

# The TRPM ion channel subfamily: molecular, biophysical and functional features

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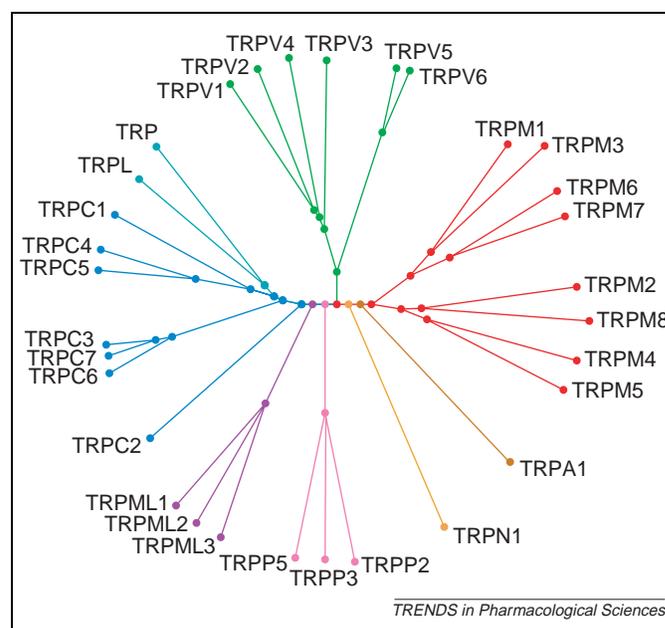
**Significant progress in the molecular and functional characterization of a subfamily of genes that encode melastatin-related transient receptor potential (TRPM) cation channels has been made during the past few years. This subgroup of the TRP superfamily of ion channels contains eight mammalian members and has isoforms in most eukaryotic organisms. The individual members of the TRPM subfamily have specific expression patterns and ion selectivity, and their specific gating and regulatory mechanisms are tailored to integrate multiple signaling pathways. The diverse functional properties of these channels have a profound effect on the regulation of ion homeostasis by mediating direct influx of  $Ca^{2+}$ , controlling  $Mg^{2+}$  entry, and determining the potential of the cell membrane. TRPM channels are involved in several physiological and pathological conditions in electrically excitable and non-excitable cells, which make them exciting targets for drug discovery.**

Transient receptor potential (TRP)-related channels are a large, diverse superfamily of proteins that are expressed in several organisms, tissues and cell types, including electrically excitable and non-excitable cells [1–5]. The TRP superfamily (Figure 1) is divided into three main subfamilies, canonical (TRPC), melastatin-related (TRPM) and vanilloid-receptor-related (TRPV), and four more-distantly related subfamilies (TRPP, TRPML, TRPN and TRPA). Despite structural similarities, the functions of the TRP channels differ widely, even among the members of the same subfamily. In this article, we focus on the TRPM subfamily of ion channels and discuss recent advances in our knowledge of their molecular and biophysical properties, and the physiological context in which they function.

## Molecular features

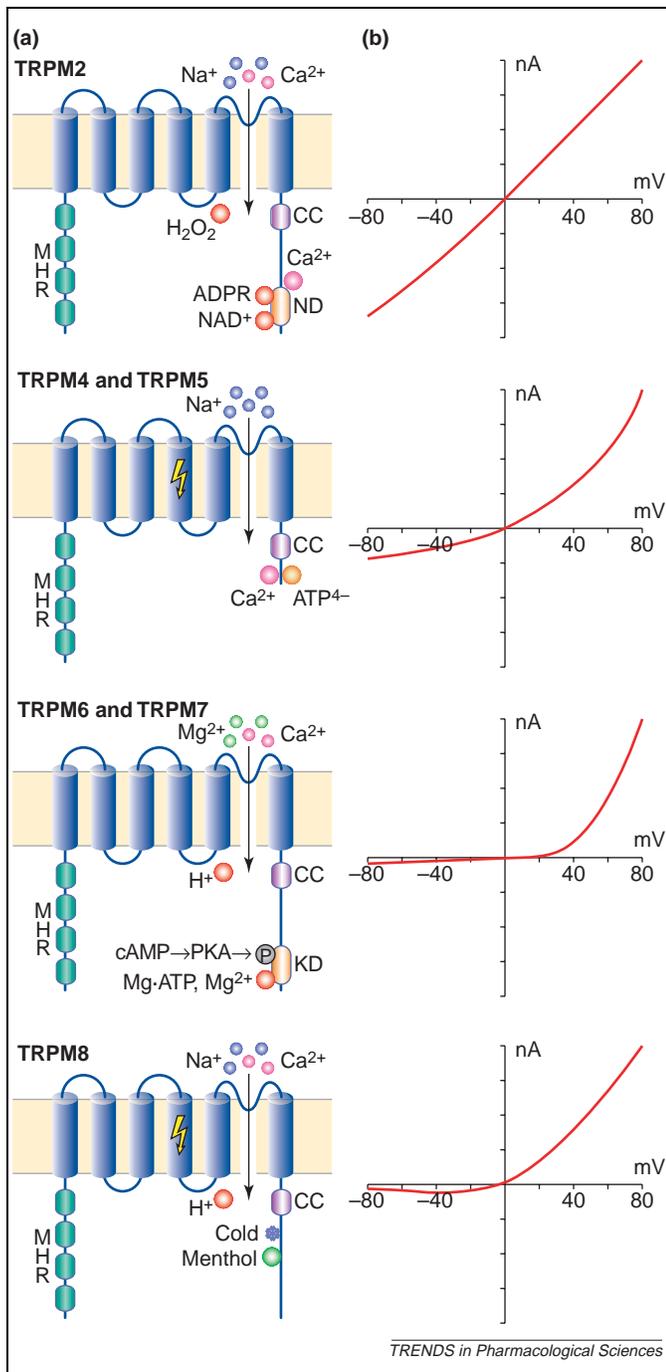
The human TRPM subfamily was named from the founding member melastatin (TRPM1) and consists of eight members that can be grouped into four pairs (TRPM1 and TRPM3; TRPM2 and TRPM8; TRPM4 and TRPM5; and TRPM6 and TRPM7). The overarching structural elements of the TRPM subfamily are similar

to those of voltage-gated channels, comprising six predicted transmembrane segments flanked by cytoplasmic N-terminal and C-terminal tails (Figure 2). The N-terminal regions of the TRPMs contain four stretches of amino acids that share some sequence similarity, but these do not represent known structural motifs and their functions remain to be established. The C-terminal sequences of the TRPM family vary in length and structure. They contain a region of coiled-coil character that is thought to participate in the assembly of TRPM proteins into multimers, with four proteins forming a tetrameric ion channel complex. The C-terminal regions of three members (TRPM2, TRPM6 and TRPM7) have enzymatic activity; TRPM2 has a nucleoside diphosphate pyrophosphatase domain [6–9] that binds specifically to and hydrolyzes ADP-ribose [7], albeit less effectively than dedicated Nudix enzymes [7,10], whereas TRPM6 and TRPM7 contain  $\alpha$ -kinase domains [11–14] with biochemical properties



**Figure 1.** Family tree of mammalian transient receptor potential (TRP) channels. The TRP superfamily of ion channels is classified into three main homologous subfamilies (TRPC, TRPV and TRPM) and more-distantly related groups (TRPML, TRPP, TRPN and TRPA). All TRP channels are characterized by six transmembrane regions with cytoplasmic N- and C-termini. Based on homology, the TRPM subfamily is divided further into four pairs of homologous channels (TRPM1 and TRPM3; TRPM2 and TRPM8; TRPM4 and TRPM5; and TRPM6 and TRPM7).

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**Figure 2.** Molecular architecture and current-voltage relationships of melastatin-related transient receptor potential (TRPM) channels. (a) The molecular domains, permeating ions and activation or regulatory mechanisms of TRPM channels are shown. The placement of most ligands and modulators at the C-terminus and the voltage-sensing elements (yellow symbol) on the fourth transmembrane segment are tentative because the exact molecular locations are unknown. Note that  $ATP^{4-}$ -mediated inhibition applies only to TRPM4 and that regulation by cAMP and protein kinase A (PKA) applies only to TRPM7. The N-terminal regions of the TRPMs contain four homology regions (MHR) (stretches of amino acids that share some sequence similarity), but these do not represent known structural motifs and their functions remain to be established. (b) Typical current-voltage relationships of whole-cell currents (see Table 1 for references that establish the biophysical properties of the channels). The pronounced outward rectification of TRPM4, TRPM5 and TRPM8 is caused by reduced open probabilities of the channels at negative potentials, whereas outward rectification of TRPM6 and TRPM7 is caused by permeation block of divalent ions at negative potentials. Abbreviations: ADPR, ADP-ribose; CC, coiled-coil region; KD, kinase domain; ND, Nudix domain.

that are nearly identical [15]. However, their downstream targets are unknown. The enzymatic domains of TRPM2 and TRPM6,7 have high homology with NUDT9, an ADP-ribose hydrolase [7,10], and PLIK, a cytosolic kinase [12], respectively. The role of the C-terminal domain is less clear in other members of the subfamily such as TRPM4 and TRPM5 because it does not extend significantly beyond the coiled-coil region.

Although the TRPM subfamily is comprised of eight genes, it seems likely that some, if not all, members have multiple protein forms. Splice variants of TRPM1, TRPM2, TRPM3, TRPM4 and TRPM5 have been identified, but little is known about their functional roles. Some might act as either alternative isoforms of the channel or function in combination with full-length versions with modified properties. A further level of complexity and flexibility of TRPM channel proteins might arise from the assembly of different TRPM channel species into heteromultimeric channels. Thus, TRPM6 and TRPM7 are known to form multimeric structures [16] and the closely related TRPM4 and TRPM5 proteins are prime candidates to do so.

During the past few years, most TRPM members have been characterized electrophysiologically in heterologous expression systems and native cells. From these studies, it is evident that the TRPM subfamily represents a heterogeneous group of ion channels with diverse selectivities, activation mechanisms, and characteristic kinetics of activation and inactivation (Table 1). TRPM1, the founding member of the subfamily, is the only TRPM channel that is not characterized electrophysiologically. The expression patterns and physiological context in which the individual TRPM subfamily members operate are as diverse as their properties. Although some channels (TRPM2 and TRPM8) are *bona fide* pathways for  $Ca^{2+}$  entry, others (TRPM4 and TRPM5) are not directly permeable to  $Ca^{2+}$  and modulate  $Ca^{2+}$  entry by shifting the membrane potential. Yet others (TRPM6 and TRPM7) are responsible primarily for  $Mg^{2+}$  homeostasis.

### TRPM1

Melastatin was identified originally in melanoma cells, where it appears to be a tumor suppressor that is downregulated in highly metastatic cells [17]. For this reason, assessment of melastatin mRNA in primary cutaneous tumors is a prognostic marker for metastasis in patients with localized malignant melanoma [18,19]. Currently, there is one report in which TRPM1 channels been assayed functionally (but not electrophysiologically) in a heterologous expression system [20]. HEK-293 cells that express TRPM1 have a higher intracellular concentration of  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ), and removal and readmission of extracellular  $Ca^{2+}$  decreases and increases  $[Ca^{2+}]_i$ , respectively. Therefore, it has been proposed that TRPM1 is a constitutively active  $Ca^{2+}$  channel. However, it is not immediately obvious how a constitutively active  $Ca^{2+}$  channel reduces the growth rate of melanoma cells. It is possible that channel expression is a secondary effect rather than a cause of the growth behavior. Alternatively, the channel might not cause  $Ca^{2+}$  influx but elevate  $[Ca^{2+}]_i$  by an unknown mechanism.

**Table 1. Properties of TRPM ion channels<sup>a</sup>**

Channel	Gating	Voltage dependent	Regulation	$\gamma$	Ion selectivity	Expression	Function	Refs
TRPM1	Constitutive?	ND	ND	ND	Nonselective?	↓ in malignant melanoma	Cell proliferation?	[17]
TRPM2	ADP-ribose, NAD, H <sub>2</sub> O <sub>2</sub>	No	↑[Ca <sup>2+</sup> ] <sub>i</sub> , ↓[Na <sup>+</sup> ] <sub>i</sub>	60 pS	Nonselective (Na <sup>+</sup> , K <sup>+</sup> , Cs <sup>+</sup> and Ca <sup>2+</sup> )	Brain, pancreas, neutrophils,	Apoptosis	[7–9]
TRPM3	Constitutive	No	↑ Osmolarity	80–120 pS	Nonselective (Na <sup>+</sup> , K <sup>+</sup> and Ca <sup>2+</sup> )	Kidney	Renal Ca <sup>2+</sup> homeostasis	[31]
TRPM4	[Ca <sup>2+</sup> ] <sub>i</sub>	Yes	↑ Decavanadate, ↓ ATP <sup>4-</sup>	25 pS	Monovalent (Na <sup>+</sup> , K <sup>+</sup> and Cs <sup>+</sup> )	Heart, liver	Membrane depolarization	[33–35,39,40]
TRPM5	[Ca <sup>2+</sup> ] <sub>i</sub>	Yes	↑ PtdIns(4,5)P <sub>2</sub>	25 pS	Monovalent (Na <sup>+</sup> , K <sup>+</sup> and Cs <sup>+</sup> )	Taste cells, pancreas,	Membrane depolarization	[34,44,45]
TRPM6	Constitutive	No	↓ Mg <sup>2+</sup>	40 pS	Divalent (Ca <sup>2+</sup> and Mg <sup>2+</sup> )	Kidney, intestine	Renal Mg <sup>2+</sup> absorption	[47]
TRPM7	Constitutive	No	↑ cAMP-PKA; ↓ Mg-ATP, Mg <sup>2+</sup> , pH	40 pS	Divalent (Ca <sup>2+</sup> , Mg <sup>2+</sup> , Zn <sup>2+</sup> and Ni <sup>2+</sup> )	Ubiquitous	Mg <sup>2+</sup> homeostasis, cell viability	[11,12,50,58,55]
TRPM8	Cold, menthol	Yes	↓ pH	83 pS	Nonselective (Na <sup>+</sup> , K <sup>+</sup> , Cs <sup>+</sup> and Ca <sup>2+</sup> )	Sensory neurons, prostate	Thermosensation	[60,61]

<sup>a</sup>Abbreviations: [Ca<sup>2+</sup>]<sub>i</sub>, intracellular Ca<sup>2+</sup> concentration;  $\gamma$ , single-channel conductance; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; ND, not determined; PKA, protein kinase A; PtdIns(4,5)P<sub>2</sub>, phosphatidylinositol (4,5)-bisphosphate; TRPM, melastatin-related transient-receptor-potential channels; ↑, upregulation; ↓, downregulation.

### TRPM2

TRPM2 channels are expressed primarily in the brain, but are detected in many other tissues, including bone marrow, spleen, heart, leukocytes, liver and lung. Native TRPM2 currents have been reported in the U937 monocyte cell line [7], neutrophils [21], microglia [22] and CRI-G1 insulinoma cells [23]. When expressed in HEK-293 cells, TRPM2 channels can be activated by elevating the intracellular concentration of ADP-ribose [7–9]. The binding site of ADP-ribose is part of the Nudix enzymatic domain at the C-terminal of the protein, but the channel can also be activated by NAD [8] and reactive oxygen species [e.g. hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)] [9,24].

TRPM2 channels give rise to linear, whole-cell currents with a reversal potential of ~0 mV. The channels carry monovalent cations such as Na<sup>+</sup>, K<sup>+</sup> and Cs<sup>+</sup>, but are also permeable to Ca<sup>2+</sup>. Although Ca<sup>2+</sup> itself does not activate TRPM2 channels, increased [Ca<sup>2+</sup>]<sub>i</sub> facilitates activation by increasing sensitivity towards ADP-ribose [7,25]. TRPM2 is also gated by ADP-ribose in excised patches, where it functions as a 60 pS cation channel with unusually long open times (in the range of several seconds). Unlike other members of the TRPM subfamily (e.g. TRPM4, TRPM5 and TRPM8), TRPM2 channels have no apparent voltage dependence. However, they undergo strong, voltage-dependent inactivation when the intracellular Na<sup>+</sup> concentration is high [7].

The physiological role of ADP-ribose is not well understood. ADP-ribose can be produced from either  $\beta$ -NAD or cADP-ribose by CD38, a multifunctional ectoenzyme that has ADP-ribosyl cyclase, cyclic ADP-ribose hydrolase and NAD glycohydrolase activities. CD38 is expressed in many cells and tissues, and is involved in the growth and differentiation of lymphoid and myeloid cells [26–28]. Many of these cells also express TRPM2 channels, which indicates that CD38 signaling might be linked with TRPM2 channel activity. Another source of ADP-ribose is ADP-ribose polymers, which are complexed by poly-ADP-ribose polymerase (PARP), and hydrolyzed by poly-ADP-ribose glycohydrolase (PARG) [29,30]. This mechanism is stimulated by DNA damage and repair, and excessive

production of ADP-ribose by this route might link the activation of TRPM2 channels with apoptotic cell death. The gating of TRPM2 channels by H<sub>2</sub>O<sub>2</sub> and other reactive oxygen species [9,24] substantiates the role of this channel in stress responses and apoptosis, but it is unclear whether H<sub>2</sub>O<sub>2</sub> gates the TRPM2 channel directly, indirectly (through the production of ADP-ribose) or both. Outside the immune system, TRPM2 channels have been identified in  $\beta$ -cells [23] in which the physiological release of insulin is controlled tightly by the metabolic state. This might indicate that  $\beta$ -NAD, which is an important factor in ATP production, could have a role in mediating TRPM2 channel activation in these cells.

### TRPM3

This protein occurs primarily in kidney and brain in humans but is not detected in mouse kidney [31,32]. Functional data for TRPM3 channels are limited to two reports in which the protein has been expressed in a heterologous system [31,32]. The two constructs used in these studies differ in length (1325 and 1555 residues), which indicates that there might be alternatively spliced variants of TRPM3. TRPM3 induces elevated basal levels of [Ca<sup>2+</sup>]<sub>i</sub>, and removal and readmission of extracellular Ca<sup>2+</sup> causes a decrease and increase in [Ca<sup>2+</sup>]<sub>i</sub>, respectively. Whole-cell recordings obtained with the shorter TRPM3 variant [31] reveal a small, constitutive current with relatively linear current–voltage characteristics and a reversal potential of ~0 mV. Ion-substitution experiments indicate that the channel is permeable to monovalent ions (Na<sup>+</sup> and Cs<sup>+</sup>) and divalent ions (Ca<sup>2+</sup> and Mg<sup>2+</sup>), and single-channel analysis reveals multiple conductance states of 80–120 pS. The TRPM3 channel is regulated by changes in osmolarity because hypotonic solutions cause an increase in channel activity.

Based on its constitutive activity, which is enhanced by extracellular hypotonicity, and its ability to permeate Ca<sup>2+</sup>, TRPM3 is proposed to be involved in renal Ca<sup>2+</sup> homeostasis [31]. It is not known whether the osmotically induced activity is relayed as a physical stimulus or is secondary to messengers generated by hypotonicity.

Therefore, it is conceivable that TRPM3 channels might be regulated by alternative mechanisms that do not rely on osmotic changes.

#### TRPM4

This channel occurs in many tissues and cell types, including electrically excitable and non-excitable cells (e.g. heart and skeletal muscle, neurons, thymocytes and liver cells). Initially, TRPM4 was described as a plasma membrane protein of 1040 residues and, based on fluorescence measurements, it was proposed to be a constitutively active,  $\text{Ca}^{2+}$ -permeable channel [20]. However, subsequent work on the 1214-residue, full-length TRPM4 (designated TRPM4b to distinguish it from the shorter TRPM4a variant) characterized the protein as a  $\text{Ca}^{2+}$ -activated, nonselective (CAN) cation channel [33]. Different laboratories report different dose–response relationships, with half-maximal  $[\text{Ca}^{2+}]_i$  values ranging from 400–800 nM to 5–10  $\mu\text{M}$  [33–35]. The reasons for these variations are unknown, but they mirror the variations observed in CAN channels in native cell types [36–38] and might reflect regulatory mechanisms that await discovery. Recent evidence shows that decavanadate causes a significant shift in the apparent  $\text{Ca}^{2+}$  dependence of TRPM4 [39].

Full-length TRPM4 carries monovalent cations ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cs}^+$ ) but is essentially impermeable to  $\text{Ca}^{2+}$ . The single-channel current–voltage relationship of TRPM4 is linear with a reversal potential of  $\sim 0$  mV and a slope conductance of  $\sim 25$  pS. Single-channel recordings also reveal that channel activity is voltage dependent because negative potentials reduce, and positive potentials increase, open probability [33] so that the steady-state current–voltage relationship is characterized by outward rectification. Subsequent work has characterized this voltage dependence [34,35]. In addition, activity of the TRPM4 channel is subject to nucleotide-mediated regulation in which free ATP, ADP and AMP inhibit channel opening [40].

Although not permeable to  $\text{Ca}^{2+}$ , TRPM4 has a significant effect on  $[\text{Ca}^{2+}]_i$  because it provides a mechanism that allows cells to depolarize in a  $\text{Ca}^{2+}$ -dependent manner [33]. In electrically non-excitable cells that lack voltage-dependent  $\text{Ca}^{2+}$  channels, TRPM4-mediated depolarization would decrease the driving force for  $\text{Ca}^{2+}$  influx through store-operated  $\text{Ca}^{2+}$  channels, whereas in excitable cells TRPM4 might be important in shaping action-potential duration and spiking frequency, thereby supporting  $\text{Ca}^{2+}$  influx through voltage-dependent  $\text{Ca}^{2+}$  channels. Thus, TRPM4 activation might either suppress or promote  $\text{Ca}^{2+}$  influx, depending on the cell type.

#### TRPM5

The gene that encodes TRPM5 was identified during functional analysis of a chromosomal region that is associated with several tumors [41]. However, it is not clear whether the channel causes tumorigenesis. Northern analyses of the expression patterns of TRPM5 yield discrepant results: one study found that expression is limited primarily to tissues involved with taste [42], whereas another found a broader distribution in several

fetal and adult tissues from humans and mice [41]. In functional studies, TRPM5 was reported originally to be a  $\text{Ca}^{2+}$ -permeable ion channel that is activated following store depletion [42]. Another study has proposed a receptor-mediated mechanism that is coupled to phospholipase C (PLC) activation and activates the channel in a  $\text{Ca}^{2+}$ -independent manner [43]. However, subsequent studies from three independent laboratories describe a  $\text{Ca}^{2+}$ -dependent activation mechanism [34,44,45]. As with TRPM4, there seems to be variability in the dose–response behavior, but all three studies find that the protein is activated directly by elevated  $[\text{Ca}^{2+}]_i$  in whole-cell recordings and in excised membrane patches. Like TRPM4, TRPM5 is a monovalent-specific ion channel with 25 pS conductance that does not permeate  $\text{Ca}^{2+}$ . The channel has a linear current–voltage relationship, but because the voltage-dependent modulation of open probability of TRPM5 is similar to that of TRPM4, the steady-state current–voltage relationship of TRPM5 currents is characterized by strong outward rectification.

Because several properties of TRPM5 ( $\text{Ca}^{2+}$ -dependent activation, selectivity, single-channel conductance and voltage dependence) are nearly identical to TRPM4, the two channels might have a similar purpose, namely to cause cell membrane depolarization. From the information available, TRPM5 appears to differ from TRPM4 in its transient nature with fast activation and inactivation kinetics and its responsiveness to  $\text{Ca}^{2+}$  release from intracellular stores (TRPM4 currents activate more slowly, inactivate less rapidly, and respond better to long-lasting  $\text{Ca}^{2+}$  entry than to  $\text{Ca}^{2+}$  release). These features make TRPM5 suited to coupling agonist-mediated  $\text{Ca}^{2+}$  release from intracellular stores with electrical activity. There is evidence exists that two native, cellular systems might benefit from such a transduction mechanism: taste receptors and pancreatic  $\beta$ -cells. In taste transduction, TRPM5 appears to be crucial in sensing sweet, bitter and amino acid tastes [42,43]. Receptors for these chemical stimuli are coupled to heterotrimeric G proteins that activate PLC and cause inositol (1,4,5)-trisphosphate [ $\text{Ins}(1,4,5)\text{P}_3$ ]-mediated  $\text{Ca}^{2+}$  release. In this scenario, TRPM5 activation might provide the necessary depolarization to trigger action potentials and activate voltage-dependent  $\text{Ca}^{2+}$  channels that, ultimately, cause transmitter release. In  $\beta$ -cells [41], a similar mechanism might link TRPM5 with insulin release triggered by PLC-coupled agonists such as acetylcholine [46], and, in glucose-induced signaling, TRPM5 might help shape the complex firing patterns of  $\beta$ -cells.

#### TRPM6

The molecular structure and electrophysiological properties of TRPM6 are related closely to those of TRPM7. Two studies that have characterized TRPM6 electrophysiologically [16,47] reveal an interesting puzzle. In cells that express TRPM6 alone, one study demonstrates that large, TRPM6-mediated currents are essentially indistinguishable from TRPM7-mediated currents; both are activated by a reduction in free  $\text{Mg}^{2+}$  and  $\text{Mg}\cdot\text{ATP}$ , and exhibit the same current–voltage relationship and selectivity for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  [47]. The other study shows that TRPM6

is retained intracellularly and that it requires co-expression of TRPM7 for translocation to the plasma membrane [16]. In this study, coexpression of TRPM6 and TRPM7 produces larger currents than TRPM7 alone and fluorescence resonance energy transfer (FRET) analysis indicates that the two proteins form heteromultimeric protein complexes.

Like TRPM7, TRPM6 is thought to be involved in  $Mg^{2+}$  transport because mutations in the gene that encodes TRPM6 are responsible for an autosomal recessive form of familial hypomagnesemia with secondary hypocalcemia [48,49]. However, unlike the ubiquitously expressed TRPM7, TRPM6 is restricted mostly to colon and kidney, and non-functional mutant forms of TRPM6 are likely to be responsible for the intestinal malabsorption and reduced renal reabsorption of  $Mg^{2+}$  in patients with these mutations. Thus, TRPM6 appears to be a key element in the transcellular transport of  $Mg^{2+}$  in colon and kidney, whereas its more ubiquitous cousin TRPM7 appears to be responsible for general cellular  $Mg^{2+}$  import in virtually every cell type. Fortunately, TRPM6-deficient patients can mitigate the disease symptoms by high-dose  $Mg^{2+}$  supplementation, possibly by compensating the TRPM6 defect by normal TRPM7 activity.

### TRPM7

Two studies that characterized TRPM7 initially arrived at different conclusions about the activation mechanism and electrophysiological properties of this channel. One report classified it as a  $Ca^{2+}$ -permeable, nonselective cation channel with a single-channel conductance of 105 pS (at +40 – +100 mV) that is activated by increasing intracellular ATP concentrations [12], and proposed that the kinase domain controlled the gating of TRPM7. A parallel study proposed that TRPM7 is a constitutively active current that is largely suppressed by high intracellular concentrations of free  $Mg^{2+}$  and  $Mg\cdot ATP$  [11]. This study also found that TRPM7 is divalent specific and carries both  $Ca^{2+}$  and  $Mg^{2+}$ , but not  $Na^{+}$ , and estimated its single-channel conductance at ~40 pS (at +60 mV). Based on these properties, native TRPM7-like currents are designated MagNuM (magnesium-nucleotide-regulated metal ion current). The strong outward rectification of TRPM7 currents is not caused by voltage dependence but reflects the permeation properties of the channel. Thus, negative potentials favor the selective inward flux of divalent ions and positive potentials enable outward movement of monovalent cations such as  $K^{+}$  and  $Cs^{+}$ .

Subsequent work largely confirms the latter characterization and reveals additional insights into selectivity and regulation of the channel, and the role of the kinase domain. Whereas  $Ca^{2+}$  and  $Mg^{2+}$  are physiologically the most relevant divalent ions that permeate TRPM7, the channel is also permeable to a range of other trace metal ions such as  $Zn^{2+}$  and  $Mn^{2+}$  in addition to toxic ions such as  $Ni^{2+}$  and  $Cd^{2+}$  [50]. The selectivity, in descending order, is  $Zn^{2+}$ ,  $Ni^{2+}$ ,  $Ba^{2+}$ ,  $Co^{2+}$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Sr^{2+}$ ,  $Cd^{2+}$  and  $Ca^{2+}$ . TRPM7 also conducts large monovalent currents in the absence of divalent charge carriers such as  $Ca^{2+}$  and  $Mg^{2+}$  [11,51]. This feature, combined with the fact that TRPM7 is activated under experimental

conditions that are used traditionally to study the store-operated  $Ca^{2+}$  current  $I_{CRAC}$ , are the basis for mistaking TRPM7 channels as  $Ca^{2+}$ -release-activated  $Ca^{2+}$  (CRAC) channels [51–53].

Although variations in the intracellular  $Mg\cdot ATP$  concentrations provide an important 'passive' regulatory mechanism of TRPM7 activity, the channels can also be modulated 'actively'. Two conflicting reports propose that TRPM7 is regulated by phosphatidylinositol (4,5)-bisphosphate [ $PtdIns(4,5)P_2$ ] [54] and cAMP signaling [55]. The first study suggests that receptor-mediated breakdown of  $PtdIns(4,5)P_2$  mediates negative-feedback inhibition of TRPM7, whereas the second study challenges this interpretation by demonstrating a complete loss of receptor-mediated  $Ins(1,4,5)P_3$  production in cells that overexpress TRPM7. Instead, the latter study reported that TRPM7 is regulated either positively or negatively by the changes in intracellular levels of cAMP that are induced by  $G_i$ - and  $G_s$ -protein-coupled receptors, respectively. This modulation requires functional protein kinase A (PKA) and a functional TRPM7 kinase domain. The kinase domain, which is not essential for channel activation, regulates both active, receptor-mediated regulation of channel activity and passive, constitutive activity because it determines the sensitivity of the channel to intracellular  $Mg^{2+}$  and  $Mg\cdot ATP$  [56]. Additionally, recent studies find that TRPM7 is also upregulated by reactive oxygen species [57] and inhibited by acidic pH [58].

The physiological relevance of TRPM7 is obvious from targeted knockout of the protein in DT40 cells, which causes these cells to stop growing and die [11]. The low-level, constitutive activity of TRPM7 is likely to represent a homeostatic mechanism that provides a constant trickle of  $Ca^{2+}$  and  $Mg^{2+}$  into the cell. Its primary function relates, apparently, to  $Mg^{2+}$  transport because supplementation of extracellular  $Mg^{2+}$  but not  $Ca^{2+}$  restores normal growth and viability of TRPM7-deficient cells [56]. Whether  $Ca^{2+}$  transport is important physiologically or whether it is incidental in enabling the larger  $Mg^{2+}$  ion to permeate is unknown. However,  $Ca^{2+}$  entry might be relevant under conditions of strong, long-lasting activation of TRPM7 (e.g. anoxia, oxidative stress and metabolic imbalance) to the point that it might cause cell death [57]. In addition to homeostatic regulation, TRPM7 can also increase and decrease the entry of  $Ca^{2+}$  and  $Mg^{2+}$  during receptor stimulation. Because the hypothesis that receptor-mediated depletion of  $PtdIns(4,5)P_2$  inhibits TRPM7 [54] is open to alternative interpretations involving the cAMP–PKA pathway [55], the  $PtdIns(4,5)P_2$  mechanism awaits additional studies that either strengthen or weaken the concept. Although the precise benefits of augmenting the entry of divalent cations through TRPM7 are unknown, enhanced  $Ca^{2+}$  and  $Mg^{2+}$  influx might deliver these ions to mitochondria. It might also modulate the activity of several cytosolic proteins including G proteins and  $Ca^{2+}$ – $Mg^{2+}$ -dependent enzymes and, even, the kinase domain of TRPM7.

### TRPM8

TRPM8 was identified originally as a prostate-specific gene [59], but was later found to be present in a subset of

cold-responsive dorsal root ganglia neurons and neurons from trigeminal ganglia [60,61]. Although the function of TRPM8 in the prostate is unknown, its presence in cold-responsive cells is consistent with a role in thermosensation and nociception [62]. Indeed, heterologously expressed TRPM8 channels are activated by cooling cells to  $\leq 24^{\circ}\text{C}$  and by cooling agents such as menthol and icilin [60,61,63]. Channel activity is regulated additionally by intracellular pH and acidification inhibits responses to cold and icilin but not menthol [64]. TRPM8 channels are permeable to  $\text{Ca}^{2+}$  and account for increases in  $[\text{Ca}^{2+}]_i$  in response to cold and menthol. They have a single-channel conductance of  $\sim 80$  pS. The current-voltage relationship of TRPM8 exhibits strong outward rectification and reverses at  $\sim 0$  mV, which is reminiscent of TRPM4 and TRPM5 and is consistent with a voltage-dependent modulation of open probability. Recently, the gating mechanism of TRPM8 has been linked to temperature-dependent modulation of the voltage dependence of the channel, where low temperatures shift the voltage dependence of TRPM8 from very positive potentials into the physiological range, and cooling compounds such as menthol and icilin mimic this behavior [65].

### Concluding remarks

Based on significant progress achieved during the past few years, we have a reasonably good understanding of the molecular and biophysical properties of most TRPM proteins. These represent a heterogeneous group of ion channels that are characterized by specific expression patterns, selectivities and activation mechanisms. Unlike other TRP subfamilies, whose members are thought to function primarily as  $\text{Ca}^{2+}$ -entry pathways, several TRPM channels are either impermeable to  $\text{Ca}^{2+}$  or function as dedicated  $\text{Mg}^{2+}$ -influx pathways. Although some TRPM channels have been identified in native cell systems, most of our knowledge stems from characterization in heterologous expression systems. Unfortunately, we lack selective inhibitors and agonists to probe pharmacologically the functions of these channels in their native tissues. However, the potential roles of TRPM channels in several physiological and pathophysiological processes have sparked the interest of the pharmaceutical industry, and several TRPM channels are the subject of rigorous drug-discovery projects. In addition to pharmacological approaches, studies that employ molecular approaches to induce cellular and organismal knockdown of TRPM channels are likely to reveal more about the function of this group of ion channels in cell physiology and disease.

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