytosol. The first luminal loop is highly N-glycosilated and undergoes proteolytic cleavage in lysosomes (42). The $NH_2$-terminus tail (*left*) contains a dileucine sorting motif that directly interacts with clathrin adaptors AP1 and AP3 (yellow circles), a stretch of polar and hydrophobic aminoacids that binds ALG-2 in a $Ca^{2+}$-dependent manner (green circles), and a poly-basic domain predicted to participate in the binding to PI(3,5)P2 (red letters). The COOH-terminus tail includes two serine residues phosphorylated by PKA (dark blue circles with white letters) (93), three cysteines that undergo palmitoylation (purple circles), and a dileucine sorting sequence that regulates AP2-dependent internalization from the plasma membrane (yellow circles).

TRPML1 directly binds PI(3,5)P2 through several positive residues located in its $NH_2$-terminus tail, thus undergoing conformational changes that favor its activation. The penta-EH-hand protein ALG-2 has also recently been identified as a TRPML1 modulator (91). ALG-2 directly binds the $NH_2$ tail of TRPML1 only in the presence of $Ca^{2+}$. Mutations in the ALG-2 binding domain significantly decreased the accumulation of enlarged lysosomes induced by TRPML1 overexpression, indicating that ALG-2 might act as a $Ca^{2+}$ sensor that mediates or regulates trafficking events dependent on TRPML1 activity. Interestingly, ALG-2 interacts with some of the positive residues implicated in the binding to PI(3,5)P2. It will be important to determine whether the cross-talk between different TRPML1 modulators may provide a temporal control for TRPML1 activation. Finally, it has been suggested that nicotinic acid adenine dinucleotide phosphate (NAADP) might act as an activator of TRPML1 promoting the release of $Ca^{2+}$ from lysosomes (99, 100). NAADP-dependent response was blocked by treatment with antibodies or siRNA against TRPML1. However, Pryor and Luzio reported that NADDP did not cause an increase in current in electrophysiology experiments on oocytes expressing TRPML1 (68). Moreover, the participation of TRPML1 in NAADP-mediated $Ca^{2+}$ release is argued by the recent identification of the two-pore family of ion channels (TPC) as bona fide NAADP effectors at endosomes and late endosomes/lysosomes (14, 16). Still, it is interesting to mention that treatment with Ned-19, a specific inhibitor of TPCs, rescues accumulation of enlarged lysosomes in MLIV cells (53), thus suggesting that TRPML1 might function as a regulator for TPCs.

## TRPML2 and TRPML3 Also Regulate Endosomal Function

In addition to TRPML1, the mucolipin family in mammals includes two other members, TRPML2 and TRPML3. Immunofluorescence and cellular fractionation techniques have established that both endogenous and recombinant TRPML3 localize mainly to early and late endosomes/lysosomes and, to a lesser extent, to the plasma membrane (40, 55, 59, 96, 97). Whole cell patch-clamp techniques in cells heterologously expressing TRPML3 revealed that the protein functions as an inwardly rectifying $Ca^{2+}$-permeable cation channel, whose activity is inhibited by acidic extracellular (or luminal) pH and increased by incubation of cells in low $Na^+$ (20, 39, 40, 59, 96). TRPML3 shows some permeability to $Fe^{2+}$ but to a lesser degree than TRPML1 or TRPML2 (27). As in the case of TRPML1, recombinant TRPML3 currents have been measured in whole endolysosomal membranes, and the activity of the channel dramatically increases in the presence of PI(3,5)P2, thus indicating that PIs are common regulators of TRPML function (28). The recent identification of several selective activators of TRPML3 will hopefully provide revealing information on the properties of the endogenous protein (33). Interestingly, gain-of-function mutations in TRPML3 result in the varitint-waddler (Va) phenotype in mice, which is characterized by hearing loss, vestibular dysfunction (circling behavior, head-bobbing, waddling), and coat color dilution (25). This Va phenotype is caused by a point mutation (V419P) in the pore region that locks the channel in an open conformation (39, 59, 96). The Va mutant causes massive

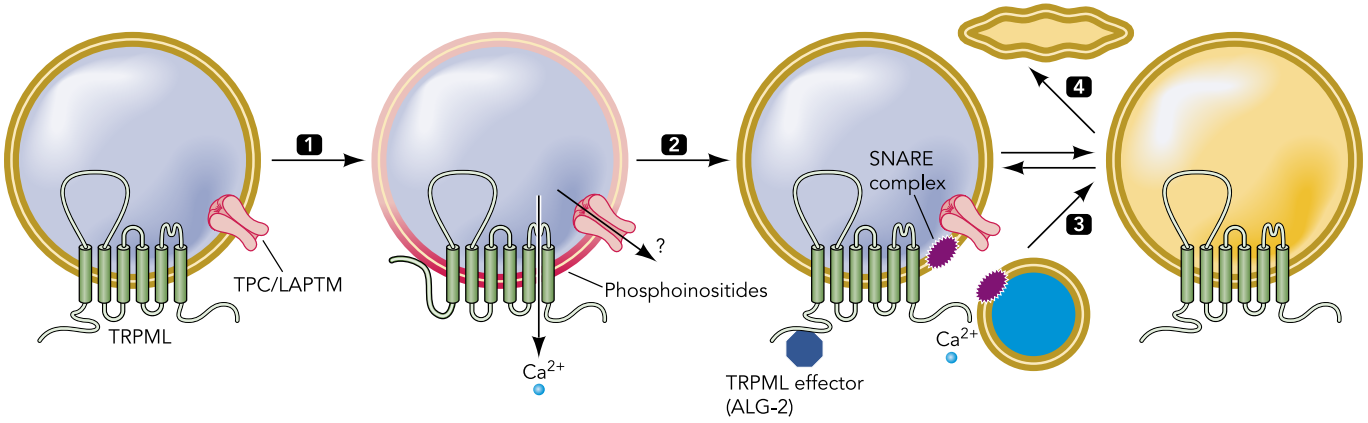


FIGURE 3. **Proposed model for TRPML activation**
TRPMLs localize to endolysosomal membranes. Under resting conditions TRPML basal activity is probably low. On stimulation (*1*) (for example by variations in luminal pH or synthesis of specific phosphoinositides as represented in the figure), TRPMLs activate and mediate release of $Ca^{2+}$ (and maybe other ions like $Fe^{2+}$) from the lumen of endosomes and lysosomes. TRPMLs might also regulate the activity of other membrane proteins such as two-pore channels or LAPTMs. Efflux of luminal $Ca^{2+}$ promotes the recruitment of specific effectors like ALG-2 (*2*) that in turn may interacts with endosomal proteins implicated in trafficking [i.e., Alix and TSG101 (62)]. Following tethering and trans-SNARE pairing, luminal $Ca^{2+}$ is also required for the final steps of endolysosomal fusion (*3*). TRPMLs may also regulate membrane retrieval from hybrid organelles (yellow), thus allowing organelle reformation (*4*) (66).

entry of $Ca^{2+}$ inside cells leading to apoptosis and cell death. It has been further proposed that death of melanocytes in the inner ear and the skin (two tissues in which the expression of TRPML3 is high) might be responsible for the hearing problems and the coat color dilution observed in varitint-waddler mice (96).

Overexpression of TRPML3 causes severe alterations in the endosomal pathway, including enlargement and clustering of endosomes, delayed epidermal growth factor receptor (EGFR) degradation, and impaired autophagosome maturation, thus suggesting that TRPML3 is an important regulator of endosomal function (41, 55). Moreover, inhibition of TRPML3 function by expression of a channel-dead dominant negative mutant (458DD/KK) or by knockdown of endogenous TRPML3 results in a significant accumulation of luminal $Ca^{2+}$ at endosomes, severe defects in endosomal acidification, and increased endosomal fusion (our laboratory's unpublished observations). Depletion of endogenous TRPML3 also affects lysosomal integrity and autophagy (41, 97). Therefore, it has been proposed that TRPML3 mediates regulated $Ca^{2+}$ release from endosomal compartments, and its function is crucial for preserving proper $Ca^{2+}$ homeostasis, endosomal pH, and membrane trafficking (55).

Similar to TRPML3, TRPML2 partially localizes to late endosomes/lysosomes when expressed in a variety of cell lines (38, 78, 86). In Hela cells, recombinant TRPML2 also distributes at the cell surface and along the tubular recycling endosomes of the Arf6-regulated pathway (26, 38). In agreement with these studies, only a fraction of endogenous TRPML2 co-localizes with LAMP1-positive vesicles, whereas the remaining protein distributes into vesicular structures that likely correspond to the early or recycling endosomes (97). The channel properties and function of TRPML2 are much less characterized than those of TRPML1 or TRPML3, albeit expression of TRPML2 in *Drosophila* S2 cells suggests that the channel displays nonselective cation permeability, which is $Ca^{2+}$-permeable and inhibited by low extracytosolic pH (49). TRPML2-mediated currents have also been recorded in endolysosomal membranes, suggesting a role for this channel at the endosomal pathway (28). This possibility is supported by recent evidence showing that expression of a dominant-negative version of TRPML2 (463DD/KK) considerably reduces recycling of internalized CD59 to the cell surface, thus indicating that TRPML2 might regulate transport of certain glycosylphosphatidylinositol-anchored proteins through the Arf6 pathway (38). In addition, depletion of endogenous TRPML2 results in the accumulation of lysosomal inclusions (97). Therefore, TRPML2 may play a role at both the early and late endocytic pathway.

In mice, two alternative-spliced variants of TRPML2 have been described that differ in the presence (long variant or TRPML2lv) or absence (short variant or TRPML2sv) of 28 residues at the $NH_2$-terminal tail of the protein (73). Although expression of TRPML2lv is very low in most analyzed mouse tissues, TRPML2sv has a predominant expression in lymphoid and kidney organs. TRPML2 is also detected in B-lymphocytes, and its expression is regulated by the Bruton's tyrosine kinase, a crucial protein in B-lymphocyte development, suggesting that TRPML2 might play a role in the regulation of immune response (50, 78). The levels of TRPML2sv (but not TRPML2lv) are considerably reduced in the TRPML1 knockout mouse, indicating that TRPML1 acts as a transcriptional regulator of TRPML2 (73). In humans, only one TRPML2 isoform, seemingly corresponding to the TRPML2lv, has been detected. Therefore, the possibility that a reduction in the levels of TRPML2 might contribute to some of the clinical manifestations of MLIV still remains uncertain.

Hetero-multimerization between the members of the TRPML family on overexpression has been detected by FRET analysis and co-immunoprecipitation (86). More recently, reconstitution of in vitro translated TRPMLs in synthetic lipid-bilayers showed that hetero-multimers display distinct electrophysiological properties compared with homo-multimers (22), indicating that interaction between TRPML proteins modifies channel function. Hetero-multimerization of recombinant TRPMLs has also proven to modulate several cellular processes regulated by TRPMLs, such as cell viability and autophagy (98). However, the formation of hetero-oligomers between endogenous TRPMLs is limited since TRPML2 and TRPML3 only partially co-localize with TRPML1 in late endosomes/lysosomes (97). Therefore, it is plausible that combinations of homo- and hetero-multimers coexist in cells, with each exhibiting unique channel properties and cellular functions. TRPMLs may also form hetero-oligomers with other endolysosomal transmembrane proteins. Co-immunoprecipitation and yeast two-hybrid assays showed that TRPML1 (but no TRPML3) interacts with a family of lysosome-associated transmembrane proteins (LAPTM) implicated in the transport of various molecules across the lysosomal membranes (92). Future studies should determine the physiological relevance of this interaction and the suitability of LAPTMs as potential therapeutic targets for MLIV.

In summary, current evidence suggests that TRPMLs mediate transient, regulated, and localized $Ca^{2+}$ efflux from acidic stores, whereas activation by pH and PIs may provide specific spatiotemporal regulation for each TRPML along the en-

dosomal pathway. Release of luminal $Ca^{2+}$ plays a pivotal role in several trafficking events such as fusion (or fission) of lysosomes with autophagosomes, late endosomes, or plasma membrane. Therefore, TRPMLs are important regulators of membrane trafficking in the endosomal pathway (FIGURE 3). TRPMLs are also permeable to $Na^+$, $K^+$, and $Fe^{2+}/Mn^{2+}$, thus suggesting that this family of proteins may contribute in additional ways to maintain vesicular ion homeostasis.

## Other TRP Channels at the Endocytic Pathway

Current evidence with respect to the role of other TRP channels in the endosomal pathway is less compelling than for TRPMLs. Still, several members from various TRP subfamilies are good candidates for future study given both their presence and/or association with endosomal membranes as well as recent findings on their physiological roles within these compartments.

Along with members of the TRPML subfamily, the TRPM2 channel is the only other known TRP channel that is found in late endosomes and lysosomes in mammals (47). TRPM2 is a chanzyme, meaning that it is a cation channel fused with an enzymatic ADP-ribose pyrophosphatase domain. TRPM2 is nonselective for cations, is permeable to $Ca^{2+}$, and has a dual function both at the plasma membrane and at lysosomes (47). At the cell surface, TRPM2 operates as a $Ca^{2+}$-influx channel allowing entry of $Ca^{2+}$ inside the cell and the regulation of numerous biological effects including insulin secretion, detection of oxidative stress and apoptosis, increased plasma membrane permeability in endothelium, and production of cytokines (37). TRPM2 channel activity is modulated by oxidative stress and ADP-ribose, which is converted into AMP by the ADP-ribose phyrophosphatase domain of TRPM2 (64, 65). Other known regulators of TRPM2 include cyclic ADP-ribose (45), reactive oxygen species (35), pH (80), intracellular $Ca^{2+}$ (64), and NAADP (7). However, it is important to point out that a recent report by Toth and Csanady indicates that physiologically relevant concentrations of NAADP or cyclic ADP-ribose do not activate TRPM2 (83). Future studies will hopefully resolve these discrepancies. Several lines of evidence suggest that TRPM2 may also function as a lysosomal $Ca^{2+}$-release channel in pancreatic beta cells (47). First, endogenous TRPM2 localizes to lysosomes in INS-1 beta cells. Second, $Ca^{2+}$ release mediated by ADP-ribose is reduced in cells from TRPM2 knockout mice or cells treated with small interfering RNA (siRNA) against TRPM2. Third, ADP-ribose-mediated $Ca^{2+}$ release is blocked by treatment with agents like bafilomycin that empty acidic $Ca^{2+}$ stores. It has been suggested that the release of intracellular $Ca^{2+}$ from lysosomes on TRPM2 activation plays a critical role in hydrogen peroxide-induced cell death in beta cells (47).

Heterologous expression of several alternative splice variants of TRPM1 revealed that all the isoforms distribute along a very dynamic network of intracellular tubular structures (61), suggesting a possible role of this channel at the endosomal pathway. TRPM1 is thought to be an indicator of melanoma aggressiveness, and its expression is severely reduced in highly metastatic cell lines (29, 30). However, the role of TRPM1 in the regulation of melanosome trafficking or biogenesis is still a matter of debate, since TRPM1 was not found to co-localize with melanosomes in SK-Mel19 cell preparations (61). In addition, TRPM1 has been implicated in pigmentation in horses (9), but no coat color alterations has been reported in TRPM1 knockout mice (44). More recently, it has been shown that mutations in TRPM1 are associated with congenital stationary night blindness in humans and that TRPM1 plays a crucial role in mediating a photo response in retinal ON bipolar cells (43).

TRPM7 might also have a potential role in intracellular trafficking. In sympathetically derived cells, TRMP7 is contained in synaptic vesicles where it may play several potential roles in vesicular content release. First, TRPM7 could serve to ionically balance neurotransmitters destined for exocytic release (46). Second, this channel might also modulate vesicular release through regulation of the membrane potential (46). Last, as proposed by Brauchi et al., on binding to $PI(4,5)P_2$ in the plasma membrane, TRPM7 is activated, allowing for conductance of ions including $Ca^{2+}$, thus acting as a critical mediator of vesicle fusion events (15).

Within the TRPV subfamily, TRPV2 is probably the best candidate so far to play a role in the endosomal pathway. After performing patch-clamp in early endosomes enlarged by expression of a hydrolysis-deficient Vps4 mutant, Saito et al. reported the presence of a calcium channel in early endosomes whose properties match those of TRPV2 (activation by 2-aminoethyldiphenyl borate and inhibition by Ruthenium Red) (72). TRPV2 current is activated by acidification and decrease in chloride luminal concentration, two processes that rapidly occur after pinching and conversion of endocytic vesicles into early endosomes. Therefore, as in the case of TRPML3, release of $Ca^{2+}$ from early endosomes mediated by TRPV2 may regulate $Ca^{2+}$-dependent fusion between endosomal membranes and proper endosomal acidification. The role of TRPV2 in trafficking was further reinforced by recent evidence showing that early phagocytosis

is impaired in TRPV2 knockout macrophages (51). TRPV2 may also function at the cell surface. Insulin accelerates exocytosis by promoting activation and translocation of TRPV2 from intracellular vesicles to the plasma membrane in a PI3K-dependent manner in pancreatic beta cells (3). Finally, TRPV2 is also activated by noxious heat and stretch and is thought to play a role as a mechano-sensor in vascular smooth muscle cells (8).

Less clear is the role of TRPV5 and TRPV6 in recycling endosomes. Both channels are constitutively active, highly $Ca^{2+}$ selective, and play an important role in $Ca^{2+}$ uptake from apical cell surfaces in the kidney (TRPV5) and in the small intestine (TRPV6) (85). Recent evidence has shown a direct interaction of TRPV5 and TRPV6 with Rab11, a small GTPase that regulates trafficking of multiple molecules from recycling endosomes to the cell surface (84). TRPV5 co-localizes with Rab11 in vesicular structures, and expression of a Rab11 mutant locked into its inactive GDP-state decreases the amount of TRPV5 and TRPV6 and the plasma membrane (84, 85). As of yet, however, there is no conclusive evidence that TRPV5 and TRPV6 are functional in recycling endosomes. Most likely, the accumulation of these channels in endosomes reflects a way for the cell to regulate their expression (and thereby activity) at the cell surface. This might also be the case of TRPC3 and TRPC5 as they mostly reside in intracellular vesicles but translocate to the cell surface in response to activation of G-protein-coupled receptors or stimulation with growth factors, respectively (11, 76).

TRPY1 (also known as YVC1) is the only member of the TRP superfamily expressed in yeast. TRPY1 is found exclusively in vacuoles, an organelle equivalent to mammalian lysosomes that in yeast constitutes the main storage for intracellular $Ca^{2+}$ (63). It has been proposed that TRPY1 functions as a mechanosensitive ion channel, releasing vacuolar $Ca^{2+}$ in response to osmotic changes in vivo (24) and to membrane stretch force under patch-clamp (101). Yeast strains deficient for TRPY1 do not show any apparent growth defects under normal or stressed conditions, but they fail to induce an increase in cytosolic $Ca^{2+}$ after hypotonic treatment. In addition, yeast overexpressing TRPY1 become more sensitive to the presence of $CaCl_2$ (but not $MgCl_2$, NaCl, or KCl) in the medium (17). Therefore, it seems clear that TRPY1 plays an important role in the regulated release of intraluminal $Ca^{2+}$ from vacuoles.

## Concluding Remarks

TRP channels at the plasma membrane chiefly mediate their effects by controlling the concentration of free intracellular calcium, which acts as a second messenger inside the cell. Recently, it has become evident that TRP channels can also regulate $Ca^{2+}$ efflux from acidic stores. This $Ca^{2+}$ release is essential for maintaining proper ion homeostasis and membrane trafficking along the endosomal pathway. Considering the increasing number of signaling molecules found to be associated with endosomal membranes, it is likely that $Ca^{2+}$ efflux also plays a crucial role in signal transduction and formation of signaling scaffolding complexes. The activity of the endosomal TRP channels can be regulated by cytosolic second messengers, by the biophysical-chemical properties of the endosomal membranes (PIs and stretch), and by the luminal composition of the endosomal organelles (pH and $Ca^{2+}$ concentration). Therefore, like their counterparts at the cell surface, endosomal TRP channels can respond to variations in their environment and integrate signals coming from the cytosol and the lumen of endosomes and lysosomes. The fact that the only TRP channel identified in yeast localizes to the vacuole and mediates $Ca^{2+}$ release strengthens the importance of this family of ion channels in the maintenance of the ionic composition of the endocytic compartments. Still, our understanding of how TRP channels function in regulating signaling and other trafficking pathways is limited. Thus, for example, not much is known regarding the ability of TRP channels to form hetero-oligomers with members of other TRP subfamilies or their interaction with endosomal regulatory proteins. In addition, it is important to point out that other families of channels, like TPCs, may also play an important role in the regulation of $Ca^{2+}$ efflux from acidic stores. Ultimately, clarifying the function of endosomal TRP channels in specific cell types will allow us to understand what role these channels play in a multitude of normal homeostatic functions, and these key discoveries will undoubtedly help us gain insight into various human diseases that currently have limited or no available treatment options. ■

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