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TRPM6 and TRPM7: A Mul-TRP-PLIK-Cation of Channel Functions

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Abstract

Unique among ion channels, TRPM6 and TRPM7 garnered much interest upon their discovery as the first ion channels to possess their own kinase domain. Soon after their identification, the two proteins were quickly linked to the regulation of magnesium homeostasis. However, study of their physiological functions in mouse and zebrafish have revealed expanding roles for these channel-kinases that include skeletogenesis and melanopore formation, thymopoiesis, cell adhesion, and neural fold closure during early development. In addition, mutations in the TRPM6 gene constitute the underlying genetic defect in hypomagnesemia with secondary hypocalcemia, a rare autosomal-recessive disease characterized by low serum magnesium accompanied by hypocalcemia. Depletion of TRPM7 expression in brain, on the other hand, proved successful in mitigating much of the cellular devastation that accompanies oxygen-glucose deprivation during ischemia. The aim of this review is to summarize the data emerging from molecular genetic, biochemical, electrophysiological, and pharmacological studies of these unique channel-kinases.

Keywords

TRPM6; TRPM7; channel; kinase; calcium; magnesium

INTRODUCTION

At the turn of the last century the ion channel and kinase superfamilies comfortably occupied separate fiefdoms. The first inkling of rebels in their midst came in the form of a correspondence from Alexey Ryazanov, Karen Pavur, and Maxim Dorokov published in *Current Biology* in 1999 in which the authors reported the identification of “melanoma kinase,” as well as other previously unidentified proteins, as “alpha-kinases,” a new class of protein kinases with little homology to conventional protein kinases [1]. Since only the sequence of the catalytic domain of melanoma kinase was reported in the “correspondence,” it wasn’t until 2001 that the aforementioned kinase would formally join the ion channel clan as TRPM7 (TRP-PLIK, LTRPC7, CHAK1), a member of the transient receptor potential (TRP) family [2–4]. In quick succession followed the identification of TRPM6 (TRP-PLIK2, LTRPC6, CHAK2, kidney kinase) as a second ion channel fused to a kinase domain [4]. With sequence homology to the putative tumor suppressor melastatin (Fig. 1), the founding member of the melastatin-like TRP (TRPM) subfamily, the two proteins were then, as they are now, enigmas, with the underlying reason why nature endowed these two ion channels with alpha-kinase domains attached to their COOH-terminal tails, remaining an unresolved mystery to this day.

CHANNEL ALPHA-KINASES

The alpha-kinase family is a recently discovered group of protein kinases that have no detectable sequence homology to conventional protein kinases (for recent reviews see [5, 6]). It is so named because of the unique mode of substrate recognition by the initial members, the *Dictyostelium* myosin II heavy chain kinases (MHCKs) and elongation factor 2 kinase (eEF-2 kinase). Unlike conventional protein kinases, which phosphorylate amino acids located within loops, turns or irregular structures, the three *Dictyostelium* MHCK A phosphorylation sites are located in the coiled-coil alpha-helical region of *Dictyostelium* myosin II's heavy chains [7, 8]. Similarly, the phosphorylation site in eEF-2 is located within a sequence predicted to be alpha-helical [5]. In addition to eEF-2 kinase, TRPM6 and TRPM7, mammals have three more alpha-kinases including alpha kinase 1 (lymphocyte alpha-kinase, LAK or ALPK1), alpha-kinase 2 (heart alpha-kinase, HAK or ALPK2), and alpha-kinase 3 (muscle alpha-kinase, MAK or ALPK3). TRPM7 was the first alpha-kinase to have the 3-dimensional structure of its catalytic kinase domain determined [9]. Surprisingly, despite the absence of visible sequence homology between TRPM7's alpha-kinase domain and conventional protein kinases, the crystal structure revealed remarkable structural conservation to eukaryotic protein kinases. The domain consists of two lobes that bind nucleotide at the interface between them. The N-terminal lobe of TRPM7 is very similar in its topology to the corresponding lobe of protein kinase A (PKA), particularly in the topological folds that make up the nucleotide binding site (P-loop) within the catalytic core. However, in contrast to PKA, the C-terminal lobe of TRPM7's kinase domain contains a zinc binding module, conserved among alpha-kinases, which is integrated into the hydrophobic core of the lobe and is predicted to be crucial for the stability of the kinase domain. Biochemical analysis of the properties of the isolated kinase domain revealed that the kinase requires Mg^{2+} or Mn^{2+} , with activity in the presence of Mn^{2+} being 2 orders of magnitude higher than in the presence of Mg^{2+} ; Ca^{2+} at concentration up to 1 mM did not affect kinase activity [10]. TRPM6's alpha-kinase domain is functional [11], and has virtually identical biochemical properties to that of TRPM7 with respect to ion and nucleotide dependence (personal communication, Alexey Ryazanov, UMDNJ-Robert Wood Johnson Medical School). TRPM7's kinase is specific for ATP and cannot use GTP as a substrate [10]. In addition, the kinase domain is able to undergo autophosphorylation and to phosphorylate myelin basic protein and histone H3 on serine and threonine residues [10]. TRPM7's kinase domain assembles into a homodimer through the exchange of a 27-residue-long N-terminal sequence that spans residues 1551 to 1577 [9]. The dimer interactions appear to play a critical role in regulating the activity of the kinase domain as well as its dimerization. Crawley and Côté demonstrated that the N-terminal segment can be divided into an 'activation sequence', encompassing residues 1553–1562 that is required for kinase activity but not dimer formation and a 'dimerization sequence' from 1563–1570 that is critical for both dimer formation and activity (Fig. 1A) [9]. The authors' speculated that the N-terminal sequence supports kinase activity by helping to position a catalytic loop in the second subunit, raising the possibility that alterations in the conformation of the exchanged N-terminal segment could regulate TRPM7 kinase activity. Intriguingly, Clark and colleagues have found that TRPM6 and TRPM7 undergo massive autophosphorylation (32 ± 4 mol/mol), with a majority of autophosphorylation sites (37 out of 46) in TRPM7 mapping to a Ser/Thr-rich region immediately NH₂-terminal of the catalytic domain (Fig. 1A) [12]. Autophosphorylation of both of these kinases strongly increases the rate of substrate recognition. Two of these phosphorylation sites reside within 'dimerization sequence' and none of them reside within the 'activation sequence', but it is not known whether phosphorylation of these sites specifically affect catalytic activity or dimerization [10]. Aside from the hypothesis that changes in intracellular free Mg^{2+} may be controlling the activity of the kinase so as to provide "real time information to {its} targets regarding alterations in cytosolic $Mg^{2+}/Mg^{2+} \cdot ATP$ levels, channel state, and/or Mg^{2+} entry," no other

signaling factors have yet been demonstrated to be involved in the regulation of the kinase [13]. With the exception of itself and its ability to transphosphorylate TRPM7, to date no unique substrates have been discovered for TRPM6 [11]. A search for endogenous substrates of TRPM7, however, uncovered annexin I, a Ca^{2+} - and phospholipid-binding protein that can promote Ca^{2+} dependent membrane fusion, as an *in vitro* substrate [14]. Interestingly, the TRPM7 phosphorylation site in annexin I was identified as Ser5, located within the N-terminal amphipathic alpha-helix of the protein. In addition, Clark and colleagues have discovered myosin IIA, IIB, and IIC can be phosphorylated by TRPM6, TRPM7, but not eEF-2 kinase [15–17]. The TRPM7 phosphorylation sites were mapped to Thr1800, Ser1803 and Ser1808, which localize within the α -helical tail of myosin IIA's heavy chain [17]. The functional implications of these phosphorylations will be discussed below, however, the fact that the annexin I and myosin II phosphorylation sites reside within alpha-helical regions may indicate that TRPM7's substrate binding site may have a conformational preference for alpha-helices.

CHANNEL PROPERTIES

TRPM7 was the first of the two channel-kinases to be functionally characterized by whole-cell and single-channel recordings. Heterologous expression of the channel produces currents over a 100–300 second time period with a characteristic steep outwardly rectifying current-voltage relationship (Fig. 1B) [2, 3]. TRPM7 currents were not altered when NaCl in the extracellular solution was substituted with $\text{CH}_3\text{SO}_3\text{Na}$, but substitution of choline-based intracellular solutions suppressed the large outward current, indicating that Cl^- does not permeate the channel and that the outward currents through TRPM7 are carried by cations [2, 3]. Single-channel currents measured at positive voltages in outside-out patches yielded a slope conductance of 105 ± 8 pS using linear regression fit from +40 to +100 mV [3]. TRPM7's inward current, which is relevant for the physiological membrane potentials found in most cells, is exceedingly small (Fig. 1B). Inward currents are not affected by substitution of choline for Na^+ and K^+ in the extracellular solution, indicating that the inward current is carried exclusively by divalent ions such as Ca^{2+} and Mg^{2+} [2]. While most divalent cations block ion fluxes through Ca^{2+} -permeable ion channels, TRPM7 exhibits high permeation of Zn^{2+} as well as other essential divalent cations, including Mg^{2+} , Mn^{2+} , Co^{2+} , and the nonphysiological or toxic metal ions Ba^{2+} , Sr^{2+} , Ni^{2+} , and Cd^{2+} [18]. In addition, TRPM7 is permeable to protons, which compete with divalents to enter the channel's pore [19, 20].

The channel properties of TRPM6, or for that matter, TRPM6/TRPM7 heteroligomers have not been as extensively investigated as for TRPM7 due to controversy over whether TRPM6 homo-oligomers form functional channels. In two sets of studies, TRPM6 was reported to be dependent upon TRPM7 for cell surface expression [11, 21]. Full-length variants of TRPM6 failed to form functional channel complexes when heterologously expressed in HEK-293 cells and *Xenopus* oocytes [21]. In contrast, Voets and colleagues were able to measure functional TRPM6 channels when the protein was heterologously expressed in HEK-293 cells [22]. Similar to TRPM7, TRPM6 constitutes a strong outwardly rectifying conductance at positive potentials with a small inward conductance at negative potentials. Likewise, TRPM6's inward current is almost exclusively carried by Mg^{2+} and/or Ca^{2+} ; the channel's pore is permeable to a range of divalents, including Ba^{2+} , Mn^{2+} , Sr^{2+} , Cd^{2+} , Ni^{2+} , and Zn^{2+} [22, 23]. The strongest evidence that TRPM6 can form functional channels independent of TRPM7 comes from work by Yue and colleagues [23]. Single channel recordings of excised patches from CHO-K1 cells transiently transfected with TRPM6 cDNA uncovered a unique single channel conductance of 83.6 pS, compared to the 40.1 pS value obtained for TRPM7 [23]. Strikingly, single channel recordings of excised patches from cells transfected with a mixture of TRPM6 and TRPM7 cDNAs revealed the 40.1 pS and 83.6 pS conductances in addition to a novel conductance of 56.6 pS, which is presumably derived from a channel

composed of both TRPM6 and TRPM7 [23]. In addition, TRPM6, TRPM7, and TRPM6/7 whole-cell currents displayed a differential permeation profiles, sensitivity to the non-selective channel blocker 2-APB (Table I), as well as to changes in extracellular pH [23]. The apparent differences in the channel properties between TRPM6, TRPM7, and TRPM6/7 will be valuable for the identification and characterization of the native currents *in vivo*.

CHANNEL MODULATION BY CATIONS, PH, AND NUCLEOTIDES

When TRPM7 was initially identified there was great speculation as to the functional significance of its kinase domain. Before the structure of the kinase domain was determined, Runnels and colleagues determined that the G1796D mutation within the GXGXXG nucleotide binding motif and mutation of C1809A/C1812A completely disrupted the catalytic activity of the isolated kinase domain, and that these same mutations within the whole protein markedly decreased whole-cell current amplitudes compared to CHO-K1 cells heterologously expressing the wildtype channel [3]. The authors therefore postulated that “TRPM7 is controlled by intracellular ATP levels and may be linked to a signal transduction cascade that modulates the channel’s kinase activity.” This hypothesis was challenged by Nadler and coworkers who made the discovery that TRPM7 is indeed regulated by levels of ATP, but instead concluded, based on a series of compelling experiments, that the nucleotide’s effect on channel conductance is independent of the catalytic activity of the kinase [2]. The authors found that TRPM7 channel activity is strongly suppressed by $\text{Mg}\cdot\text{ATP}$ in the millimolar range, with nearly complete suppression of currents at 6 mM $\text{Mg}\cdot\text{ATP}$ [2]. Under their experimental conditions the concentration of free Mg^{2+} varied from 670 to 800 μM , such that nearly all of the available ATP was essentially in the physiological $\text{Mg}\cdot\text{ATP}$ form. Strikingly, cells perfused with four different Mg^{2+} -nucleotide triphosphates revealed that both ATP and GTP efficiently suppressed activation of TRPM7 currents, whereas CTP and ITP were less effective [2]. They also found that intracellular Mg^{2+} alone could suppress TRPM7 current [2]. ATP by itself, however, was without effect [24]. The authors analyzed endogenous currents in several cultured cell lines that exhibited a current-voltage relationship similar to those observed in HEK-293 cells overexpressing TRPM7, possessed comparable permeation properties, and that were similarly regulated by $\text{Mg}\cdot\text{ATP}$, and designated the currents as magnesium•nucleotide-regulated metal ion currents or MagNuM. Prakriya and Lewis characterized similar currents and named the channel involved in this activity, MIC, for magnesium-inhibited cation channel [25]. Later studies by Kozak and Cahalan, however, revealed Mg^{2+} is not unique in its ability to inhibit MagNuM/MIC, as Ba^{2+} , Sr^{2+} , Zn^{2+} and Mn^{2+} can substitute for Mg^{2+} , causing complete inhibition of channel conductance [26]. Since both Mg^{2+} and $\text{Mg}^{2+}\cdot\text{ATP}$ can inhibit TRPM7 conductance, this caused controversy over which species was the more dominant regulator of the channel [24, 26]. Demeuse and colleagues performed analysis of the effects of Mg^{2+} , $\text{Mg}^{2+}\cdot\text{ATP}$, and other Mg^{2+} -nucleotide phosphates on TRPM7 conductance [24]. The half-maximal inhibition derived from a dose-response curve where the concentration of free Mg^{2+} was allowed to vary was $720\pm 79\ \mu\text{M}$. Intriguingly, when intracellular solutions were buffered to physiological levels of Mg^{2+} ($\sim 800\ \mu\text{M}$), both ATP and ADP potentiated inhibition of TRPM7 currents, whereas AMP was much less effective. While ATP and ADP were the most potent of all of the nucleotides tested, other purine- and pyrimidine-based nucleoside triphosphates and diphosphates inhibited TRPM7 conductance as well, with ITP being the least effective [24]. Thus, the prevailing view is the Mg^{2+} and $\text{Mg}^{2+}\cdot\text{ATP}$ work in synergy to suppress TRPM7 conductance *in vivo*. Nevertheless, the role of the kinase domain in controlling TRPM7 channel function continued to be debated.

Results from several labs supported the conclusion by Nadler and colleagues that the catalytic activity of TRPM7’s kinase was not required for channel activity. Schmitz and coworkers functionally characterized two kinase-inactivating point mutants TRPM7-

K1648R and TRPM7-G1799D as well as a truncation mutant lacking the kinase domain (TRPM7- Δ -kinase) and found that their heterologous expression in HEK-293 cells yielded whole-cell currents similar to the wildtype channel, albeit the measured current amplitudes of TRPM7- Δ -kinase were significantly reduced [13]. Lys1648 is implicated in catalysis and is conserved in classical kinases, whereas, Gly1799 may be involved in peptide substrate binding or orientation of the substrate-binding loop [9, 27]. Matsushita and coworkers similarly found that heterologous expression of the “kinase-inactive” TRPM7-D1775A point mutant, which is predicted to have disabled binding of Mg^{2+} to the kinase, in HEK-293 cells yielded whole-cell current amplitudes similar to that obtained using the wildtype channel [27]. Interestingly, TRPM7-K1648R and TRPM7-G1799D exhibited reduced sensitivity to inhibition by Mg^{2+} compared to the wildtype channel, whereas the sensitivity of TRPM7- Δ -kinase to Mg^{2+} was enhanced. A later study by Demeuse and colleagues revealed a similar rank order for the sensitivity of TRPM7-K1648R, TRPM7-G1799D, and TRPM7- Δ -kinase to inhibition by Mg^{2+} -nucleotides compared to the wildtype channel [24]. In apparent contrast, Matsushita and coworkers detected no difference between the sensitivities of the TRPM7-D1775A mutant and the wildtype channel to inhibition by Mg^{2+} [27]. However, this may simply be due to the fact that they did not test Mg^{2+} concentrations lower than 4 mM in their experiments. Although, they did find that deletion of the kinase domain results in expression of an inactive channel. Su and colleagues similarly reported that deletion of the kinase domain results in an inactive channel [28]. Demeuse’s measurements of the effects of Mg^{2+} and Mg^{2+} •ATP on TRPM7 channel conductance suggested that the channel may possess two distinct binding sites for Mg^{2+} and Mg^{2+} •ATP, the latter being located within the channel’s endogenous kinase domain and the former closer to the inner mouth of the channel [24]. This notion is supported by the fact that all three “kinase-inactive” mutants retain sensitivity to inhibition by Mg^{2+} [24, 27]. Their prediction of a binding site for Mg^{2+} located intracellularly near the channel’s pore is supported by a striking series of experiments by Kozak and colleagues [29]. The authors had observed that inhibition of the presumed native TRPM7 current (MagNum/MIC), by Mg^{2+} and other divalent metal cations such as Ba^{2+} , Sr^{2+} , Zn^{2+} and Mn^{2+} was voltage-independent. Perfusion of a high concentration of Mg^{2+} into cells inhibited the current more slowly than would be expected for diffusion of the cation into the cytosol (<1 min), making it unlikely that Mg^{2+} , or the other divalent cations, inhibits conductance by direct ion channel pore block [30]. Kozak and colleagues discovered that application of external NH_4^+ stimulates TRPM7 channel activity [29]. NH_4^+ is in equilibrium with its freely membrane-permeant neutral form NH_3 . As NH_3 enters the cell it captures a proton, thereby alkalinizing the internal pH. Conversely, weak acids including acetate and propionate, which can diffuse through the membrane in neutral form, acidify the cytosol by the release of their protons, produced inhibition of the channel [29]. The authors’ posited that inhibition of the current at low cytoplasmic pH likely occurs by screening the negatively charged head groups on phosphatidylinositol 4,5-bisphosphate (PIP₂) or another acidic phospholipid involved in gating the channel. TRPM6 is also inhibited in a voltage-independent manner by internal Mg^{2+} , as might be expected given the significant sequence similarity between the two channel-kinases [22]. However, TRPM6 is distinct in its regulation by nucleotides. ATP, but not GTP and CTP, inhibits TRPM6 currents [31]. TRPM6 mutants harboring amino acid substitutions predicted to disable ATP-binding (TRPM6-T1851A & TRPM6-T1851D) or catalysis (TRPM6-K1804R) exhibited a comparable sensitivity to inhibition of their currents by ATP. Surprisingly, mutation of G1955D within the GXG(A)XXG loop of TRPM6’s kinase domain, disrupted the ability of the channel to be inhibited by ATP, without affecting its capacity to bind the nucleotide. This result supports the notion that some form ATP-dependent conformational coupling independent of kinase activity occurs for both TRPM6 and TRPM7 [13, 31]. In the case of TRPM6, compelling evidence suggests that this coupling could potentially be modulated by direct protein-protein interactions. Cao and colleagues identified RACK1, a scaffold protein originally discovered as an adaptor for protein kinase C (PKC), as a TRPM6

interacting protein [32]. The RACK1 binding site within TRPM6 is restricted to the region between positions 1857 and 1885, encompassing the 5th to 8th β -sheet of the catalytic domain. Transfection of increasing amounts of RACK1 into TRPM6 expressing cells diminished current densities, whereas knockdown of RACK1 potentiated channel activity. Interestingly, RACK1 did not affect whole-cell current densities of cells expressing the kinase-inactive TRPM6-K1804R mutant or of cells expressing truncated TRPM6 lacking the kinase domain (TRPM6- Δ -kinase). The authors identified Thr1851 as an autophosphorylation site for the kinase. Substitution of T1851D (a mutation that mimics phosphorylation) in TRPM6 did not alter the sensitivity of the channel to inhibition by RACK1, however, the T1851A mutation rendered the channel insensitive to inhibition by the protein. Intriguingly, TRPM6-T1851A displayed reduced sensitivity to inhibition by Mg^{2+} compared to the WT channel. In addition, pretreatment of cells with phorbol 12-myristate 13-acetate-PMA (PMA), an activator of PKC, prevented the inhibitory effect of RACK1 on TRPM6 current. These data suggest that RACK1 functions as a mediator linking autophosphorylation of the α -kinase domain to channel activity. The same group also identified repressor of estrogen receptor activity (REA) as a TRPM6-associated protein [33]. REA interacts with the α -kinase domain in the same region as RACK1, however RACK1 does not appear to compete for binding of REA to TRPM6. Like RACK1, REA also inhibits the channel activity of TRPM6 but not the phosphotransferase-deficient mutant (TRPM6-K1804R). The inhibitory effect of REA on TRPM6 channel activity can be prevented by pretreatment of cells with PMA as well as by 17 β -estradiol, which dissociated the interaction between TRPM6 and REA. However, unlike RACK1, which also interacts with TRPM7's α -kinase domain, REA appears to be selective only for TRPM6. Thus, in living cells the regulation of both these channels by protein-protein interactions, phosphorylation, and cations, including Mg^{2+} , Mg^{2+} -nucleotides, and changes in intracellular pH are likely to be exceedingly complex.

RECEPTOR CONTROL OF CHANNEL ACTIVITY

One of the avenues leading to the discovery of TRPM7 was the channel's identification through a yeast two-hybrid screen using the COOH-terminal tail of phospholipase C- β_1 (PLC β_1) as "bait" [3]. The kinase domain of TRPM7 directly associates with the C2 domain of PLC, which motivated the authors to name the channel TRP-PLIK, for transient receptor potential phospholipase C interacting kinase [34]. Experiments by two separate groups have shown that activation of receptors coupled to PLC potently inhibits TRPM7 channel activity by a mechanism that is thought to involve hydrolysis of PLC's substrate PIP_2 [34, 35]. Indeed, application of antibodies against PIP_2 inhibit TRPM7 channel activity, which can be recovered upon washout, and direct application of PIP_2 to inside-out patches stimulates channel activity after channel rundown [34]. Takezawa and colleagues reported that in HEK-293 cells TRPM7 currents are inhibited by the G_i signaling pathway and facilitated by cAMP, concluding that TRPM7 channel activity is up- and down-regulated through its endogenous kinase in a cAMP- and PKA-dependent manner [36]. In contrast to results by Runnels and colleagues, Takezawa and coworkers were unable to detect inhibition of TRPM7 by G_q -dependent activation of PLC. However, in N1E-115/TRPM7 cells, which overexpressed the channel by 2–3 fold, Langelag and colleagues observed that application of the PLC agonist, bradykinin, caused a dramatic decrease in whole-cell currents [35]. Other PLC-coupled agonists, including thrombin receptor activating peptide and lysophosphatidic acid, also inhibited the currents, although to a lesser extent. However, their study added a new wrinkle to our understanding of receptor-mediated regulation of the TRPM7 channel. Langelag and coworkers observed that compared to parental N1E-115 cells, which produced a transient (< 60 sec) increase in cytosolic Ca^{2+} in response to bradykinin stimulation, the increased expression of the TRPM7 channel engendered cells capable of producing an additional sustained peak in Ca^{2+} following the

initial Ca^{2+} transient evoked by bradykinin. How could bradykinin cause both a TRPM7-dependent increase in sustained Ca^{2+} , while at the same time produce inhibition of whole-cell currents? The authors observed that although bradykinin caused inhibition of TRPM7 currents using whole-cell electrophysiology, current recordings in the perforated patch configuration, which provides electrical access to the cell without disturbing the cytosolic composition, revealed that bradykinin increased TRPM7 currents. Under perforated patch conditions stimulation of PLC caused a reversible decrease in its substrate PIP_2 , whereas in whole-cell conditions application of bradykinin caused an irreversible decrease in PIP_2 levels. Thus, the more pronounced hydrolysis of PIP_2 in the whole-cell configuration appeared to mask a signaling mechanism downstream of PLC-coupled receptors that activates the channel. Interestingly, application of the adenylyl cyclase activator forskolin together with the phosphodiesterase inhibitor isobutylmethylxanthine, which increased cAMP levels, failed to stimulate TRPM7 currents or increase Ca^{2+} influx in cells in which TRPM7 is moderately overexpressed 2–3 fold [35]. In addition, neither prostaglandin E1, which activated G_s to cause a rapid and sustained increase in cAMP, nor nitroprusside, which increased cGMP, had any effect on Ca^{2+} levels. Langelag and colleagues speculated “the opposing effect of PIP_2 depletion on PLC-mediated activation may reflect a subtle feedback mechanism whereby ongoing loss of PIP_2 counteracts or limits TRPM7 activation.” Thus, the mechanism(s) that are involved in receptor-mediated activation of TRPM7 remain unclear.

There is some evidence that plasma membrane expression of the channel may be stimulated in response to fluid flow [37]. This effect appears distinct from the inhibition of TRPM7 current produced under hypotonic conditions, which is due to molecular crowding of solutes (Mg^{2+} , spermine) that affect channel activity [38]. However, unlike other TRP channels, receptor-mediated enrichment of TRPM7 onto the plasma membrane has not been reported. In contrast, TRPM6 channel activity can be stimulated by epidermal growth factor (EGF) [39, 40]. Application of EGF to HEK-293 cells expressing TRPM6 increased surface expression of the channel and increased whole-cell current amplitudes [40]. The increase in TRPM6 currents by EGF was shown to be dependent upon the activation of Src family tyrosine kinases and its effector Rac1 [40]. Interestingly, TRPM7 channel activity was not stimulated by the same EGF treatment, suggesting the receptor control of the two channels as well as their biological functions may be distinct [40].

THE ROLES OF TRPM6 AND TRPM7 IN MAGNESIUM HOMEOSTASIS AND CELL GROWTH

Whole body regulation of magnesium homeostasis is an equilibrium between dietary magnesium absorption and urinary excretion in which changes in intake are balanced by changes in urinary magnesium reabsorption, principally by passive paracellular reabsorption of magnesium in the loop of Henle and active transcellular reabsorption of the divalent cation in the distal convoluted tubule [41]. Cases of familial hypomagnesemia have been linked to chromosome 9q [41], and in 2002 two separate groups identified *TRPM6* as the gene responsible for this rare autosomal disorder [42, 43]. Familial hypomagnesemia with secondary hypocalcemia (HSH) is characterized by very low magnesium and low calcium serum levels. Affected individuals exhibit neurologic symptoms of hypomagnesemic hypocalcemia, including seizures and muscle spasm, shortly after birth, which can be corrected by oral magnesium supplementation. It has been reported that HSH is a disorder of abnormal magnesium absorption in the intestine, rather than a disorder of renal magnesium wasting. However, intravenous magnesium loading studies on six individuals with HSH revealed that they begin to leak magnesium at an ultrafiltered concentration of 0.7–0.9 $\text{mg}\cdot\text{dl}^{-1}$ compared with a concentration of 1.4 $\text{mg}\cdot\text{dl}^{-1}$ in normal individuals [43]. Indeed, northern analysis of mouse and human RNA revealed high TRPM6 expression in the kidney

and to a lesser extent in the colon [4, 43]. RT-PCR using RNA from various rat tissues yielded PCR amplification products in intestine and kidney, with strongest amplification occurring in the distal convoluted tubule and to a lesser extent in the proximal convoluted tubule. Expression was also assessed by *in situ* hybridization in various human tissues, revealing *TRPM6* mRNA in colon epithelial cells, duodenum, jejunum and ileum as well as in distal renal tubule cells [42]. Voets and colleagues raised an antibody against TRPM6; immunofluorescence staining was observed in the superficial cortex of the mouse kidney, revealing that TRPM6 expression was restricted to the distal convoluted tubule, where it is localized along the apical membrane [22]. Thus the tissue distribution of TRPM6 is consistent with its involvement in magnesium absorption in intestine and kidney. Importantly, subsequent work has shown that TRPM6 is a target for regulation of magnesium reabsorption by magnesiotropic hormones, such as EGF and 17- β -estradiol [39, 44].

While a majority of the mutations in individuals affected with HSH are either nonsense or frameshift mutations easily compatible with a loss-of-function phenotype, one missense mutation entails the exchange of a highly conserved serine for a leucine at amino acid position 141 [42]. Surprisingly, Chubanov and colleagues showed that the S141L mutation disables the ability of TRPM6 to form multimers with TRPM7 for efficient trafficking to the plasma membrane, suggesting a role for TRPM7 in whole-body magnesium homeostasis as well [21]. However, unlike TRPM6, TRPM7 is more widely expressed [2–4]. While significantly expressed in the distal convoluted tubule, it is at varying levels in all other nephron segments [21]. Owing to the high sequence similarity between TRPM6 and TRPM7, the identification of *TRPM6* as a gene mutated in HSH, and TRPM7's nearly ubiquitous expression raised the question of whether TRPM7 was also involved in cellular magnesium homeostasis [42].

Chicken DT40 B cells made deficient in TRPM7 via cre-lox-mediated ablation of the *TRPM7* gene (DT40-KO) go into growth arrest and die after a few days in culture [2]. Strikingly, supplementing their growth media with 10–25 mM Mg^{2+} (but not Ca^{2+} , Mn^{2+} , or Zn^{2+}) permits the knockout cells to survive and grow in culture [13]. Reexpression of human TRPM7 as well as a phosphotransferase-deficient mutant TRPM7-K1648R was also capable of reversing the growth arrest phenotype [13]. Consistent with reports that TRPM6 by itself cannot form functional channels, expression of human TRPM6 in TRPM7 deficient DT 40 cells did not restore the ability of these cells to proliferate [11]. In support for a role for TRPM7 as a key constituent of the Mg^{2+} uptake mechanism involved in the homeostatic regulation of vertebrate cellular Mg^{2+} , total cellular Mg^{2+} of TRPM7 deficient cells grown in regular media normalized to that of cells grown in 15 mM supplemental Mg^{2+} was reduced, but could be restored by reexpression of the human TRPM7 as well as the kinase-inactive TRPM7-K1648R mutant [13]. The fact that provision of supplemental Mg^{2+} allowed TRPM7-deficient cells to accumulate Mg^{2+} and proliferate suggests the existence of Mg^{2+} uptake mechanism(s) other than TRPM7. Indeed, overexpression of the plasma-membrane Mg^{2+} transporter SLC41A2 is also capable of reversing the growth arrest phenotype, albeit not as efficiently as Mg^{2+} supplementation alone [45]. The identification of multiple macromolecules capable of transporting Mg^{2+} suggests that, as is the case for regulation of levels of cellular Ca^{2+} , homeostatic control of Mg^{2+} is complex (for a recent review see [46]). The regulatory process for Mg^{2+} is likely to vary according to circumstance (e.g. proliferating versus non-proliferating) as well as by the differential expression of the respective molecules in different cell types and tissues. For example, deletion of *TRPM7* in murine thymocytes and T-lymphocytes did not affect the average concentration of total cellular Mg^{2+} normalized to K^+ compared to wildtype cells nor Mg^{2+} uptake as assessed using the Mg^{2+} -indicator KMG104AM [47]. Similarly, depletion of TRPM7 in HEK-293 cells does not alter the concentration of free Mg^{2+} compared to a

control cell line [48]. However, these studies did not investigate whether up-regulation of other Mg^{2+} transporters/channels compensated for the loss or depletion of TRPM7 from cells. A compensatory mechanism is known to be operative, at least in some cell types, as Zhou and colleagues recently showed that the Mg^{2+} transporter MagT1 is up-regulated in HEK-293 cells when the concentration of extracellular Mg^{2+} in the growth media is reduced [49]. Thus, for future studies it will be important to test whether other Mg^{2+} transporters or channels can counterbalance loss or depletion of TRPM7 from cells.

How does loss of TRPM7 interfere with cell proliferation? Several studies have found that depletion of TRPM7 by RNA interference in normal as well as cancer cells interferes with cell growth [50–54]. A TRPM7-like MagNum current is similar through all phases of the cell cycle in RBL-2H3 cells, with the exception of G1 when the current is nearly twice as large [55]. The growth arrest caused by deletion of TRPM7 from chicken DT40 B cells can be rescued by stable expression of a constitutively active form of phosphoinositide-3-kinase [56]. Consistent with a role for TRPM7 in growth and cell proliferation, ablation of *TRPM7* interfered with activation of the mTOR pathway [56]. However, the inability of Mg^{2+} supplementation to rescue loss of cell growth in human retinoblastoma and gastric adenocarcinoma cells caused by depletion of TRPM7 by RNA interference has led to the alternative view that TRPM7-mediated Ca^{2+} influx may be involved in controlling this cellular process [52, 54]. Further complicating the understanding of the role of TRPM7 in controlling cell proliferation is the observation by Inoue and Xiong that silencing of TRPM7 promotes cell proliferation and nitric oxide production of vascular endothelial cells via upregulation of nitric oxide synthase in an ERK-dependent manner [57]. Although TRPM7 may be ubiquitously expressed, its impact on cell proliferation may be pleiotropic, depending upon the tissue or cell type in which it is found. Thus, additional work is required to clarify TRPM7's role in cellular proliferation and cell growth, as well as the impact that the channel's conductance of Mg^{2+} and Ca^{2+} has on this process.

RESULTS FROM ANIMAL STUDIES

There are 34 separate mutations in *TRPM6* reported to cause the autosomal recessive HSH that results in electrolyte abnormalities shortly after birth [58]. Individuals affected with HSH develop seizures, muscle spasms and tetany, but otherwise develop normally if treated with magnesium supplements. Thus it was surprising that Walder and colleagues revealed that mice defective in *TRPM6* showed embryonic mortality and neural tube defects [58]. Cross of *TRPM6*^{+/-} mice yielded a total of 125 pups that were genotyped at weaning; however, no homozygous null mice were identified. *TRPM6*^{+/-} dams fed a high Mg^{2+} diet during mating, pregnancy and lactation mated to *TRPM6*^{+/-} males produced only four *TRPM6*^{-/-} offspring included among the 105 weaned offspring. Interestingly, the 60 day mortality of the *TRPM6*^{+/+} was 0, whereas that of the *TRPM6*^{+/-} mice was 4.7%. Prior to death the *TRPM6*^{+/-} mice moved with a waddling gait, and their coat appeared dull with their hair follicles standing straight up, indicating that the heterozygous state can also produce a phenotype. In addition, the concentration of Mg^{2+} in plasma was lower in *TRPM6*^{+/-} mice compared to *TRPM6*^{+/+} mice of either gender, consistent with TRPM6's ascribed function as a regulator of bodily magnesium homeostasis.

Results from animal studies for TRPM7 were no less surprising. Deletion of *TRPM7* from mice disrupted embryonic development, with the mice dying before day 7.5 of embryogenesis [47, 59]. The embryonic lethality caused by loss of TRPM7 may have been predictable given that ablation of *TRPM7* from chicken DT40 cells disabled cells proliferation [13], however, Jin and colleagues did not discover evidence that deletion of TRPM7 affected acute uptake of Mg^{2+} or the maintenance of total cellular Mg^{2+} [47]. Thus, the reason why loss of *TRPM7* disrupts embryonic development remains unclear.

Interestingly, tissue-specific deletion of *TRPM7* in the T cell lineage using a lck-Cre line disrupted thymopoiesis (the differentiation of thymocytes into mature T-cells), characterized by a developmental block of thymocytes at the double-negative stage and a progressive depletion of thymic medullary cells [47]. *TRPM7*-deficient thymocytes exhibited dysregulated synthesis of many growth factors that are necessary for the differentiation and maintenance of thymic epithelial cells. The thymic medullary cells lost signal transducer and activator of transcription 3 (STAT3) activity, which presumably accounted for depletion of the growth factors when *TRPM7* was deleted in thymocytes [47].

The phenotypes produced by disruption of *TRPM7* in zebrafish were no less startling. Elizondo and colleagues identified *TRPM7* as the gene disrupted in the *nutria*^{*124e2*} and *touchstone* (*tct*) mutants [60]. The *nutria*^{*124e2*} mutant was named for its small size, odd shape, and tendency to swim near the surface [60]. *Nutria*^{*124e2*} are comparable in size to wild-type siblings until 2–5 days post fertilization, but during later development they exhibit mineralization within mesonephric tubules, a severe growth deficit, and gross alterations in skeletal development that include accelerated endochondral ossification, delayed intramembranous ossification, as well as skeletal deformities [60]. In addition, both *tct* and *nutria* mutants exhibited melanophore deficiencies and touch unresponsiveness prior to hatching, which can be phenocopied by injection of wild-type embryos with a *TRPM7* splice-blocking morpholino oligonucleotide [60]. However, the *nutria* mutant exhibited a weaker embryonic melanophore phenotype than *tct* mutants [60]. Sequencing of *TRPM7* cDNAs from the various mutants revealed a premature stop codon at residue 1545 in the severe *tct*^{*124e1*} allele, whereas *tct*^{*b508*} mutant had a 68 nucleotide deletion (a.a. 1410–1432), comprising a single exon, that resulted in a frameshift, 16 novel amino acids, and a premature stop codon [60]. Both the *tct*^{*b722*} and *nutria*^{*124e2*} mutant did not have any changes to their open reading frames (Robert Cornell, University of Iowa, personal communication). While it is apparent a disruption of *TRPM7* kinase activity has occurred for the more severe mutants possessing premature stop codons, to what extent *TRPM7*'s channel activity has been additionally compromised is not known. The loss of melanophores in *TRPM7* mutant embryos is dependent on melanin synthesis, as application of 1-phenyl-2-thiourea (PTU), a copper chelator that inhibits the copper-dependent enzyme tyrosinase, prevents the death of melanophores in *tct*^{*b508*} heterozygotes [61]. Interestingly, supplemental Mg²⁺ was also able partially rescue melanophore development [60, 61]. However, whether *TRPM7* exerts its effect in melanophores at the plasma membrane or intracellularly, as may be suggested by the ability of PTU to prevent cell death, remains to be determined. Indeed, there is evidence that *TRPM7* may not function at the plasma membrane in all instances. Krapivinsky and colleagues demonstrated that *TRPM7* resides in the membrane of synaptic vesicles of sympathetic neurons, where it forms molecular complexes with the synaptic vesicle proteins synapsin I and synaptotagmin I, and directly interacts with snapin [62]. Knockdown of *TRPM7* by RNA interference or by targeted peptide interference of *TRPM7*'s interaction with snapin resulted in the attenuation of neurotransmitter quantal size and probability of neurotransmitter release in response to neuronal stimulation [62]. Similar effects were observed in rat pheochromocytoma PC12 cells, leading the authors to conclude that the conductance of cations by *TRPM7* across the vesicle membrane is important to vesicle fusion [63].

THE ROLES OF THE CHANNEL-KINASES IN DISEASE

Mutations in *TRPM6* are clearly linked to familial HSH, however, it is not clear whether disruption of *TRPM6* may also produce neural tube defects in humans similar to that was observed in *TRPM6*^{*-/-*} homozygote mice [58]. For *TRPM7*, only one single nucleotide polymorphism (SNP) has been associated with disease. Hermosura and colleagues identified a *TRPM7* variant with the T1482I missense mutation in a subset of patients that have

Guamanian amyotrophic lateral sclerosis (ALS-G) or parkinsonism dementia (PD-G) [64]. This polymorphism, however, was not associated with amyotrophic lateral sclerosis-parkinsonism dementia complex (ALS/PDC) in the Kii peninsula of Japan [65]. In addition, the T1482I polymorphism has been associated with an elevated risk of both adenomatous and hyperplastic polyps [66]. The T1482I mutation does not appear to affect the basal catalytic activity of the kinase, however Thr1482 has been reported to be a site of autophosphorylation whose mutation to isoleucine renders the channel more sensitive to inhibition by free Mg^{2+} [64]. This conclusion was disputed by a second study in which no apparent difference in the sensitivities of the wildtype and mutant channels to Mg^{2+} inhibition were observed [24]. However, work by Clark and colleagues verified that Thr1482 appears to be a bona fide autophosphorylation site [12].

The most striking example of the role of TRPM7 in disease comes from the collective work by Tymianski, MacDonald and colleagues [67–69]. Cultured cortical neurons subjected to oxygen glucose deprivation (OGD) produced a Ca^{2+} -permeable nonselective cation conductance termed I_{OGD} [67]. I_{OGD} was activated by reactive oxygen/nitrogen species, and promoted neuronal Ca^{2+} overload and anoxic death [67]. I_{OGD} exhibited a similar current-voltage relationship and sensitivity to TRPM7 channels, leading Aarts and colleagues to evaluate whether I_{OGD} corresponds to TRPM7 [67]. Transfection of small interfering RNA (siRNA) targeting TRPM7 reduced I_{OGD} in primary cultured neurons, reduced Ca^{2+} uptake, and reduced cell death [67]. A second study by Wei and colleagues provided evidence that TRPM7 underlies the previously described calcium-sensing nonselective cation channel (I_{csNSC}), which becomes transiently activated in cultured and isolated hippocampal neurons when the concentration of extracellular divalents is reduced [69]. Transient periods of brain ischemia are characterized by substantial decreases in extracellular Ca^{2+} and Mg^{2+} , suggesting a parallel mechanism by which TRPM7 currents are potentiated to contribute to neuronal death during transient brain ischemia [69]. While depletion of TRPM7 in cultured neurons was protective against OGD *in vitro*, would suppression of TRPM7 expression provide the same protection *in vivo*? The question was answered by Sun and colleagues who showed that lowering TRPM7 protein levels prevents delayed neuronal death in brain ischemia [68]. TRPM7 expression was suppressed in CA1 neurons by intrahippocampal injections of viral vectors bearing short hairpin RNA specific for TRPM7, which had no negative effect on animal survival, neuronal and dendritic morphology, neuronal excitability, or synaptic plasticity, but did effectively make neurons resistant to ischemic death after brain ischemia [68]. Cell toxicity may be due to more than Ca^{2+} overload alone, as TRPM7-mediated zinc-induced neurotoxicity may also play a role [70]. Although there is no evidence that polymorphisms in the *TRPM7* gene confer risk to stroke [71], TRPM7 appears to be a potentially attractive target for the treatment of brain ischemia.

In addition to offering protection against neuron death during ischemia, suppression of TRPM7 expression in cardiac fibroblasts reduced TRPM7 current and Ca^{2+} influx in atrial fibroblasts as well as significantly reduced basal atrial fibrillation fibroblast differentiation [72]. Du and colleagues also determined that atrial fibroblasts from atrial fibrillation patients showed upregulation of both TRPM7 currents and Ca^{2+} influx, and were more prone to myofibroblast differentiation. Interestingly, TGF- β 1 induced differentiation of cultured human atrial fibroblasts was well correlated with an increase of TRPM7 expression induced by TGF- β 1, suggesting that the higher TGF- β 1 levels that occur during atrial fibrillation may be involved in stimulating TRPM7 upregulation [72].

TRPM7 may also contribute to other forms of cardiovascular disease. Studies from the group led by Touyz have suggested that dysregulation of vascular TRPM6 and/or TRPM7 may contribute to disrupted cellular magnesium handling in hypertension [73]. TRPM6 and TRPM7 are expressed vascular smooth muscle cells (VSMC) [74, 75]. Expression of

TRPM7 messenger RNA is upregulated in VSMC over several hours by challenging cells with angiotensin II and aldosterone [74]. In addition a ~160 kDa protein product (below the 220 kDa expected molecular weight of TRPM7) was recognized by an antibody targeting TRPM7 and its levels in VSMC were similarly upregulated by angiotensin II stimulation [74]. In keeping with TRPM7's assumed role in Mg^{2+} homeostasis, transfection of siRNA targeting TRPM7 decreased VSMC free Mg^{2+} levels [74]. Intracellular Mg^{2+} depletion has been suggested to play a role in vascular dysfunction in hypertension [75]. Levels of TRPM7 were reduced in spontaneously hypertensive rats [75]. Thus, it will be important to test for a role of TRPM7 in hypertension, using available TRPM7 mouse models.

Finally, TRPM7 has been associated with cancer, where it may be influencing cell proliferation as well as cell migration. TRPM7 has been found to be overexpressed in breast adenocarcinoma, where its expression appears to correlate with tumor grade, proliferative index Ki67, and tumor size [51]. TRPM7 expression has been detected in other tumor cell lines [54, 76], but whether a similar correlation between TRPM7 expression and tumor grade exists for other cancers has not been investigated.

TRPM6 & TRPM7 PHARMACOLOGY

Several molecules have been identified that block TRPM6 and TRPM7 channel activity (Table I), however, only one molecule, rottlerin, has been identified as a TRPM7 kinase inhibitor [10]. Rottlerin is also equally effective against TRPM6 (personal communication, Alexey Ryazanov, UMDNJ-Robert Wood Johnson Medical School). The development of more specific protein kinase inhibitors for the two proteins would be helpful for studies aimed at identifying functions as well as substrates for the two channel-kinases. The non-specific channel inhibitor 2-APB is unique in that it increases TRPM6 channel activity at concentrations less than 500 μM , and potentiates TRPM7 channel activity at concentrations greater than 1.5 mM [23]. Such differential effects on the channels will be extremely useful for identifying native currents in isolated cells. The trivalents La^{3+} and Gd^{3+} block TRPM7 channel activity (Table I), and are predicted to block TRPM6 currents as well, since pore domains of the two channels appear to function similarly [19, 77–79]. It is important to note that the studies characterizing the effect of Gd^{3+} on inward currents were conducted using divalent-free extracellular solutions, whereas the experiments with La^{3+} were not. Thus, the apparent higher blocking efficiency of Gd^{3+} over La^{3+} likely reflects the fact that Mg^{2+} and Ca^{2+} were not present in the extracellular solution to act as competitive inhibitors for binding of Gd^{3+} to the channel's pore. Given that depletion of the TRPM7 protein is predicted to slow the progression of cardiac fibrosis and reduce anoxic neuronal death during stroke [68, 72], the development of specific inhibitors against the channel could be of key clinical importance. Chen and colleagues recently showed that knockdown of TRPM7 in HEK-293 cells reduces cell death in response to cell stress [80]. The authors identified NDGA, AA861, and MK886 as TRPM7 channel blockers and showed that these molecules can be employed to attenuate cell death under the same conditions [80]. NDGA, AA861, and MK886 were originally characterized as inhibitors of 5-lipoxygenase. Chen and colleagues provided evidence that 5-lipoxygenase is not regulating the TRPM7 channel, indicating that blockade of its enzymatic activity is not required for the channel inhibitory effects of NDGA, AA861, and MK886 on the channel [80].

TRPM7 AND CELL MOTILITY

One of the earliest observations made regarding TRPM7 was that the channel's overexpression in HEK-293 cells elicits cell rounding, loss of adhesion, and eventual cell death [2]. Su and colleagues investigated this phenomenon, finding that overexpression of TRPM7 produces cell rounding by stimulating the activity of the Ca^{2+} -dependent protease

m-calpain [28]. Surprisingly, activation of the protease was accomplished without raising cytoplasmic Ca^{2+} concentration, leaving it unclear how TRPM7 was able to activate m-calpain. While overexpression of the channel caused cell rounding, knockdown of TRPM7 by RNA interference produced the opposite effect, increasing the adhesion, spreading and motility of HEK-293 cells [28]. In a follow-up study Su and colleagues revealed that the cell rounding elicited by TRPM7 overexpression was initiated by a stress response, finding that activation of m-calpain was dependent upon the production of reactive oxygen and nitrogen species and the concomitant stimulation of p38 MAPK and c-Jun N-Terminal Kinase (JNK) [48]. Further evidence that TRPM7 was involved in cell adhesion was provided by Clark and colleagues who revealed that modest overexpression of TRPM7, as well as a kinase-inactive mutant, in N1E-115 neuroblastoma cells increased cell adhesion and cell spreading, the opposite effect of what was observed when the channel was overexpressed in HEK-293 cells [15]. Surprisingly, overexpression of TRPM7, but not the kinase-inactive mutant, in neuroblastoma line treated with bradykinin (which has been shown to activate the channel and increase TRPM7-mediated Ca^{2+} influx [35]), stimulated the formation of adhesive structures reminiscent of podosomes [15]. Consistent with a role for TRPM7 in regulation of these cytoskeletal structures, TRPM7 was found to localize to membrane ruffles and the podosome-like structures [15]. Clark and colleagues hypothesized that because TRPM7 is a member of the alpha-kinase family, with notable homology to myosin heavy chain kinases from *Dictyostelium*, it may affect actomyosin remodeling and podosome assembly by directly coupling to and phosphorylating components within the actomyosin cytoskeleton [15]. Immunoprecipitation of TRPM7 from N1E-115 cell lysates copurified β -actin as well as the myosin IIA heavy chain. The association of myosin IIA with the channel was significantly enhanced by bradykinin stimulation and could be reversed by chelation of cellular Ca^{2+} by BAPTA or EDTA [15]. An *in vitro* kinase assay employing immunopurified TRPM7 revealed that phosphorylation of myosin IIA, but not β -actin, was enhanced by bradykinin stimulation [15]. In addition, an *in vitro* kinase domain using a myosin tail fragment expressed as a GST-fusion protein in *Escherichia coli* as a substrate, demonstrated that immunoaffinity-purified WT but not kinase-dead TRPM7 efficiently phosphorylated recombinant myosin IIA. The TRPM7 phosphorylation sites on myosin IIA were mapped to Thr1800, Ser1803 and Ser1808, whose mutation to Ala and Asp respectively, lead to an increase and a decrease in myosin IIA incorporation into the actomyosin cytoskeleton [17]. Interestingly, the association between TRPM7 and the myosin IIA heavy chain required an active kinase domain [15], leading to a compelling model in which bradykinin-stimulated Ca^{2+} influx mediated by TRPM7 instigates massive autophosphorylation of the channel-kinase that is required for the association and subsequent phosphorylation of myosin IIA, promoting local relaxation of the actomyosin cytoskeleton [12]. Evidence that TRPM7 may be involved in the formation of high-calcium microdomains (termed 'calcium flickers') comes from work by Wei and colleagues who found that knockdown of TRPM7 by RNA interference reduces the number of calcium flickers elicited by platelet-derived growth factor (PDGF), which consequently disrupted the turning of migrating WI-38 fibroblasts in response to this growth factor [81]. Additional evidence pointing to a role for TRPM7-mediated Ca^{2+} influx in cell migration comes from work by Chen and colleagues, who demonstrated that knockdown of TRPM7 reduces cell motility and bradykinin-stimulated Ca^{2+} influx in 5–8F human nasopharyngeal carcinoma cells [82]. Nevertheless, it's possible that PDGF-mediated Mg^{2+} -influx may also play a role, as Abed and Moreau have shown that silencing of TRPM7 expression in osteoblasts by RNA interference prevented the induction of Mg^{2+} influx and cell migration by PDGF [83]. Similar to what has been observed for TRPM7's role in magnesium homeostasis, the effect of TRPM7's channel and kinase domain on cell adhesion and cell migration may also be cell specific. Additional work is therefore required to uncover the complicated role of TRPM7 in cell motility.

CONCLUSIONS

The last ten years ushered in a revolution of our understanding of the biological roles of TRPM6 and TRPM7, and the next decade promises even more exciting revelations, especially given the development of new animal models of the channel-kinases. The interconnectivity between the channel and kinase has made it extremely difficult to tease apart the specific functions of each. For example, the kinase domain (but apparently not catalytic activity) appears crucial to the support of channel function; likewise the kinase may similarly depend upon the activity of the channel [12, 15]. Thus, biological studies employing point mutants affecting the channel or kinase domain should be interpreted with caution, but are clearly the way forward, and should hopefully aid in unraveling of the riddle of why it was so essential these two activities become physically linked during vertebrate evolution.

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Fig. 1.

Domain Structure and Current-Voltage Relationship of TRPM6 & TRPM7. (A) TRPM6 and TRPM7 contain both channel (blue) and alpha-kinase domains (KIN). The typical TRP domain is present directly COOH-terminal to the last transmembrane domain. Following the TRP domain is the coiled-coil domain (CC), which is assumed to be important to channel assembly [86]. Just before the alpha-kinase domain is a Ser/Thr-rich region (S/T) containing multiple autophosphorylation sites that are proposed to play a role in substrate recognition [12]. The cytoplasmic NH₂-terminus contains a region with high sequence similarity to melastatin (TRPM1), the founding member of the melastatin-related TRP (TRPM) family. The function of the TRPM (melastatin-like TRP) region is not known with the exception that residues within this domain (87–326) interact with the synaptic protein snapin [62]. **(B)** Current-voltage relationship of whole-cell currents typically displayed by TRPM6 and TRPM7. Shown is a representative current-voltage relationship of whole-cell current obtained in HEK-293 cells expressing mouse TRPM7. TRPM6- and TRPM7-mediated currents are characterized by pronounced outward rectification with a small inward current in the presence of normal external concentrations of Ca²⁺ and Mg²⁺.

Table I

Pharmacology of TRPM6 & TRPM7 channel blockers & activators

TRPM6				TRPM7		
Molecule	Concentration	Effect	Ref.	Molecule	Concentration	Effect
2-APB	380 ± 22 μM (i) 205 ± 10 μM (o) EC50	activate	[23]	2-APB	205 ± 10 μM (o) EC50	blocks < 1mM activates > 1.5mM
ruthenium red	10 μM	blocks inward monovalent	[22]	La ³⁺	2 mM	blocks, 2mM 97% (i) and 37% (o)
rotterlin	100 μM	completely blocks kinase activity (Alexey Ryazanov, UMDNJ-RWJMS, personal communication)		Gd ³⁺	10 μM (i)	partial block
				Gd ³⁺	10 μM (o)	no effect
				Gd ³⁺	2.4±0.45 μM (i) EC50	locks
				U73122	10 μM	50% block
				MnTBAP	0.2 mM	blocks by 50 min
				SP600126	20 μM	blocks by 63%
				spermine	20 mM	complete voltage-dependent block
				carvacrol	306 ± 65 μM EC50	blocks
				NDGA	10 μM	complete block
				AA861	10 μM	complete block
				MK886	20 μM	complete block
				rotterlin	100 μM	complete block of kinase activity

Note: (i) and (o) refer to inward and outward. Data is only presented for the heterologously expressed channels.