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REVIEW

# TRP channels of intracellular membranes

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**Abstract**

Ion channels are classically understood to regulate the flux of ions across the plasma membrane in response to a variety of environmental and intracellular cues. Ion channels serve a number of functions in intracellular membranes as well. These channels may be temporarily localized to intracellular membranes as a function of their biosynthetic or secretory pathways, i.e., *en route* to their destination location. Intracellular membrane ion channels may also be located in the endocytic pathways, either being recycled back to the plasma membrane or targeted to the lysosome for degradation. Several channels do participate in intracellular signal transduction; the most well known example is the inositol 1,4,5-trisphosphate receptor ($IP_3R$) in the endoplasmic reticulum. Some organellar intracellular membrane channels are required for the ionic homeostasis of their residing organelles. Several newly-discovered intracellular membrane $Ca^{2+}$ channels actually play active roles in membrane trafficking. Transient receptor potential (TRP) proteins are a superfamily (28 members in mammal) of $Ca^{2+}$-permeable channels with diverse tissue distribution, subcellular localization, and physiological functions. Almost all mammalian TRP channels studied thus far, like their ancestor yeast TRP channel (TRPY1) that localizes to the vacuole compartment, are also (in addition to their plasma membrane localization) found to be localized to intracellular membranes. Accumulated evidence suggests that intracellularly-localized TRP channels actively participate in regulating membrane traffic, signal transduction, and vesicular ion homeostasis. This review aims to provide a summary of these recent works. The discussion will also be extended to the basic membrane and electrical properties of the TRP-residing compartments.

**Keywords:** endosomes, intracellular channel, lysosomes, membrane traffic, TRP channel, TRPML.

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## TRP channels and organelles

### Overview of TRP channels

Transient receptor potential (TRP) is a cation channel superfamily with diverse physiological functions including thermosensation and mechanosensation (excellently reviewed in Refs. Clapham 2003; Montell 2005; Nilius *et al.* 2007). Mammalian TRPs can be divided into six subfamilies: TRPC(1–7), TRPV(1–6), TRPM(1–8), TRPA(1), TRPP(1–3), and TRPML(1–3) (Fig. 1). In past years, extensive efforts have been made to elucidate three basic aspects of TRP channels: the channel pore properties, the activation (gating) mechanisms, and the channels' subcellular localization. Of these, the biophysical properties of the TRP channel pore have been best characterized. When activated, most TRPs conduct $Ca^{2+}$, as well as $Na^+$ and $K^+$ ions. The activation/gating mechanisms, however, have not been described in detail for most TRPs. In sensory cells such as somatosensory neurons, a subset of TRP channels (so-called 'sensory' TRPs) are activated by a variety of environmental cues such as temperature, mechanical force, and plant-derived volatiles (reviewed in Refs. Clapham 2003; Montell 2005; Nilius *et al.* 2007). Many of the sensory TRPs, along with the

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*Abbreviations used*: AM, acetoxymethyl; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; CaM, Calmodulin; CICR, $Ca^{2+}$-induced $Ca^{2+}$ release; EPSP, excitatory postsynaptic potential; ER, endoplasmic reticulum; $IP_3R$, inositol 1,4,5-trisphosphate receptor; LEL, late endosomal and lysosomal; LROs, lysosome-related organelles; MHCII, major histocompatibility complex II; ML4, type IV mucolipidosis; NAADP, nicotinic acid adenine dinucleotide phosphate; PIP, phosphoinositide; PM, plasma membrane; RyR, ryanodine receptor; SG, secretory granule; SNARE, soluble NSF attachment protein receptors; SR, sarcoplasmic reticulum; SV, secretory vesicle; SyV, synaptic vesicle; TG, thapsigargin; TGN, *trans*-Golgi network; TRP, transient receptor potential.

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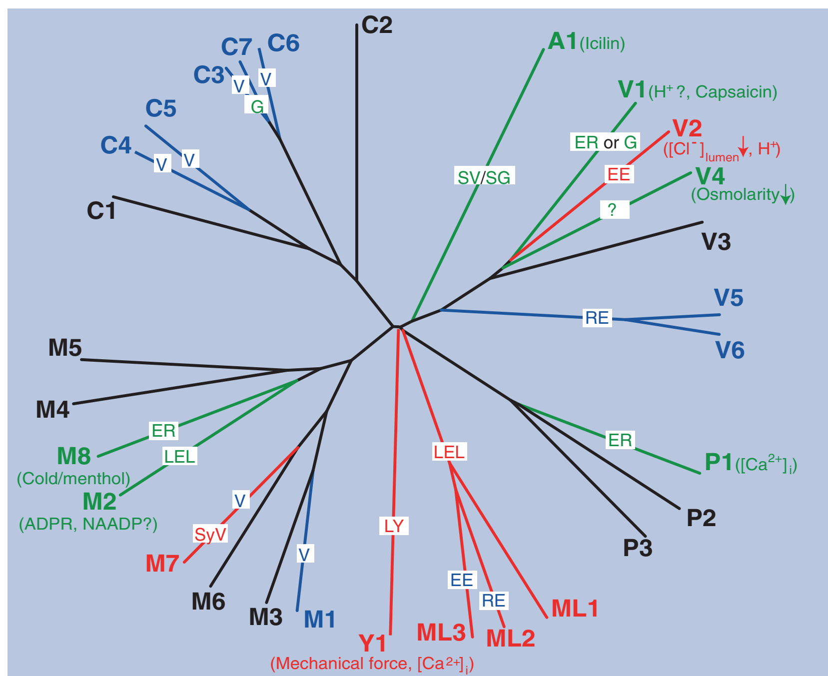


**Fig. 1** Intracellular location and putative activation mechanisms of TRP channels. TRPs can be divided into six groups (TRPC, TRPV, TRPM, TRPA, TRPML, and TRPP). TRPML1-3, TRPV2, and TRPY1 (yeast TRP *yvc1*), and TRPM7 (in red) are likely to play active roles in membrane traffic and exocytosis. TRPM2, TRPM8, TRPV1, TRPP1, TRPA1, and TRPV4 (in green) have been shown to be active in intracellular membranes and may play roles in intracellular signal transduction. TRPC3-6, TRPMV5/6, TRPM1, TRPM7, and TRPML2/3 (in blue) have been shown to undergo regulated exocytosis. Intracellular localization of other TRPs (in black) has not been well documented.

non-sensory TRPs, are also expressed in non-sensory tissues. Here the endogenous cellular cues activating the TRPs are largely unknown, although ligands of the G protein-coupled receptor and RTK (receptor tyrosine kinase) families reportedly activate or modulate TRPs in heterologous systems (reviewed in Ref. Ramsey *et al.* 2006). Initially thought to be solely plasma membrane (PM) channels mediating $Ca^{2+}$ entry, almost all mammalian TRPs studied thus far have also been found to be localized to intracellular vesicular membranes (Turner *et al.* 2003; Bezzerides *et al.* 2004; Krapivinsky *et al.* 2006; Dong *et al.* 2008; Lange *et al.* 2009). Despite the fact that TRP channel activity can be recorded in the plasma membrane using functional assays, and that TRP proteins can be detected in the plasma membrane using surface labeling techniques, the majority of heterologously-expressed and many of the endogenous TRP proteins are located intracellularly (for example see Refs Turner *et al.* 2003; Bezzerides *et al.* 2004; Krapivinsky *et al.* 2006; Dong *et al.* 2008; Lange *et al.* 2009; Oancea *et al.* 2009). The molecular identities of these intracellular TRP-residing compartments are poorly defined. It remains unclear whether TRPs appear in these compartments as intermediates of biosynthetic pathways being shuttled to their ultimate destinations, or if they are active participants in signal transduction and/or membrane trafficking. If the latter is true, the larger question arises as to how TRPs function in vesicles and are activated and/or modulated by cellular cues. TRPs localized to the plasma membrane (PM TRPs) are modulated by G protein signaling, lipid signaling, and protein phosphorylation and de-phosphorylation events (Clapham 2003; Ramsey *et al.* 2006). It is not known if vesicular intracellular membrane TRPs are modulated by these same signaling molecules. It is also not known whether intracellular membrane TRPs are modulated by luminal factors. Given that the chemicophysical properties of intracellular membranes differ significantly from those of the plasma membrane (Watson and Pessin 2001), it is of interest whether vesicular TRPs function in the secretory/endocytic pathways in a similar fashion to their plasma membrane counterparts. In this review, we will mainly discuss TRP channels whose residing compartments have been reasonably defined, especially those for whom channel activities have been measured in their residing compartments.

## Overview of intracellular organelles

In most mammalian cells there are at least eight different types of intracellular organelles, which can be arbitrarily divided into two groups (Michelangeli *et al.* 2005). The first endocytic, secretory and autophagic group (group I) includes the endoplasmic reticulum (ER), the Golgi apparatus, secretory vesicles/granules, endosomes, autophagosomes, and lysosomes (see Fig. 2). These group I organelles exchange materials with each other, either directly by complete or partial ('kiss and run') membrane fusion, or with the aid of intermediate transport vesicles that bud off (membrane fission) from the donor membrane and migrate to the target membrane (Piper and Luzio 2004; Roth 2004; Luzio *et al.* 2007b; Stenmark 2009). In specialized cell types, there are also cell-type-specific vesicular compartments derived from endocytic and secretory pathways. For

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example melanosomes, which release melanin in response to light, are specialized lysosome-related organelles (LROs) in melanocytes (Blott and Griffiths 2002). In neurons, synaptic vesicles that release neurotransmitters upon electrochemical stimulation are derived from endosomes (Suudhof 2008). The second group of intracellular organelles includes mitochondria, peroxisomes, and the nucleus. Both group I and group II organelles function as intracellular $Ca^{2+}$ stores with the luminal $Ca^{2+}$ concentration ($[Ca^{2+}]_{lumen}$) ranging from micromolar (μM) to millimolar (mM) values, 10- to 10,000-fold higher than the level of resting cytosolic $Ca^{2+}$($[Ca^{2+}]_{cyt}$, ∼ 100 nM) (Michelangeli *et al.* 2005). The $Ca^{2+}$ gradient in each of these organelles is actively established and maintained by various $Ca^{2+}$ pumps and/or secondary $Ca^{2+}$ transporters (Fig. 2; see reviews Camello *et al.* 2002; Michelangeli *et al.* 2005).

### Role of $Ca^{2+}$ in signal transduction and organellar homeostasis

Intra-organellar $Ca^{2+}$ release (efflux) has been shown to have an important role in signal transduction. For example, the inositol 1,4,5- trisphosphate receptor ($IP_3R$) and the ryanodine receptor (RyR) in the ER and sarcoplasmic reticulum (SR), respectively, couple numerous extracellular signals to intracellular events such as hormone secretion and muscle contraction (Berridge *et al.* 2003). Alternatively other second messengers, such as nicotinic acid adenine dinucleotide phosphate (NAADP) and sphingolipid-derived messengers, may induce $Ca^{2+}$ release from intracellular stores via novel mechanisms (Berridge *et al.* 2003; Zhang and Li 2007; Calcraft *et al.* 2009). Intra-organellar $Ca^{2+}$ release, effecting a reduction of $[Ca^{2+}]_{lumen}$, may also modulate the function of organelles. For example, a reduction of $[Ca^{2+}]_{lumen}$ may affect the protein folding and lipid synthesis in the ER (Corbett and Michalak 2000).

### $Ca^{2+}$-dependence of membrane traffic

Numerous *in vitro* and *in vivo* studies suggest that $Ca^{2+}$ release (efflux) from group I organelles (ER, Golgi, endosomes, and lysosomes) is essential for membrane trafficking, fusion and fission (Burgoyne and Clague 2003; Hay 2007; Luzio *et al.* 2007b). The basic steps of fusion (tethering, docking, priming, and bilayer fusion) and the fusion machinery (SNAREs, phosphoinositides, and Rabs) involved are similar for fusion between intracellular membranes and for plasma membrane exocytosis (Martens and McMahon 2008; Suudhof 2008), suggesting that a similar mechanism may regulate both processes. $Ca^{2+}$-dependence has been well documented for regulated exocytotic events, i.e. fusion of intracellular organelles such as synaptic vesicles, secretory vesicles/granules, or lysosomes with the plasma membrane (Reddy *et al.* 2001; Blott and Griffiths 2002; Suudhof 2008). On the other hand, intracellular membrane traffic has been traditionally classified to be 'constitutive' (Roth 2004; Hay 2007). Evidence now exists, however, suggesting that intracellular traffic is also highly regulated. The final trigger of intracellular membrane fusion is also likely to be a rise of $[Ca^{2+}]_{cyt}$, whose amplitude and duration might be in a scale different from fusion at plasma membrane (Burgoyne and Clague 2003; Hay 2007). Both *in vitro* and *in vivo* studies suggest that $[Ca^{2+}]_{cyt}$ in the vicinity of organelles plays a critical role in most, but not all, fusion events during the biosynthetic, secretory and endocytic pathways (Holroyd *et al.* 1999; Luzio *et al.* 2000, 2007b; Pryor *et al.* 2000; Chen *et al.* 2002; Burgoyne and Clague 2003; Hay 2007). Using cell extracts from yeast or mammalian cells, *in vitro* fusion (content mixing) has been successfully reconstituted between various endosomal compartments: early endosome-early endosome, late endosome – late endosome, late endosome – lysosome, and lysosome-lysosome (Peters and Mayer 1998; Holroyd *et al.* 1999; Pryor *et al.* 2000). In all cases, fusion is inhibited by 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA), but not EGTA. Although both are strong chelators for $Ca^{2+}$, BAPTA binds $Ca^{2+}$ ions at least one hundred times faster than EGTA (Chen *et al.* 2002; Hay 2007). The increased sensitivity of endosomal fusion to BAPTA versus EGTA has been widely interpreted as evidence to support that intraluminal $Ca^{2+}$ release is essential for triggering intracellular membrane fusion, and that the putative action site is extremely close (estimated to be < 20 nm) to the $Ca^{2+}$ release site (Pryor *et al.* 2000; Chen *et al.* 2002; Hay 2007). Using membrane permeable forms of chelators, i.e., BAPTA-acetoxymethyl (AM) ester and EGTA-AM, it was shown that, in intact mammalian cells, intraluminal $Ca^{2+}$ release is required for many steps of intracellular transport. For example, anterograde intra-Golgi transport and retrograde endosome-to-*trans* Golgi network (TGN) transport require intraluminal $Ca^{2+}$ release (Chen *et al.* 2002; Burgoyne and Clague 2003; Hay 2007). The $Ca^{2+}$-sensitive transport steps in the secretory and endocytotic pathways are summarized in Fig. 2 (white arrows). Upon localized juxta-organellar $Ca^{2+}$ elevation, two kinds of $Ca^{2+}$ sensor proteins are activated to initiate membrane fusion. Synaptotagmins (Syts), a family of proteins with C2-type $Ca^{2+}$ binding sites, are involved in the exocytosis of secretory vesicles, secretory granules, synaptic vesicles and lysosomes (Reddy *et al.* 2001; Hay 2007; Luzio *et al.* 2007b; Suudhof 2008). Calmodulin (CaM), an EF-hand cytosolic protein, has been shown to play important roles in multiple transport steps of secretory (for example, intra-Golgi transport) and endocytic pathways (for example, early and late endosomal fusions) (Peters and Mayer 1998; Burgoyne and Clague 2003; Hay 2007). How CaM is recruited to various intracellular vesicles is still not clear.

Juxta-organellar luminal $Ca^{2+}$ release also regulates membrane fission/budding events (Hay 2007; Luzio *et al.* 2007a,b). Fission events share many common mechanisms

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with fusion events, although the outcome of fission or budding is to extract, rather than add, membrane. For example, Rab proteins and phosphoinositides (PIPs) can regulate both membrane fission and fusion (Roth 2004). Membrane fission is also dependent upon $Ca^{2+}$ (Hay 2007; Luzio *et al.* 2007a,b). For instance BAPTA-AM, but not EGTA-AM, inhibited vesicle budding *in vitro* (Ahluwalia *et al.* 2001). The $Ca^{2+}$ effector protein involved in the fission process is not known. In the TGN, both neuronal calcium sensor 1 and Arf proteins are implicated (Hay 2007).

Although the importance of organellar $Ca^{2+}$ release in signal transduction, organelle homeostasis, and membrane traffic has been established, the molecular identities of the $Ca^{2+}$ release proteins resident in these compartments have remained elusive. Furthermore, the mechanisms that activate the release channels are largely unknown. As many TRP proteins are $Ca^{2+}$-permeable channels localized in the membranes of vesicles involved in the secretory and endocytic pathways, they are natural candidates to mediate organellar $Ca^{2+}$ release. A list of TRP channels with their reported intracellular locations and potential functions is provided in Fig. 1 and will be discussed in detail below.

## TRP channels in the endocytic and autophagic pathways

### Biogenesis of endosomes and lysosomes

The primary functions of endosomes and lysosomes (endolysosomes, collectively) are degradation, membrane trafficking, protein transport and signal transduction (for review, see Refs. Berridge *et al.* 2003; Luzio *et al.* 2007b). Endosomes are endocytotic vesicles derived from the plasma membrane (Fig. 2). Primary endocytic vesicles undergo maturation and trafficking to become early endosomes initially and late endosomes or multi-vesicular bodies later with a time scale of minutes or tens of minutes (Maxfield and McGraw 2004). Derived from late endosomes, lysosomes are membrane-enclosed compartments containing hydrolases for the intracellular digestion of macro-molecules (Luzio *et al.* 2007b). Lysosomes may fuse with late endosomes or multi-vesicular bodies to degrade macro-molecules (for example, the ligand-bound epidermal growth factor receptor) that are taken up from the plasma membrane via endocytosis (Luzio *et al.* 2007b). Alternatively, lysosomes may fuse with obsolete parts of the cell via autophagy (Luzio *et al.* 2007b). In both cases, ingested materials are degraded to obtain energy and to

**Fig. 2** Intracellular TRP channels in the secretory and endocytic pathways. Intracellular compartments undergo membrane fusion and fission/budding. There are two kinds of membrane fusions: 'kiss and run' and complete fusion. In some steps, transport vesicles fission off from the source membranes and fuse with the target membranes to delivery cargos. $Ca^{2+}$-sensitive membrane fusion and fission steps are indicated with white arrows. The molecular identities of intracellular compartments are defined by specific recruitment of small G proteins (Rab and Arf GTPases) and the composition of phosphoinositides (PIPs). The luminal ionic ($H^+$ and $Ca^{2+}$) composition is indicated for each organelle. (a) The Biosynthetic Pathway. Essentially all TRPs (labeled in mixed color) in the biosynthetic pathway may be present in the ER (pH 7.2; $[Ca^{2+}]_{ER} \sim 0.7$ mM; PI(4)P + PI(4,5)$P_2$) and the Golgi apparatus (*trans*-Golgi network; TGN; pH 6.4; $[Ca^{2+}]_{Golgi} \sim 0.3$–0.7 mM; PI(4)P + PI(4,5)$P_2$). The $Ca^{2+}$ gradients are established by the thapsigargin (TG)-sensitive SERCA (sarco/endoplasmic reticulum $Ca^{2+}$) pump in the ER, and by both SERCA and TG-insensitive SPCA (secretory pathway $Ca^{2+}$) pumps in the Golgi. TRPV1 is reportedly functional in the ER or Golgi. There are intermediate transport vesicles derived from the ER and the Golgi apparatus. These transport vesicles may deliver cargos to early endosomes (EE; pH 6.0; $[Ca^{2+}]_{EE} \sim 0.003$–2 mM; PI(3)P; Rab5), late endosomes (LE; pH 5.5; $[Ca^{2+}]_{LE} \sim 0.5$ mM; PI(3)P + PI(3,5)$P_2$; Rab7 and Rab9), or the PM through secretory vesicles (SV; pH6.4) and/or secretory granules (SG; pH 6.4). $[Ca^{2+}]_{EE}$ changes significantly during the maturation of early EE, dropping from 2 mM in the primary endocytic vesicles to $\sim 0.003$ mM 20 min after endocytosis (Gerasimenko *et al.* 1998). SVs and SGs deliver the newly synthesized TRP channels to the PM. (b) The Endocytic Pathway. EEs are derived from the primary endocytic vesicles after endocytosis. In addition to the late endocytic pathway, contents in the EE can also be sorted into recycling endosomes (RE; pH 6.4; $[Ca^{2+}]_{RE} \sim 0.003$–2 mM; PI(3)P + PI(4)P + PI(4,5)$P_2$; Rab11/Rab4), which are subsequently recycled back to the PM. TRP channels may be detected in the EE and RE as cargos during this cycle of endocytosis and recycling. In addition, TRPV2, TRPML2, and TRPML3 may play active roles in the early endocytic pathways. TRPV2 is activated by low pH and a reduction of intra-endosomal [$Cl^-$]. The channel activity of TRPML2 (in RE) may regulate the activation of small GTPase Arf6, an important regulator of the recycling pathway. The activation mechanism of TRPML3 (in EE) is still not known. In sympathetic neurons, TRPM7 is localized in synaptic vesicles (SyV; pH 6.4) that are derived from EEs. The channel activity of TRPM7 plays a role in controlling neurotransmitter release. In EEs, intra-endosomal $Ca^{2+}$ release may activate $Ca^{2+}$ sensor proteins such as Synaptotagmin (Syt) and calmodulin (CaM). Subsequently, homotypic and heterotypic fusion events occur. In the late endocytic pathways, late endosomes (LEs) may 'kiss and run' or completely fuse with other LE or lysosomes (LY; pH 4.5; $[Ca^{2+}]_{LY} \sim 0.5$ mM; PI(3)P + PI(3,5)$P_2$; Rab7). TRPML1-3 channels are predominantly localized in LEs and LYs. Activation of TRPML channels by unidentified cellular cues may induce intralysosomal $Ca^{2+}$ release. LEs, LYs, or hybrids of LEs and LYs, will then undergo calmodulin (CaM)- or synaptotagmin (Syt)-s dependent membrane fusion or fission/budding. Membrane proteins enter the degradation pathway following membrane invagination to form multi-vesicular bodies (MVB) in LEs. The inward budding of internal vesicles into MVBs is a $Ca^{2+}$-dependent process. In addition, MVBs may also undergo $Ca^{2+}$-dependent exocytosis to release internal vesicles (exosomes) (Savina *et al.* 2003). Retrograde (retromer) transport vesicles (TVs), derived from EEs, LEs, or LYs upon membrane fission, transport lipids and proteins retrogradely to the TGN. In addition to fusion with LEs, LYs can also undergo fusion with autophagosomes (APs) to form autolysosomes (ALs), or with the PM, i.e., lysosomal exocytosis. TRPM2 in LEL compartments is activated by ADPR, and likely by NAADP as well.

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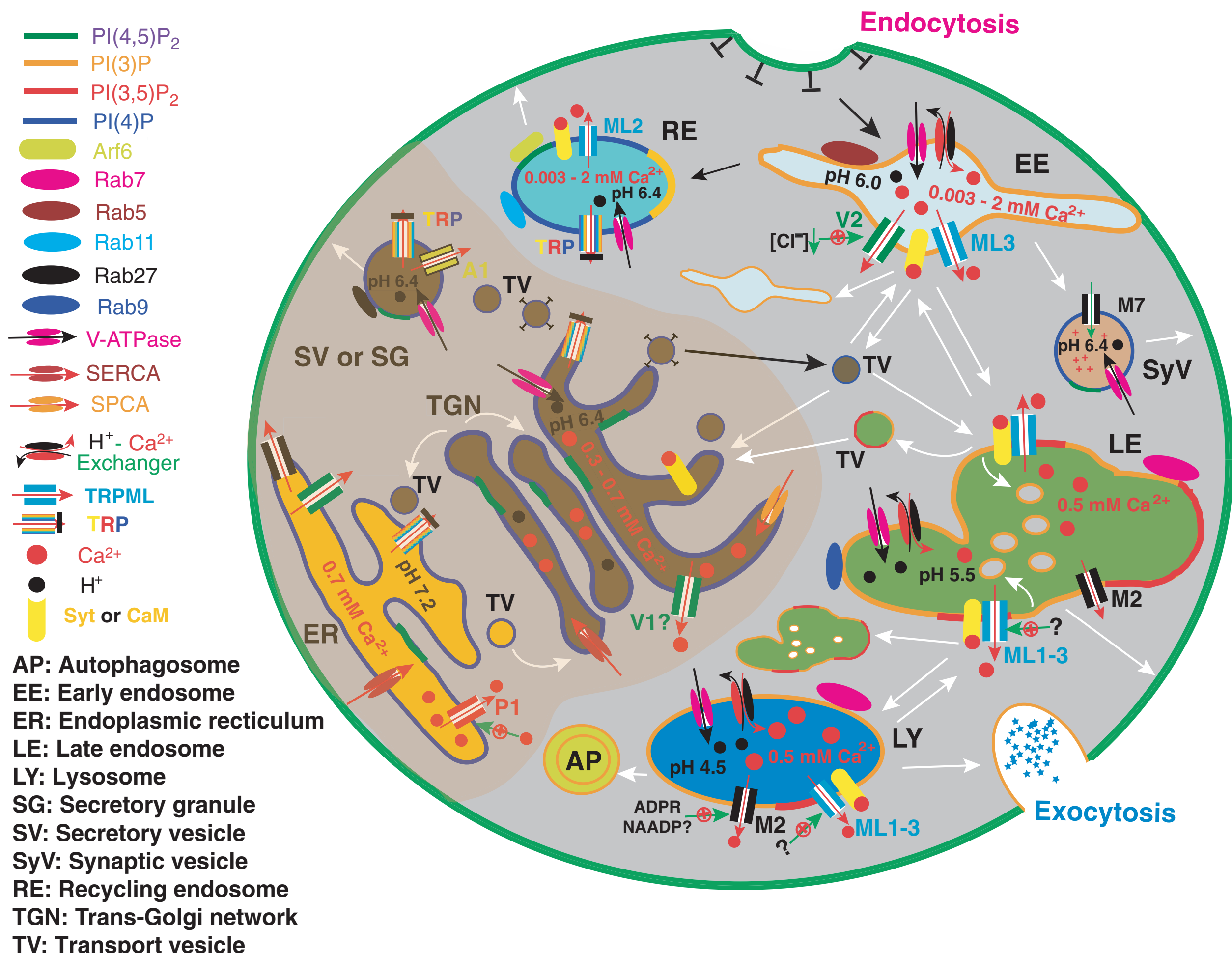
recycle building materials which are then released into the cytosol or delivered to the TGN via a retrograde route. Internalized membrane proteins that are not targeted for degradation, for example, transferrin receptor, are recycled back to the plasma membrane. In this case, the protein cargo is sorted into the recycling endosome (Fig. 2).

### Structural and regulatory mechanisms of membrane traffic

To control the direction and specificity of transport, a collection of structural and signaling proteins are recruited during various stages of the endocytic pathway. The fusion of two distinct vesicular compartments requires the contact of one v-SNARE (soluble NSF attachment protein receptors) and two or three t-SNARE proteins (Martens and McMahon 2008). SNAREs and their accessory proteins are regulated by both G proteins and lipid signaling (Roth 2004; Stenmark 2009). Rabs are small GTPases that are involved in mediating the identification and transport of organelles (review see Ref. Stenmark 2009). There are more than sixty Rabs in the secretory and endocytic pathways. The mechanisms for recruiting specific Rabs are not clear, although the cargos involved are known to play a crucial role (Stenmark 2009). The GTP-GDP cycle controls the recruitment of Rabs and their effector proteins to vesicles (Stenmark 2009). To control the transport specificity, various lipid kinases and phosphatases are often recruited to generate a set of organelle-specific PIPs (Roth 2004; Poccia and Larijani 2009). Rabs and PIPs collaboratively determine the vesicular identities (see Fig. 2). For example, Rab5 and PI(3)P define early endosomes; Rab7 and PI(3,5)$P_2$ define late endosomes (Roth 2004; Poccia and Larijani 2009). Dysregulated Rab signaling or PIP levels result in defective membrane trafficking (Roth 2004; Poccia and Larijani 2009; Stenmark 2009). While Rabs and PIPs can determine the direction of membrane traffic, the final step(s), i.e., the fusion of two vesicular compartments, depends on a brief increase in juxta-organellar [$Ca^{2+}$](Luzio *et al.* 2007a,b).

### Ionic composition and electrical properties of endolysosomes

The properties of endo-lysosomes (see Fig. 3) fit well with their primary functions. For example, the degradative

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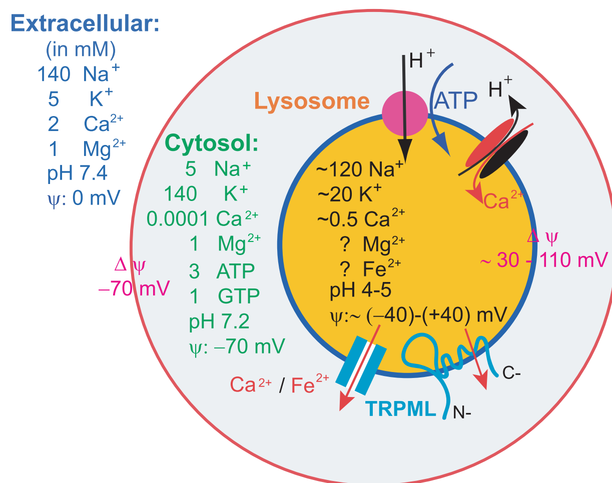


**Fig. 3** Electrical properties of lysosomes. While the ionic compositions of extracellular space and cytosol have been well established, ion concentrations in the lumen of lysosome are not clear. The luminal pH is between 4 and 5 and is established by a V-type ATPase. The $[Ca^{2+}]_{LY}$ is ~ 0.5 mM, which is maintained by an unidentified $H^+$-$Ca^{2+}$ exchanger. The resting membrane potential ($\Delta\varphi$; cytosol relative to lumen or extracellular) of the cell is ~ –70 mV (cytoplasmic-side negative). Based on studies on synaptic vesicles and phagosomes, the membrane potential across the lysosomal membrane is estimated to be ~ +30 to +110 mV (luminal-side positive).

functions of hydrolases are dependent on an acidic environment. A salient feature of endosomes and lysosomes is a low luminal pH (pH ~5–6 for endosomes and ~4–5 for lysosomes), which is established by a vacuolar (V)-type $H^+$-ATPase (Luzio *et al.* 2007b). As mentioned previously, endo-lysosomes are also intracellular $Ca^{2+}$ stores with the luminal $Ca^{2+}$ concentration ($[Ca^{2+}]_{lumen}$) estimated to be ~0.5 mM for lysosomes (Christensen *et al.* 2002), and varying from 0.003 mM to 2 mM for early endosomes (from 2 mM in the primary endocytotic vesicles to ~ 0.003 mM after 20 min post-endocytosis) (Gerasimenko *et al.* 1998) (Fig. 2). The proton ($H^+$) gradient helps in establishing the $Ca^{2+}$ gradient in the endo-lysosome, presumably through a $H^+$-$Ca^{2+}$ exchanger (Christensen *et al.* 2002; Michelangeli *et al.* 2005; Luzio *et al.* 2007b). Inhibition of the V-ATPase by bafilomycin A1 leads to depletion of endo-lysosomal $Ca^{2+}$ stores. The electrical potentials across the membranes of endosomes and lysosome are not precisely known. Based on studies of synaptic vesicles (Van der Kloot 2003) and phagosomes (Steinberg *et al.* 2007), transmembrane potentials are presumed to be positive in the lumen (relative to the cytosol) and likely to be between +30 and +110 mV (see Fig. 3). The positive potential of endo-lysosomes provides a driving force for $Ca^{2+}$ release into the cytosol. Keeping the basic properties and primary functions of endosomes and lysosomes in mind, we now will discuss the function of TRP channels found in the endo-lysosomal membrane.

### TRPV2 in early endosomes

Initially reported to be a temperature-activated channel in somatosensory neurons (Caterina *et al.* 1999), TRPV2 is also localized to intracellular vesicles (Kanzaki *et al.* 1999). A proteomic study revealing the presence of TRPV2 in the early endosome led Saito *et al.* (Saito *et al.* 2007) to test the hypothesis that TRPV2 is an endosomal $Ca^{2+}$ channel. Using an elegantly modified patch-clamp technique, Saito *et al.* successfully measured endogenous ionic currents ($I_{EE}$) in the isolated enlarged early endosome, which was made possible after endosomal fusion was genetically promoted (Saito *et al.* 2007). $I_{EE}$ is activated by reductions in the luminal pH and $Cl^-$ concentration, which usually occur within 20 min post-endocytosis (Gerasimenko *et al.* 1998). The pharmacological properties of $I_{EE}$ resemble those of $I_{TRPV2}$ studied in heterologous systems. For example 2-aminoethoxydiphenyl borate, a known activator and RuR, a known blocker of $I_{TRPV2}$, enhanced and inhibited $I_{EE}$, respectively. Saito *et al.* proposed that activation of TRPV2 leads to $Ca^{2+}$-dependent fusion between endosomal membranes. To ultimately prove the hypothesis, more experiments are necessary. For example, if TRPV2 indeed is an endosomal $Ca^{2+}$ channel important for endosomal fusion, $I_{EE}$ is expected to be augmented by over-expression of TRPV2, but reduced by a decrease or depletion of endogenous TRPV2 expression. In addition, inactivation of TRPV2 is expected to alter the size and morphology of early endosomes.

### TRPY1 in the yeast vacuole

The only TRP-like protein in yeast is the TRPY1 (also called *yvc1*) protein (Palmer *et al.* 2001; Denis and Cyert 2002). TRPY1 is localized in the yeast vacuole, which is equivalent to the mammalian lysosome. In a series of elegant studies, patch-clamp recordings were performed directly on isolated yeast vacuoles. It was found that mechanical force or cytoplasmic $Ca^{2+}$ could directly activate TRPY1 (Palmer *et al.* 2001; Zhou *et al.* 2003). $I_{TRPY1}$ is a large-conductance inwardly rectifying $Ca^{2+}$-permeable current. Using the aequorin $Ca^{2+}$ reporter, Denis and Cyert have demonstrated that TRPY1 mediates the vacuolar $Ca^{2+}$ release upon hyperosmotic shock (Denis and Cyert 2002). Collectively, these works have provided the most convincing evidence that TRPY1 is a physiologically important channel for intracellular $Ca^{2+}$ release.

### TRPML channels in late endosomal, lysosomal and other endocytic compartments

#### *Tissue distribution and subcellular localization*

Unlike other TRP channels, TRPML proteins are ideal candidate endo-lysosomal $Ca^{2+}$ channels, as cells which lack TRPML1 exhibit enlarged endo-lysosomes (Chen *et al.* 1998; Piper and Luzio 2004). There are three TRPML proteins in mammals (TRPML1–3, also called MCOLN1–3)

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(Puertollano and Kiselyov 2009). TRPML1 is expressed in most tissues (Slaugenhaupt 2002; Cheng *et al.* 2010) and co-localizes exclusively with late endosomal and lysosomal (LEL) markers (Dong *et al.* 2009; Puertollano and Kiselyov 2009; Cheng *et al.* 2010) (see Fig. 1). The LEL localization of TRPML1 is also supported by gradient fractionation studies on both endogenous and heterologously-expressed proteins (Kim *et al.* 2009; Zeevi *et al.* 2009). In addition, TRPML2 and TRPML3 exhibit more restrictive tissue distribution patterns, but are also located in the LEL compartment (Cuajungco and Samie 2008; Puertollano and Kiselyov 2009; Zeevi *et al.* 2009). TRPML2 and TRPML3 are also found in recycling endosomes (Arf6-positive pathway) and early endosomes, respectively (Karacsonyi *et al.* 2007; Kim *et al.* 2009; Martina *et al.* 2009). TRPML distribution and localization are reviewed in detail recently (Puertollano and Kiselyov 2009; Cheng *et al.* 2010).

### *Pore properties of TRPML channels*

All three TRPMLs have been shown to be $Ca^{2+}$-permeable (LaPlante *et al.* 2002; Xu *et al.* 2007; Dong *et al.* 2008; Kim *et al.* 2008, 2009), making them candidates to be lysosomal $Ca^{2+}$ release channels. The first electrophysiologically characterized wild-type TRPML channel was TRPML3 (Kim *et al.* 2007, 2008; Xu *et al.* 2007). Although the majority of heterologously-expressed TRPML3 proteins are vesicular, a small portion of them are able to traffic to the plasma membrane and give rise to whole-cell currents ($I_{TRPML3}$) (Grimm *et al.* 2007; Xu *et al.* 2007; Cuajungco and Samie 2008; Nagata *et al.* 2008; Kim *et al.* 2009; Martina *et al.* 2009; Puertollano and Kiselyov 2009). $I_{TRPML3}$ is an inwardly-rectifying $Ca^{2+}$-permeable current that is inhibited by low extracellular (analogous to the luminal side) pH but increased by $Na^+$ manipulation (removal followed by re-addition) (Kim *et al.* 2007, 2008). Mutations in the mouse *TRPML3* (*A419P*) result in the *varitint-waddler* (*Va*) phenotype (Cuajungco and Samie 2008; Puertollano and Kiselyov 2009). *Va* mice are deaf, exhibit circling behavior and have pigmentation defects. Compared with wild-type TRPML3, much larger TRPML3-mediated currents are seen in cells expressing $TRPML3^{A419P}$ ($TRPML3^{Va}$). The $TRPML3^{Va}$ channel exhibits similar pore properties as wild-type TRPML3, but with altered gating behavior, suggesting that *Va* is a channel gain-of-function mutation (Grimm *et al.* 2007; Kim *et al.* 2007, 2008; Xu *et al.* 2007; Cuajungco and Samie 2008; Nagata *et al.* 2008; Puertollano and Kiselyov 2009). By artificially introducing a *Va*-like mutation in the analogous position of TRPML1 (V432P), Xu *et al.* were able to characterize the pore properties of TRPML1 (Xu *et al.* 2007). Like TRPML3, $TRPML1^{Va}$ is an inwardly rectifying $Ca^{2+}$-permeable but proton-impermeable channel. But, unlike $I_{TRPML3}$, $I_{TRPML1\text{-}Va}$ is potentiated by low pH. Similarly, $I_{TRPML2\text{-}Va}$ is also a proton-potentiated inwardly rectifying $Ca^{2+}$-permeable current (Dong *et al.* 2008). It remains a possibility that *Va*-like mutations change the pore properties of TRPML1 and TRPML2, although this is least likely. Recently, Dong *et al.* developed a patch-clamp method to record currents directly from isolated endo-lysosomes which were pharmacologically enlarged (from 0.1–0.5 to 2–3 μm) using the small molecule compound vacuolin-1 (Dong *et al.* 2008). With this method, Dong *et al.* were able to record $I_{TRPML1}$ in enlarged endo-lysosomes. Lysosomal $I_{TRPML1}$, although much smaller in amplitude than $I_{TRPML1\text{-}Va}$, largely resembles $I_{TRPML1\text{-}Va}$, suggesting that although TRPML1 is likely to be gated by other, unidentified, cellular cues, the activating mutation is still a valid approach for characterizing the pore properties of TRPML1 and TRPML2. In summary, TRPMLs constitute a family of inwardly rectifying, $Ca^{2+}$-permeant but proton-impermeant cation channels. TRPML channel properties are reviewed in detail recently (Puertollano and Kiselyov 2009; Cheng *et al.* 2010).

### *Dual roles of TRPMLs in membrane trafficking: fusion and fission*

The role of the TRPMLs in post-endocytic membrane trafficking and organelle dynamics in the late endocytic pathway has been extensively studied both *in vitro* and *in vivo* (reviewed in Refs. Puertollano and Kiselyov 2009; Cheng *et al.* 2010). Mutations in human *TRPML1* cause type IV mucolipidosis (ML4), a devastating neurodegenerative disease in young children (Slaugenhaupt 2002). ML4 patients exhibit motor defects, mental retardation, retinal degeneration, and iron-deficiency anemia. Loss-of-function studies revealed a role of TRPML1 in membrane fission from the LEL compartment. Fibroblasts derived from ML4 patients (ML4 cells), expressing loss-of-function TRPML1 mutations, exhibit enlarged, swollen lysosome-like vacuoles (Chen *et al.* 1998; Slaugenhaupt 2002). Endocytic delivery to lysosomes involves direct fusion between late endosomes and lysosomes to produce late endosome–lysosome hybrid organelles, from which the lysosomes are re-formed (Luzio *et al.* 2000, 2007b; Pryor *et al.* 2000). Genetic studies of the *cup-5* mutant, a *C. elegans* orthologue of mammalian TRPMLs, reveal that the enlarged vacuoles of *cup-5* cells contain markers for both late endosomes and lysosomes and are likely to be hybrid organelles (Treusch *et al.* 2004). Mechanistically, enlarged endo-lysosomes could result from uncontrolled and excessive fusion, defective membrane fission, or impaired organellar osmoregulation (Luzio *et al.* 2007a). In most cases, enlarged endosomes and lysosomes are suggestive of defective trafficking. For example, dysregulation of Rabs and PIPs, two essential regulators of membrane trafficking, often leads to similar phenotypes, i.e., enlarged vacuoles containing hybrid markers (Roth 2004; Poccia and Larijani 2009; Stenmark 2009). While overactive Rab5 causes enlarged endosomes, loss-of-function mutations of PIKfyve/Fab1, a LEL-specific lipid kinase synthesizing $PI(3,5)P_2$ from PI(3)P, causes enlarged LEL compartments.

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ML4 cells also accumulate a variety of undigested lipids and water-soluble substances in the LEL compartment (Chen *et al.* 1998). Lipid accumulation may result from the defective degradation of cellular components and/or disruptions in membrane trafficking. Defective degradation could in turn be because of either a lack of specific hydrolase enzymes, as revealed in most lysosome storage diseases (Puertollano and Kiselyov 2009), or an impaired ion homeostasis (especially $H^+$) of organelles. However, in TRPML1-deficient (TRPML1$^{-/-}$) cells, accumulated lipids and storage materials are of heterogeneous origins (Chen *et al.* 1998; Treusch *et al.* 2004; Venugopal *et al.* 2007; Venkatachalam *et al.* 2008), suggesting that the defects might be related to the homeostasis of LEL compartments and/or disruption of broad spectrum lipid metabolism. The hydrolase-catalyzed lysosomal degradation of storage materials, however, is largely unaffected by the TRPML1 defect in ML4 cells (Chen *et al.* 1998). Thus the most likely defect of ML4 cells is membrane trafficking. Consistent with this, the use of labeled lipids such as fluorescent lactosylceramide in pulse-chase experiments demonstrated a delay in the retrograde transport of lipids from lysosomes to the TGN, consistent with the role of TRPML1 in late endocytic pathway trafficking (Chen *et al.* 1998; Pryor *et al.* 2006; Thompson *et al.* 2007). Although lipid accumulation phenotype may be caused by secondary effects because of the chronic accumulation of undigested lipids (Miedel *et al.* 2008), the lipid accumulation in ML4 cells, however, can be acutely rescued by introduction of the wild-type TRPML1 gene (Pryor *et al.* 2006). Thus the defective lipid exit from the LEL compartment may be a direct consequence of TRPML1-deficiency.

The simplest model to reconcile most results in the literature is that the formation of transport vesicles from the LEL compartment to the TGN, and the reformation of lysosomes from the late endosome-lysosome hybrid organelles, are blocked in ML4 or cup-5 cells (Piper and Luzio 2004; Treusch *et al.* 2004; Thompson *et al.* 2007). In other words, TRPML1 may be required for the biogenesis of lysosomes and LEL-derived transport vesicles. Consistent with this idea, TRPML1 gene expression is significantly elevated when lysosome biogenesis is induced (Sardiello *et al.* 2009). As membrane fission is $Ca^{2+}$-dependent, it is likely that TRPML1 and, more specifically, its $Ca^{2+}$ conduction, is required for the membrane fission from LEL compartments or late endosome-lysosome hybrids. Notably, intraluminal $Ca^{2+}$ release has been demonstrated to play an essential role in the *in vitro* re-formation of lysosomes from endosome–lysosome hybrids (Pryor *et al.* 2000; Luzio *et al.* 2007a). Consistent with the requirement of TRPML1 in lipid migration from late endosomes and lysosomes to the TGN, $Ca^{2+}$ has been shown to be required for the formation and stabilization of specific transport vesicles (Ahluwalia *et al.* 2001; Luzio *et al.* 2007a,b).

TRPML1 is also involved in the membrane fusion of endo-lysosomes. Both the transport of fluid-phase markers to lysosomes and the lysosomal degradation of internalized growth factor receptors are delayed in TRPML1$^{-/-}$ cells (Treusch *et al.* 2004; Thompson *et al.* 2007), suggesting a defect in trafficking of endocytosed materials into the LEL compartment. These findings could indicate that the $Ca^{2+}$ permeability of TRPML1 is required for the $Ca^{2+}$-dependent membrane fusion between early endocytic compartments, or for the formation of transport vesicles from early to late endosomes. In addition to its interaction with the late endosome, a lysosome can also incompletely ('kiss-and-run') or completely fuse with the plasma membrane (Luzio *et al.* 2007b) resulting in the exocytosis of lysosomal contents (lysosomal exocytosis). This process has been implicated in cellular waste elimination, membrane repair, and neurotransmitter release (Reddy *et al.* 2001; Zhang *et al.* 2007). Lysosomal exocytosis is triggered by a rise of $[Ca^{2+}]_{cyt}$ and the subsequent binding of $Ca^{2+}$ to the C2 domains of synaptotagmin VII localized in the lysosomal membrane.

The lumen of the lysosome is a major sources of the $Ca^{2+}$ involved in lysosomal exocytosis (Luzio *et al.* 2007a,b). The mechanisms underlying lysosomal $Ca^{2+}$ release, however, are still unclear. Moreover, the molecular identities of the putative lysosomal $Ca^{2+}$ release channels remain elusive. Lysosomal exocytosis is reduced in ML4 cells (LaPlante *et al.* 2006) but increased in HEK cells expressing gain-of-function TRPML1 mutations (Dong *et al.* 2009). Increased lysosomal exocytosis results in increased cell surface expression of TRPML1, which may explain the measurable whole-cell current of TRPML1$^{Va}$ (Dong *et al.* 2009). To elaborate this hypothesis, it is necessary to investigate whether or not the blockade of lysosomal exocytosis reduces $I_{TRPML1\text{-}Va}$ amplitude. Consistent with a role of TRPML1 in exocytosis, shRNA knockdown of TRPML1 leads to the reduced transport of major histocompatibility complex II (MHCII) to the plasma membrane in macrophages (Thompson *et al.* 2007). In summary, TRPML1 participates in multiple transport steps of late endocytic pathways by its involvement in the mechanisms of both membrane fusion and fission.

TRPML2 and TRPML3 appear to play similar roles to TRPML1 in post-endocytic membrane trafficking. Zeevi *et al.* recently reported that siRNA knockdown of endogenous TRPML2 in HEK or Hela cells results in inclusion bodies in the LEL compartment (Zeevi *et al.* 2009). Inactivation of TRPML2 by a dominant-negative approach reduced recycling of internalized plasma membrane proteins back to the plasma membrane (Karacsonyi *et al.* 2007). Conversely, over-expression of TRPML2 caused a constitutive activation of Arf6, leading to increased exocytosis (Karacsonyi *et al.* 2007). These studies suggest that TRPML2 is required for membrane fusion in the early endocytic pathways, and membrane fusion and fission in the

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late endocytic pathways. Consistent with a positive role of TRPML3 in the late endocytic pathways, siRNA knockdown of TRPML3 leads to inclusion bodies in the LEL compartment of HEK and Hela cells (Zeevi *et al.* 2009). Two recent studies report that over-expression of TRPML3 results in enlarged endo-lysosomes, decreased endocytosis, and reduced lysosomal degradation (Kim *et al.* 2009; Martina *et al.* 2009). These results initially appear inconsistent with Zeevi *et al.*'s TRPML3 findings and the proposed functions for TRPML1 and TRPML2. These apparent disparities can be reconciled if we consider the dual functions of TRPMLs in membrane fusion and fission. As mentioned above, enlarged endo-lysosomes might result from either excessive fusion or reduced fission. TRPML3 is expressed in both early endosomes and the LEL compartment (Kim *et al.* 2009; Martina *et al.* 2009). Dysregulated organellar $Ca^{2+}$ release resulting from TRPML3 over-expression may possibly increase membrane fusion events in the early and/or late endocytic pathways. The vacuolar phenotype resulting from over-expression may not be limited to TRPML3. Indeed, we have observed enlarged LEL compartments in HEK cells expressing high levels of TRPML1 (Cheng and Xu, unpublished observation; also see Ref. Vergarajauregui *et al.* 2009). An over-expression of C-terminal, but not N-terminal, enhanced green fluorescence protein fusion constructs of TRPML1 or TRPML2 in B-lymphocytes was reported to cause enlarged LEL compartments (Song *et al.* 2006). These C-terminal fusion constructs appear to exhibit high levels of expression in B-lymphocytes (personal communication with Scharenberg A.). Consistent with a role of TRPML3 in membrane fusion, TRPML3 over-expression also leads to an increased number of autolysosomes, which results from the fusion of autophagosomes and lysosomes (Kim *et al.* 2009). TRPMLs may thus play important roles in membrane traffic by regulating both $Ca^{2+}$-dependent membrane fusion and fission. As different $Ca^{2+}$ sensors and effectors are implicated in fusion and fission, selective inhibitors of $Ca^{2+}$ sensors may prove informative regarding the nature (effecting fusion vs. fission) of the defects leading to enlarged endolysosomes.

A key open question is how TRPML channels are regulated by various cytosolic and luminal factors, and/or proteins and lipids in the endo-lysosomal membranes, especially those which are known to be involved in endo-lysosomal trafficking. In addition to $Ca^{2+}$, PIPs and G proteins have been found to regulate intracellular trafficking (Roth 2004; Stenmark 2009). It is conceivable that these signaling cascades may be involved in relaying extracellular signals to those intracellular pathways that mediate membrane trafficking. Signaling by lipid and G proteins is known to modulate plasma membrane TRP channels (Clapham 2003; Montell 2005; Nilius *et al.* 2007); it is likely that PIPs and Rabs regulate membrane traffic by modulating vesicular TRPMLs. Direct evidence to support this hypothesis is still lacking.

### *TRPMLs in signal transduction*

TRPML1 has been proposed to be an important participant in lysosome-mediated signal transduction. As mentioned previously, $Ca^{2+}$ release from lysosomes or lysosome-related acidic stores, similar to $Ca^{2+}$ release from the ER, plays an indispensable role in the transduction of many extracellular signals (Galione *et al.* 2009). For example, endothelin-1 and integrin ligands mobilize lysosome-related $Ca^{2+}$ stores in smooth muscle cells (Galione *et al.* 2009). Accumulated evidence suggests that NAADP may act as a common second messenger downstream of these receptors, and that the putative NAADP receptor is likely to be found in lysosomes (Zhang and Li 2007; Calcraft *et al.* 2009; Galione *et al.* 2009). As $Ca^{2+}$ channels in the LEL compartment, TRPMLs are natural candidates to mediate the NAADP response. By reconstituting lysosomal membranes into a lipid bilayer, Zhang *et al.* recently reported that NAADP up-regulates a cationic current that is dependent on TRPML1 expression (Zhang and Li 2007). The pharmacological properties of $I_{NAADP}$ in the lipid bilayer are similar to those of the putative NAADP receptor. However, $I_{NAADP}$ is a $Cs^{+}$-permeable current and exhibits a linear I-V curve, two properties that are inconsistent with $I_{TRPML1}$. It is possible that other lysosomal membrane proteins may form heteromultimers with TRPML1, leading to a novel current. For example, TPC2 (two pore calcium channel protein 2) protein has been recently shown to mediate NAADP-induced $Ca^{2+}$ release from lysosomes (Calcraft *et al.* 2009; Galione *et al.* 2009). Future work may reveal the relative contributions of TRPML1, TRPM2 (see following), and TPC2 to the endogenous NAADP response in different cell types.

### *TRPMLs in vesicular ion homeostasis*

In the endo-lysosome system, $H^{+}$, $Ca^{2+}$, and membrane fusion have been found to be interconnected (Luzio *et al.* 2007a,b). In addition to $Ca^{2+}$, TRPML channels are permeable to other cations in the LEL lumen (Fig. 3) and thus may have functions distinct from $Ca^{2+}$ signaling. Although these findings are highly controversial, ML4 or TRPML1 knockdown cells (TRPML1$^{-/-}$) cells appear to have an overly acidified pH in LEL compartments (Soyombo *et al.* 2006; Miedel *et al.* 2008). Given that the TRPML1$^{Va}$ channel exhibits no permeability to protons, it is not immediately clear what may cause lysosomal hyperacidification in the absence of TRPML1. Although the TRPML3 channel is not proton-permeable and is indeed inhibited by low pH, over-expression of TRPML3 results in the alkalization of endo-lysosomes (Martina *et al.* 2009). Therefore, it is likely that endo-lysosomal acidification is secondary to $Ca^{2+}$ release (see Ref. (Cheng *et al.* 2010) for discussion).

TRPML1 and TRPML2, but not TRPML3, are also permeable to $Fe^{2+}$, $Mn^{2+}$, and other heavy trace metals (Dong *et al.* 2008). ML4 cells exhibit a cytosolic $Fe^{2+}$ deficiency and a concurrent lysosomal $Fe^{2+}$ overload, suggesting that

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the iron efflux pathway is blocked in ML4 cells and that TRPML1 is essential for lysosomal $Fe^{2+}$ release (Dong *et al.* 2008). Under oxidative conditions, lysosomal $Fe^{2+}$ overload may dramatically increase the production of reactive hydroxyl radicals ($OH^-$; Fenton reaction), which in turn facilitate the formation of lipofuscin (also called aging pigment) (Kurz *et al.* 2008).

In summary, TRPMLs participate in multiple endo-lysosome-mediated functions including signal transduction, ionic homeostasis, and more than one aspect of membrane trafficking. A major challenge of TRPML research is to understand how one single TRPML protein can play such diverse roles. Multiple ionic conductances in a single membrane channel may certainly contribute to multifaceted functions. In addition to their divalent permeability, TRPMLs are also permeable to $Na^+$ and $K^+$ (Xu *et al.* 2007). TRPMLs may therefore regulate organelle dynamics by regulating endo-lysosomal membrane potentials. Rabs and PIPs exist in 'microdomains' in the membranes of endo-lysosomes, participating in multiple functions by recruiting distinct effector proteins (Poccia and Larijani 2009; Stenmark 2009). TRPMLs may differentially associate with Rabs, PIPs, and $Ca^{2+}$ sensors (for example, CaM, Syt, and ALG-2), giving them the ability to generate multiple cellular outputs.

### TRPM2 in the LEL compartment

Initially characterized as a plasma membrane channel gated by free cytosolic ADP-ribose (ADPR) (Perraud *et al.* 2001), recent evidence suggests that TRPM2 is also localized in the LEL compartment (Lange *et al.* 2009). Rather than simply being cargo in the degradative pathway, TRPM2 can function as a lysosomal $Ca^{2+}$ release channel in response to cytosolic ADP-ribose (Lange *et al.* 2009). Plasma membrane TRPM2 is reportedly activated by NAADP (Beck *et al.* 2006). It is conceivable that TRPM2 also has a role in lysosomal NAADP signaling (see above). In addition, plasma membrane TRPM2 is activated by increases in $[Ca^{2+}]_i$ (Du *et al.* 2009). Thus, TRPM2 might mediate $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) in the LEL compartment, analogous to $IP_3R$/RyR in the ER/SR (Berridge *et al.* 2003). Although CICR has not been demonstrated in mammalian LELs, such mechanism has been proposed to exist in yeast vacuoles (Palmer *et al.* 2001).

### TRP channels in recycling endosomes

Like the transferrin receptor, plasma membrane TRP channels constantly undergo endocytosis and enter the recycling pathway (Maxfield and McGraw 2004). The steady-state location of TRPs is determined by the balance of endocytosis and membrane insertion, which is under tight regulation by a variety of extracellular signals and cellular cues (Maxfield and McGraw 2004). TRPV5 and TRPV6 are $Ca^{2+}$-selective channels involved in $Ca^{2+}$ transport in the kidney (van de Graaf *et al.* 2006). In epithelial cells, TRPV5 and 6 are found to be localized to recycling endosomes and physically interact with Rab11, a small G protein that is predominantly localized in recycling endosomes (van de Graaf *et al.* 2006). Locking Rab11 in an inactive GDP-bound state results in the reduced surface expression of TRPV5 and 6 (van de Graaf *et al.* 2006).

Several TRPs have been shown to undergo regulated exocytosis. In hippocampal neurons, TRPC5 is localized in the intracellular vesicles of neurites (Bezzerides *et al.* 2004). Using total internal reflection fluorescence microscopy to image events within 100 nm of the plasma membrane, Berrerides *et al.* demonstrated that, in response to growth factor stimulation, TRPC5-residing vesicles undergo rapid insertion into the plasma membrane (Bezzerides *et al.* 2004). Similarly TRPC3, residing in vesicle-associated membrane protein 2-positive compartments, was reported to undergo G protein-coupled receptor-stimulated translocation to the plasma membrane (Singh *et al.* 2004). In response to mechanical shear stress, TRPM7-residing vesicles undergo translocation to the plasma membrane of vascular smooth muscle cells (Oancea *et al.* 2006). TRPV1 is found in intracellular vesicles and physically interacts with the vesicular proteins Snapin and Synaptotagmin IX (Morenilla-Palao *et al.* 2004). Upon stimulation, TRPV1-containing vesicles undergo protein kinase C-dependent exocytosis, allowing a novel form of channel regulation (Morenilla-Palao *et al.* 2004). Although the molecular identities of these vesicles are poorly defined, they can be tentatively classified as belonging to an intracellular pool that is derived from recycling endosomes.

Are these vesicular TRPs functional? It was shown that activation of TRPC3 by diacylglycerol may further increase the cell surface expression of the channel (Singh *et al.* 2004). It is not clear whether diacylglycerol was activating plasma membrane or vesicular TRPC3, or perhaps both. Conducting this experiment in the presence of a membrane-impermeable TRPC3 inhibitor (not reported yet) would prove informative. If vesicular TRPs were purely cargo that did not actively participate in vesicular trafficking, it would be important that they keep inactive during transport to avoid the misregulation of membrane traffic. There are several reasons to suggest that this might be the case. The lipid composition of intracellular membranes is quite different from that of the plasma membrane. Many TRPs require $PI(4,5)P_2$ for their function (Ramsey *et al.* 2006). Although abundant in the plasma membrane, this lipid is usually excluded from endocytic vesicles (Roth 2004; Poccia and Larijani 2009). In addition, most TRPs are inhibited by low pH (Clapham 2003), such as that found in many vesicles. Therefore, it is likely that even the presence of agonist may not sufficiently activate TRPs in the intracellular reserve pool. This hypothesis could be tested by measuring the $[Ca^{2+}]_{cyt}$ response upon stimulating the cells using membrane-permeable agonists (if available) in the absence of extracellular $Ca^{2+}$. As intracellular vesicles may

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be heavily dependent on extracellular $Ca^{2+}$ to fill/refill their $Ca^{2+}$ stores, caution is necessary regarding to the time course of such experiments.

## TRP channels in the biosynthetic/secretory pathway

Like all other plasma membrane proteins, TRPs have to go through various stages of biosynthetic pathways before they reach their destination. For example, in order to carry out normal functions, plasma membrane TRPs are glycosylated in the ER and Golgi apparatus (Cohen 2006). Recent works, however, indicate that several TRPs are constitutively localized in the ER and Golgi and, more importantly, can be activated in these compartments by their agonists (Koulen *et al.* 2002; Turner *et al.* 2003; Thebault *et al.* 2005; Prasad *et al.* 2008). As multiple transport steps in the ER and the Golgi are regulated by intraluminal $Ca^{2+}$ release (Ahluwalia *et al.* 2001; Hay 2007), these studies point to active roles of TRPs in ER or Golgi-mediated signal transduction and/or membrane trafficking.

$Ca^{2+}$ release from organelles in the secretory pathways (ER and Golgi) is important for signal transduction (Berridge *et al.* 2003). For example, the 'classic' $Ca^{2+}$ release channels, i.e., $IP_3$Rs/RyRs in the ER/SR, couple numerous extracellular signals to intracellular events such as hormone secretion (Berridge *et al.* 2003). Several TRP $Ca^{2+}$ channels (see below) in the ER and Golgi may also be activated to completely or partially deplete the $Ca^{2+}$ stores, which may be coupled with unidentified cellular events. A most likely possibility is that TRP-mediated ER $Ca^{2+}$ release activates $IP_3$R/RyR-mediated CICR (Berridge *et al.* 2003). In addition, there exist poorly characterized 'ER leak' pathways that are known to be important in regulating ER $Ca^{2+}$ homeostasis, although their molecular identities are not known (Camello *et al.* 2002; Berridge *et al.* 2003).

$Ca^{2+}$ release from the ER and the Golgi is also important for membrane trafficking. *In vivo* studies revealed distinct sensitivities to BAPTA-AM versus EGTA-AM for many steps of membrane trafficking in the secretory pathways including, for example, anterograde intra-Golgi transport and retrograde endosome-to-TGN transport (Ahluwalia *et al.* 2001; Chen *et al.* 2002; Burgoyne and Clague 2003; Hay 2007). Inhibitory effects by BAPTA but not EGTA were also observed in cell-free intra-Golgi transport assays (Porat and Elazar 2000). CaM inhibitors have also been shown to block the intra-Golgi transport (Porat and Elazar 2000; Ahluwalia *et al.* 2001; Chen *et al.* 2002). Therefore, it is likely that uncharacterized $Ca^{2+}$ release channels in the ER or Golgi may be activated by trafficking cues to regulate membrane fusion/fission events in the secretory pathways.

### TRPP1 channels in the endoplasmic reticulum

TRPP1 (also called polycystin-2, PC2, and TRPP2) is one of two genes mutated in polycystic kidney disease (Zhou 2009). TRPP1 is localized to the ciliary membranes of kidney epithelial cells where it mediates fluid flow-induced $Ca^{2+}$ influx (Nilius *et al.* 2007). However, both heterologously-expressed and endogenous TRPP1 proteins are also found to be localized to the ER (Koulen *et al.* 2002; Geng *et al.* 2008). By reconstitution into the lipid bilayer, TRPP1 was shown to be activated by $Ca^{2+}$ on the cytoplasmic side (Koulen *et al.* 2002; Geng *et al.* 2008). TRPP1 may therefore function as a calcium-induced calcium release channel like the $IP_3$R and the RyR, participating in signal amplification. Consistent with this, receptor-mediated $IP_3$-dependent ER $Ca^{2+}$ release is increased by the over-expression of wild-type, but not the disease-causing mutant TRPP1 (Koulen *et al.* 2002; Geng *et al.* 2008). In TRPP1-deficient cells, the basal $[Ca^{2+}]_{cyt}$ is significantly lower than in control cells (Geng *et al.* 2008). In contrast, over-expression of TRPP1 leads to an increase of $[Ca^{2+}]_{cyt}$ and a concurrent decrease of ER $Ca^{2+}$ content (Geng *et al.* 2008). These results suggest that TRPP1 may function as an ER leak channel. TRPP1 is also found to interact with Syntaxin-5, an ER and Golgi-associated t-SNARE; this interaction inhibits the $I_{TRPP1}$(Geng *et al.* 2008). While $Ca^{2+}$ is presumed to regulate SNARE complex formation, this study suggests that SNARE proteins can in turn regulate $Ca^{2+}$ release channels.

### TRPM8 in the endoplasmic reticulum

TRPM8 is a cold- and menthol-activated channel in the plasma membrane of somatosensory neurons (Ramsey *et al.* 2006). In human prostate epithelial cells, however, TRPM8 is highly localized to the ER (Thebault *et al.* 2005). In the absence of extracellular $Ca^{2+}$, activation of TRPM8 by menthol or cold induces $[Ca^{2+}]_i$ increases that is sensitive to thapsigargin (TG), suggesting that the ER store is the source of the $Ca^{2+}$ release (Thebault *et al.* 2005). TRPM8-agonist-inducced ER $Ca^{2+}$ store depletion results in the activation of the store-operated $Ca^{2+}$ release-activated $Ca^{2+}$ channel (Thebault *et al.* 2005). Therefore, in some cell types, TRPM8 may function as an ER $Ca^{2+}$ release channel similar to $IP_3$R and RyR. It is not clear whether TRPM8 can function as a $Ca^{2+}$ release channel in the ER of other cell types, such as sensory neurons. Although the cellular mechanism activating TRPM8 in the ER is not known, it is conceivable that endogenous menthol-like molecules exist, functioning as novel $Ca^{2+}$-mobilizing second messengers.

### TRPV1 in the endoplasmic reticulum and the *trans*-Golgi network

TRPV1 is a true sensory channel that is expressed in somatosensory neurons and is activated by heat, protons, and capsaicin (Clapham 2003). Subcellular localization studies suggest that both heterologously-expressed and endogenous TRPV1 are also localized to the ER and Golgi compartments (Turner *et al.* 2003). Activation of TRPV1 by

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capsaicin induces $Ca^{2+}$ release from an $IP_3$-sensitive but TG-insensitive store. While ER $Ca^{2+}$ stores are maintained by the TG-sensitive SERCA (sarco/endoplasmic reticulum $Ca^{2+}$) pump, $Ca^{2+}$ gradients in the Golgi are established by both SERCA and the TG-insensitive SPCA (secretory pathway $Ca^{2+}$) pump. Like the ER, the Golgi also contains functional $IP_3Rs$ (Michelangeli *et al.* 2005). Because of the TG-insensitivity of TRPV1-induced $Ca^{2+}$ release, the most likely location for TRPV1 is in SPCA-positive Golgi compartments. Mobilization of TRPV1-containing $Ca^{2+}$ stores, however, is not sufficient to activate $Ca^{2+}$ release-activated $Ca^{2+}$ channels (Turner *et al.* 2003). Nevertheless, the evidence sufficiently supports a role of TRPV1 in intracellular $Ca^{2+}$ release.

The endogenous agonist of TRPV1 in intracellular membranes has remained elusive. As the Golgi is acidic relative to the ER (Fig. 1), transport of TRPV1-positive vesicles from the ER to the Golgi may readily activate or sensitize TRPV1. Anandamide, a weak agonist of TRPV1, is known to induce intracellular $Ca^{2+}$ release via a phospholipase C-independent mechanism (Felder *et al.* 1993). TRPV1 in the Golgi compartments may serve as a natural candidate for this release.

### TRPA1 in secretory vesicles and secretory granules

TRPA1 is expressed in somatosensory neurons and responds to a variety of noxious sensory compounds (Ramsey *et al.* 2006). In mast cells, TRPA1 is localized in secretory vesicles (SVs) and secretory granules (SGs) and physically interacts with vesicular proteins in SGs (Prasad *et al.* 2008). When heterologously expressed in HEK cells, TRPA1 can mediate intracellular $Ca^{2+}$ release, presumably from SVs, in response to the TRPA1 agonist icilin (Prasad *et al.* 2008). As intracellular $Ca^{2+}$ activates TRPA1 (Zurborg *et al.* 2007), TRPA1 may function as another CICR-like channel in SV or SG. As SVs or SGs undergo $Ca^{2+}$-regulated exocytosis, this mechanism may allow a feed-forward induction of cell-surface expression of TRPA1, contributing to a sudden increase in the $I_{TRPA1}$.

### Other TRP channels in the endoplasmic reticulum, *trans*-Golgi network, secretory vesicles and granules

All plasma membrane TRP channels need to go through the biosynthetic pathways, i.e. ER, TGN, and secretory vesicles/granules before being inserted into the plasma membrane. Are these nascent TRP channels functional in these compartments? As many synthetic and natural products have been found to activate specific TRPs in the plasma membrane (Ramsey *et al.* 2006), for these TRPs, these relatively selective agonists may be useful for future studies to reveal whether they are functional in intracellular compartments.

All four TRPs mentioned above (TRPP1, TRPM8, TRPV1, and TRPA1) are active intracellularly. Is intracellular activation a general feature for TRPs in the biosynthetic pathways? While $PI(4,5)P_2$ is a limiting factor to prevent the activation of TRPs in the recycling pathways, the ER and the Golgi do contain significant amounts of $PI(4,5)P_2$ in their membranes (Roth 2004). There are factors, however, that may prevent the full functionality of TRPs in the ER and Golgi membranes. For example, the lipid bilayers of the ER and the Golgi favor proteins with shorter transmembrane domains than those of the plasma membrane (Watson and Pessin 2001). In addition, several TRPs require post-translational modification such as glycosylation for function (Cohen 2006). Nevertheless, as all four TRPs studied so far are active intracellularly, it is conceivable that TRPs in the biosynthetic pathways might be active. Although activation of many other vesicular TRPs (Fig. 1) has not been reported, some of them, for example, TRPC3 and TRPC7, have been shown to actively participate in trafficking/exocytosis (Lavender *et al.* 2008).

## TRP channels in the cell-type specific compartments

Several TRPs are found in cell-type specific vesicular compartments, for example, LROs and synaptic vesicles (SyVs). These may participate in cell-type specific functions in these compartments independent of their general roles.

### TRPM7 in synaptic vesicles

TRPM7 is a protein with two functional domains: a cation channel module and a kinase domain (Ramsey *et al.* 2006). Under physiological conditions, $I_{TRPM7}$ is outwardly rectifying with an inward non-selective ($Ca^{2+}$, $Mg^{2+}$, $Na^{+}$) conductance and an outward monovalent conductance (Clapham 2003). Although $I_{TRPM7}$-like current can be recorded in almost every cell, in sympathetic neurons and PC12 cells, TRPM7 is found to be mainly localized in acetylcholine-containing SyVs (Krapivinsky *et al.* 2006; Brauchi *et al.* 2008). Consistent with its vesicular localization, Kapivinsky *et al.* provided additional biochemical evidence that TRPM7 forms a molecular complex with the fusion machinery components snapsin, a SNARE protein, and synaptotagmin I (Krapivinsky *et al.* 2006). Electrophysiological analyses revealed that both amplitude and frequency of excitatory postsynaptic potential (EPSP) are reduced in neurons transfected with TRPM7-specific siRNA or dominant-negative TRPM7 constructs. Further analysis suggest that the reduced EPSP amplitude is because of a smaller quantal size of neurotransmitter release, which reflects a reduced amount of acetylcholine release in a single fusion event (fusion of a SyV with the plasma membrane). As TRPM7 is outwardly rectifying, one possibility is that TRPM7 may provide counter ions for the release of positively-charged neurotransmitters (Krapivinsky *et al.* 2006). However, vesicular $I_{TRPM7}$ is presumed to exhibit a linear I-V as low pH from the luminal side may potentiate the inward (cations flowing out the vesicular lumen) conductance (Jiang *et al.* 2005). An alternative possibility is that the $I_{TRPM7}$ controls the ionic

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homeostasis and membrane potential of the SyV, which may indirectly affect the neurotransmitter release. The role of TRPM7 in controlling transmitter release probability and/or frequency has been confirmed using a direct assay for the fusion of acidic vesicles with the plasma membrane (Brauchi *et al.* 2008). In this assay, total internal reflection fluorescence microscopy was employed to monitor the fluorescent reporter pHluorin, which is quenched in the acidic lumen of the vesicle but increases in fluorescence upon exposure to extracellular pH, thus indicating fusion of the vesicle with the plasma membrane. Reduced EPSP amplitude may cause a portion of the fusion events to fall out of the assay detection limit, which might indirectly cause the reduced frequency phenotype. It is also possible that TRPM7 may play a direct role in controlling the probability of transmitter release. For example, the $Ca^{2+}$ permeability of TRPM7 and its association with synaptotagmin may allow TRPM7 to regulate membrane fusion. A $Ca^{2+}$-impermeable TRPM7 pore mutant with intact outward conductance would allow a test of this possibility. Finally, the kinase domain of TRPM7 appears to play a role in vesicle mobility (Brauchi *et al.* 2008). Thus TRPM7 may be a key regulator of multiple steps of exocytosis involved in neurotransmitter release.

The key question remains unanswered is how TRPM7 is activated in the SyV. Synaptic, like endocytic, vesicles are deprived of the $PI(4,5)P_2$ that is abundant in the plasma membrane (Krapivinsky *et al.* 2006). $PI(4,5)P_2$ binding activates TRPM7 in the plasma membrane (Runnels *et al.* 2002). One interesting possibility is that when a SyV is tethered to the plasma membrane via the SNARE complex, the cytoplasmic portion of TRPM7 is exposed to $PI(4,5)P_2$ from the inner leaf of the plasma membrane, and is activated. $PIP_2$ is indispensable for exocytosis and neurotransmission (Suudhof 2008). Upon $Ca^{2+}$ binding, the cytoplasmic part of the synaptotagmins may interact with $PIP_2$ in the plasma membrane, and this interaction is important for the membrane penetration of synaptotagmin and subsequent vesicle fusion (Bai *et al.* 2004). As TRPM7 has been shown to complex with synaptotagmin I (Krapivinsky *et al.* 2006), it is possible that TRPM7-mediated $Ca^{2+}$ release further enhances the interaction of synaptotagmin and the plasma membrane which is necessary for bilayer fusion.

### TRPs in lysosome-related organelles

TRPMLs are also localized in cell-type specific LROs, which resemble LEL compartments but have distinct morphologies, content, and functions (Blott and Griffiths 2002). For example, TRPML3 is localized in melanosomes of melanocytes (Xu *et al.* 2007). In response to light stimulation, melanosomes undergo rapid translocation to the plasma membrane and release melanin (Blott and Griffiths 2002). The pigmentation defects seen in the *Va* mice could indicate a role of TRPML3 in melanin release. However, *Va* is a severe gain-of-function phenotype and may cause melanocyte cell death because of $Ca^{2+}$ overload in the cytosol. To dissect the mechanism underlying the pigmentation defect, it is necessary to conduct loss-of-function studies of TRPML3 using, for example, TRPML3 knockout mice. Rab38 and Rab27 are two small G proteins that co-localize with TRPML3 in the melanosomes (Stenmark 2009). Mice with mutations of Rab38 and Rab27 exhibit pigmentation phenotypes (Blott and Griffiths 2002). This is suggestive of an interaction between Rab proteins and TRPML3 being involved in the regulation of melanosome trafficking. Recently, Oancea *et al.* reported that TRPM1, although not localized in melanosomes, can regulate the melanin content and pigmentation in melanocytes (Oancea *et al.* 2009). It is intriguing how vesicular TRPs indirectly regulate functions of LROs.

Gastric parietal cells contains a specialized vesicular compartment that is involved in the regulated transport of vesicles containing an $H^+/K^+$-ATPase for acid secretion into the gastric lumen (Slaugenhaupt 2002; Puertollano and Kiselyov 2009). ML4 patients exhibit reduced gastric acid secretion, suggesting that TRPML1 might be required for the translocation (Slaugenhaupt 2002). In addition, TRPML1, and probably TRPML2 as well, is localized in the MHCII compartments of antigen presenting cells such as macrophages and B-lymphocytes (Song *et al.* 2006; Thompson *et al.* 2007). Following endocytosis or phagocytosis of antigen receptors, MHCII compartments undergo translocation to the plasma membrane (Thompson *et al.* 2007). Over-expression of TRPML1 or TRPML2 results in enlarged compartments that are positive for MHCII (Song *et al.* 2006). Consistent with this trafficking defect, knockdown of TRPML1 results in a delayed translocation of MHCII compartments to the plasma membrane in cultured macrophages.

## Summary, perspectives, and future directions

Many TRP channels have been found to be localized to intracellular vesicles and to interact with a variety of vesicular proteins. Functional studies suggest that many intracellularly-localized TRPs are not simply passive cargo, but instead play active roles in membrane fusion and fission, signal transduction, and vesicular homeostasis. In the future, we hope to see the advancement of our knowledge of intracellular TRPs in the following areas:

(i) Real-time live imaging methods will be used to capture the local $Ca^{2+}$ transients mediated by release from vesicles. These seemingly spontaneous events might be able to be correlated with the membrane fusion events, which can be monitored with fluorescence imaging approaches. $Ca^{2+}$ release could possibly be altered by genetic or pharmacological manipulation of the vesicular proteins involved in membrane fusion and fission.

(ii) Organellar identities of TRP-resident compartments will be defined for most TRPs. It will be revealed whether TRPs are also in group II organelles.

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(iii) More information will be provided for the electric properties of intracellular compartments and vesicles. Ion imaging methods will be applied to accurately measure luminal ion concentrations at both basal and stimulated states.

(iv) Molecular identification will be performed of $Ca^{2+}$ release channels and respective activation mechanisms. Agonists and antagonists of intracellular TRPs may provide useful tools for altering membrane traffic.

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