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REVIEW

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Emerging roles of TRPM6/TRPM7 channel kinase signal transduction complexes

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Abstract Investigations into *Drosophila* mutants with impaired vision due to mutations in the transient receptor potential gene (*trp*) initiated a systematic search for TRP homologs in other species, finally leading to the discovery of a whole new family of plasma membrane cation channels involved in multiple physiological processes. Among the recently discovered TRP cation channels two homologous proteins, TRPM6 and TRPM7, display unique domain compositions and biophysical properties. These remarkable genes are vital for Mg^{2+} homeostasis in vertebrates and, if disrupted, lead to cell death or human disease.

Keywords Transient receptor potential channel · Melastatin · Kinase · Calcium · Magnesium · TRPM6 · TRPM7

Introduction

Genetic screening in *Drosophila melanogaster* for proteins involved in light-induced Ca^{2+} influx into photosensitive cells resulted in the discovery of the transient receptor potential (TRP) cation channel family (Hardie 2001; Minke and Cook 2002; Montell 2001; Montell et al. 2002a). According to primary sequence similarity, the members of the TRP gene family are classified into six subfamilies: TRPCs (seven canonical or classical TRPs), TRPVs (six vanilloid receptor and related proteins), TRPMs (eight TRP proteins homologous to the first cloned mammalian subfamily member, melastatin; Montell et al. 2002a,b; Clapham et al. 2003), TRPA (a single gene is present in mammalian genomes, ANKTM1; Tominaga and Caterina 2004), and two groups of more distantly related proteins, TRPMLs (three proteins defined by the initially discovered gene mucolipin 1; Slaugenhaupt 2002) and TRPPs (polycystic kidney disease 2 related proteins; Cantiello 2004). The current manuscript focuses on recent progress made in the field of TRPM cation channels. In particular, we highlight recent data obtained for two unique proteins, the channel-kinases TRPM6 and TRPM7. Other aspects of TRP cation channels were recently addressed in a number of excellent review articles cited above.

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Heteromerization of TRPC and TRPV channel subunits as an intrinsic mechanism for diversification of channel function

Significant progress has been made to define biophysical properties, regulation and compositions of TRPC and TRPV channel complexes. It is commonly accepted now, that the three *Drosophila* TRP proteins, TRP, TRPL, TRPγ and their mammalian TRPC relatives mediate cation entry in response to phospholipase C activation (Clapham 2003; Gudermann et al. 2004a,b; Moran et al. 2004; Nilius and Voets 2004). Recently, evidence has been obtained that TRPL may also be critical for fluid transport by *Drosophila* Malpighian (renal) tubules (Macpherson et al. 2005). The TRPV1-4 subfamily members represent Ca^{2+}-permeable cation channels involved in the perception of physical and chemical stimuli, such as temperature, pH and mechanical stress (Benham et al. 2003; Clapham 2003; Nilius and Voets 2004). TRPV5 and TRPV6 have been identified as channels responsible for transcellular Ca^{2+} transport in the small intestine, kidney and placenta (den Dekker et al. 2003; Peng et al. 2003).

Despite their disparate biophysical and regulatory properties, TRP channel complexes share a common architecture similar to that of voltage-gated cation channels (Hofmann et al. 2000). Firstly, TRP proteins contain six transmembrane domains (S1–S6) flanked by cytoplasmic

N- and C-termini. Secondly, TRPC and TRPV subunits form homo- and heterotetramers, thereby contributing a hydrophobic loop between the S5–S6 segments of each subunit to a common putative channel pore.

Most notably, TRP channel subunits do not associate arbitrarily. Two labs have independently demonstrated that mammalian TRPCs assemble exclusively within two subfamilies, TRPC3/6/7 and TRPC1/4/5, irrespective of whether they are expressed heterologously or studied in native environments like brain synaptosomes (Strübing et al. 2001; Goel et al. 2002; Hofmann et al. 2002; Schaefer et al. 2002). In contrast to these findings, Strübing et al. described a more complex mode of TRPC assembly in microsomes from rat embryonic brain (Strübing et al. 2003). It was found that TRPC3 or TRPC6 subunits assemble with TRPC1, 4, or 5 in embryonic brain (Strübing et al. 2003). Moreover, the presence of TRPC1 appears to be essential for the assembly of TRPC(1+4/5+3/6) complexes (Strübing et al. 2003).

There is evidence that within the TRPV subfamily, heteromeric interactions are only detectable between TRPV1 and TRPV2, and between TRPV5 and TRPV6 subunits, while TRPV3 and TRPV4 assembly is restricted to homooligomeric complexes (Hoenderop et al. 2003; Hellwig et al. 2005).

The molecular mechanisms governing the assembly of functional TRP protein complexes are still elusive. The following domains have been suggested to be required for oligomerization:

1. The highly conserved TRP domain, located in the intracellular C-terminus immediately downstream of the S6 transmembrane helix as shown for TRPV1 (Garcia-Sanz et al. 2004)
2. The third and fourth ankyrin repeats positioned in N-terminus of TRPV6 (Erler et al. 2004)
3. The N- and C-termini for TRPV5 and TRPV4 complexes (Chang et al. 2004; Hellwig et al. 2005)
4. The transmembrane segments for TRPV1 subunits (Hellwig et al. 2005)

At present, a unifying structural principle underlying TRP channel multimerization still remains elusive.

The physiological relevance of heteromultimerization among TRP proteins is only incompletely understood. The biophysical characterization of *D. melanogaster* TRP/TRPL, TRPL/TRPγ complexes and mammalian TRPV5/6, TRPC1/5 or TRPC1/4 oligomers displayed novel and unique properties when compared to their homomultimeric counterparts (Hoenderop et al. 2003; Strübing et al. 2001, 2003; Xu et al. 1997, 2000). Yet, genetic analyses in *D. melanogaster* and *C. elegans* have highlighted an essential role of heteromerization of TRPV subunits for an appropriate subcellular targeting of their complexes in polarized sensory cells (Gong et al. 2004; Hardie, 2001; Tobin et al. 2002; Xu et al. 2000). Taken together, an increasing body of experimental data indicates that heteromerization of channel subunits is an important mechanism for the regulation of TRP channel function in vivo.

The TRPM subfamily: a diverse group of cation channels

The first member of the TRPM gene subfamily, *gon-2*, was identified during the characterization of loss-of-function alleles in *Caenorhabditis elegans* with impaired post-embryonic mitotic cell division of gonadal precursor cells (Sun and Lambie 1997; Church and Lambie 2003; West et al. 2001). Interestingly, *C. elegans* contains two other *gon-2*-like genes (GTL-1, GTL-2) and a group of more distantly related proteins (Fig. 1), while *D. melanogaster*, as well as other insects, are equipped with only one copy of the melastatin-related gene (Fig. 1). The biological functions of these invertebrate TRPMs are still elusive.

The mammalian TRPM subfamily consists of eight genes (TRPM1–8 respectively). Like TRPC and TRPV channel subunits, TRPMs contain six putative transmembrane helices. Surprisingly, the long (more than 800 amino acids) intracellular N-termini of TRPMs do not display any obvious sequence similarity to other proteins. Moreover, three members of the TRPM family, i.e., TRPM2, TRPM6 and TRPM7, differ from other ion channels because they harbor enzyme domains in their C-termini and represent prototypes of a new protein family of enzyme-coupled ion channels. Thus, the NUDT9 domain in TRPM2 was shown to have ADP-ribose pyrophosphatase activity (Perraud et al. 2001), while the C-termini of TRPM7 and TRPM6 contain a serine/threonine protein kinase domain resembling that of elongation factor 2 (eEF-2) kinase and other α-kinases (Chubanov et al. 2004; Drennan and Ryazanov 2004; Nadler et al. 2001; Riazanova et al. 2001; Runnels et al. 2001, 2002; Schlingmann et al. 2002; Walder et al. 2002; Yamaguchi et al. 2001).

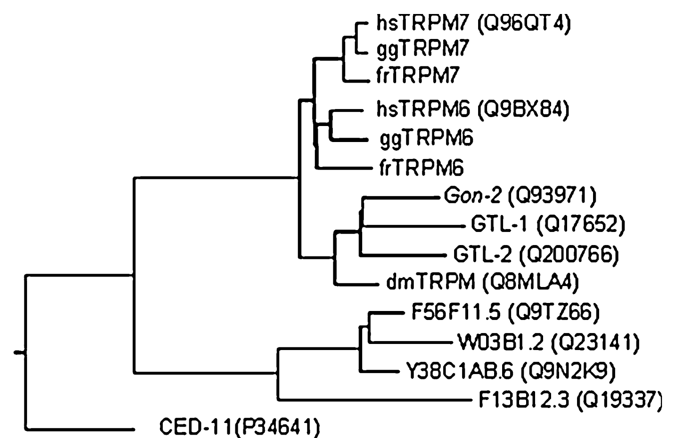

Fig. 1 Phylogenetic analysis of vertebrate channel kinases, and related proteins in *C. elegans* and *D. melanogaster*. The phylogenetic family tree was obtained by multiple sequence alignment (Clustal algorithm, DNAstar) for TRPM6 and TRPM7 proteins of *Homo sapiens*, *Gallus gallus*, *Fugu rubripes* (hsTRPM6, hsTRPM7, ggTRPM6, ggTRPM7, frTRPM6, and frTRPM7 respectively), *D. melanogaster* TRPM (dmTRPM) and *C. elegans* TRPM-related proteins (*Gon-2*, GTL-1, GTL-2, F56F11.5, W03B1.2, Y38C1AB.6, F13B12.3, and CED-11). The ggTRPM6, ggTRPM7, frTRPM6, and frTRPM7 genes are annotated in the Ensemble database as ENSGALG00000015154, ENSGALG00000005835, SINFRUG00000143648 and SINFRUG 00000141791 respectively. The relevant accession numbers of the Swiss–Prot database are shown in parentheses when available.

The TRPMs are characterized by very special ion permeation properties and modes of regulation (Fleig and Penner 2004). Thus, TRPM2, TRPM3 and TRPM8 are Ca^{2+} permeable nonselective cation channels, which differ substantially with respect to their activation stimuli (Chuang et al. 2004; Grimm et al. 2003; Hara et al. 2002; Lee et al. 2003; McKemy et al. 2002; Peier et al. 2002; Perraud et al. 2001; Sano et al. 2001; Wehage et al. 2002). TRPM8 is activated by low temperature (Chuang et al. 2004; Voets et al. 2004a), while TRPM2 is stimulated by intracellular ADP-ribose, $NAD^{+}$and oxidative stress (Hara et al. 2002; Perraud et al. 2001, 2003). TRPM3 appears to be activated by hypoosmotic cell swelling, rises of intracellular Ca^{2+} and by D-erythro-sphingosine (Grimm et al. 2003, 2004; Lee et al. 2003). Two other TRPM subfamily members, TRPM4 and TRPM5, are mainly permeable for monovalent cations, gated by increases in intracellular Ca^{2+} and exhibit a pronounced voltage modulation (Hofmann et al. 2003; Launay et al. 2002; Liu and Liman, 2003; Nilius et al. 2003; Prawitt et al. 2003). Intriguingly, mice with a genetically disrupted TRPM5 gene display impaired sweet, bitter and umami taste perception (Zhang et al. 2003b), but the precise role of TRPM5 in taste receptor cells is still under debate (Hofmann et al. 2003; Perez et al. 2002; Prawitt et al. 2003; Zhang et al. 2003b). Compared to the TRPM proteins mentioned so far, TRPM6 and TRPM7 are characterized by highly unusual permeation properties in that they conduct a range of essential and toxic divalent metal ions including Mg^{2+} and Ca^{2+} (Monteilh-Zoller et al. 2003; Nadler et al. 2001). Thus, the current view is that members of the TRPM subfamily are highly divergent in domain composition, biophysical properties and activation mechanisms when compared to their relatives, TRPCs and TRPVs.

TRPM6 and TRPM7 channel subunits and their complexes

As briefly mentioned before, two genes of the TRPM subfamily, channel-kinases TRPM6 and TRPM7, revealed features, which are unique within the TRP gene family. The domain composition of TRPM6 and TRPM7 raises a set of intriguing questions. Do they primary function in vivo as cation channels, as kinases, or both? When and why have these bi-functional proteins been generated during evolution? A phylogenetic analysis (Fig. 1) demonstrates that none of the invertebrate relatives of mammalian channel-kinases harbor amino acid sequence motifs similar to those of other known enzymes. However, two genes encoding channel-kinases, orthologs of mammalian TRPM6 and TRPM7, have been predicted in the genomes of birds (*Gallus gallus*) and fish (*Fugu rubripes*) later in evolution (Fig. 1). Thus, these two unique genes were generated before the divergence of fish and land vertebrates, i.e., more than 450 million years ago.

Another remarkable feature of TRPM6 and TRPM7 genes is the expression of alternatively spliced variants lacking the internal exons coding for the hexahelical transmembrane domains (Chubanov et al. 2004; Runnels et al. 2001, 2002). Originally, such a splice variant, containing only the N-terminus and the kinase domain from TRPM7, was discovered in the course of a yeast two-hybrid screening for proteins interacting with the C2 domain of PLCβ isoforms (PLCβ-interacting kinase, PLIK; Runnels et al. 2001, 2002). A set of splice variants derived from the TRPM6 gene, homologous to PLIK, has been cloned recently (Chubanov et al. 2004). The different isoforms were called M6-kinases 1, 2, and 3 due to their homology to melastatin-related TRP proteins within the N-termini and the presence of a C-terminal protein kinase domain (Chubanov et al. 2004). Accordingly, two different classes of proteins can be derived from TRPM6 and TRPM7 genes, TRP-related channel-kinases and M-kinases. So far, a physiological role for M-kinases is still unknown. In contrast to TRPM7, the human TRPM6 gene displays a more complex organisation of its promoter region: three alternative 5′ exons (1A, 1B and 1C) are spliced in-frame to a common second exon (Fig. 2; Chubanov et al. 2004). The close proximity of the 5′ exons within a 700-bp genomic region suggests that a single core promoter with alternative transcription start sites governs the expression of TRPM6 isoforms (Fig. 2).

Characterization of TRPM7 in heterologous expression systems (Fig. 3) revealed that TRPM7 is a constitutively active cation channel, which is suppressed by intracellular free Mg^{2+} ($[Mg^{2+}]_i$) and Mg·ATP ($[Mg{\cdot}ATP]_i$; Monteilh-Zoller et al. 2003; Nadler et al. 2001; Runnels et al. 2001; Schmitz et al. 2003). Consequently, it was postulated that variations in $[Mg^{2+}]_i$ and $[Mg{\cdot}ATP]_i$ are major physiological mechanisms controlling TRPM7 channel activity (Monteilh-Zoller et al. 2003; Nadler et al. 2001; Schmitz et al. 2003). TRPM7 is permeable for a broad range of divalent cations, including trace metals, such as Zn^{2+}, Co^{2+}, and Mn^{2+}. Importantly, in contrast to other TRPs, TRPM7 is slightly more permeable for Mg^{2+} than for Ca^{2+} (Monteilh-Zoller et al. 2003; Nadler et al. 2001; Schmitz et al. 2003). In the absence of divalent cations in the extracellular solution, TRPM7 conducts monovalent cations, such as Na^{+} (Monteilh-Zoller et al. 2003; Nadler et al. 2001; Schmitz et al. 2003). Channel properties of TRPM6 were found to be indistinguishable from those of TRPM7 (Voets et al. 2004b).

A recent report provided first evidence that the N-terminal domain of annexin 1 is a physiological substrate of the TRPM7 kinase domain (Dorovkov and Ryazanov 2004). Annexin 1 is a member of a conserved family of Ca^{2+} and lipid binding proteins. The N-terminal region of annexins is unique and vital for a specific function of each family member (Hayes and Moss 2004; Moss and Morgan 2004). In the case of annexin 1, the N-terminus is involved in a Ca^{2+}-dependent interaction of the protein with S100A11 (Moss and Morgan 2004). S100 proteins are essential for cytoskeletal dynamics, cell proliferation and ion channel trafficking. Peptides proteolytically derived from the N-terminal sequence of annexin 1 are responsible for its anti-inflammatory activity (Lewit-Bentley et al. 2000; Moss and Morgan 2004; Perretti and Flower 2004). Consequently, it was proposed (Dorovkov and Ryazanov 2004) that the

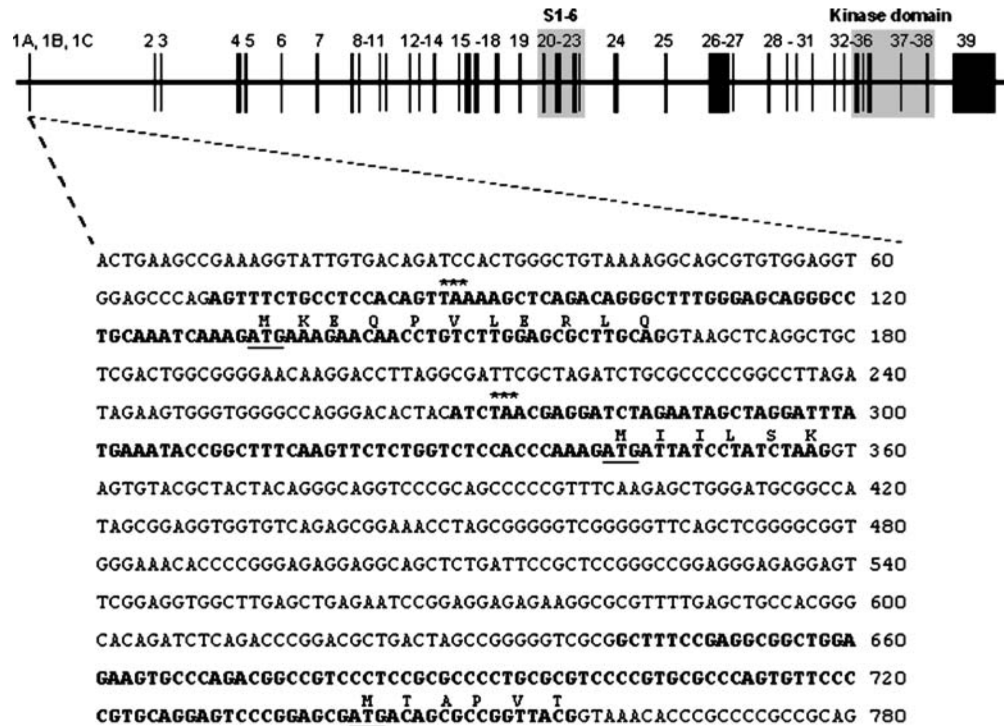

Fig. 2 Intron–exon structure of the human TRPM6 gene. In total, 39 exons (numbered *1–39*) are mapped to the genomic region of human chromosome 9 according to the TRPM6 cDNA variants cloned (Chubanov et al. 2004). A *gray background* highlights exons, encoding predicted transmembrane domains (S1–S6) and the kinase domain. An *insert* shows a zoomed genomic region (780 base pairs), encompassing the position of alternative 5′ exons. The sequence of exons 1A (70–165 base pairs), 1B (269–358 base pairs) and 1C (642–758 base pairs) is depicted in *bold*. Amino acid sequences obtained by translation from the start ATG codon (*underlined*) are shown above the corresponding nucleotide sequences. Note the presence of STOP codons (*asterisks*) in the 5′-untranslated regions of exons 1A and 1B.

TRPM7-mediated phosphorylation of annexin-1 might interfere with such a physiological process.

As mentioned earlier, the kinase domain of TRPM7 directly associates with the C2 domain of phosospholipase C (PLCβ1-3, and PLCγ1 isoforms; Runnels et al. 2001, 2002). So far, the biological role of this interaction has remained elusive. According to Runnels et al. phosphatidylinositol 4,5-biphosphate (PIP_2), the endogenous substrate of PLC, is required for TRPM7 channel activity and receptor-mediated activation of PLC decreases TRPM7 activity due to local hydrolysis of PIP_2 (Runnels et al. 2002). On the contrary, Takezawa et al. demonstrated that

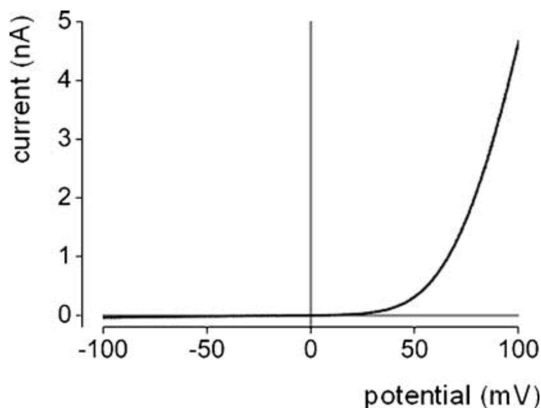

Fig. 3 Current–voltage relationship of whole-cell currents typically displayed by TRPM7. A representative current–voltage relationship of whole-cell currents obtained in HEK293 cells expressing mouse TRPM7 is shown. TRPM7-mediated currents are characterized by pronounced outward rectification under the following conditions of free concentrations: intracellular—10 nM Mg^{2+} and 10 nM Ca^{2+}, and extracellular—1 mM Mg^{2+} and 2 mM Ca^{2+}. The outward current is Cs^{+}-driven.

TRPM7, heterologously expressed in HEK293 cells, abolished activation of PLCβ via muscarinic and thrombin receptors coupled to G_q proteins (Takezawa et al. 2004). Moreover, TRPM7 channel activity was found to be positively modulated by receptors coupled to the G_s/cAMP/PKA signaling cascade. The kinase domain of TRPM7 was essential for PKA-dependent potentiation (Takezawa et al. 2004).

Finally, there is recent evidence to show that TRPM7 forms heterooligomeric channel complexes with the closely-related TRPM6 protein in different expression systems such as HEK293 cells and *Xenopus* oocytes (Chubanov et al. 2004). A number of independent approaches were employed to demonstrate the biochemical interaction of TRPM6 and TRPM7. Yet, the most important observation was that TRPM7 could modulate the subcellular distribution of TRPM6. Thus, TRPM6 expressed alone was not detectable on the cell surface, whereas co-expression with TRPM7 resulted in trafficking of TRPM6 to the plasma membrane. The interaction of TRPM6 with TRPM7 was specific, since none of the other TRPM channel subunits (TRPM1, 2, 3, 4, 5, 8) revealed any sign of heteromerization with channel-kinases (Chubanov et al. 2004). It should be noted, however, that other investigators recently showed that in heterologous expression systems TRPM6 alone was able to form homooligomeric channel complexes with biophysical properties identical to those of TRPM7 (Voets et al. 2004b).

TRPM7 was found to be a ubiquitously expressed cation channel (Nadler et al. 2001; Runnels et al. 2001), while TRPM6 displayed a more restricted expression pattern, which included epithelial cells of the renal convoluted tubule and of the intestine (Chubanov et al. 2004; Schlingmann et al. 2002; Voets et al. 2004b; Walder et al. 2002). Therefore,

TRPM6 expression will invariably occur on the background of the partner TRPM7 subunits, and the physiological significance of such a coexpression was revealed by the analysis of loss-of-function mutations in the human TRPM6 gene diagnosed in patients with hypomagnesemia with secondary hypocalcemia (HSH) and will be discussed below (Chubanov et al. 2004). According to primary sequence similarity, the eight TRPM proteins fall into four distinct groups: TRPM6/7, TRPM1/3, TRPM4/5, TRPM2/8. Based on the specific interaction of TRPM6 with TRPM7, it is tempting to speculate that heteromeric complexes can only be formed within but not beyond these groups.

A vital role of TRPM6 and TRPM7 for Mg^{2+} homeostasis

The unique structural and functional characteristics of TRPM6 and TRPM7 would suggest that their roles in vivo differ substantially from those of other TRP proteins. In fact, two independent lines of evidence indicate a vital role of channel-kinases in Mg^{2+} homeostasis. Firstly, it was demonstrated that DT40 chicken lymphocytes lacking the TRPM7 gene are not viable (Schmitz et al. 2003). However, supplementation of the cell culture medium with high levels of Mg^{2+} (but not Ca^{2+}) restores the viability of the mutant DT40 cells (Schmitz et al. 2003). Importantly, the mammalian TRPM7 (wild type protein as well as a kinase-dead mutant) was able to rescue the mutant DT40 cell line cultured in normal, physiological Mg^{2+}concentrations (Schmitz et al. 2003). It would be interesting to know whether mammalian or chicken TRPM6 can also substitute for the mutant TRPM7 in DT40 cells.

The second line of evidence stressing the role of channel-kinases for Mg^{2+} homeostasis was obtained by analysis of an autosomal recessive disorder, hypomagnesemia with secondary hypocalcemia (HSH), characterized by low serum Mg^{2+} levels due to defective intestinal absorption and/or renal Mg^{2+} wasting (Schlingmann et al. 2002; Walder et al. 2002). It was discovered that HSH is caused by mutations in the TRPM6 gene, underscoring its essential role in active transcellular Mg^{2+} transport (Schlingmann et al. 2002; Walder et al. 2002). Most mutations of the human TRPM6 gene, diagnosed in HSH patients, are either nonsense mutations or result in the deletion of splice sites, thereby generating mRNAs with premature stop codons (Schlingmann et al. 2002; Walder et al. 2002). Transcripts containing premature stop codons undergo degradation via nonsense-mediated mRNA decay (Holbrook et al. 2004), easily explaining a loss-of-function phenotype of patients with mutations in TRPM6. However, one inactivating mutation diagnosed in an HSH patient results in the exchange of the highly conserved S141 for an L in TRPM6 (Schlingmann et al. 2002). Using the heterologous expression systems HEK293 cells and *Xenopus* oocytes, it was demonstrated that the mutation specifically impaired TRPM6/7 channel complex formation and, consequently, TRPM6(S141L) was retained in intracellular membrane compartments (Chubanov et al. 2004). Since S141 is a conserved amino acid residue in TRPM proteins, one may speculate that a corresponding S to L missense mutation in TRPM7 would also affect the trafficking competence of this ion channel. In fact, TRPM7(S138L) was also found to be retained intracellularly (Chubanov et al. 2004).

Interestingly, four out of five loss-of-function mutations in the *C. elegans* TRPM gene, *Gon-2,* are located in the N-terminal part of the protein (West et al. 2001). In the *Gon-2* (dx22) allele, a conserved G427 is affected, which corresponds to G144 in TRPM6 and is located close to the critical S141 mutated in an HSH patient. In aggregate, experiments with TRPM6(S141L) strengthen the notion of a crucial role of the conserved N-terminal region for TRPM function as proposed for *Gon-2* (West et al. 2001), TRPM1 (Xu et al. 2001), TRPM2 (Zhang et al. 2003a) and TRPM4A/TRPM4B isoforms (Launay et al. 2002; Xu et al. 2001).

At present, our mechanistic understanding of the molecular events controlling Mg^{2+} homeostasis at the cellular level have eluded detailed analysis (Dai et al. 2001; Konrad et al. 2004; Quamme and de Rouffignac 2000). Mg^{2+} plays a vital role in virtually all cellular pathways as a co-factor of many enzymes, an essential structural element of proteins and nucleic acids and a modulator of ion channels (Grubbs 2002; Konrad and Weber 2003; Konrad et al. 2004; Romani and Maguire 2002; Wolf et al. 2003). While free $[Mg^{2+}]_i$ was estimated to be between 0.5 and 1 mM, the total Mg^{2+} content in the majority of cells was calculated to be in the range of 14–20 mM (Grubbs 2002; Konrad and Weber 2003; Konrad et al. 2004; Romani and Maguire 2002; Wolf et al. 2003). Intracellular Mg^{2+} is mostly bound to ATP, other phosphonucleotides, phospholipids and proteins (Grubbs 2002; Romani and Scarpa 2000). Mammalian cells lack a substantial transmembrane chemical gradient for ionized Mg^{2+}, since the plasma concentration of Mg^{2+} in most species is in the range of 0.9–1 mM of which about 50% is bound to albumin and other molecules. Nevertheless, Mg^{2+} ions move into the cell primary driven by the electrical gradient. A variety of hormonal and metabolic stimuli tightly regulate $[Mg^{2+}]_i$, a process involving at least two types of transport systems in the plasma membrane: extrusion of the cation by putative Na^+/Mg^{2+} or/and H^+/Mg^{2+} exchangers and its entry via Mg^{2+}-permeable cation channels (Grubbs 2002; Konrad and Weber 2003; Konrad et al. 2004; Romani and Maguire 2002; Wolf et al. 2003).

As mentioned above, TRPM7 is permeable to Mg^{2+} and its channel activity is controlled by $[Mg^{2+}]_i$ and $[Mg{\cdot}ATP]_i$. These features are well suited for a protein which is responsible for Mg^{2+} influx into vertebrate cells (Monteilh-Zoller et al. 2003; Nadler et al. 2001; Schmitz et al. 2003). The crucial question is whether the permeability of TRPM7/6 complexes to Ca^{2+} can be neglected, and whether channel-kinase mediated entry of Ca^{2+} also plays a physiological role. In fact, there are a number of reports showing that non-selective cation channels responsible for influx of Ca^{2+} in vivo are also permeable for Mg^{2+}. For instance, two genes essential for sensory physiology, TRPV1 and *Drosophila* TRPL, were found to be only slightly more permeable to Ca^{2+} than to Mg^{2+} (Caterina et al. 1997; Reuss et al. 1997). Finally, two recent reports suggest that TRPM7-mediated

Ca^{2+} influx is involved in anoxic neuronal cell death and in regulation of the cell cycle of human retinoblastoma cells (Aarts et al. 2003; Hanano et al. 2004). Thus, additional experiments are required to elucidate whether the biological role of TRPM6 and TRPM7 channels is restricted to Mg^{2+} homeostasis.

In conclusion, the discovery and functional characterization of the melastatin-related TRP cation channels substantially extended our knowledge about the biological role of TRP proteins, especially about the cellular mechanisms governing Mg^{2+} homeostasis of vertebrate cells. Additional efforts are necessary in order to elucidate the in vivo functions of these extraordinary cation channels.

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