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Organellar TRP channels

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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 those in lysosomes, organellar TRPs respond to compartment-derived signals to control membrane trafficking, signal transduction, and organelle function. Complementing mouse and human genetics and high-resolution structural approaches, physiological studies employing natural agonists and synthetic inhibitors have become critical in resolving the in vivo functions of metabotropic, sensory, and organellar TRPs.

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]. On the basis of sequence homology, mammalian TRPs can be divided into six subfamilies: TRPC1–TRPC7 (C for canonical), TRPV1–TRPV6 (V for vanilloid), TRPM1–TRPM8 (M for melastatin), TRPA1 (A for Ankyrin), TRPML1–TRPML3 (ML for mucolipin), and TRPP1–TRPP3 (P for polycystin) (Table 1). A common feature of TRP channels is the homotetrameric assembly of subunits containing six transmembrane segments (S1–S6, Fig. 1a)[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 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, owing to their crucial roles in both sensory and signal transduction[7]. Exploration of the biological functions of TRPs relies heavily on human genetics (channelopathies) studies and reverse mouse genetics studies. TRP-knockout mouse studies have 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 because of their various activation mechanisms and 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 (Table 1). The biophysical properties of TRP channels are usually established by heterologous overexpression and whole-cell and/or 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] (Fig. 1 and Box 1). Individually, each of these approaches has 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 structures, 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[24]. Liposome reconstitution studies have indicated that some TRPs (for example, TRPV1 and TRPM8) are activated directly by thermal stimulation[25], and mutagenesis analyses suggest that thermosensitivity and chemosensitivity can be segregated in specific TRPs[26]. Some physical factors (for example, light and hypotonicity) activate TRPs indirectly through derived chemical signals[27–29]. Chemical 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 juxtaorganellar[30]. Second, Na^{+} flux reduces transmembrane voltage potential either across the plasma or organellar membrane[20]. Third, some TRPs (for example, 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[31,32] but can be reduced[33] or accommodated as partially hydrated ions within the large TRP pore[13,14,22,34,35].

TRP channel protomers have six transmembrane segments (S1–S6), with N- and C-terminal domains facing the cytosol (Fig. 1a). S1–S4 form a voltage-sensor-like domain (VSLD; Fig. 1d). However, whereas many TRP channels are weakly modulated by voltage, the VSLD may not be the primary determinant for voltage sensitivity in most TRPs[10,36]. Instead, VSLD may serve as a 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, the S4–S5 linker, the TRP domain, and

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Table 1 | Summary of TRP family members

TRP	Subgroup	Activation	Subcellular distribution	Genetic phenotypes	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	↓ Nonimage-forming photoreception	
V1	S, M, O	$Heat_{>42\,°C}$, H^+, vanilloids, 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 morphogenesis; 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, SF-51, MK6-83	LELs, TVs	Neuro- and retinal degeneration, muscle dystrophy, hypochlorhydria	Mucolipidosis type IV
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	Autosomal dominant polycystic kidney disease (ADPKD)
P2	O	Calmidazolium	Cilia	Renal, retinal, and intestinal defects	
P3	O		Cilia		

Subgroups: S, sensory TRPs, M, metabotripic TRPs, O, organellar TRPs. Activation: 2-APB, 2-aminoethoxydiphenyl borate; 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; ML-SA, mucolipin synthetic agonist; ML-SI, mucolipin synthetic inhibitor; MK6-83, 5-methyl-N-[2-(1-piperidinyl)phenyl]-2-thiophenesulfonamide; PS, pregnenolone sulfate; SF-51, 2-[2-oxo-2-(2,2,4-trimethylquinolin-1-yl)ethyl]isoindole-1,3-dione. Subcellular localization: ER, endoplasmic reticulum; LEL, late endosome and lysosome; PM, plasma membrane; TV, tubulovesicles.

the intracellular N- and C-terminal domains, may be involved in ligand binding and in coupling ligand binding to opening of the channel gate (Fig. 1d).

The TRP selectivity filter is formed by a pore loop between S5 and S6 (ref. [34,37,38]) (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+} (ref. [10,13,14,22,35,38]). 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, which have 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

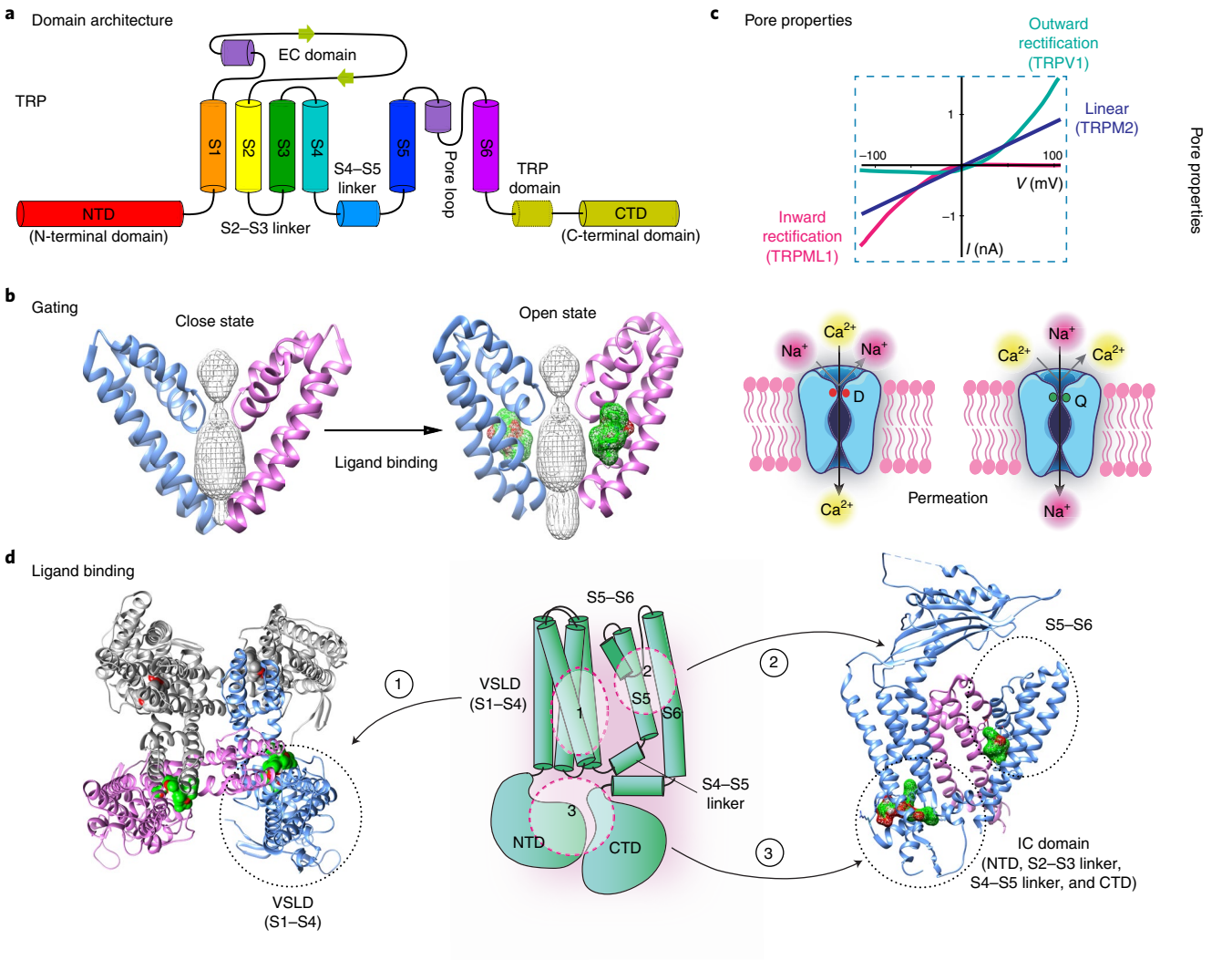


Fig. 1 | Architecture and functional elements of a TRP channel. Structural biology analyses reveal critical domains and residues involved in different channel properties. **a**, TRPs are cation channels with six transmembrane segments (S1–S6) and N- and C-terminal domains facing the cytosol. **b**, Ligand binding to the S5–S6 domain leads to opening the lower S6 gate. **c**, Pore properties of TRP channels. Top, representative TRP current–voltage (*I*–*V*) traces. Bottom, the pore loop between S5 and S6 forms the selectivity filter and upper gate of the channel. Negatively charged residues (for example, Asp541 in TRPV6, shown in red in 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 residues (for example, Gln977 in TRPM4, shown in green in the right panel) form binding sites that favor monovalent cations over Ca^{2+}. **d**, Ligand binding sites are localized in S1–S4 VSLD (site 1), S5–S6 region (site 2, shown in agonist-bound cryo-EM structures of TRP in inset), or intracellular (IC) domains (site 3, also shown in inset). 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. NTD, N-terminal domain; CTD, C-terminal domain.

monovalent permeation (Fig. 1c)[39,40]. In contrast, TRPM4 and TRPM5 have very low P_{Ca}/P_{Na} (< 0.05) due to a ring of glutamine residues in the selectivity filter that preferentially bind monovalent ions (Fig. 1c)[36–38]. 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[10,11,13,14,22,35,41].

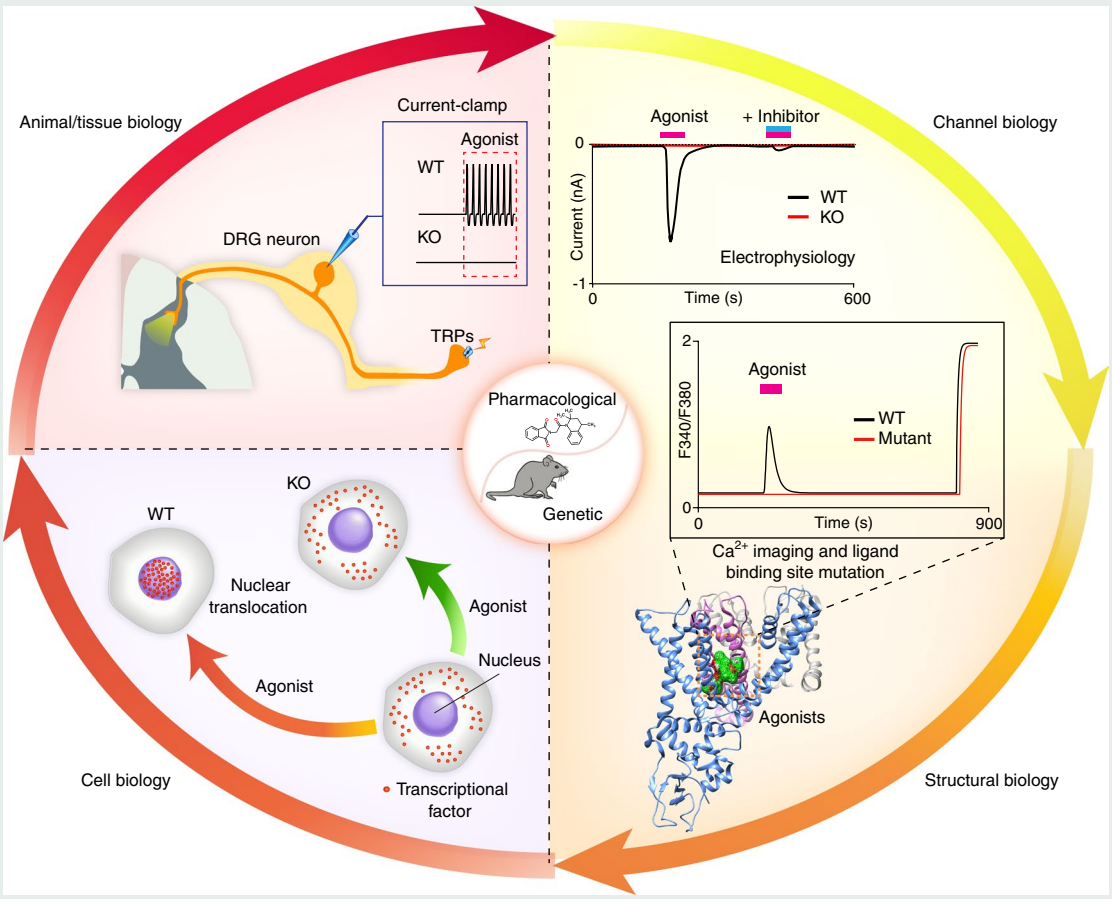
There are one or two activation gates in TRPs. The lower activation gate is found in all TRPs and is formed by the S6 helices (Fig. 1)[10,22], similarly to that in voltage-gated K^+ channels. Mutations affecting the lower gate can result in constitutively active TRP channels[10,42]. The binding of agonists to different regions of the channel may cause conformational changes that converge at the S6 gate, leading to channel opening[12,14,38]. The upper gate is present only in some TRPs (for example, 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[10,19,43]. The coupling mechanisms between the two gates remain to be established.

Many endogenous and synthetic TRP agonists bind to the VSLD. Comparison of apo-state 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[10,11,14,44] (Fig. 1d). Binding of intracellular ligands (for example, Ca^{2+}, phosphatidylinositol bisphosphate (PIP_2), or ATP) to or phosphorylation events in cytoplasmic regions, such as the N-terminal domains (for example, ankyrin repeats in TRPC, TRPV, and TRPA; TRPM-homology-regions and pre-S1 domains), S2–S3 linkers, or C-terminal domains (for example, coiled-coil domain)[10,11,14,44], also 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 have revealed that some agonists, such as mucolipin synthetic

Box 1

Research in the TRP channel field has benefited enormously from the use of integrated approaches, such that the same modulators used in channel studies and high-resolution structures are expected to produce TRP-specific effects in cellular, tissue, and behavioral assays. Phenotypes at the animal level may be dampened by compensatory mechanisms in knockout mice, or they may be due to indirect GOF effects in transgenic mice. For example, in TRPC6-knockout mice, other TRPCs are upregulated as a compensatory mechanism, resulting in a paradoxical increase of neurotransmitter-induced arterial contractility[8,98]. Hence, the complementary use of biochemical and genetic approaches provides a safeguard against complications produced by pharmacological off-target effects or genetic compensation issues. In fact, the consistency in temperature and pain phenotypes observed in TRPV1 knockout and in pharmacological inhibition studies has provided great confidence in both sets of findings[19].

The integration of these multiple approaches is illustrated below, in clockwise direction. Agonist-evoked inward currents, measured at negative voltages, are abolished in TRP-knockout cells or by synthetic inhibitors, suggesting the specificity of the response (top right). Structural biology analyses reveal critical domains and residues as agonist binding sites, and mutations in the binding site abolish agonist-induced Ca^{2+} imaging response (middle and bottom right). Cellular functions of TRPs (for example, agonist activation of TRPML1 leads to nuclear translocation of transcription factor TFEB in WT, but not knockout cells) are examined (bottom left). These are complemented with organismal biology and physiology studies; for example, agonist (e.g., capsaicin for TRPV1) application increases firing frequency in WT but not TRP-knockout DRG neurons (top left).



agonist 1 (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[35,45] (Fig. 1d). Hence, multiple ligand-binding sites and gating mechanisms seem to exist in TRPs.

Functional classification of TRPs

Ion channels are commonly classified on the basis of their selectivity and/or gating mechanisms. TRPs are generally cation nonselective, and the same stimulus may activate a subset of TRPs in different subfamilies (for example, temperature activation of TRPV1–4, TRPM2–5, TRPM8, and TRPC5)[8]. Therefore, a more informative classification of TRPs is based on physiological function and endogenous activation mechanism, leading to three subgroups: metabotropic, sensory, and organellar TRPs (Table 1). Each functional subgroup contains members from individual TRP subfamilies. Because a given TRP can be involved in multiple functions and many TRPs are activated by both environmental and cellular signals, some TRPs belong to more than one functional group; 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

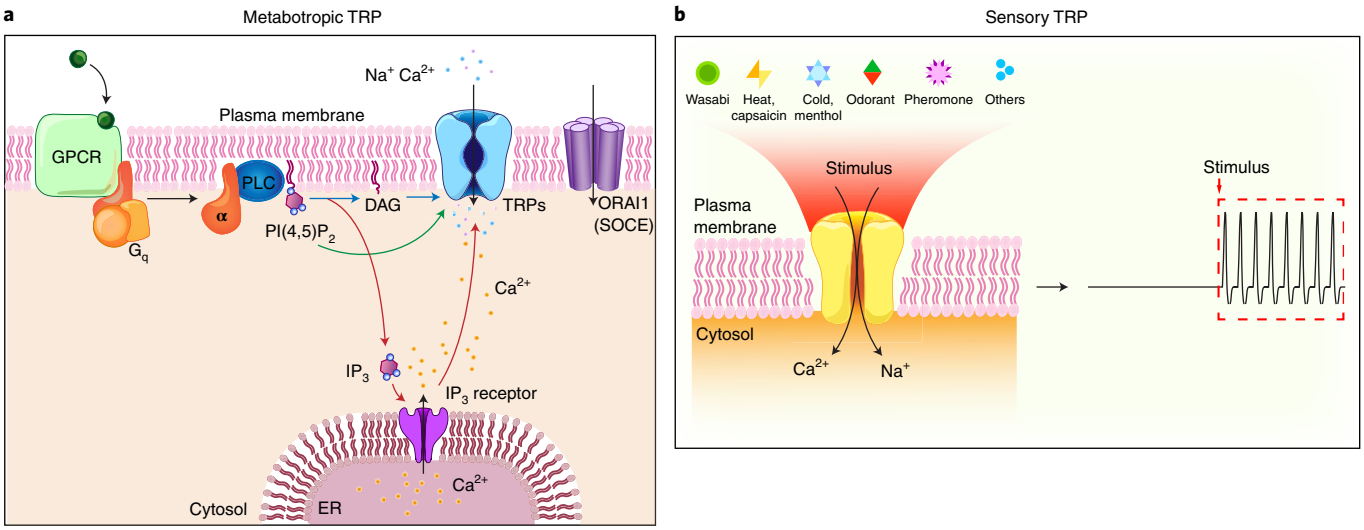


Fig. 2 | Metabotropic and sensory TRPs. a, Metabotropic TRPs couple extracellular cues to biology. Excellular neuroendocrine signals (e.g., neurotransmitters) act on GPCRs. Metabotropic TRPs are signal transducers in cells that also express PLC-dependent GPCRs. Activation of G_q-coupled receptors stimulates 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 IP_3 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. **b**, Sensory TRPs couple environmental cues to biology. Sensory TRPs are activated by environmental signals such as light, temperature change, osmomechanical force, and plant-derived compounds, pain- and/or 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.

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 heart, smooth muscle, and kidney podocytes; they are regulated by sympathetic and parasympathetic transmitters, and they mediate agonist-induced Ca^{2+}-entry pathways[4–7]. Activation of G_q-coupled receptors stimulates PLC activity, resulting in hydrolysis of $PI(4,5)P_2$ into DAG and inositol trisphosphate (IP_3); IP_3 then induces Ca^{2+} release from the endoplasmic reticulum (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 pores are formed by the ORAI proteins[46,47].

Sensory TRPs. Animals detect environmental cues such as light, temperature change, osmomechanical force, and natural compounds, including pain- or itch-inducing chemicals, tastants, and pheromones[19]. These physical and chemical signals increase the membrane excitability of various sensory cells (for example, dorsal root ganglion (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)[48]. Other TRPs involved in sensory functions include TRPM8, a cold and menthol receptor found in DRG neurons[49,50], and TRPA1, a temperature receptor for the spice wasabi (allylisothiocyanate (AITC))[51,52] (Table 1). Other thermosensors include TRPM3 in DRG neurons[53], TRPM2 in sympathetic and central neurons[54,55], and TRPV3 and TRPV4 in keratinocytes[56].

TRPV1 is also sensitive to itch-inducing substances, such as histamine, and injury-evoked inflammatory mediators (for example, bradykinin) through indirect receptor-dependent mechanisms[19]. In taste receptor cells, tastants activate TRPM4 and TRPM5, and they induce membrane depolarization through GPCR taste receptors[57]. TRPC2 in mouse vomeronasal organ (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, and these sensory TRPs are also metabotropic (metabotropic sensory TRPs).

Organellar TRPs. Organellar TRPs comprise 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-controlled immunohistochemistry[20]. More importantly, organelle electrophysiology and organelle-targeted Ca^{2+} imaging provide validation for the functions of organellar TRPs[23,58,59]. The key members in this group are the TRPMLs, which are activated by compartment-specific intracellular cues[58]. TRPML1–3 and TRPP1–3 are distantly related TRPs, whose channel functions remained mysterious until the development of organelle-specific patch–clamp techniques[32,60]. 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, and metabotropic TRPs 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 osmomechanical force, may also regulate organellar TRPs[61–63].

Most organellar TRPs function either in biosynthetic and secretory pathways (in the ER and Golgi), or in the endocytic pathway (for example, in endosomes and lysosomes[20]). Confirmed and candidate (i.e., not yet functionally validated by organelle electrophysiology) organellar TRPs include TRPV1 and TRPP1, which are known to be localized on the ER membranes, and TRPC3–5, TRPV5 and TRPV6, 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[64]. Their Ca^{2+} permeability allows organellar TRPs to contribute to cellular signal transduction by causing changes in cytoplasmic Ca^{2+} levels[30]. Importantly, Ca^{2+} release by organellar TRP channels may increase juxtaorganellar Ca^{2+} levels, specifically affecting the dynamics and 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 'noncommitted' 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[61]. 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[61]. Hence, TRPY1 has dual sensory (activated by an environmental signal) and organellar (vacuole) functionalities[61].

TRPML1 in the lysosome. Lysosomes degrade cargo materials delivered via endocytosis or autophagy, converting them into catabolites and building blocks (for example, amino acids)[65,66]. The degradation products are transported out of the lysosomes via vesicular trafficking and catabolite exporters[23,67]. All these lysosome trafficking steps are regulated by various intracellular signals produced according to the status of luminal cargos and products[23,67]. 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 juxtalysosomal Ca^{2+} levels, may differentially regulate trafficking of individual lysosomes[59,68].

TRPML1 (also called MCOLN1) channels are the major Ca^{2+}-permeable channels in the lysosomes of all cell types. TRPML1-knockout cells exhibit defective lysosomal membrane fusion and fission[23,69]. Whole-endolysosome patch–clamp studies using artificially enlarged lysosomes[58] suggest that TRPML1 may conduct Ca^{2+}, Fe^{2+}, Zn^{2+}, Na^{+}, and K^{+} across lysosomal membranes[32]. By lysosome-targeted genetically encoded Ca^{2+} sensors, TRPML1 was shown to mediate Ca^{2+} release from lysosomes in intact cells[59,70].

TRPML1 channels are activated by cellular cues that regulate lysosome trafficking and function. Phosphatidylinositol 3,5-biphosphate ($PI(3,5)P_2$), a lysosome-specific phosphoinositide, was the first endogenous signal identified for TRPML1, activating it at a physiological, low-nanomolar range concentration[12,58]. $PI(3,5)P_2$ binds to positively charged residues in the N-terminus region of TRPML1, resulting in opening of the S6 gate through the S2–S3 linker[12]. $PI(3,5)P_2$ levels may increase transiently before fusion of two lysosomes[71] and during phagocytic uptake of large particles[72]. Hence, $PI(3,5)P_2$ dynamics likely serve as the cellular cue that activates lysosomal TRPML1.

Several synthetic small-molecule TRPML agonists and inhibitors have been identified[59,73]. Although synthetic agonists activate TRPML1 independently of endogenous cues[35,59], they provide useful tools to probe the channel's cellular functions. The agonist ML-SA1 binds directly to the S5–S6 region of the channel[35], where it may exert a direct force on the S6 gate[35,45]. 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)[74] (Fig. 3) in wild-type but not TRPML1-knockout cells[72]. Retrograde movement of lysosomes to the perinuclear region, which is required for autophagosome–lysosome fusion, is increased with TRPML1 overexpression or synthetic agonism through Ca^{2+} sensor EF-hand-protein apoptosis-linked gene 2 (ALG-2)[75], but reduced by TRPML1 knockout, synthetic inhibition, or $PI(3,5)P_2$ deficiency[75] (Fig. 3). Hence, TRPML1 may allow cellular cues, such as lysosomal lipids, to control or regulate lysosomal trafficking by triggering an increase in juxtalysosomal 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[63]. Activation of TRPML1 triggers the 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)[63]. Activation of TFEB is sufficient to promote mitophagy, removing damaged mitochondria to reduce excessive ROS in the cell[63].

The regulation of TRPML1 by endogenous cellular cues and synthetic modulators illuminates how organellar TRPs are involved in different cellular processes. Although regulation by lipids and ROS is a general feature of TRPs[7], colocalization 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 (for example, ROS) can also act as an intracellular agonist for an organellar TRP such as TRPML1 (ref. [63,76]). The specific cellular process regulated by TRPML1 is determined by both activating signals and downstream effectors (for example, Ca^{2+} sensors) (Fig. 3). Given that synthetic agonists activate TRPML1 independent of $PI(3,5)P_2$ and ROS[12,59,63], it is likely that additional endogenous agonists for TRPML1 exist. It has been demonstrated that sensitivity to ROS, but not to $PI(3,5)P_2$, is required for TFEB activation upon mitochondrial damage[63]. Finally, while the major lysosomal membrane potential regulators are likely the two-pore channels (TPCs)[77], cellular cues that affect lysosomal membrane potential may also regulate lysosome function in a TRPML1-dependent manner[23,58], given that TRPML1 currents are strongly rectifying (Fig. 1c).

TRPML2 and TRPML3 in the endosomes and lysosomes. Both TRPML2 and TRPML3 (TRPML2/3) are localized in lysosomes, but only in certain cell types[23,69,78]. TRPML1 and TRPML3 play complementary roles in cells expressing both channels, such as intestinal enterocytes and cochlear hair cells[79]. $PI(3,5)P_2$ activates all three TRPMLs[58], and it is thus 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[62].

TRPML2/3 are also functional in early and recycling endosomes in some cell types[23,69,78]. For example, TRPML3 is the primary endosomal channel in some macrophage-type cells[78], 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[23,69].

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[80,] 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[81]. TV exocytosis is essential for gastric acid secretion in response to neurotransmitter histamine[80,81]. In this context, the organellar TRPML1 also acts as a receptor-operated channel. Loss-of-function mutations of TRPML1 underlie

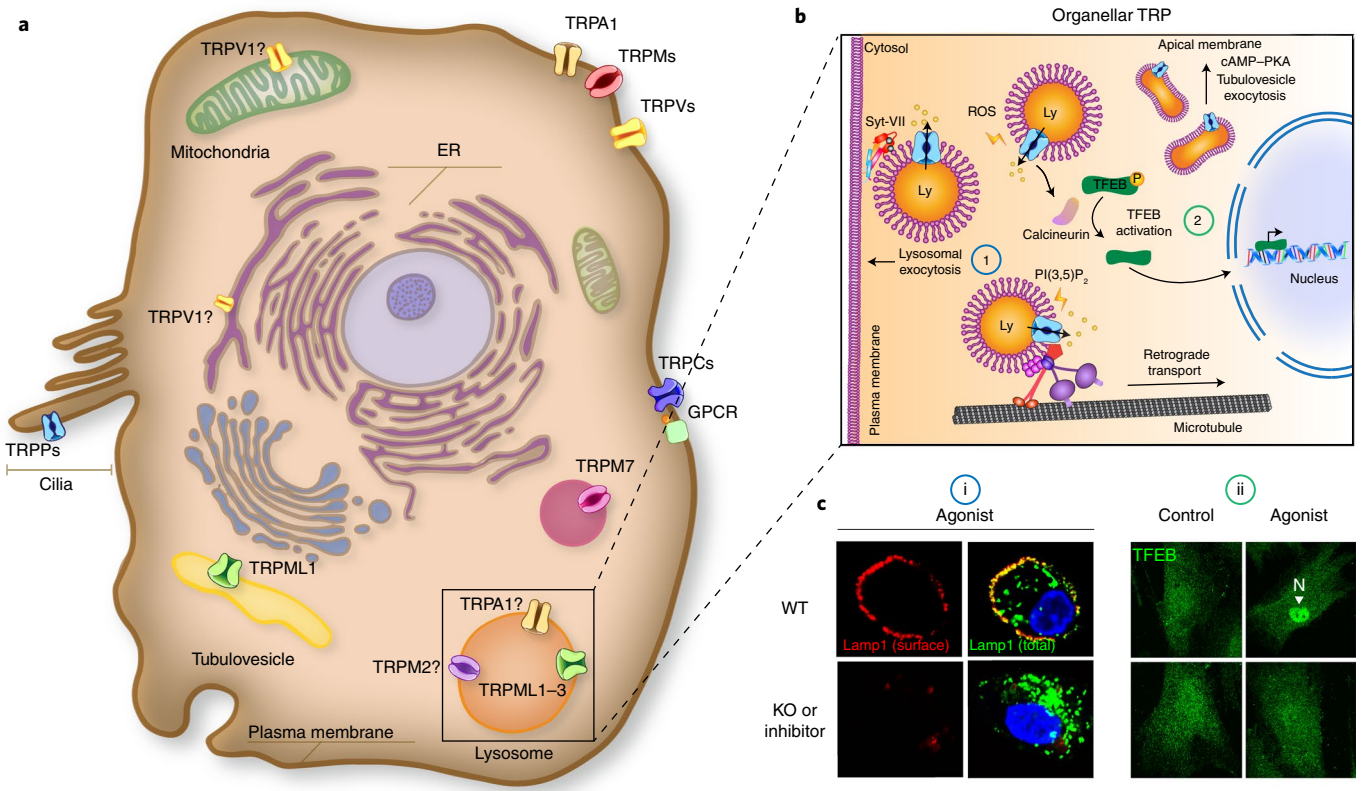


Fig. 3 | Organellar TRP channels. a, Though most TRPs are located at the plasma membrane, TRPMLs are localized in intracellular endosomes and lysosomes, and TRPPs are localized in primary cilia. In addition, TRPV1 is localized in the ER and possibly mitochondria, and TRPM7 is localized in M7-like vesicles. 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 (for example, ROS and $PI(3,5)P_2$) or synthetic agonists induce TRPML1-mediated lysosomal Ca^{2+} release to trigger lysosomal exocytosis (1 in figure), retrograde transport, and TFEB nuclear translocation (2 in figure). In the parietal cells, histamine-induced cAMP/protein kinase A signaling activates TV-localized TRPML1, increasing TV trafficking and exocytosis. Ly, lysosome. **c**, Activation of TRPML1 triggers lysosomal exocytosis, detected by the surface expression of LAMP1 proteins in wild-type but not in TRPML1-knockout (KO) cells (**i**); images are modified from ref. [63] with permission. Agonist activation of TRPML1 leads to nuclear translocation of TFEB (green), a transcription factor for lysosome biogenesis and autophagy, in wild-type but not TRPML1-knockout cells (**ii**); images are modified from ref. [72] with permission. N, nucleus.

type IV mucolipidosis (ML-IV), a genetic disease in which patients experience hyposecretion of gastric acid[23,82]. Consistently with these clinical observations, in mice, knockout or inhibition of TRPML1 suppresses gastric acid secretion, whereas TRPML1 overexpression or activation augments gastric acid secretion[81,83].

TRPPs in cilia. Primary cilia are isolated cellular organelles in eukaryotic cells[84]. TRPP1 (also called PKD2) and TRPP2 (PKD2L1) channels are expressed in primary cilia, and their channel physiology characteristics were established in whole-cilium recordings with or without synthetic channel modulators[60]. However, cilium-specific TRPP-activating cues are yet to be identified.

Noncommitted 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 noncommitted organellar TRPs.

TRPM2 and TRPA1 in the lysosomes of specialized cells. In DRG neurons, TRPA1 is localized in the lysosomes that mediate lysosomal Ca^{2+} release in response to agonist AITC[85]. 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[85]. TRPA1-mediated lysosomal Ca^{2+} release promotes the exocytosis of neuropeptide-containing, dense-core vesicles[85]. 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[86]. In both cases, lysosomal Ca^{2+} release regulates the same functions as their plasma membrane counterparts. To dissect the organellar and plasma membrane functions of dually localized TRPs, it would be 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, 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^{+} (ref [8,87,88]). However, TRPM7 is also localized in the so-called M7-like vesicles[31] and in the synaptic vesicles of sympathetic neurons[89]. Consistently with such intracellular localization, TRPM7 mediates Zn^{2+} release from intracellular vesicles in response to ROS elevation[31]. Other organellar TRPs (for example, TRPML1) may also contribute to heavy metal release from intracellular vesicles such as lysosomes[32]. As organellar TRPs may also

mediate vesicular Ca^{2+} and Na^{+} release, selective metal chelators (for example, the Ca^{2+} chelator BAPTA) may enable distinguishing the actions of the different permeant ions[20]. Additionally, knock-in mutations with altered Ca^{2+}, Fe^{2+}, and 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 as 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[20,90]. For example, activation of TRPV1 using exogenous native agonists increased cytosolic Ca^{2+} but decreased ER luminal Ca^{2+} (ref. [20,90]), suggesting that organellar TRPV1 might regulate luminal Ca^{2+} homeostasis.

Organellar TRPs in disease and therapeutics

More than a dozen inheritable diseases are associated with gain-of-function (GOF) or loss-of-function mutations in TRPs (Table 1). Loss-of-function mutations in TRPML1 cause ML-IV, a neurodegenerative lysosome storage disease[23,82]. Given their diverse biological functions and the technical feasibility of high-throughput screening of TRP modulators based on Ca^{2+} imaging, pharmaceutical companies are currently 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 patients with ML-IV who have partial loss of TRPML1 activity[91]. There are more than 50 lysosome storage diseases (LSDs), and many exhibit lysosomal trafficking defects at the cellular level similar to those seen in ML-IV[23,92]; thus, TRPML agonists could be used to upregulate organelle function in those LSDs[23,59]. For example, in Niemann–Pick type C disease, TRPML1-mediated lysosomal Ca^{2+} release and lysosomal trafficking are partially blocked[59]. Similarly, TRPML1 activity is reduced in $PI(3,5)P_2$-deficient cells, which may cause lysosomal trafficking defects and storage[93]. Furthermore, lysosomal trafficking is defective in many common neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases[92]. Mutations in hydrolases or exporters can 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 generating a vicious cycle[23,59]. Hence, TRPML1 channel dysregulation in the lysosome may be a primary cause of secondary storage in many lysosomal diseases. If so, increasing TRPML1 activity could break the vicious cycle and facilitate lysosomal trafficking to clear lysosomal storage[23,59]. Indeed, TRPML1 overexpression or agonism increases cholesterol clearance in cells derived from patients with Niemann–Pick type C disease[23,59]. TFEB overexpression also induces cellular clearance in most LSDs, except ML-IV[94]. Hence, TRPML1 and TFEB may constitute a cellular clearance program for lysosomal storage[23,95]. 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, atomic-resolution TRP structures are providing 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, 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 through which TRPs are activated by cellular cues in vivo are not well understood. Furthermore, how organellar TRPs regulate organelle function remains largely unknown. In the future, we expect to see more knockout-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 knockout and inhibition. Thus, an open question in the field is which activation mechanisms are physiologically relevant in vivo. For example, TRPV1 inhibitors increased core body temperature in clinical trials[21], but such effect might not be related to the thermosensitivity of TRPV1 per se; more likely, it involves TRPV1 activation by visceral nonthermal cues[96]. The availability of genome-editing technologies such as CRISPR–Cas9 (ref. [97]) has made it possible to study the biological functions of specific modes of TRP modulation. Future studies taking advantage of mouse genetics in combination with biochemical manipulations should determine the in vivo relevance of TRP channel activation by different environmental and cellular signals.

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Competing interests

The authors declare no competing interests.

Additional information

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