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Review

TRPM6 and TRPM7—Gatekeepers of human magnesium metabolism

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Abstract

Human magnesium homeostasis primarily depends on the balance between intestinal absorption and renal excretion. Magnesium transport processes in both organ systems – next to passive paracellular magnesium flux – involve active transcellular magnesium transport consisting of an apical uptake into the epithelial cell and a basolateral extrusion into the interstitium. Whereas the mechanism of basolateral magnesium extrusion remains unknown, recent molecular genetic studies in patients with hereditary hypomagnesemia helped gain insight into the molecular nature of apical magnesium entry into intestinal brush border and renal tubular epithelial cells. Patients with Hypomagnesemia with Secondary Hypocalcemia (HSH), a primary defect in intestinal magnesium absorption, were found to carry mutations in TRPM6, a member of the melastatin-related subfamily of transient receptor potential (TRP) ion channels. Before, a close homologue of TRPM6, TRPM7, had been characterized as a magnesium and calcium permeable ion channel vital for cellular magnesium homeostasis. Both proteins share the unique feature of an ion channel fused to a kinase domain with homology to the family of atypical alpha kinases. The aim of this review is to summarize the data emerging from clinical and molecular genetic studies as well as from electrophysiologic and biochemical studies on these fascinating two new proteins and their role in human magnesium metabolism.

Keywords: Magnesium; Hypomagnesemia; TRPM6; TRPM7; Channel kinase; Transient receptor potential channel

1. Introduction

The TRPM subgroup, named after its founding member melastatin, of the transient receptor potential (TRP) family of cation channels comprises eight members which exhibit an interesting diversity of cation permeation characteristics and activation mechanisms. Within the TRPM subfamily, two members, TRPM6 (Chak2) and TRPM7 (ChaK1, TRP-PLIK, LTRPC7), share the unique feature of a kinase domain belonging to the atypical family of alpha-kinases fused to the ion channel domain. Indeed, TRPM6 was initially cloned by Ryazanova and colleagues who screened for homologues of eukaryotic elongation factor 2 kinase [1].

Before, its close homologue TRPM7 had been functionally characterized as a divalent cation permeable cation channel yielding outwardly rectifying currents when overexpressed in heterologous expression systems [2,3]. Whereas one study initially suggested a more nonselective conduction of sodium and calcium [2], another report showed selectivity for calcium and magnesium [3]. Controversy also existed towards TRPM7 gating mechanisms as well as the role of the kinase domain for ion channel function. More recent reports argue for a regulation of TRPM7 channel function by intracellular magnesium and magnesium-nucleotides which is modulated by TRPM7 phosphotransferase activity [4–6]. TRPM7 was shown to be crucial for cellular magnesium homeostasis as targeted gene deletion of TRPM7 in cell lines leads to intracellular magnesium deficiency and growth arrest [4].

The critical role of TRPM6 for epithelial magnesium transport became evident when loss-of-function mutations in the TRPM6 gene were discovered in patients with a rare form of hereditary hypomagnesemia called hypomagnesemia with secondary hypocalcemia (HSH). HSH patients suffer from a

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primary defect in intestinal magnesium absorption and present with cerebral convulsions due to profound hypomagnesemia early in infancy. In addition to disturbed intestinal magnesium uptake, they also show renal magnesium wasting originating from impaired magnesium reabsorption in the distal convoluted tubule (DCT) [7]. In contrast to its ubiquitiously expressed homologue TRPM7, TRPM6 was found to be intensely expressed in intestine and kidney but also in lung and testis [8,9]. Recent studies point to a regulation of TRPM6 expression by nutritional magnesium [9]. Despite its obvious importance in transepithelial magnesium reabsorption, data on functional characteristics of TRPM6 ion channels are still controversial. Two groups succeeded in heterologous expression of TRPM6 and describe whole cell currents strongly resembling the currents observed for TRPM7 by Nadler and colleagues [10,11]. In contrast, two other groups showed that functional expression of TRPM6 in the cell membrane requires co-expression with TRPM7 thus suggesting the formation of heteromeric ion channels [8,12]. Therefore, although closely related, TRPM7 and TRPM6 clearly serve distinct roles in human cellular and epithelial magnesium transport.

2. Magnesium transport in intestine and kidney

Magnesium homeostasis in humans primarily depends on the balance between intestinal uptake and renal excretion. Magnesium deficiency can result from reduced dietary intake, intestinal malabsorption or renal loss. The control of body magnesium homeostasis primarily resides in the kidney tubules.

In the intestine, physiologic studies indicate two different transport systems for magnesium acting in a parallel fashion: An active transcellular transport and a passive paracellular pathway [13,14]. The saturable transcellular uptake consists of an apical entry into the epithelial cell through a specific ion channel and a not yet defined basolateral extrusion mechanism which probably couples magnesium export to sodium influx [15]. Whereas at relatively low, physiologic intraluminal concentrations magnesium is primarily taken up via the active transcellular route, passive paracellular magnesium absorption linearly increases with rising intraluminal concentrations. Together the two transport processes yield a curvilinear kinetic for intestinal magnesium absorption [16] (Fig. 1).

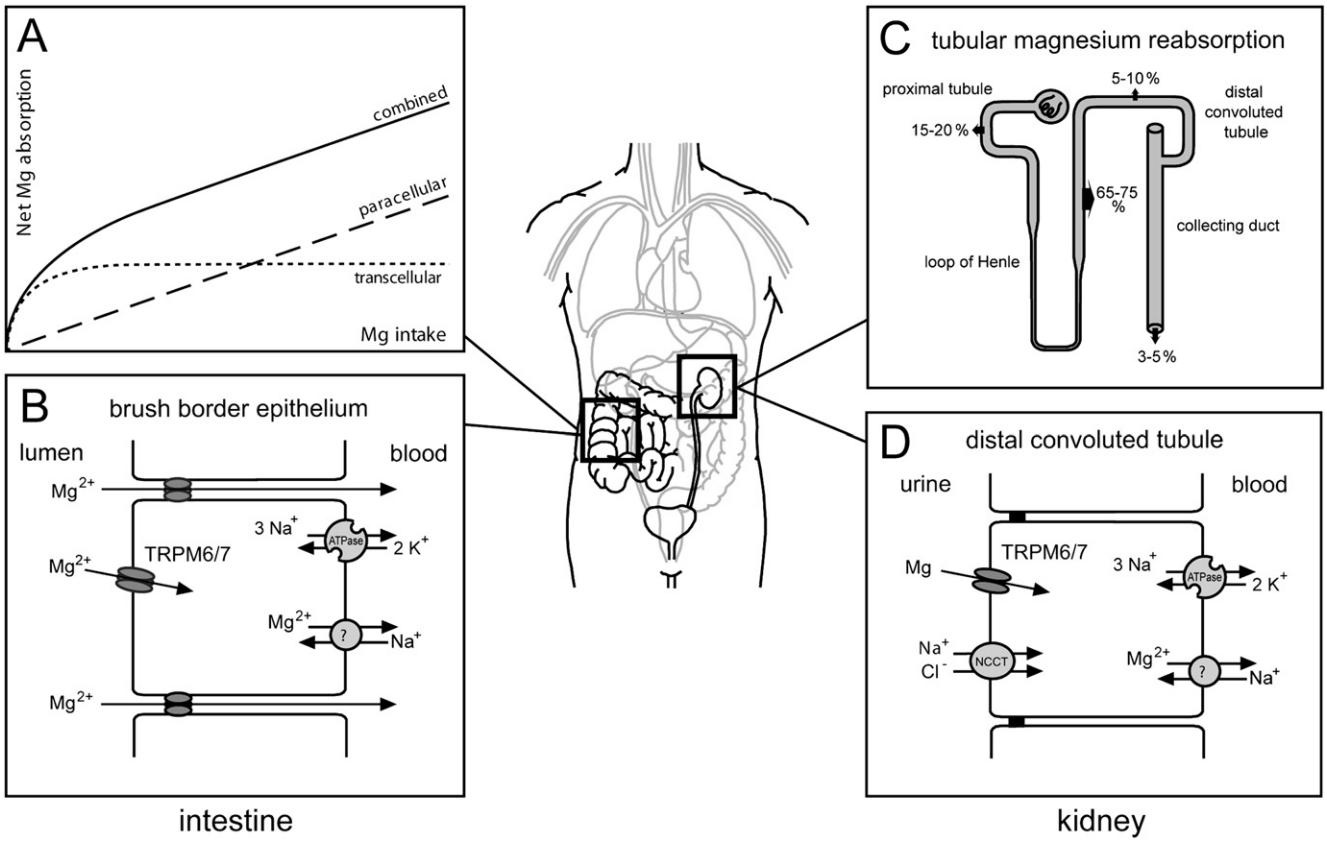

Fig. 1. Epithelial magnesium transport in intestine and kidney. (A) Intestinal absorption follows a curvilinear kinetic resulting from two transport mechanisms: a saturable transcellular transport (dotted line) which is of functional importance at low intraluminal concentrations and a paracellular passive transport (dashed line) linearly rising with intraluminal magnesium concentrations. (B) TRPM6 is a component of the active transcellular pathway as HSH patients are able to compensate for their genetic defect by high oral magnesium intake. (C) Magnesium transport along the nephron. Though only a minority of filtered magnesium is reabsorbed in the distal convoluted tubule (DCT), this nephron segment determines the final urinary magnesium concentration, as no reabsorption takes place beyond this segment. (D) The DCT reabsorbs magnesium in a transcellular fashion, consisting of an apical entry into the DCT cell through a magnesium-selective ion channel, probably consisting of TRPM6/TRPM7 heteromers, and a basolateral extrusion step of unknown molecular identity.

In the kidney, different transport pathways for magnesium exist serially arranged along the nephron [17]. The vast majority of filtered magnesium is reabsorbed in the thick ascending limb of Henle's loop (TALH) via the paracellular route. This process is thought to be mediated by a specific divalent cation permeant pore formed by proteins of the claudin family of tight junction proteins. The first component of the paracellular pathway, paracellin-1 (*PCLN1*) or claudin-16 (*CLDN16*), was discovered by Simon and coworkers, who identified loss-of-function mutations leading to a combined form of urinary magnesium and calcium wasting called familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) [18]. Very recently, Konrad and colleagues reported the involvement of a second member of the claudin family, claudin-19 (*CLDN19*), in TALH mediated paracellular calcium and magnesium reabsorption by identifying CLDN19 mutations in patients with a renal phenotype indistinguishable from patients affected with claudin-16 mutations, but with an additional severe ocular affection [19].

Only around 10% of filtered magnesium is reabsorbed in the DCT. However, the DCT determines the final urinary magnesium concentration, as no reabsorption takes place beyond this segment [20]. Magnesium reabsorption in the DCT is active and transcellular in nature and critically influenced by active salt transport and cellular energy metabolism [21]. Transcellular magnesium transport as in the intestine consists of an apical entry step driven by the favorable electrochemical gradient probably through a magnesium selective ion channel and an active extrusion step on the basolateral side against the electrochemical gradient (Fig. 1).

Molecular genetic studies in patients with hereditary renal magnesium wasting identified several proteins influencing DCT mediated magnesium reabsorption. For example, patients with loss-of-function mutations in the sodium chloride cotransporter NCCT (*SLC12A3*) in addition to salt wasting with hypokalemic alkalosis also exhibit renal magnesium wasting and hypocalciuria [22]. The combination of magnesium wasting together with decreased calcium excretion appears to be pathognomonic for impaired DCT function. Also patients with mutations in the Na-K-ATPase gamma-subunit as well as patients with a defect in a mitochondrial tRNA both show impaired magnesium reabsorption in the DCT and hypocalciuria [21,23]. Despite this progress, the molecular components directly involved in the transcellular pathway in the DCT remained unknown until the discovery of TRPM6 as a component of the apical epithelial magnesium channel. Unfortunately, the molecular identity of basolateral magnesium extrusion is still unresolved.

3. Hypomagnesemia with secondary hypocalcemia (HSH)

Primary hypomagnesemia with secondary hypocalcemia is a rare autosomal-recessive disease characterized by extremely low serum magnesium levels (~0.2 mmol/L) accompanied by hypocalcemia (~1.6 mmol/L). It was first described by Paunier and colleagues in 1968 [24]. The hypocalcemia is thought to result from an inhibition of parathyroid hormone synthesis and release from the parathyroid gland in the presence of profound hypomagnesemia [25].

Affected patients typically manifest during the first months of life. As magnesium is freely exchanged at the placenta level, magnesium supplementation during intrauterine development is warranted. After birth, however, magnesium levels are progressively depleted until magnesium deficiency becomes clinically manifest after several weeks to months. The clinical picture at initial presentation usually consists of generalized seizures. Tetany or muscle spasms are observed only in very few cases.

Relief of clinical symptoms and normalization of calcium homeostasis is assured by immediate administration of magnesium via the intravenous route followed by lifelong oral substitution of high doses of magnesium [26]. Serum magnesium levels usually fail to reach normal values under oral substitution and remain in the subnormal range (around 0.5–0.6 mmol/L). Delay in diagnosis may lead to severe neurological deficits or can even be fatal. Daily oral magnesium doses greatly vary between patients with a mean dose equivalent to four times the recommended daily amounts. The main side effect of high oral magnesium supplementation is pronounced diarrhea which is observed in a considerable number of patients [27].

Contrasting with all other known hereditary disorders of magnesium homeostasis, pathophysiologic studies in affected patients using radioactive magnesium isotopes pointed to a primary defect in intestinal magnesium absorption [28,29]. The presence of an additional renal magnesium leak in HSH was controversially discussed [29–31]. At last, renal magnesium wasting in HSH patients was clearly demonstrated by using magnesium loading tests [7].

As magnesium uptake studies using radioactive magnesium isotopes are not available on a routine basis, measurement of urinary magnesium excretion remains the predominant diagnostic tool in clinical practice. Physiologically, the kidney, in the presence of hypomagnesemia, aims at preserving magnesium by lowering fractional excretions below 0.5–1%, with the physiological range being 3–5% [32]. Rodriguez-Soriano and coworkers suggest a renal threshold for magnesium with virtually absent renal magnesium excretion when serum magnesium levels decrease below 0.7 mmol/L (equivalent to ~0.5 mmol/L (~1.2 mg/dl) ultrafiltrable magnesium) [33]. Therefore, in HSH patients with serum magnesium levels almost continuously in the subnormal range, fractional excretions would be expected to be below 1% presuming an intact renal magnesium conservation. Indeed, at initial manifestation during profound hypomagnesemia (S_{Mg} around 0.2 mmol/L), fractional magnesium excretions are found to be low in HSH patients. In contrast, during oral magnesium supplementation, fractional magnesium excretions are inadequately increased considering that patients are still overtly hypomagnesemic [27]. Therefore, a significant renal magnesium leak clearly contributes to the pathogenesis of the disease and probably prevents reaching physiologic serum values under adequate therapy in most cases.

By using a DNA pooling strategy, Walder and co-workers mapped a gene locus for HSH on chromosome 9q22 [34]. A positional candidate gene approach then identified mutations in *TRPM6* as the underlying genetic defect in HSH [7,35].

4. TRPM6 and TRPM7

Expression studies detected the presence of TRPM6 along the entire gastrointestinal tract as well as in kidney predominantly in the distal convoluted tubule (DCT) [35]. The expected apical expression in the distal convoluted tubule was confirmed by immunohistochemistry which showed a complete co-localization with the sodium–chloride cotransporter NCCT and also with parvalbumin and calbindin-D_{28K}, two cytosolic proteins that may act as intracellular magnesium buffers [10].

The *TRPM6* gene comprises 39 exons and codes for large protein of up to 2022 amino acids. A variety of splice variants has been identified including three alternative first exons [8]. However, the biological relevance of these multiple variants remains unknown. The spectrum of mutations identified in HSH includes various stop mutations, splice site mutations, frame shift mutations, and deletions of exons (Fig. 2). These mutations are thought to lead to a truncated TRPM6 protein and a complete loss-of-function [7,35,36]. Mutations leading to premature stops of translation are distributed over the entire *TRPM6* gene. From several single amino acid mutations only two, S141L in the N-terminus of the TRPM6 protein, and P1017R in the putative pore region were functionally characterized (see below) [8,35,37].

TRPM6 belongs to the TRPM subfamily of transient receptor potential (TRP) ion channels named after its founding member melastatin. The eight members of the family are characterized by a long conserved N-terminus of unknown function as well as an extended C-terminus. Three TRPM cation channels exhibit a C-terminal enzyme domain for which these proteins have been termed "chanzymes" [38]. The phylogenetic analysis of the TRPM subfamily based on amino acid sequence similarities indicates four distinct groups of which one consists of TRPM6 and its closest homologue TRPM7. Both proteins share the unique feature of a C-terminal serine/threonine protein

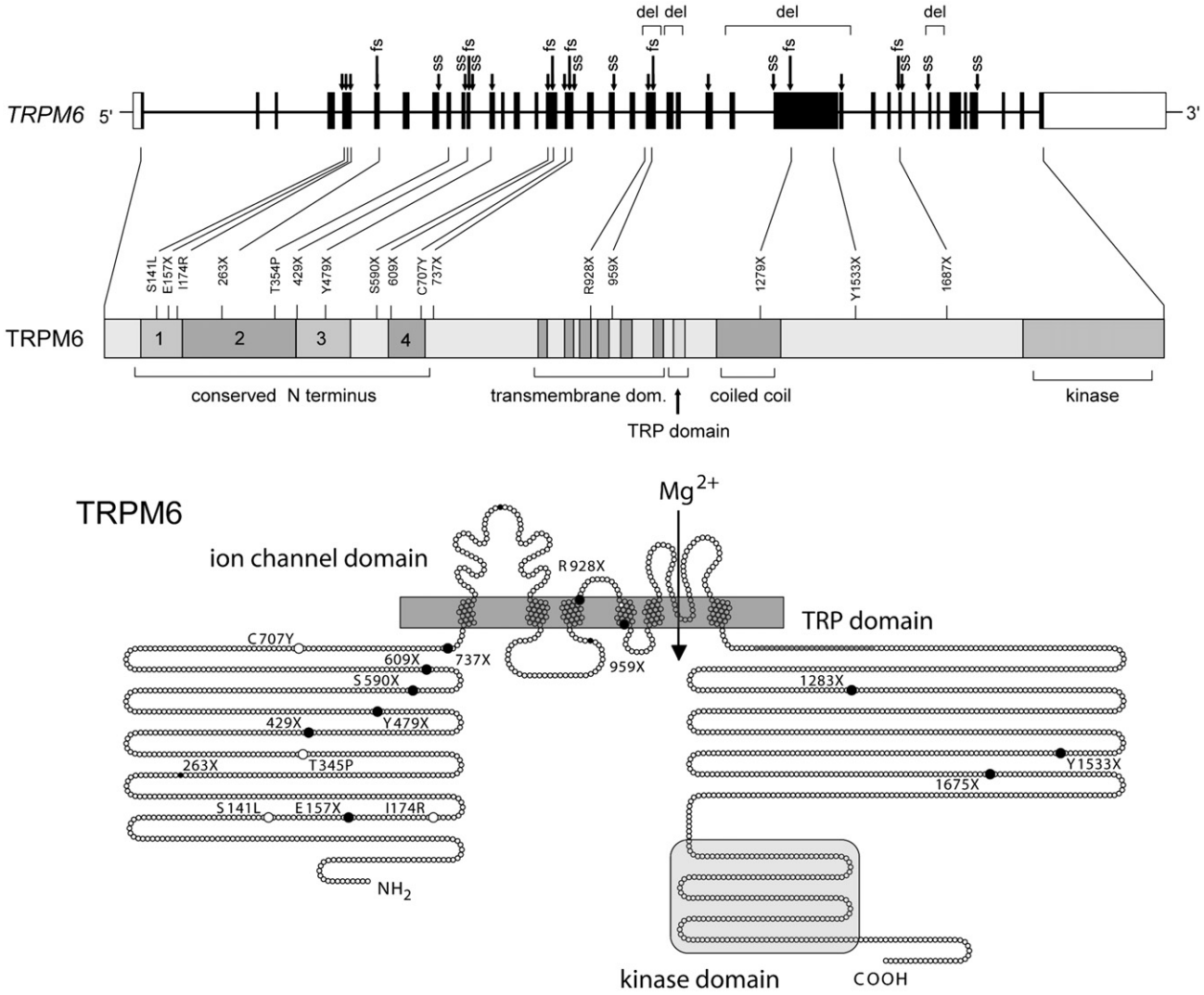

Fig. 2. TRPM6 Gene and Protein. *TRPM6* consists of 39 Exons spanning 167 kb of genomic sequence and coding for a protein of 2022 amino acids. The TRPM6 protein harbors an ion channel region with six transmembrane domains and a putative pore region between the fifth and sixth transmembrane domain, a long N-terminus conserved within the TRPM family, the TRP domain of unknown function located c-terminally of the ion channel domain, and a c-terminal kinase domain with sequence similarity to the atypical alpha kinases. *TRPM6* mutations identified so far are indicated together with the corresponding amino acid exchanges in the TRPM6 protein.

kinase domain resembling those of elongation factor 2 (eEF-2) kinase and other atypical alpha kinases [1].

Significant progress towards a functional characterization of the two highly homologous channel kinases was recently made by electrophysiological as well as cell biological studies on TRPM7.

Electrophysiological characterization of TRPM7 in heterologous expression systems demonstrated that TRPM7 is a constitutively active cation channel which is inhibited by intracellular free magnesium and magnesium ATP [2,3,39]. Consequently, it was postulated that variations in intracellular magnesium and magnesium ATP are key physiological mechanisms controlling TRPM7 channel activity [3,4,39]. TRPM7 is permeable to a broad range of divalent cations, including trace metals, such as zinc, cobalt, and manganese. In contrast to other TRPs, TRPM7 was shown to be slightly more permeable for magnesium than for calcium [3,4,39]. In the absence of divalent cations in the extracellular solution, TRPM7 conducts monovalent cations such as sodium [3,4,39].

More insight into the functional role of vertebrate TRPM7 at the cellular level has been obtained using the chicken B lymphocyte cell line DT40. DT40 cells are characterized by the possibility for efficient gene targeting, a high frequency of homologous recombinations and a stable phenotype, and, therefore, are regarded as a useful model for genetic manipulations in a cell system. It was shown recently that DT40 cells lacking the chicken TRPM7 gene are not viable [4]. However, supplementation of the cell culture medium with high levels of Mg2+, but not Ca2+, restores the viability of mutant DT40 cells [4]. Importantly, mammalian TRPM7 variants (wild type protein as well as a kinase-dead mutant) were able to rescue the mutant DT40 cell line cultured at physiological Mg2+ concentrations [4]. Together, these data indicate a vital role of TRPM7 in Mg2+ homeostasis in vertebrate cells.

Recent data controversially describe the role of the kinase domain for TRPM7 ion channel function. While abolishing phosphotransferase activity does obviously not impede channel activation, deletion of the kinase moiety leads to a suppression of ion channel activity at physiologic intracellular magnesium levels [4]. Therefore, Schmitz and colleagues conclude that ion channel inhibition by intracellular magnesium is influenced by the coordinated action of ion channel and phosphotransferase activity [40]. Therefore, TRPM7 could potentially serve both as a magnesium uptake mechanism and a magnesium sensor [40]. Another study recently found TRPM7 completely inactive upon deletion of the kinase domain, probably, due to trafficking incompetence of the truncated cation channel [5]. The same study identified two major sites of autophosphorylation of TRPM7 by its kinase domain (Ser1511 and Ser1567). However, mutation of phosphorylation sites as well as of the katalytic center of kinase left TRPM7 channel activity unaffected pointing to a critical role of kinase structure rather than katalytic activity for channel function [5]. Further studies are required to reconcile this discrepancy.

But what is the physiological role of ion channel and phosphotransferase interplay? Recently, annexin 1 was identified by Ryazanovs group as a first substrate of TRPM7 kinase [41]. Annexin 1 belongs to a family of calcium- and phospholipid-binding proteins, that were originally identified as endogenous mediators of the anti-inflammatory actions of glucocorticoids, but are also implicated in the regulation of cell growth and apoptosis [42,43]. Interestingly, phosphorylation by TRPM7 kinase occurs at a conserved serine residue (Ser5) in the N terminus of annexin 1, a site that is possibly specific for TRPM7 kinase [41]. The N terminus of annexin 1 is thought to mediate its interaction with protein ligands and membranes. As both proteins, annexin 1 and TRPM7, have been linked to processes of cell survival and cell growth [42,44], the authors speculate that the regulation of cell death and proliferation by TRPM7 involves the phosphorylation of annexin 1 by TRPM7 kinase.

The implication of TRPM7 in cellular growth and differentiation is substantiated by the observations of Clark and coworkers who identified myosin IIA heavy chain as a further substrate of TRPM7 phosphotransferase. By interaction with the actomyosin cytoskeleton, TRPM7 was shown to regulate cell spreading and the formation of cell–cell adhesions [45].

The discovery of potentially differentially phosphorylated substrates of TRPM6 and TRPM7 kinases will certainly be of key importance to understand the physiological role of the chanzymes in vivo.

5. Functional characterization of TRPM6

The discovery of TRPM6 mutations in HSH together with the functional data obtained for TRPM7 instigated a functional characterization of TRPM6. However, functional data obtained for TRPM6 are discussed controversially.

Two groups succeeded in functionally expressing TRPM6 in heterologous expression systems [10,11]. In a collaborative effort, the groups of B. Nilius, Leuven, and R. Bindels, Nijmegen, successfully expressed functional TRPM6 channels in HEK293 cells and showed a somewhat higher permeability for magnesium when compared with TRPM7 [10]. They describe large outwardly-rectifying whole cell currents resembling the currents observed for TRPM7 by Nadler and colleagues [10]. The current–voltage profile was shown to originate from small inward currents at negative potentials carried by divalent cations (i.e. calcium and magnesium), and large outward currents at positive potentials reflecting efflux of monovalent cations (i.e. sodium). In addition, extracellular divalent cations provoke a block of inward currents carried by monovalent cations. As magnesium more efficiently blocks monovalent inward currents than calcium, Voets and colleagues conclude that the TRPM6 pore shows a higher affinity to magnesium than calcium potentially indicating magnesium selectivity [10]. Influx of magnesium through TRPM6 was also demonstrated after intracellular depletion by increases in intracellular magnesium using the fluorescent probe mag-fura-2 [10]. Furthermore, as shown for TRPM7, TRPM6-induced currents exhibit a marked sensitivity to intracellular magnesium at physiologic concentrations (0.5 mmol/L).

Besides these characteristics shared between TRPM6 and TRPM7, Li and colleagues report a number of electrophysio-

logic and biochemical differences between TRPM6 and TRPM7 including divalent cation permeation profile, pH-sensitivity and inhibition by 2-APB with TRPM6 being less permeable for nickel, less sensitive to extracellular pH and activated rather than inhibited by 2-APB [11]. Most remarkably, single channel conductance of TRPM6 with 84 pS is more than twice compared to that reported for TRPM7 (~40 pS) [11].

Further support for divalent cation permeation through TRPM6 monomers originate from a study of Topala and coworkers who demonstrated modified TRPM6 permeation characteristics by single amino acid mutations within the putative TRPM6 pore [46]. Changing E^{1029} within the putative selectivity filter of the TRPM pore between transmembrane segments five and six (TRPM6 ^{1028}GEIDVC^{1033}) resulted in changes in cation permeation profile, sensitivity to ruthenium red, and pore diameter as assessed by permeation of methyl ammonium derivates [46]. Moreover, E^{1024} and D^{1031} were identified as potential determinants of cation permeation as neutralization of both residues to alanine resulted in non-functional channels and D^{1031} showed a dominant-negative effect upon co-expression with wild-type TRPM6 [46].

In contrast, other groups failed to observe measurable currents upon TRPM6 expression. Instead, heterologous expression in different expression systems including mammalian cells and *Xenopus* oocytes consistently showed an intracellular retention of the TRPM6 protein. In line with this observation, TRPM6 fails to rescue the lethal effect of TRPM7 deficiency in the DT40 cell line [12]. In fact, several lines of evidence support an essential role of TRPM7 for TRPM6 surface expression:

Co-expression with TRPM7 in HEK293 cells results in the incorporation of TRPM6 into channel complexes at the plasma membrane as detected by a TRPM6-specific polyclonal antibody, using GFP-fusion constructs and immunoblotting [8,12]. The direct and specific interaction between both proteins was also confirmed by a static FRET (fluorescence resonance energy transfer) strategy [8] as well as by co-immunoprecipitation of epitope-tagged TRPM6/7 constructs [12].

Next, electrophysiological data on TRPM6/7 heteromultimerization obtained in *Xenopus* oocytes as well as in mammalian cells shows that co-expression of TRPM6 with TRPM7 results in a significant amplification of TRPM7-induced currents [8,36] (Fig. 3). Currents obtained in *Xenopus* oocytes exhibit the typical features observed for TRPM7 in mammalian cells with pronounced outward rectification at positive potentials. However, the reversal voltage of around −30 mV points to the contribution to the whole cell currents of endogenously expressed chloride channels putatively activated by calcium entry. Therefore, oocyte currents are thought to provide a rather indirect, but nevertheless sensitive read out of divalent cation fluxes through the TRPM6/7 channel complex (Fig. 3).

Further support for the concept of TRPM6/7 heteromultimerization originates from the analysis of the TRPM6 S141L mutant observed in HSH. Using immunofluorescence studies, this mutant was found to abrogate the interaction between TRPM6 and TRPM7 resulting in intracellular retention of mutant TRPM6 S141L. The failure of TRPM6 S141L to form heteromultimers with TRPM7 was also demonstrated using the FRET

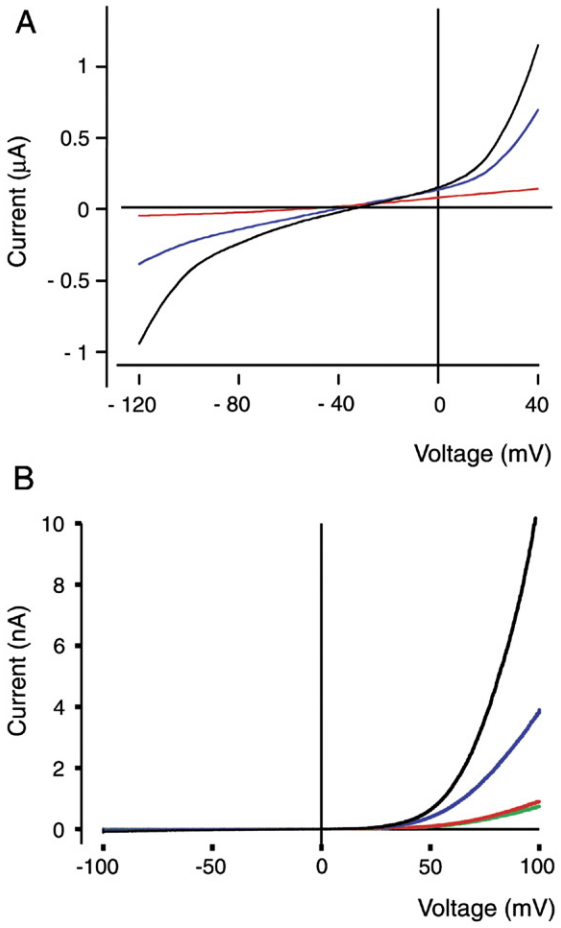

Fig. 3. Electrophysiological characterization of TRPM6/TRPM7. (A) Two-electrode voltage clamp analysis of *Xenopus* oocytes injected with 10 ng of cRNA encoding TRPM6 (red curve), TRPM7 (blue curve), or 10 ng of TRPM6 cRNA together with 10 ng of TRPM7 cRNA (black curve). Co-expression of TRPM6 with TRPM7 results in a significant amplification of TRPM7-induced currents. (B) Patch-clamp analysis of TRPM6/7 in HEK293 cells stablely transfected using an ecdysone inducible gene expression system (Invitrogen). Without induction, small endogenous TRPM7 currents are observed (green curve). TRPM6 induction does not result in a significant increase in currents (red curve), whereas induction of TRPM7 results in the development of large currents with outward rectification at positive potentials (blue curve). Additional transient expression of TRPM6 in the TRPM7 cell line leads to a significant amplification of currents (black curve).

technique. In the *Xenopus* oocyte system, the mutant in contrast to wildtype TRPM6 failed to increase TRPM7 induced currents [8]. Therefore, the S141L mutation is thought to specifically interfere with TRPM6/7 heteromultimerization presumably in the endoplasmic reticulum. The most convincing evidence for direct TRPM6/-7 interaction finally arises from the recent observation that another TRPM6 mutation identified in HSH (P1017R) exerts a strong dominant negative effect on TRPM7 function after heterologous expression in *Xenopus* oocytes and mammalian cells [37].

Our findings were recently corroborated by two other groups of investigators, thus lending further credence to the principal concept emerging from our data on TRPM6 mutations in HSH patients that TRPM6 and TRPM7 are functionally non-redundant and play unique and indispensable biological roles

despite their high degree of homology. In DT 40 B-lymphocytes in which TRPM7 was deleted causing growth arrest, TRPM7 deficiency cannot be complemented by heterologously expressed TRPM6, and the latter protein requires TRPM7 for cell surface expression [12]. Similarly, overexpression of TRPM6 did not mimic the morphological changes of cells (loss of adhesion and cell rounding) that were visible when TRPM7 was expressed [47].

Surprisingly, coexpression of TRPM6 with TRPM7 results in an inhibitory effect on cell growth at low extracellular magnesium levels which is not observed at physiologic magnesium concentrations in the culture medium [12]. The same study suggested that TRPM6 modulates TRPM7 activity via phosphorylation as TRPM6 kinase was found to cross-phosphorylate TRPM7 but not vice versa. Interestingly, a naturally occurring TRPM7 variant (T1482I) was identified recently lacking a putative threonine phosphorylation site [48]. TRPM7 T1482I was shown to exhibit an altered sensitivity to intracellular magnesium resulting in decreased currents at low intracellular magnesium levels. The authors therefore suggest that TRPM7 sensitivity to intracellular magnesium is modulated by phosphorylation at that threonine residue [48]. As T1482 lies in a serine and threonine rich region between TRPM7 channel and kinase domains which is not conserved in TRPM6, Schmitz and colleagues hypothesize that it could also be the target of unidirectional cross-phosphorylation of TRPM7 by TRPM6 [12]. These data together point to a model in which TRPM6 modulates TRPM7 activity by cross-phosphorylation which in turn alters the sensitivity of TRPM7 to intracellular magnesium. However, if this modulation of TRPM7 activity by TRPM6 is of physiological relevance in vivo has to be determined in future studies.

Interestingly, Li and colleagues next to comparing TRPM6 evoked currents with those observed for TRPM7 also performed electrophysiological recordings after co-expression of both channel kinases [11]. They provided several lines of evidence indicating that TRPM6, TRPM7, and TRPM6/7 channels are biophysically and pharmacologically distinguishable, and that TRPM6, TRPM7, and TRPM6/7 form distinct ion channels [11]. In accord with our previous observation, the TRPM6-S141L mutant did not produce functional channels.

After coexpression of TRPM6 and TRPM7 Li and colleagues observed a unique single channel conductance of 56 pS in outside-out patches compared to 84 pS vs. 40 pS conductances observed for TRPM6 and TRPM7, respectively, a finding indeed pointing to the formation of functional heteromers with specific pore characteristics [11]. Moreover, the finding of a single intermediate conductance for TRPM6/7 heteromers might even suggest a specific stoichiometry of the tetramer. In addition, TRPM6/7 heteromers were shown to possess distinct, more or less “intermediate” characteristics concerning the sensitivity to protons and 2-ABP as well as specific cation permeation profile. While the permeability to magnesium or calcium is very similar between the three different types of channels, they differ most strikingly in their relative permeability to nickel [11]. Such “intermediate” properties have well been described for other heteromultimeric ion channels including TRPV5/6 [49].

As to now, we are unable to offer plausible explanations for the discrepant findings which deserve further investigation. The above cited studies are altogether based on data obtained in heterologous over-expression systems, and, subsequently, do not necessarily reflect physiological properties of epithelial magnesium channels in vivo. Indeed, as suggested by Topala et al., surface expression of TRPM6 might be influenced by the presence of endogenous TRPM7 in HEK293 cells. In this context, the data of Li and colleagues, who suggest the possibility of TRPM6 homomer as well as TRPM6/7 heteromer formation at least in such artificial systems, may also be interpreted as an effect of different degrees of TRPM7 expression on TRPM6 function. Although we cannot exclude, that a specific overexpression strategy or chemical chaperone may overcome an intracellular retention of TRPM6, the specific interaction of TRPM6 with TRPM7, reproduced in different expression systems, most likely reflects physiologic TRPM6 function as a subunit of TRPM6/7 channel complexes. Additional studies are surely required to validate the functional significance of TRPM6/7 assembly in native cells.

6. Regulation of TRPM6 and TRPM7 in vivo

Whereas TRPM7 putatively serves as an ubiquitous cellular magnesium uptake mechanism, the hypomagnesemic phenotype of HSH patients together with the expression pattern of TRPM6 suggest that TRPM6 – potentially in cooperation with TRPM7 – accomplishes the uptake of magnesium through the epithelial barrier in intestine and kidney. One can easily imagine that this specialized function requires distinct qualities in activation and regulation of the ion channel complex. Recent data by the Bindels group indeed point to a differential regulation of the two channel kinases in vivo [9,50]. Dietary magnesium restriction results in magnesium deficiency and hypomagnesemia in mice which in turn leads to magnesium conservation in the kidney. This physiological renal response to hypomagnesemia goes along with an upregulated expression of TRPM6 in the DCT [9]. In contrast, renal TRPM7 expression is unaffected by magnesium deficiency. In the intestine, TRPM6 expression appears to be positively regulated by dietary magnesium content as increasing dietary magnesium leads to increased TRPM6 mRNA expression in mice [9]. Furthermore, TRPM6 expression in mouse kidney was also found to be regulated by 17beta-estradiol but not by parathyroid hormone or 1,25-hydroxy-vitamin D. In line with these data, TRPM6 expression was shown to be significantly reduced in ovariectomized rats and normalized after 17beta-estradiol treatment [9].

Renal magnesium handling in the DCT depends on acid–base status in a way that metabolic acidosis leads to renal magnesium wasting whereas metabolic alkalosis preserves magnesium via increased distal tubular reabsorption [15]. This might (at least in part) be explained by a downregulation of TRPM6 expression going along with renal magnesium wasting in mice subjected to metabolic acidosis by ammonium chloride loading [50]. Metabolic alkalosis provoked by sodium bicarbonate lead to the opposite effects in mice [50].

7. Summary

In conclusion, the identification of TRPM6 and TRPM7 for the first time provides insight into cellular magnesium transport at the molecular level. The vital role of TRPM7 for cellular magnesium homeostasis, the phenotype of HSH patients with defective TRPM6, and the rapidly growing set of functional data obtained for both TRPM proteins point to a critical role of the two channel kinases for cellular magnesium uptake. Currently, data concerning the relevance of TRPM6 homomer formation for epithelial magnesium transport in vivo is still inconclusive. Meanwhile, TRPM6 heteromer formation with TRPM7 has been observed by several independent groups and distinct properties of TRPM6/7 heteromers concerning biochemical and electrophysiological properties have been described. Moreover, a mutual interaction of both TRPM members putatively via their kinase domains appears to be critical for the magnesium sensitivity of the channel complex and for the putative negative feed-back regulation by intracellular magnesium. As data on TRPM6 homo- and/or heteromer formation mainly originate from artificial overexpression systems, future studies will have to address the functional significance of TRPM6/7 assembly for epithelial magnesium transport in vivo. For this purpose, the combined genetic and functional analysis of TRPM6 mutants observed in HSH patients represents a favourable opportunity.

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