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Organellar TRP Channels

Xiaoli Zhang[1,#], **Meiqin Hu**[1,2,#], **Yexin Yang**[1], **Haoxing Xu**[1,*]

[1]Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA

[2]Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou 310014, China

Abstract

Mammalian transient receptor potential (TRP) channels mediate Ca^{2+} flux and voltage changes across membranes in response to environmental and cellular signals. At the plasma membrane, sensory TRPs act as neuronal detectors of physical and chemical environmental signals, and receptor-operated (metabotropic) TRPs decode extracellular neuroendocrine cues to control body homeostasis. In intracellular membranes, such as lysosomes, organellar TRPs respond to compartment-derived signals to control membrane trafficking, signal transduction, and organelle function. Complementing mouse genetics, human genetics, and high-resolution structural studies, physiological studies employing natural agonists and synthetic inhibitors have become critical to resolving the *in vivo* functions of metabotropic, sensory, and organellar TRPs.

Introduction

Transient receptor potential (TRP) was initially identified as a receptor-operated sensory cation channel required for sustained light responses in *Drosophila*[1–3]. Subsequent homology cloning revealed a superfamily of cation channels in mammals[4–8]. Based on sequence homology, mammalian TRPs can be divided into 6 subfamilies: TRPC1–7 (C for canonical), TRPV1–6 (V for vanilloid), TRPM1–8 (M for melastatin), TRPA1 (A for Ankyrin), TRPML1–3 (ML for mucolipin), and TRPP1–3 (P for polycystin) (see Table 1). A common feature of TRP channels is the homotetrameric assembly of subunits containing six transmembrane segments (S1–6; see Fig. 1)[9–14]. Most TRPs are Ca^{2+}-permeable cation channels, and virtually all are gated by chemical signals, ranging from environmental sensory signals to extracellular neurotransmitters and intracellular messengers[8,15]. Hence, TRPs can be considered as a superfamily of ligand-gated cation channels, although some TRPs can also be gated by physical signals, such as temperature, mechanical force, and voltage[8].

TRP channels have been intensively studied for over 20 years, due to their crucial roles in both sensory and signal transduction[7]. Exploration of the biological functions of TRPs relies heavily on human genetics (channelopathy studies) and reverse mouse genetics. TRP

*To whom correspondence should be addressed: haoxingx@umich.edu.
#These authors contributed equally to the work.

knockout (KO) mouse studies revealed the essential roles of TRPs in temperature and pain sensation (TRPV1), pheromone detection (TRPC2), taste sensation (TRPM5), and innate fear responses (TRPC5)[7,16–19]. The cellular functions of TRPs are extremely diverse, partly due to their diverse activation mechaniams and different subcellular localizations[20]: most TRPs are expressed at the plasma membrane, whereas others may function as organellar channels that actively participate in signal transduction and organelle biology (see Table 1).

The biophysical properties of TRP channels are usually established in heterologous overexpression systems using whole-cell and whole-organelle patch clamps[8] (Box 1). Ca^{2+} imaging assays have also been useful to study TRP channel physiology in intact cells and have allowed high-throughput screening of small molecule modulators[15,21]. High-resolution structural studies and the use of chemical modulators have further improved the reliability of channel characterization and become critical to resolving the *in vivo* functions of TRPs[22,23] (Box 1 & Fig. 1). Individually, each of these approaches have inherent limitations, and it is thus essential to integrate different types of studies (Box 1).

In this review, we summarize our current knowledge of TRP channels, focusing in particular on the least-known functional group, the organellar TRPs, to bring together findings from studies on channel modulation, atomic structure, cell biology, animal physiology, and disease.

Physiology and architecture of TRP channels

TRPs are Ca^{2+}-flux channels that can be activated by both physical and chemical signals[7]. How physical factors, such as temperature and mechanical force, activate TRPs is not yet known, though the domains and residues from TRPV1 involved in the temperature response have been identified[25]. Liposome reconstitution studies have indicated that some TRPs, e.g., TRPV1 and TRPM8, are activated directly by thermal stimulation[26], and mutagenesis analyses suggest that thermosensitivity and chemosensitivity can be segregated in specific TRPs[27]. Some physical factors, e.g. light and hypotonicity, activate TRPs indirectly, through derived chemical signals[28–30]. Chemcial signals, either environmental cues or intracellular messengers, may activate TRPs by binding directly to channel proteins[10] (Fig. 1d). When activated, TRP channels can permeate at least three cation groups, contributing to their diverse cellular functions. First, Ca^{2+} permeation results in changes in cytoplasmic Ca^{2+} levels, either global or juxta-organellar[31]. Second, Na^{+} flux reduces transmembrane voltage potential either across the plasma or organellar membrane[20]. Third, some TRPs (e.g., TRPM7 and TRPML1) are permeable to metal ions such as Mg^{2+}, Zn^{2+}, and Fe^{2+}, whose dehydration energy is too high for non-TRP ion channels[32,33] but can be reduced[34] or accommodated as partially hydrated ions within the large TRP pore[13,14,22,35,36].

TRP channel protomers have 6 transmembrane segments (S1–S6) with N- and C- terminal domains facing the cytosol (Fig. 1a). The S1–S4 form a voltage-sensor-like domain (VSLD; Fig. 1d). However, although many TRP channels are weakly modulated by voltage, the VSLD may not be the primary determinant for voltage sensitivity in most TRPs[10,37]. Instead, VSLD may serve as the ligand-binding domain for many TRPs[22]. The S5–S6 domain forms the cationic selectivity filter and channel activation gate (Fig. 1). In some

TRPs such as TRPMLs and TRPPs, the large S1–S2 extracellular domain may also contribute to Ca^{2+} permeation[12]. Several intracellular domains, including the S2–S3 linker, S4–S5 linker, the TRP domain, intracellular N- and C- terminal doamins, may be involved in ligand binding and coupling of ligand-binding to opening of the channel gate (Fig. 1d).

The TRP selectivity filter is formed by a pore loop between S5 and S6[35,38,39] (Fig. 1). The pore size at the selectivity filter ranges from 2 to 8 Å, allowing the passage of dehydrated or partially-hydrated Na^+ and Ca^{2+} [10,13,14,22,36,39]. The broad range of Ca^{2+} permeability to Na^+ permeability ratios (P_{Ca}/P_{Na}) among TRP channels can be attributed to selectivity filter features. For example, in TRPV5 and TRPV6 channels, with high P_{Ca}/P_{Na} (>100), four aspartate residues in the selectivity filter region of each subunit form a high-affinity Ca^{2+}-binding site that excludes monovalent permeation (Fig. 1c)[40,41]. Conversely, TRPM4 and TRPM5 have very low P_{Ca}/P_{Na} (< 0.05), due to a ring of glutamine residues in the selectivity filter that bind preferentially monovalent ions (Fig. 1c)[37–39]. TRPs with P_{Ca}/P_{Na} in the 1~10 range have intermediate-affinity Ca^{2+} binding sites in the selectivity filter, composed of negatively-charged residues[10,11,13,14,22,36,42].

There are one or two activation gates in TRPs. The lower activation gate is found in all TRPs, and is formed by the S6 helices (Fig. 1)[10,22], similarly to that in voltage-gated K^+ channels. Mutations affecting the lower gate can result in constitutively active TRP channels[10,43]. The binding of agonists to different regions of the channel may cause conformational changes that converge at the S6 gate, leading to channel opening[12,14,39]. The upper gate is present only in some TRPs (e.g., TRPV1 and TRPC5), and is formed by the pore loop involved in selectivity[10,22]. Binding of extracellular ligands such as H^+ and toxin opens the upper gate of these TRPs[10,19,44]. The coupling mechanisms between the two gates remain to be established.

Many endogenous and synthetic TRP agonists bind to the VSLD. Comparison of apo- and ligand-bound structures revealed that ligand binding causes conformational changes in the S4–S5 linker region, which in turn can interact directly with the S6 helices (lower gate) in another subunit (a so-called domain swap) or indirectly, through the TRP domain that is present in most TRPs and lies parallel to the membrane[10,11,14,45] (Fig. 1d). Binding of intracellular ligands (e.g., Ca^{2+}, PIP_2, or ATP) to or phosphorylation events in cytoplasmic regions, such as the N-terminal domains (e.g., ankyrin repeats in TRPC, -V, and -A, TRPM-homology-regions and pre-S1 domains), S2–S3 linkers, or C-terminal domains (e.g. coiled-coil domain)[10,11,14,45], control S6 gating through the S4–S5 linker or TRP domain (Fig. 1d). Thus, the S4–S5 linker, similar to that in other tetrameric channels, and the TRP domain are essential for coupling ligand binding and gating in most TRPs. However, recent studies revealed that some other agonists, e.g., ML-SA1 for TRPML1 and TRPML3, bind to the S5–S6 region, where they could exert direct force on the S6 gate, and possibly also the upper gate[36,46] (Fig. 1d). Hence, there exist multiple ligand-binding sites and gating mechanisms in TRPs.

Functional classification of TRPs

Ion channels are commonly classified based on their selectivity and/or gating mechanisms. TRPs are generally cation non-selective, and the same activing stimulus may activate a subset of TRPs in different sequence homology-based subfamilies, e.g., temperature activation of TRPV1–4, TRPM2–5, 8, and TRPC5[8]. Therefore, TRPs can be more informatively classified, based on their physiological function and endogenous activation mechanism, into three subgroups: metabotropic, sensory, and organellar TRPs (Table 1). Each functional subgroup contains members from individual TRP subfamilies. Because individual TRPs can be involved in multiple functions, and many TRPs are activated by both environmental and cellular signals, some TRPs belong to more than function groups. For instance, the fly TRP has both sensory (activated by light) and metabotropic (coupled to rhodopsin) functionality[3].

Metabotropic TRPs.

Most animal tissues and organs are regulated by both nervous and endocrine systems, and neuroendocrine signals need to be transduced at the cellular level[8]. Metabotropic TRPs are signal transducers in cells that express phospholipase (PLC)-dependent G protein-coupled receptors (GPCRs). This group includes TRPCs and members from each of the other subfamilies (Table 1). TRPC channels are expressed in the heart, smooth muscle, and kidney podocytes; they are regulated by sympathetic and parasympathetic transmitters, and mediate agonist-induced Ca^{2+}-entry pathways[4–7]. Activation of Gq-coupled receptors stimulates PLC activity, resulting in hydrolysis of $PI(4,5)P_2$ into DAG and IP_3; IP_3 then induces Ca^{2+} release from the ER through IP_3 receptors (Fig. 2a). Such PLC-dependent signal transduction mechanisms then activate metabotrophic TRPs and store-operated Ca^{2+} entry channels, whose pore are formed by the Orai proteins[47,48].

Sensory TRPs.

Animals detect environmental cues such as light, temperature change, osmo-mechanical force, and natural compounds, pain/itch-inducing chemicals, tastants, and pheromones[19]. These physical and chemical signals increase the membrane excitability of various sensory cells (e.g. DRG neurons, taste receptor cells, hair cells, and retinal ganglion cells) through sensory TRPs (Fig. 2b). This group includes the founding member of the TRPV subfamily, TRPV1, discovered as a cation channel activated by somatosensory cues such as heat and capsaicin (an alkaloid in chili peppers that produces a burning sensation)[49]. Other TRPs involved in sensory functions include TRPM8, a cold and menthol receptor found in DRG neurons[50,51], and TRPA1, a temperature receptor for the spice wasabi (allylisothiocyanate, AITC)[52,53](Table 1). Other thermosensors include TRPM3 in DRG neurons[54], TRPM2 in sympathetic and central neurons[55,56], and TRPV3/4 in keratinocytes[57].

TRPV1 is also sensitive to itch-inducing substances such as histamine and injury-evoked inflammatory mediators (e.g., bradykinin) through indirect receptor-dependent mechanisms[19]. In taste receptor cells, tastants activate TRPM4/5 and membrane depolarization through GPCR taste receptors[58]. TRPC2 in mouse VNO neurons is activated by pheromones through GPCR pheromone receptors[16]. In these cases, GPCRs, but not

TRPs, are the direct targets of sensory signals, these sensory TRPs are also metabotropic: metabotrophic sensory TRPs.

Organellar TRPs.

This is the least-understood group among TRP channels. Most TRPs observed in intracellular locations are thought to be plasma membrane channels going through biosynthetic or secretory processes, en route to their final destination[20]. The intracellular localization of organellar TRPs is usually demonstrated by overexpression of GFP fusions, tag-knock-in studies, and knockout (KO)-controlled immunohistochemistry[20]. More importantly, organelle electrophysiology and organelle-targeted Ca^{2+} imaging provide validation for the functions of organellar TRPs[23,59,60]. The key members in this group are the TRPMLs, which are activated by compartment-specific intracellular cues[59]. TRPML1–3 and TRPP1–3 are distantly related TRPs, whose channel functions remained mysterious until the development of organelle-specific patch-clamp techniques[33,61]. In the remainder of this review, we discuss the roles of organellar TRPs in decoding cellular signals.

Organellar TRPs couple intracellular cues to biology

Sensory TRPs are activated by physical and chemical environmental signals; metabotropic TRPs are activated by extracellular neuroendocrine signals. Similarly, cellular signals generated in the cytoplasm and other cellular compartments are thought to activate organellar TRPs. Notably, the same environmental signals that activate sensory TRPs, such as oxidants, pH, and osmo-mechanical force, may also regulate organellar TRPs[62–64].

Most organellar TRPs function either in biosynthetic/secretory pathways (in the endoplasmic reticulum (ER) and Golgi), or in the endocytic pathway (e.g. in endosomes and lysosomes[20]). Candidate (if not functionally validated by organell electrophysiology yet) and confirmed organellar TRPs include TRPV1 and TRPP1, which are known to be localized on the ER membranes; and TRPC3–5, TRPV5–6, TRPM2, TRPM8, TRPA1, and TRPML1–3, which are present in secretory vesicles, early and recycling endosomes, and lysosomes (Table 1). All those organelles are intracellular Ca^{2+} stores, with luminal Ca^{2+} concentrations ranging from 0.3 to 0.7 mM[65]. Their Ca^{2+} permeability allows organellar TRPs to contribute to cellular signal transduction, by causing global changes in cytoplasmic Ca^{2+} levels[31]. More importantly, Ca^{2+} release by organellar TRP channels may increase juxta-organellar Ca^{2+} levels, specifically affecting the dynamics anf function of the organelles[20]. In addition, changes in membrane potential across organellar membranes may also affect organellar functions[23].

Organellar TRPs can be divided into two groups: those predominantly localized in the organellar membranes, referred to as “committed” organellar TRPs; and those dually localized at plasma membrane and organellar membranes, referred to as “non-committed” organellar TRPs. We review our current knowledge of the members in each group below.

Committed organellar TRPs

TRPY1 in the yeast vacuole.

TRPY1, the only TRP-like protein in yeast, is localized in the membrane of the vacuole, which is equivalent to the mammalian lysosome[62]. In response to osmotic shock, changes in cytoplasmic ionic strength and/or mechanical force on the vacuolar membrane, activate TRPY1 to mediate vacuolar Ca^{2+} release and possibly vacuolar membrane fission[62]. Hence, TRPY1 is has dual sensory (activated by an environmental signal) and organellar (vacuole) functionalities[62].

TRPML1 in the lysosome.

Lysosomes degrade cargo materials delivered via endocytosis or autophagy, converting them into catabolites and building blocks (e.g. amino acids) for reuse by the cell[66,67]. The degradation products are transported out of the lysosomes via vesicular trafficking and catabolite exporters[23,68]. All these lysosome trafficking steps, including cargo and enzyme import, lysosomal degradation, and catabolite export, are regulated by various intracellular signals produced according to the status of luminal cargos and products[23,68]. Both lumen-to-cytosol and cytosol-to-lumen signals need to be decoded[23]. As lysosomes are highly heterogeneous, compartment-specific intracellular signals such as changes in lysosomal lipid composition, membrane potential, and juxta-lysosomal Ca^{2+} levels, may differentially regulate trafficking of individual lysosomes[60,69].

TRPML1 (a.k.a MCOLN1) channels are the major Ca^{2+}-permeable channels in the lysosomes of all cell types. TRPML1 KO cells exhibit defective lysosomal membrane fusion and fission[23,70]. Whole-endolysosome patch-clamp studies using artificially-enlarged lysosomes[59], suggested that TRPML1 may conduct Ca^{2+}, Fe^{2+}, Zn^{2+}, Na^{+}, and K^{+} across lysosomal membranes[33]. Employing lysosome-targeted genetically encoded Ca^{2+} sensors, it was shown that TRPML1 mediates Ca^{2+} release from lysosomes in intact cells[60,71].

TRPML1 channels are activated by cellular cues that regulate lysosome trafficking and function. $PI(3,5)P_2$, a lysosome-specific phosphoinositide, was the first endogenous signal identified for lysosomal TRPML1, activating it in a physiological, low-nanomolar range[12,59]. $PI(3,5)P_2$ binds to positively-charged amino acid residues in the N-terminus region of the channel, resulting in opening of the S6 gate through the S2–S3 linker [12]. $PI(3,5)P_2$ levels may increase transiently prior to fusion of two lysosomes[72], and during phagocytic uptake of large particles[73]. Hence, it is likely $PI(3,5)P_2$ dynamics serves as the cellular cue that activates lysosomal TRPML1.

Several synthetic small-molecule TRPML agonists and inhibitors have been identified[60,74]. Although synthetic agonists activate TRPML1 independent of endogenous cues[36,60], they provide useful tools to probe the channel's cellular functions. ML-SA1 is an agonist that binds directly to the S5–S6 region of the channel[36], where the bound compound may exert a direct force on the S6 gate[36,46]. In macrophages, acute ML-SA1 treatment induces the fusion of lysosomes with the plasma membrane, i.e., lysosomal exocytosis, through activation of Ca^{2+} sensor synaptotagmin VII (Syt-VII)[75] (see Fig. 3) in wild-type but not TRPML1 KO cells[73]. Moreover, retrograde movement of lysosomes to the perinuclear

region, which is required for autophagosome-lysosome fusion, is increased with TRPML1 over-expression or synthetic agonism through Ca^{2+} sensor EF-hand-protein ALG2[76], but reduced by TRPML1 KO, synthetic inhibition or $PI(3,5)P_2$ deficiency[76] (see Fig. 2). Hence, activation of TRPML1 may allow cellular cues such as lysosomal lipids to control/regulate lysosomal trafficking by triggering increase in juxta-lysosomal Ca^{2+}.

TRPML1 is also directly activated by reactive oxygen species (ROS), which are released from mitochondria under stress conditions to activate autophagosome and lysosome biogenesis[64]. Activation of TRPML1 triggers Ca^{2+} sensor calcineurin, which in turn promotes the nuclear translocation of transcription factor EB (TFEB), a master regulator of autophagy and lysosome function (Fig. 3)[64]. Activation of TFEB is sufficient to promote mitophagy, removing damaged mitochondria to reduce excessive ROS in the cell[64].

The regulation of TRPML1 by endogenous cellular cues and synthetic modulators can illuminate how organellar TRPs are involved in different cellular processes. Although regulation by lipids and ROS is a general feature of TRPs[7], co-localization of the membrane-delimited activating signal and channel within the same compartment defines a specific organelle function for such regulation. Hence, a signal that acts as an environmental cue for a sensory TRP (e.g., ROS) can also act as an intracellular agonist for an organellar TRP such as TRPML1[64,77]. The specific cellular process regulated by TRPML1 is determined by both activating signals and downstream effectors, e.g., Ca^{2+} sensors (see Fig. 3). Given that synthetic agonists activate TRPML1 independent of $PI(3,5)P_2$ and ROS[12,60,64], it is likely that additional endogenous agonists for TRPML1 exist. It has been demonstrated that ROS sensitivity, but not $PI(3,5)P_2$ sensitivity, is required for the TFEB activation upon mitochondria damage[64]. Finally, although the major lysosomal $\Delta\psi$ regulator is likely two-repeat (2× 6TM) TPC channels[78], cellular cues that affect lysosomal membrane potential may also regulate lysosome function in a TRPML1-dependent manner [23,59], given that TRPML1 currents are strongly rectifying (see Fig. 1c).

TRPML2/3 in the endosomes and lysosomes.

Both TRPML2 and TRPML3 are also localized in the lysosomes, but only in certain cell types[23,70,79]. TRPML1 and TRPML3 play complementary roles in cells expressing both channels, such as intestinal enterocytes and cochlear hair cells[80]. As $PI(3,5)P_2$ activates all three TRPMLs[59], it is possible that $PI(3,5)P_2$ serves as the endogenous cellular cue that activates lysosomal TRPML2/3 channels. In the bladder epithelial cells, TRPML3 is activated by lysosomal alkalization to mediate exosome release and bacterial extrusion[63].

TRPML2 and TRPML3 also function in early and recycling endosomes of certain cell types[23,70,79]. For example, TRPML3 is the primary endosomal channel in some macrophage types[79], but the cellular signals that activate early-endosomal TRPML3 are still unknown. Moreover, early endosomal functions of TRPML2/3 are not firmly established; lysosomal defects associated with the lack of TRPML2/3 could indirectly affect the functions of early endosomes, such as endocytosis and endosome-autophagosome fusion[23,70].

TRPML1 in tubulovesicles.

In specialized cell types, TRPMLs are also expressed in endosome- or lysosome-related vesicles. For example, TRPML1 is expressed in tubulovesicles (TVs), the specialized organelles of acid-secreting gastric parietal cells[81], and is required for TV Ca^{2+} release that triggers TV exocytosis in response to cAMP signaling downstream of histamine, a neurotransmitter that induces gastric acid secretion[82]. TV exocytosis is essential for gastric acid secretion in response to neurotransmitter histamine[81,82]. In this context, the organellar TRPML1 also acts as a receptor-operated channel. Clinically, loss-of-function mutations of TRPML1 underlie type IV mucolipidosis (ML-IV), a genetic disease in which patients experience hyposecretion of gastric acid[23,83]. In concordance, TRPML1 KO or inhibition suppresses gastric acid secretion, while TRPML1 overexpression or activation augments gastric acid secretion in mice[82,84].

TRPPs in cilia.

Primary cilia are isolated cellular organelles in eukaryotic cells[85]. TRPP1 (a.k.a, PKD2) and TRPP2 (PKD2L1) channels are expressed in primary cilia, and their channel physiology characteristics were established in whole-cilium recordings with and without synthetic channel modulators[61]. However, cilium-specific TRPP-activating cues are yet to be identified.

Non-committed organellar TRPs

Most intracellularly-localized TRPs are thought to be plasma membrane channels that are temporarily associated with intracellular membranes as they go through their biosynthetic or secretory pathways[20]. However, several TRPs have been shown to be functional in intracellular membranes, and hence referred to as non-committed organellar TRPs.

TRPM2 and TRPA1 in the lysosomes of specialized cells.

In Dorsal Root Ganglion (DRG) neurons, TRPA1 is localized in the lysosomes that mediate lysosomal Ca^{2+} release in response to AITC[86]. However, TRPA1 is not present in the lysosomes of TRPA1-transfected HEK293 cells, suggesting that TRPA1 is targeted to lysosomes via a DRG neuron-specific mechanism[86]. TRPA1-mediated lysosomal Ca^{2+} release promotes the exocytosis of neuropeptide-containing, dense-core vesicles[86]. Likewise, in pancreatic β cells, ROS may generate another intracellular signal, ADP-ribose, which then activates lysosome-localized TRPM2, triggering lysosomal Ca^{2+} release to evoke insulin secretion[87]. In both cases, lysosomal Ca^{2+} release regulates the same functions as their plasma membrane counterparts. To separate organellar from plasma membrane functions of dually-localized TRPs, it is necessary to genetically modify the organelle-targeting motif and/or to develop membrane-permeable (organelle targeting) and impermeable channel inhibitors. It remains unknown whether lysosomal TRPA1 and TRPM2 are activated by organelle-specific luminal or cytosolic factors to regulate specific lysosome functions.

TRPM7 in vesicles.

TRPM7, ubiquitously expressed at the plasma membrane of most cell types, is permeable to both Mg^{2+} and Zn^{2+}, in addition to Ca^{2+} and Na^{+} [8,88,89]. However, TRPM7 is also localized in the so-called M7-like vesicles[32] and in the synaptic vesicles of sympathetic neurons[90]. Consistent with the intracellular localization, TRPM7 mediates Zn^{2+} release from intracellular vesicles in response to ROS elevation[32]. Other organellar TRPs, e.g., TRPML1, may also contribute to heavy metal release from intracellular vesicles such as lysosomes[33]. As organellar TRPs may also mediate vesicular Ca^{2+}/Na^{+} release, selective metal chelators (e.g., Ca^{2+} chelator BAPTA) may enable the actions of permeant ions to be distinguished[20]. Additionally, knock-in mutations with altered $Ca^{2+}/Fe^{2+}/Zn^{2+}$ selectivity produced by genome editing tools may help to separate these functions.

TRPs in the ER.

ER-localized TRPs in the biosynthetic pathway are expected to be inactive since they lack glycosylation modifications that take place in the Golgi after ER exit [20]. However, several TRPs, including TRPV1, TRPM8, and TRPP1, may be functionally expressed in the ER [20,91]. For example, activation of TRPV1 using exogenous native agonists increased cytosolic Ca^{2+}, but decreased ER luminal Ca^{2+} [20,91]. The latter result suggests that the high surface/volume ratio in intracellular organelles may confer a regulation of luminal ionic homeostasis by organellar TRPs.

Organellar TRPs in disease and therapeutics

More than a dozen inheritable diseases are associated with gain-of-function (GOF) or loss-of-function TRP mutations (see Table 1). Among them, loss-of-function mutations in TRPML1 cause ML-IV, a neurodegenerative lysosome storage disease[23,83]. Given their diverse biological functions and the technical feasibility of developing high-throughput screening of TRP modulators based on Ca^{2+} imaging, pharmaceutical companies are pursuing TRPs, including TRPV1 and TRPA1 as potential drug targets[15,21].

The demonstrated roles of organellar TRPs in regulating organelle function suggest that small molecule TRP modulators may boost organelle function. For instance, TRPML agonists can potentially enhance lysosome function in ML-IV patients with partial loss of TRPML1 activity[92]. There are more than 50 lysosome storage diseases (LSDs), many of which at the cellular level exhibit lysosomal trafficking defects similar to those seen in ML-IV[23,93]; thus, TRPML agonists may be used to up-regulate organelle function in LSDs characterized by lysosome impairment[23,60]. For instance, in Niemann-Pick type C disease, TRPML1-mediated lysosomal Ca^{2+} release and lysosomal trafficking are partially blocked[60]. Likewise, in PI(3,5)P_2-deficient cells, TRPML1 activity is also reduced, which may cause lysosomal trafficking defects and storage[94]. Furthermore, lysosomal trafficking is also defective in many common neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases[93]. In some lysosomal diseases, mutations in hydrolases or exporters disrupt lysosomal storage, which in turn affects lysosomal degradation and trafficking, leading to accumulation of secondary materials in that organelle ("secondary lysosome storage") and, ultimately, a vicious cycle[23,60]. Hence, TRPML1 channel dysregulation in the

lysosome may be a primary cause of secondary storage in many lysosomal diseases. If so, then increasing TRPML1 activity could break the vicious cycle and facilitate lysosomal trafficking to clear lysosomal storage[23,60]. Indeed, TRPML1 overexpression or TRPML1 agonism increases cholesterol clearance in Niemann-Pick type C patient-derived cells[23,60]. Similarly to TRPML1, TFEB overexpression also induces cellular clearance in most LSDs, except ML-IV[95]. Hence, TRPML1 and TFEB may constitute a cellular clearance program for lysosomal storage[23,96]. However, the *in vivo* efficacy of TRPML1 agonists has not been reported.

Future directions

After 20 years of intensive research, we have reached a nearly complete characterization of basic TRP channel physiology. With the advent of cryo-EM development, more atomic-resolution TRP structures are available to provide mechanistic insights into the selectivity and activation of TRPs, which may lead to the development of potent, selective agents, both for research and therapeutic purposes. The list of identified extracellular and intracellular, physical and chemical signals that activate TRPs continues to grow, specific channel modulators have been developed, and various genetically-engineered mice (with global or local/conditional knockout and knockin of TRPs) are available for research applications. Despite such progress, the mechanisms by which TRPs are activated by cellular cues *in vivo* are not well understood. Furthermore, how organellar TRPs regulate organelle function remain largely unknown. In the future, we expect to see more KO-controlled *in vitro* studies and an expansion of *in vivo* animal studies using TRP modulators.

TRPs are activated by polymodal signals *in vitro*, and there are often multiple phenotypes associated with TRP KO and inhibition. Thus, an open question in the TRP channel field is which activation mechanisms are physiologically relevant *in vivo*. For example, although TRPV1 inhibitors increased core body temperature in clinical trials[21], such effect might not be related to the thermosensitivity of TRPV1 *per se*, but more likely it involves TRPV1 activation by visceral non-thermal cues[97]. The availability of genome-editing technologies, such as CRISPR/Cas9[98], has made it possible to study the biological functions of specific modes of TRP modulation. In Conclusion, TRPs are activated by both environmental and cellular signals, but the *in vivo* relevance of such activation awaits further studies taking advantage of mouse genetics in combination with biochemical manipulations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in the TRP channel field has benefited enormously from the use of an integrated approach, such that the same channel modulators used in the channel studies and high-resolution structures are expected to produce TRP-specific effects in cellular, tissue and behavioral analyses (see Box Fig. 1). Phenotypes at the animal level may be dampened by compensatory mechanisms in KO mice, or be due to indirect gain-of-function effects in transgenic mice. For example, in TRPC6 KO mice, other TRPCs are upregulated in a compensatory mechanism, resulting in a paradoxical increase in neurotransmitter-induced arterial contractility[8,24]. Hence, the complementary use of biochemical and genetic approaches provides a safeguard against complications produced by pharmacological off-target effects and genetic compensation issues when each is used alone, respectively. Therefore, the fact that consistent temperature and pain phenotypes are observed across TRPV1 KO and pharmacological inhibition studies, has provided great confidence in the findings[19].

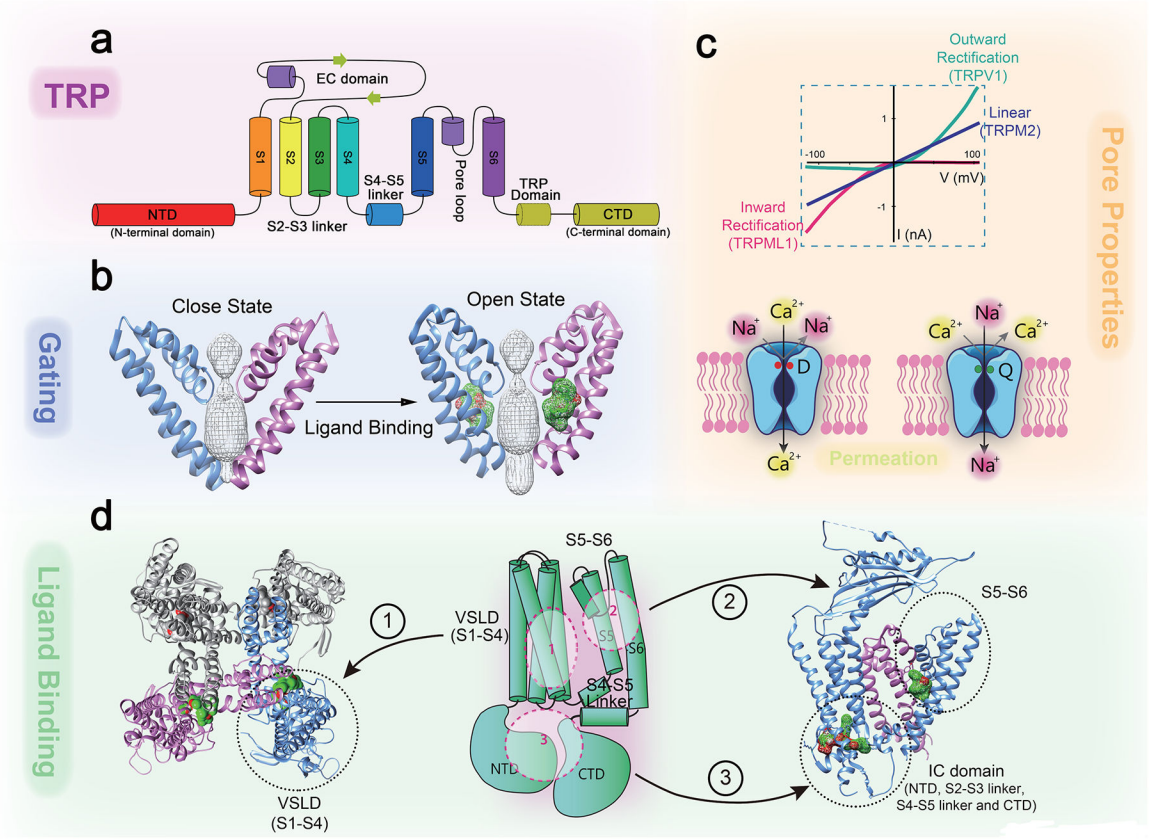


Fig. 1. Architecture and functional elements of a TRP channel.

Structural biology analyses reveal critical domains and amino acid residues for Ca^{2+} permeation and selectivity, and allow visualization of elements such as selectivity filter, activation gates, agonist binding sites, and explain gating-coupling machinery. **a.** TRPs are 6 transmembrane (TM, S1–S6) cation channels with N- and C- terminal domains facing the cytosol. **b**, Ligand binding to the S5–S6 domain leads to opening the lower S6 gate. **c.** Pore properties of TRP channels. Upper panel: representative TRP current-voltage (I-V) traces. Lower panels: the pore-loop between S5 and S6 forms the selectivity filter and upper gate of the channel. Negatively charged residues (e.g. Asp541 in TRPV6; red, the left panel) form the high-affinity Ca^{2+}-binding sites required for Ca^{2+} permeation and selectivity. In TRPs with very low P_{Ca}, specific neutral and polar amino acid residues (e.g., Gln977 in TRPM4; green, the righ panel) form binding sites that favor monovalent cations over Ca^{2+}. **d.** Ligand binding sites are localized in S1–4 VSLD (site 1), S5–S6 region (site 2; see agonist-bound cryo-EM structures of TRP in the zoomed-in image), or intracellular (IC) domains (site 3; see agonist-bound cryo-EM structures of TRP in the zoomed-in image). The S4–S5 linker and TRP domain act as the binding-gating coupling machinery that interacts to pull the S6 gate open upon ligand-binding.

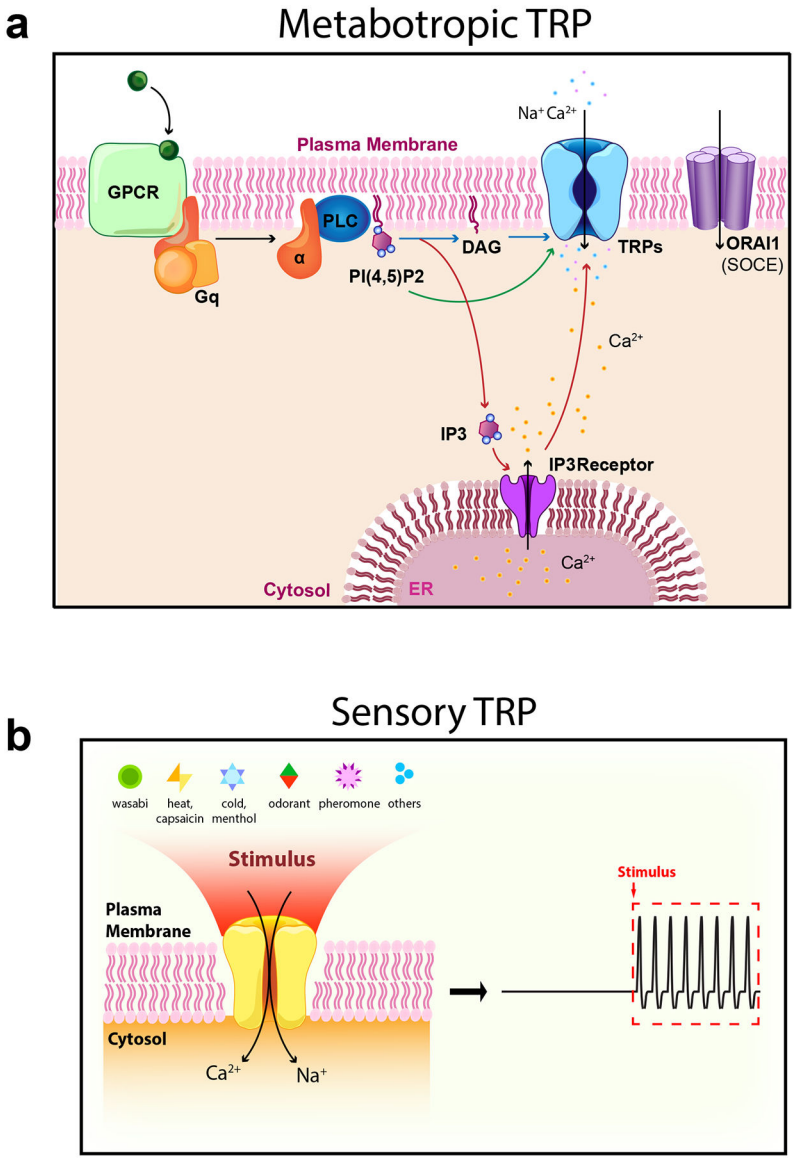


Fig 2. Metabotropic and sensory TRPs.

a. Metabotropic TRPs couple extracellular cues to biology. Excellular neuroendocrine signals, e. g., neurotransmitters, act on G protein-coupled receptors (GPCRs). Metabotropic TRPs are signal transducers in cells that also express phospholipase (PLC)-dependent GPCRs. Activation of Gq-coupled receptors stimulates PLC activity, which hydrolyze $PI(4,5)P_2$ into DAG and IP_3. IP_3 then induces Ca^{2+} release from the ER through IP3 receptors. PLC-dependent signal transduction mechanisms activate metabotropic TRPs, as well as store-operated Ca^{2+} entry channels, whose pore-forming subunits are Orai proteins. Details see Table 1. **b.** Sensory TRPs couple environmental cues to biology. Sensory TRPs are activated by environmental signals such as light, temperature change, osmo-mechanical force, and plant-derived compounds, pain-/itch-inducing chemicals, tastants, and pheromones. These physical and chemical signals activate sensory TRP-mediated Na^+ entry, increasing the membrane excitability of various sensory cells, including DRG neurons, taste

receptor cells, hair cells, and retinal ganglion cells through sensory TRPs. Details see Table 1.

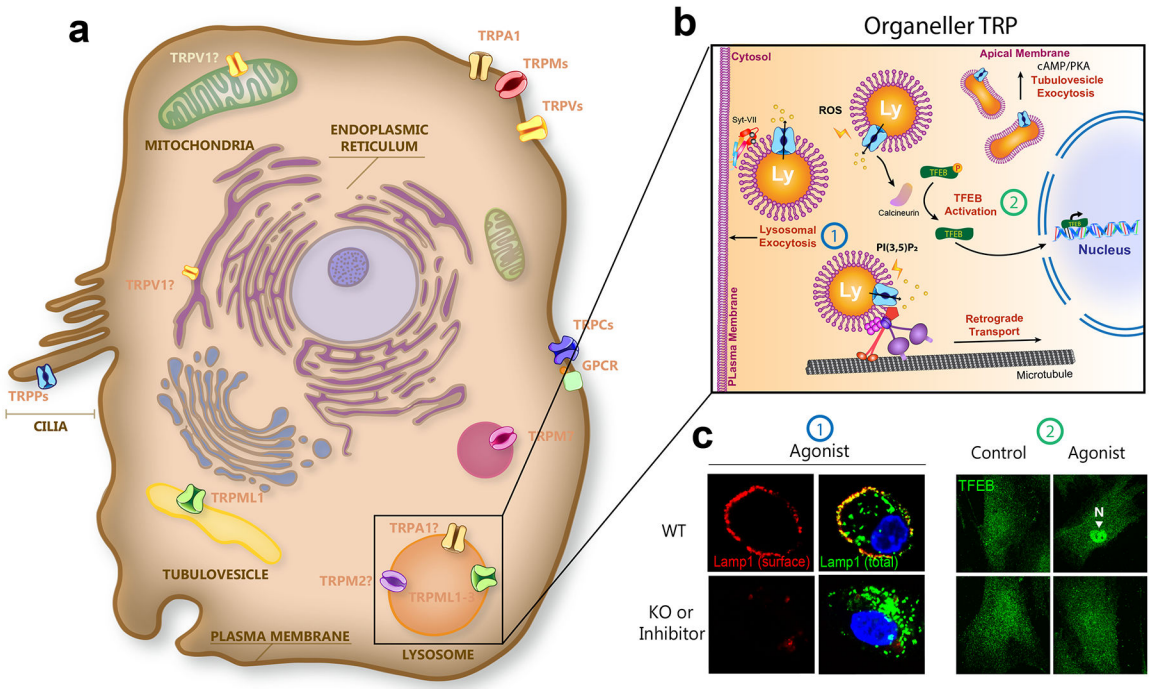
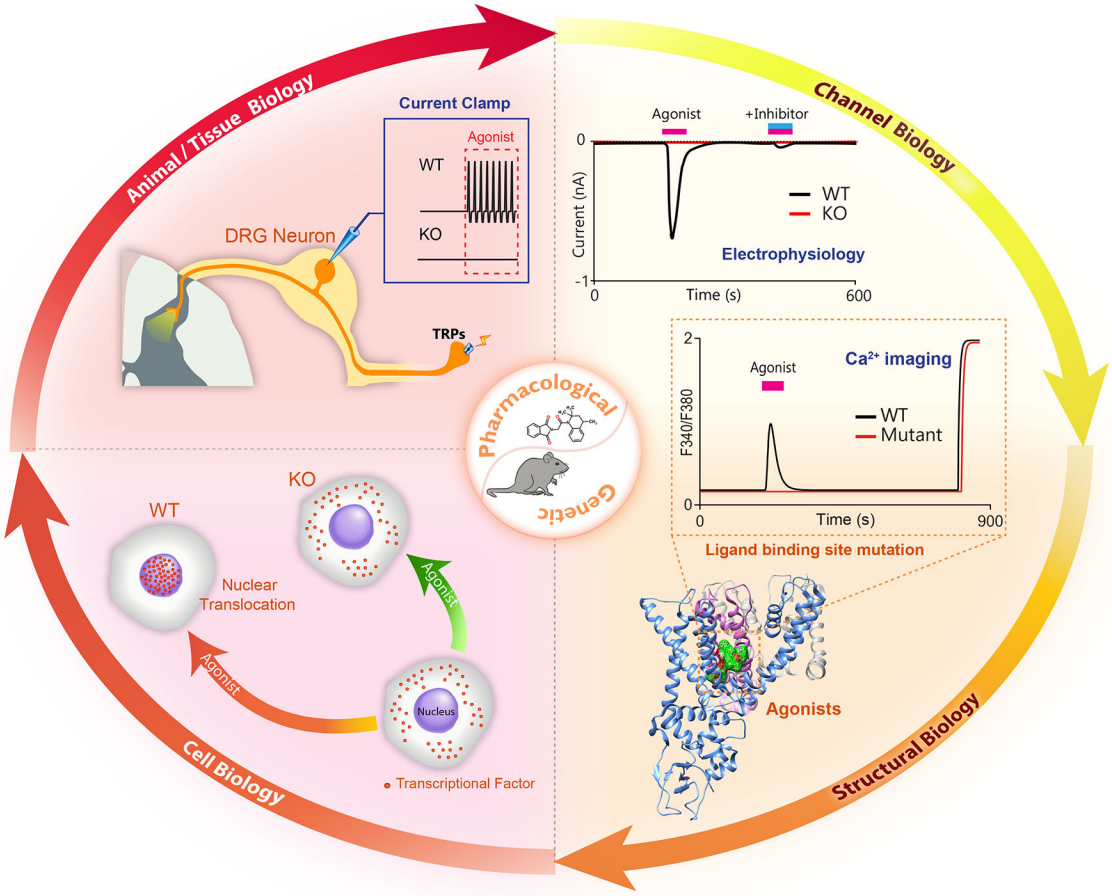


Fig. 3. Organellar TRP channels.

a. Though most TRPs are located at the plasma membrane, TRPMLs are localized in intracellular endosomes and lysosomes, and TRPPs are localized in primary cilia. In addition, TRPV1 is localized in the ER and possibly mitochondria, and TRPM7 is localized in M7-like vesicles. In specialized cell types, TRPA1 and TRPM2 are functionally expressed in lysosomes. In parietal cells, TRPML1 is an organellar TRP that functions in both lysosomes and tubulovesicles (TVs). **b.** Endogenous (e.g. ROS and $PI(3,5)P_2$) or synthetic agonists induce TRPML1-mediated lysosomal Ca^{2+} release to trigger lysosomal exocytosis (example 1), retrograde transport, and TFEB nuclear translocation (example 2). In the parietal cells, histamine-induced cAMP/protein kinase A signaling activates TV-localized TRPML1, increasing TV trafficking and exocytosis. **c.** In example 1, activation of TRPML1 triggers lysosomal exocytosis, detected by the surface expression of Lamp1 proteins in WT, but not in TRPML1 KO cells. Images are modified with permission from ref.[64]; In example 2, agonist activation of TRPML1 leads to nuclear translocation of TFEB (green), a transcription factor for lysosome biogenesis and autophagy in WT, but not TRPML1 KO cells. Images are modified with permission from ref. [102].



Box Fig. 1. An integrated approach to study TRPs.

An integrated approach to establish functions of TRPs with respect to channel physiology, cell and tissue physiology, and organismal biology, is illustrated below, in anticlockwise direction. Top right, agonist-evoked inward currents (measured at negative voltages) are abolished in TRP KO cells or by synthetic inhibitors, suggestive of the specificity of the response. Bottom right, structural biology analyses reveal critical domains and amino acid residues as agonist binding sites. Middle right, corresponding mutations in the binding sites abolished agonist-induced Ca^{2+} imaging responses. Bottom left, cell biological functions of TRPs (e.g. agonist activation of TRPML1 leads to nuclear translocation of transcription factor TFEB in WT, but not KO cells). Top left, animal biology and physiology of TRPs. For example, agonist (e.g., capsaicin for TRPV1) application increases firing frequency in WT, but not TRP KO, DRG neurons.

Table 1.

TRP summary table.

TRP	Subgroup[$]	Activation	Subcellular distribution[#]	Genetic phenotypes	Human disease/Drug target
C1			PM	↓ Salivation	
C2	S, M	Pheromones, DAG	PM	↓ Pheromone detection	
C3	M	GPCR-PLC, DAG	PM, Secretory vesicles, Mitochondria	Ataxia; motor coordination defects	
C4	M	GPCR-PLC, Englerin A	PM, Secretory vesicles	Impaired vascular function	
C5	S, M	GPCR-PLC, $Cool_{25–37\ °C}$, Englerin A	PM, Secretory vesicles	↓ Anxiety behaviors	
C6	S, M	GPCR-PLC, DAG	PM	↑ Arterial contractility	FSGS (GOF)
C7	S, M	GPCR-PLC, DAG	PM	↓ Non-image forming photoreception	
V1	S, M, O	$Heat_{>42\ °C}$, H^+, Vanolloids, DkTx	PM, ER, Mitochondria	↓ nociception; ↓ thermal hyperalgesia; ↓ bladder function	Analgesia (clinical trial)
V2	S, O	$Heat_{>52\ °C}$, 2-APB	PM, EE (?)	Susceptibility to bacterial infection	
V3	S	$Warm_{30–35\ °C}$, 2-APB, Carvacrol, Incensole	LELs (?), PM	Abnormal hair morpho-genesis; compromised skin barrier	Olmsted syndrome (GOF)
V4	S	Hypotonicity, $Warm_{24–38°C}$, 4α-PDD	PM	↑ Bone density, altered urinary function	CMT type 2C, skeletal dysplasias (GOF); pulmonary edema (clinical trial)
V5			PM, Secretory vesicles	↓ Renal Ca^{2+} absorption; hypercalciuria; kidney stones	
V6			PM, Secretory vesicles	Defective intestinal Ca^{2+} absorption; osteopenia; infertility	
A1	S, M, O	ROS, 4-HNE, AITC	PM, LELs	Defective chemosensation	Familial episodic pain syndrome (GOF); chronic pain (clinical trial)
M1	M, O		Melanosomes	Impaired vision	Congenital stationary night blindness
M2	S, O	ROS, $Warm_{>35\ °C}$, ADPR	PM, LELs	↓ Inflammation response	
M3	S	$Heat_{>40\ °C}$, PS, Sphingolipids	PM	Defective thermosensation	
M4	S, M	Tastants, Ca^{2+}	PM	Defective gustation	Brugada syndrome; familial heart block 1 (GOF)
M5	S, M	Tastants, $Heat_{>35\ °C}$, Ca^{2+}	PM	Defective gustation	
M6	O (?)		PM, M7-like vesicles (?)	Embryonic lethal	Heritable hypomagnesaemia
M7	O	ROS	PM, M7-like vesicles	Embryonic lethal	
M8	S, O	$Cool_{<25\ °C}$, Menthol, Icilin	PM, ER	Defective cold sensation	Analgesia (clinical trial)
ML1	O	$PI(3,5)P_2$, ROS, ML-SAs, SF51, Mk-683	LELs, TVs	Neuro- and retinal degeneration, muscle dystrophy, hypochlorhydria	Mucolipidosis type IV

TRP	Subgroup[$]	Activation	Subcellular distribution[#]	Genetic phenotypes	Human disease/Drug target
ML2	O	$PI(3,5)P_2$, ROS, ML-SAs	Recycling endosomes, LELs	Impaired innate immune responses	
ML3	O	$PI(3,5)P_2$, ML-SAs, SFs	Early endosomes, LELs	Varitint-Waddler (GOF)	
P1	O		Cilia, ER	Embryonic lethal	ADPKD
P2	O	Calmidazolium	Cilia	Renal, retinal, and intestinal defects.	
P3	O		Cilia		

Abbreviations: 4-HNE, 4-hydroxynonenal; 4α-PDD, 4α-phorbol 12–13-didecanoate; ADPKD, autosomal dominant polycystic kidney disease; ADPR, cyclic ADP ribose; AITC, allyl isothiocyanate; DkTx, double-knot toxin; DVT, decavanadate; FSGS, familial focal segmental glomerulosclerosis; GPCR, G-protein coupled receptor; IC, intracellular; ML-SA, mucolipin synthetic agonist; ML-SI, mucolipin synthetic inhibitor; LEL, late endosome and lysosome; PM, plasma membrane; PS, pregnenolone sulfate; ROS, reactive oxygen species; TV, tubulovesicles.

[$]S, sensory TRPs, M, metabotripic TRPs, O, organellar TRPs.