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Dual-Function Ion Channel/Protein Kinases: Novel Components of Vertebrate Magnesium Regulatory Mechanisms

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ABSTRACT

Although magnesium is the dominant divalent intracellular cation and is required for the function of diverse types of enzymes that participate in virtually every cellular process, the molecular mechanisms that regulate its homeostasis are poorly understood. Electrophysiologic and biochemical investigations of a novel dual-function ion channel/kinase protein have recently converged with the identification of the gene locus for an autosomal recessive form of inherited hypomagnesemia to provide new insight into vertebrate magnesium regulatory mechanisms. **(*Pediatr Res* 55: 734–737, 2004)**

Magnesium (Mg^{2+}) is the dominant divalent intracellular cation, present at several mM total concentration including ~10 mM sequestered in organelles (especially mitochondria), 2–5 mM in complex with ATP in the cytosol, 0.5 mM as free Mg^{2+}, and trace amounts complexed with enzymes (reviewed in Refs. 1 and 2). Mg^{2+}'s importance to cell function is underscored by its involvement in the catalytic mechanisms of a tremendous variety of enzymes, including every enzyme that catalyzes a reaction requiring a nucleotide co-factor. Although studies of Mg^{2+} metabolism in bacterial and yeast strains have allowed the identification of several proteins involved in Mg^{2+} transport (3–6), only a single homologue of these proteins has been found in humans, and it seems to be a mitochondrial Mg^{2+} transporter (7). Studies of Mg^{2+} fluxes of human cells have indicated the presence of one or more plasma membrane Mg^{2+} active transport mechanisms, but the protein(s) responsible for these fluxes has not been identified (1, 2). As a consequence, despite its abundance and biologic importance, the molecular mechanisms that regulate Mg^{2+} homeostasis in the cells of humans and other vertebrates have remained largely unknown. However, recent developments in understanding the function of two novel ion channels of the TRPM family suggest that these proteins are critical regulators of our body's access to environmental Mg^{2+} and our body's cell's access to body-fluid Mg^{2+}.

The completion of various model organism genomes along with the human genome has accelerated research in ion channel biology by allowing the identification of many new ion channels by sequence analysis. This strategy has led to a particularly large expansion of the TRP (transient receptor potential) superfamily of ion channels (8), members of which share significant amino acid similarity primarily over TM-spanning regions. Within the TRP superfamily, five subtypes of channels are distinguishable on the basis of conservation of domains outside the TM-spanning regions, and these have now been designated TRPC, TRPV, TRPM, TRPML, and TRP-PKD (see Ref. 8). TRPM (transient receptor potential cation channel superfamily, melastatin subfamily) members are notable for their conserved domain structure, including a cassette of canonical N-terminal, transmembrane-spanning, and coiled coil regions that facilitated the identification of the various human members by sequence alignment (see Fig. 1*A*) and for their diverse gating mechanisms and permeation properties (reviewed in Ref. 9).

Two TRPM family members, subsequently designated TRPM6 and TRPM7, drew immediate interest from electrophysiologists and biochemists upon their discovery, as se-

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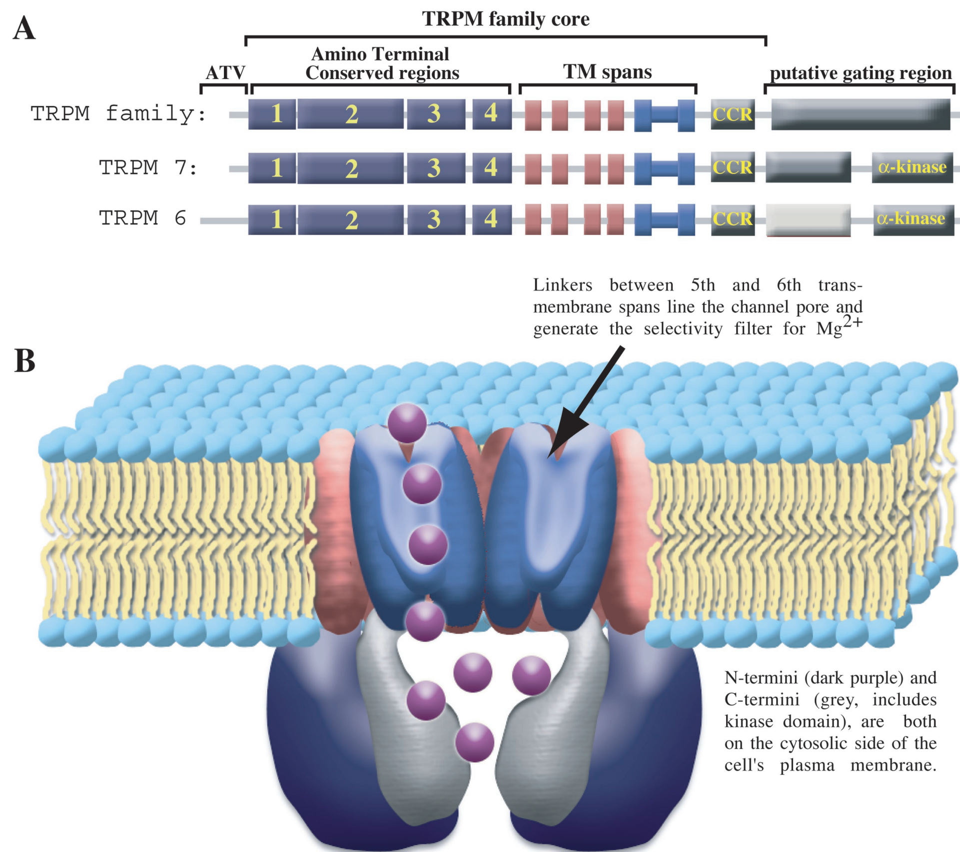

Figure 1. (*A*) Schematic structure of TRPM family channels TRPM6 and TRPM7, respectively. TRPM6 and TRPM7 are highly homologous except for the region between the TM spans and the α-kinase domain. Amino terminal variable region (ATV) is a short region at the N-termini of TRPM channels that is specific to each member. The amino terminal conserved region defines the TRPM family and is composed of four conserved subdomains separated by short linker regions of variable length in different TRPM members. Coiled coil region (CCR) is a region of highly coiled coil character positioned just C-terminal to the sixth TM span in all TRPM members. (*B*) Model for the three-dimensional organization of TRPM family channels. The model represents a channel transected in half (a single channel is most likely made of four TRPM subunits, all of the same type) and bent slightly open in the plane of the plasma membrane for illustrative purposes. The N- and C-termini both are intracellular, and they are pictured in red; the fifth and sixth TM spans and linker region, which form the channel pore, are pictured in blue. Dark purple spheres represent permeating Mg^{2+}, which would create a local domain of high free $[Mg^{2+}]$ around the cytosolic channel structures upon channel opening. On the basis of the functional analyses available for TRPM2 and TRPM7, the C-terminal regions of TRPM channels seem to be critical for the control of channel gating.

quence analyses suggested that they should function not only as ion channels but also as protein kinases (Fig. 1*A*), a combination unique among all known proteins. The combined conclusions from several groups' initial analyses of TRPM7 indicated that TRPM7's ion channel domain is capable of permeating some combination of Ca^{2+}, Mg^{2+}, and/or Na^{+} and that its gating is controlled by intracellular concentrations of Mg^{2+} or Mg^{2+}/nucleotide complexes, G-proteins, and turnover of a specific membrane lipid (10–15). Insights into the role of the kinase domain in TRPM7 function were less forthcoming, as the activity of the kinase domain of TRPM7 was initially reported as required for channel gating, yet it did not seem to be involved in channel suppression by several types of manipulations (13–15). A crystal structure for the isolated TRPM7 kinase domain was also determined, providing an important tool for structure/function analyses of the kinase domain's phosphotransferase activity but no further clues to its connection to TRPM7 channel domain function. During the same period, little progress was made in electrophysiologic and biochemical studies of TRPM6, a situation that persists to the present and that is most likely due to difficulties with surface expression and/or lack of constitutive gating of recombinant homomultimeric TRPM6 channels. However, the isolated kinase domain of TRPM6 was expressed and its phosphotransferase activity was characterized (16), with the available data indicating that it has biochemical properties nearly identical to those of the TRPM7 kinase domain.

Although providing important information regarding the molecular properties of TRPM6 and TRPM7, the electrophysiologic and biochemical data described above offered no exceptional insight into the biologic function of these proteins. However, shortly after the initial data on TRPM7's ion channel function were reported, two groups using a candidate gene approach to identify the locus for an autosomal recessive form of hypomagnesemia began to focus on the TRPM6 gene, which mapped into a genomic region shown to contain the disease locus (17). Both groups found one or more mutations likely to inactivate TRPM6 in both alleles of TRPM6 genes from affected patients (18, 19). In marked contrast to patients who

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have an alternative form of inherited hypomagnesemia and exhibit defects in paracellular Mg^{2+} absorption as a result of defects in the tight junction protein Paracellin-1, analyses of the urinary fractional excretion of Mg^{2+} of patients with this disorder demonstrated that they excrete inappropriately large amounts of Mg^{2+} as a result of a defect in transcellular Mg^{2+} uptake in the distal convoluted tubule (19). Consistent with this, analysis of TRPM6 transcripts indicated expression primarily in intestine and kidney within regions previously shown to be involved in active transcellular Mg^{2+} uptake (18, 19). Taking note of these data along with the capacity of Mg^{2+} supplementation to complement defects in Mg^{2+} uptake in lower organisms, our laboratory evaluated whether supplemental Mg^{2+} could similarly complement cultured cells rendered TRPM7 deficient by conditional gene targeting. Remarkably, supplemental Mg^{2+} provided in amounts ~10- to 20-fold above typical levels in cell culture media allowed TRPM7-deficient cells to grow seemingly normally, and the use of supplemental Mg^{2+} in subsequent experiments allowed us to generate additional cell lines rendered stably TRPM7 deficient and engineered to express various types of mutant TRPM7 channels (20). Studies using these cell lines subsequently established that TRPM7 deficiency is associated with profound cellular Mg^{2+} deficiency under standard culture conditions and helped to demonstrate the existence of a functional coupling between TRPM7 channel and kinase domains by showing that expression of TRPM7 channels lacking a kinase domain results in altered Mg^{2+} homeostasis and growth (20).

What type of biologic roles might be envisioned for TRPM6 and TRPM7 given the accumulated new data? Previous reports indicated that patients with primary hypomagnesemia of the type linked to deficient TRPM6 function are able to live normal lives if they receive oral Mg^{2+} supplementation sufficient to maintain near-normal serum Mg^{2+}. On this basis, the capacity of Mg^{2+} supplementation to rescue deficiency of TRPM7 in cultured cells and the high degree of protein sequence homology of TRPM6 and TRPM7, it seems likely that both proteins have roles involving transmembrane uptake of Mg^{2+}. However, these roles seem to be physiologically distinct: the selective expression of TRPM6 within kidney and intestine along with the organismal Mg^{2+}-deficient phenotype of TRPM6-deficient humans suggests that TRPM6 regulates Mg^{2+} uptake from the external environment to the extracellular fluids of an organism; similarly, the ubiquitous expression of TRPM7 and the profound cellular Mg^{2+} deficiency that occurs in TRPM7-deficient cells in culture suggest that it has an analogous role in the regulation of Mg^{2+} uptake from extracellular fluids to the intracellular milieu of cells. The recent data from our laboratory addressing the relationship between channel and kinase domains of TRPM7 further suggest how the dual channel and kinase domains of these proteins might work together (Fig. 1*B*): the channel domain permeates Mg^{2+} and influences the phosphotransferase activity of the kinase domain either indirectly through the resulting local changes in Mg^{2+} and/or, alternatively, directly through conformational changes induced by channel gating. Whereas many channels are known to be regulated *via* phosphorylation by physically associated protein kinases, to our knowledge, a reverse relationship in which ion flow through a channel regulates a closely associated kinase has not been previously described. Because protein kinases act to encode information as a stable biochemical change informative to other components of a cell, the role of the protein kinase domains of TRPM6 and TRPM7 would presumably be to transfer information regarding the channel's gating or cell's Mg^{2+} status to a (the) downstream molecular or protein targets. Such a mechanism has the important implication that these channels may function both as a Mg^{2+} uptake mechanism and as a form of Mg^{2+} sensor.

What are the implications of these new results for cell biologists? Several unresolved issues stand out as critical to a better understanding of TRPM6 and TRPM7 function and their respective roles in vertebrate cell biology. Certainly functional characterization of TRPM6 using a combination of biochemical and patch-clamp approaches is a prerequisite to a better understanding of TRPM6 and TRPM7 structure/function relationships. Another major issue is whether TRPM6 and TRPM7 are themselves major cellular bulk Mg^{2+} uptake mechanisms or instead function primarily or solely as Mg^{2+} sensors. Of relevance to the latter possibility is that the existence of a ubiquitous Mg^{2+} sensor has been proposed on the basis of previous physiologic investigations of Mg^{2+} metabolism (21–23), although these studies did not provide insight into what the connection between the proposed sensing mechanism and Mg^{2+} uptake and efflux mechanisms might be. Whether TRPM6 or TRPM7 represents the sensing mechanism reported in those studies is not yet known, but this is clearly an area of interest for future work. A related aspect of this issue is the identities of direct substrates, pathways, and genes that are targets of TRPM7 phosphotransferase activity. An obvious set of potential downstream targets of information from the TRPM6/7 kinases would be Mg^{2+} efflux transporters, on the basis of the simple rationale that it would be advantageous to a cell to coordinate Mg^{2+} uptake and efflux so as to ensure that efflux occurs only under conditions when Mg^{2+} is available in sufficient amounts extracellularly. If this is the case, then identification of TRPM6 and TRPM7 may facilitate the identification and functional characterization of these proteins. Finally, TRPM7 function has been reported to be rapidly inhibited by G-proteins and phosphoinositide turnover (12, 15), suggesting that regulation of Mg^{2+} fluxes *via* alteration of TRPM7-mediated Mg^{2+} entry might be occurring during diverse types of signaling events. What purpose(s) this might serve is (are) presently unclear, but alterations of subplasmalemmal Mg^{2+} could potentially affect any process involving the action of enzymes that require Mg^{2+} for activity (including, *e.g.* many types of protein kinases), processes involving Mg^{2+}-binding lipids (Mg^{2+} binds a variety of negatively charged lipid moieties), mitochondrial ATP production (Mg^{2+} is an important internal mitochondrial cation, and substantial Mg^{2+} is complexed with ATP), and the actions of ion channels whose gating is Mg^{2+} regulated (reviewed in Refs. 1 and 2). Future work will no doubt begin to clarify each of the above issues and enhance our understanding of how these novel proteins function in vertebrate cell biology.

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Note Added in Proof. A recent electrophysiologic characterization of TRPM6 suggests that it behaves quite similarly to TRPM7 in terms of capacity to permeate Mg^{2+} and regulation by Mg^{2+}, consistent with its also serving as a Mg^{2+} uptake channel (24).

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