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TRANSPORT PHYSIOLOGY

Regulation of magnesium reabsorption in DCT

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Abstract The distal convoluted tubule (DCT) is the shortest segment of the nephron and consists of an early (DCT1) and late part (DCT2). Here, several transport proteins, like the thiazide-sensitive NaCl cotransporter (NCC) and the epithelial magnesium (Mg^{2+}) channel (TRPM6), are exclusively expressed. This makes the DCT the major site of active transcellular Mg^{2+} reabsorption determining the final excretion in the urine. Following the Mg^{2+} influx via the apically localized TRPM6, intracellular Mg^{2+} diffuses to the basolateral membrane where it is extruded to the blood compartment via still-unidentified Mg^{2+} transporters. Recent years have witnessed multiple breakthroughs in the field of transcellular Mg^{2+} reabsorption. Epidermal growth factor and estrogen were identified as magnesiotropic hormones by their effect on TRPM6 activity. Intracellularly, receptor of activated protein kinase C 1 and adenosine triphosphate were shown to inhibit TRPM6 activity through its α-kinase domain. Furthermore, dysregulation or malfunction of transcellular Mg^{2+} reabsorption in DCT has been associated with renal Mg^{2+} wasting. Mutations in TRPM6 are responsible for hypomagnesemia with secondary hypocalcemia. A defect in the γ-subunit of the Na^+/K^+-adenosine triphosphatase causes isolated dominant hypomagnesemia resulting from renal Mg^{2+} wasting. Moreover, in Gitelman's syndrome, mutations in NCC also result in impaired transcellular Mg^{2+} reabsorption in DCT. This review highlights our recently obtained knowledge concerning the molecular regulation of transcellular Mg^{2+} reabsorption.

Keywords TRPM6 · Nephron · Distal convoluted tubule (DCT) · Transcellular magnesium reabsorption · Hypomagnesemia

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Introduction

The distal convoluted tubule

The renal distal tubule comprises anatomically discrete segments (Fig. 1), including the thick ascending limb of Henle (TAL) and the distal convoluted tubule (DCT), and ends in the connecting tubule (CNT) [65]. In rodents and humans, but not in rabbit, the segment of DCT can be further subdivided into an early (DCT1) and late (DCT2) portion [29–31]. The DCT plays an important role in fine-tuning renal excretion of sodium (Na^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}). Consequently, several transport proteins for these cations are specifically present in this nephron segment and are, therefore, frequently used as markers for DCT cells (Table 1). First of all, a thiazide-sensitive NaCl cotransporter (NCC) is a well-recognized marker for DCT [40]. NCC is the major apical Na^+ transporter in this nephron segment. Its expression begins abruptly at the junction between TAL and DCT and ends at the junction between DCT and CNT [3]. Moreover, along the DCT, the abundance of NCC protein is high in the DCT1 cells and gradually decreases in the DCT2 cells [30, 31, 66]. Another apical transporter, the amiloride-sensitive epithelial Na^+ channel (ENaC), is also present in DCT2 but to a much lesser extent. ENaC is predominantly expressed in CNT and cortical collecting duct (CCD) [31]. On the

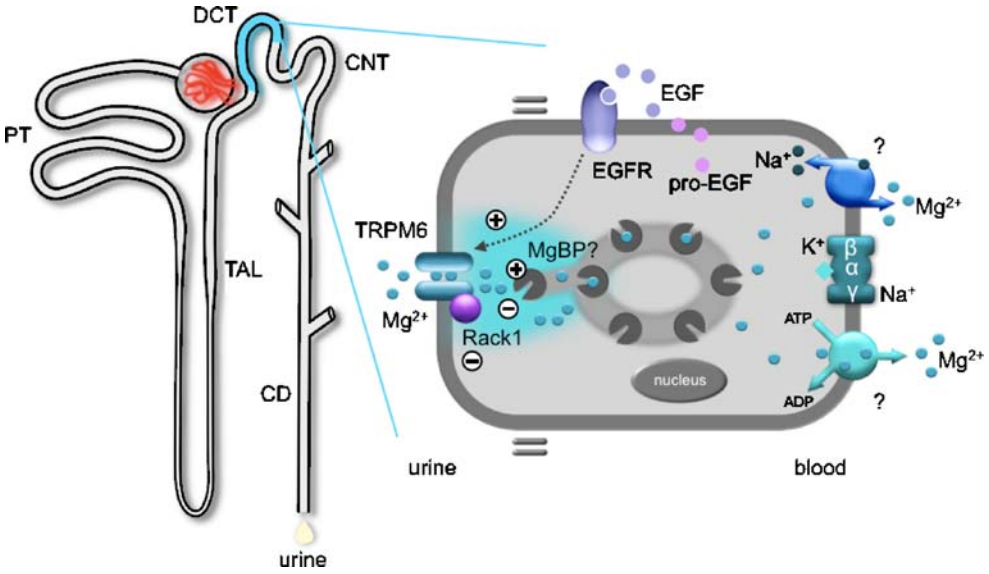

Fig. 1 Model of transcellular Mg^{2+} reabsorption. The renal distal tubule in the nephron comprises anatomically discrete segments, including the thick ascending limb of the loop of Henle (*TAL*) and the distal convoluted tubule (*DCT*) that ends in the connecting tubule (*CNT*). DCT plays an important role in fine-tuning renal excretion of Mg^{2+}. The epithelial Mg^{2+} channel (*TRPM6*) is predominantly expressed in DCT1 with a lower abundance in DCT2. TRPM6 co-localizes with NCC and parvalbumin in DCT1 and with calbindin-D_{28K} in DCT2. Mg^{2+} influx via TRPM6 is controlled by the luminal and intracellular free Mg^{2+}. Another intracellular TRPM6-associated protein is RACK1, which can inhibit TRPM6 activity by binding to its α-kinase domain. After Mg^{2+} influx via TRPM6, intracellular Mg^{2+} may be buffered and transported by a putative Mg^{2+}-binding protein (*MgBP*). The basolateral membrane harbors epidermal growth factor receptor (*EGFR*), Na^+/K^+-ATPase, and possibly a Na^+/Mg^{2+} exchanger and Mg^{2+}-ATPase. The molecular identities of Na^+/Mg^{2+} exchanger and Mg^{2+}-ATPase are still elusive. EGFR activation by EGF can enhance Mg^{2+} influx by increasing TRPM6 membrane expression. FXYD2 may bind as γ-subunit with α- and β-subunit of Na^+/K^+-ATPase. A detailed mechanism by which FXYD2 regulates transcellular Mg^{2+} reabsorption is still under investigation

other hand, the Na^+/K^+-adenosine triphosphatase (ATPase) is consistently present at the basolateral membrane of the DCT. In addition, DCT1 is the major site of transcellular Mg^{2+} reabsorption. This notion is supported by the fact that the epithelial Mg^{2+} channel (TRPM6) is predominantly expressed in DCT1 with a lower abundance in DCT2 [66]. Furthermore, TRPM6 co-localizes with parvalbumin in DCT1 and with calbindin-D_{28K} (CaBP28) in DCT2 [66]. Both parvalbumin and CaBP28 are cytoplasmic binding proteins for Ca^{2+} and Mg^{2+} with distinct distribution patterns.

Table 1 Distribution of transporters in DCT segment of the nephron

	TAL	DCT1	DCT2	CNT	CCD
Na^+ transporters					
NCC [30, 31, 66]		+++	++		
ENaC [31]			+	++	+++
γ-Na^+/K^+ ATPase [2, 41]	+++	+	+	+	
Mg^{2+} transporters					
TRPM6 [66]		+++	++		
Ca^{2+} transporters					
TRPV5 [30]			+++	++	
Calbindin-D_{28K} [30, 31]		+	+++	++	++
Parvalbumin [30, 31, 66]	+	+++			
NCX1/PMCA1b [30, 31]		+	+++	++	

Protein abundance: + weak, ++ intermediate, +++ strong

DCT distal convoluted tubule, *DCT1* early DCT, *DCT2* late DCT, *CNT* connecting tubule, *CCD* cortical collecting duct, NCC thiazide-sensitive NaCl cotransporter, *ENaC* the amiloride-sensitive epithelial Na^+ channel, *TRPV5* transient receptor potential vanilloid 5, *TRPM6* transient receptor potential melastatin 6, *NCX1* Na^+/Ca^{2+} exchanger, *PMCA1b* Ca^{2+}-ATPase

Parvalbumin is strongly expressed in DCT1 and only weakly in TAL and DCT2 [30, 31, 66]. Given that parvalbumin co-localizes with TRPM6 in DCT1, it could imply that parvalbumin may function as a Mg^{2+}-binding protein. Furthermore, the abundance of CaBP28 is low in DCT1 but high in DCT2, suggesting a minor role for CaBP28 in Mg^{2+} reabsorption in DCT. Furthermore, Ca^{2+} transport proteins are confined to the DCT2 and CNT. Here, the epithelial Ca^{2+} channel TRPV5 is detected, and its expression pattern changes from prominent apical location in DCT2 to a progressively weaker apical to cytoplasmic location in CNT [30]. This suggests that DCT2 is the major site for transcellular Ca^{2+} reabsorption. CaBP28 primarily co-localizes with TRPV5 in DCT2, suggesting a critical role in transcellular Ca^{2+} reabsorption. On the basolateral side of DCT and CNT, the Na^+/Ca^{2+} exchanger (NCX) and Ca^{2+}-ATPase (PMCA1b) can be found. Both proteins have their highest abundance in DCT2 and CNT and a weaker expression in DCT1 [30, 31]. Thus, DCT contains all the ion transporters to facilitate the transcellular movement of cations, where DCT1 is critical in fine-tuning Mg^{2+} and DCT2 for Ca^{2+} handling. So far, the process of transcellular Ca^{2+} reabsorption has been investigated in great detail [19, 64]. In contrast, research on the molecular mechanism of transcellular Mg^{2+} reabsorption in DCT is still at an early stage. This could in part be due to the lack of specific blockers of Mg^{2+} reabsorption or an easy and reliable way to measure Mg^{2+} concentrations with fluorescent dyes or isotopes. Thus far, research on the molecular defect underlying inherited forms of hypomagnesemia has been instrumental in the elucidation of transport proteins important in transcellular Mg^{2+} reabsorption. This review highlights the recent breakthroughs in this field of research.

DCT is important in transcellular Mg^{2+} reabsorption in the kidney

In the nephron, the majority (~85%) of filtered Mg^{2+} is reabsorbed in the proximal tubule and TAL via passive transport through the tight junctions. DCT reabsorbs ~10% of the filtered Mg^{2+}, which is 70–80% of that delivered from the loop of Henle (Fig. 1) [12]. Using micropuncture and microperfusion studies of the superficial nephron, it has been revealed that net Mg^{2+} reabsorption in DCT is essentially unidirectional since no secretion of Mg^{2+} into the lumen has been reported [12]. Accordingly, this segment is critical in determining the final urinary excretion. In general, blood Mg^{2+} levels are maintained between 0.7 and 1.1 mM [46], and intracellular Mg^{2+} concentration is ~0.6 mM being buffered by adenosine triphosphate (ATP) [12, 62]. The luminal Mg^{2+} concentration in DCT, however, is around 0.2–0.7 mM [12], which is lower than the plasma and intracellular Mg^{2+} concentration. In addition, using immortalized mouse DCT cells, Dai and coworkers have demonstrated that the negative membrane potential directly determines the Mg^{2+} influx rate. Indeed, depolarization of the membrane diminished Mg^{2+} uptake in these cells [10]. This data would be in line with the operation of a voltage-driven Mg^{2+} permeable channel to support the entry of Mg^{2+} across the apical membrane [11]. Not long ago, this hypothesis was confirmed as being correct. Patients with hypomagnesemia with secondary hypocalcemia (HSH), a primary defect in intestinal and renal Mg^{2+} absorption, were found to carry mutations in TRPM6 [47, 50, 67]. TRPM6 is the first epithelial Mg^{2+}-permeable channel predominantly present in the luminal membrane of epithelial cells in kidney and intestine [47, 50, 67]. In concert with TRPM6, the apical Na^+ transporter NCC is indirectly involved in the transcellular movement of Mg^{2+} in DCT [38]. The evidence for this notion came from a NCC knockout mouse. Inactivation of the NCC gene resulted in a mouse model resembling Gitelman's syndrome [31], a human disease characterized by mild renal Na^+ wasting, hypocalciuria, hypomagnesemia, and hypokalemic alkalosis. Although it is still unclear how NCC dysfunction precisely leads to hypomagnesemia, their study provides further evidence to support the DCT as the major site for transcellular Mg^{2+} reabsorption. Following the Mg^{2+} influx in DCT cells, intracellular Mg^{2+} will be transported to the basolateral domain for extrusion to the blood compartment. A putative Na^+/Mg^{2+} exchanger and Mg^{2+}-ATPase have been proposed as candidates for the extrusion process [12]. So far, the molecular identities of these basolateral Mg^{2+} transporters are unknown. Furthermore, the basolateral membrane also harbors the receptors for magnesiotropic hormones, such as epidermal growth factor (EGF) and 17-β-estradiol [14, 15].

Mg^{2+} influx in DCT through TRPM6

TRPM6 as the gatekeeper of transcellular Mg^{2+} reabsorption

TRPM6 is the sixth member in the melastatin subfamily of transient receptor potential (TRP) ion channels. TRPM6 consists of 39 exons spanning 167 kb of genomic sequence and coding for a protein of 2,022 amino acids [47, 50, 67]. The TRPM6 protein has six transmembrane-spanning domains, in which the putative pore region is between the fifth and sixth domains. It also has a long amino (N)-terminus and a carboxyl (C)-terminus. TRPM6 has an α-kinase domain in the C-terminus. This kinase domain plays an important role in regulating TRPM6 activity. Further, TRPM6 also displays unique electrophysiological properties. When heterogeneously expressed in HEK293 cells, TRPM6 constitutes a channel that is characterized by extremely small inward but large outward currents [66].

External divalent cations such as Mg^{2+} and Ca^{2+} are permeable to TRPM6 and at the same time block monovalent cations permeating through the pore of the channel [66]. TRPM6 displays strong outward rectification, has a 5-fold higher affinity for Mg^{2+} than for Ca^{2+}, and is blocked in a voltage-dependent manner by ruthenium red [66]. In other Ca^{2+}-selective channels, there is a 10–1,000 lower affinity for Mg^{2+} than for Ca^{2+} [18], which further favors the TRPM6 identity as Mg^{2+}-permeable channel. The permeation rank order determined from the inward current amplitude at −80 mV is $Ba^{2+} \geq Ni^{2+} > Mg^{2+} > Ca^{2+}$. Single-channel conductance of homomeric TRPM6 channel is 84±2 pS [28]. Amino acid residues E1024, I1030, and D1031 seem important for channel function and account in the pore region for TRPM6 permeation properties [61]. In addition, Li et al. provided evidence that E1029 is also critical in regulating TRPM6 permeability [27]. It is remarkable that neutralization of the single amino acid residue, E1024Q, largely eliminates divalent permeation, converting the divalent selective TRPM6 to a virtually monovalent selective channel, and at the same time abolishes external pH sensitivity [27]. However, the Mg^{2+} selectivity filter of TRPM6 is still unclear. So far, two candidate regions in TRPM6 have been proposed as Mg^{2+} selectivity filters, including ^{1028}GEIDVC^{1033} [61] and ^{1024}EVYAGE^{1029} [27]. The missense mutations in TRPM6 identified in HSH lead to nonfunctional, mostly truncated, TRPM6 proteins [50, 67]. Collectively, these studies identified TRPM6 as a Mg^{2+}-permeable ion channel.

α-Kinase domain is critical in TRPM6 activity

TRPM6 is a channel kinase with an α-kinase domain fused to its C-terminus. These kinases display little sequence similarity with conventional protein kinases. Although α-kinases can predominantly phosphorylate residues present in α-helices [48], the α-kinase of TRPM6 is able to autophosphorylate the channel at several autophosphorylation sites [8]. Additionally, the autophosphorylation of the Ser/Thr-rich domain in the α-kinase is critical in the subsequent phosphorylation of protein substrates by providing access of the catalytic domain to the substrate [8]. The TRPM6 α-kinase domain is also a regulatory site for TRPM6 channel activity. For instance, deletion of the α-kinase domain reduces the TRPM6 current [59]. Furthermore, we have identified receptor of activated protein kinase C 1 (RACK1) as the first TRPM6-associated protein that inhibits TRPM6 channel activity depending on the phosphorylation of the threonine residue at position 1851 (T1851) in the α-kinase domain. RACK1 is a scaffold protein that binds phosphorylated protein kinase C (PKC) [45], by which RACK1 can bring the activated PKC into contact with its various substrates. Using the glutathione S-transferase (GST)-pull down technology, Cao and colleagues revealed that RACK1 is one of the associated proteins of TRPM6. RACK1 co-localizes with TRPM6 and NCC in DCT cells, suggesting a role for RACK1 in renal Mg^{2+} handling. Indeed, overexpressed RACK1 reduces TRPM6 currents in an α-kinase activity-dependent manner. Given that T1851 is crucial for the Mg^{2+}-dependent autophosphorylation of TRPM6 [6] and channel activity, these data indicate that RACK1 confers, at least in part, the Mg^{2+} sensitivity of TRPM6 channel activity. Taken together, RACK1 is an intracellular TRPM6-associated protein that mediates TRMP6 channel activity and thus renal Mg^{2+} handling through the TRPM6 α-kinase domain.

Besides RACK1, intracellular ATP also regulates TRPM6 activity via its α-kinase domain [59]. In the TRPM6 α-kinase region, there is a conserved ATP-binding pocket [59]. By binding to this ATP-binding pocket, both Na^{+}-ATP and Mg^{2+}-ATP inhibited TRPM6 currents in a dose-dependent manner with a comparable IC_{50} of ~1.3 mM [59]. Mutation of the conserved glycine residue in the ATP-binding pocket prevented the inhibitory effect of ATP on TRPM6 channel activity [59]. Interestingly, the α-kinase domain, but not its activity, is essential for ATP-induced inhibition on TRPM6 [59]. This result supports the critical role for the α-kinase domain in TRPM6 regulation. In contrast to ATP, Na^{+}-CTP or Na^{+}-GTP did not affect the TRPM6 current in transfected HEK293 cells [59], indicating the unique role of ATP in regulating the channel. However, it is still unknown to what extent and how ATP can modulate the transcellular Mg^{2+} reabsorption in DCT.

Regulation of TRPM6 by magnesiotropic hormones

Hormones primarily controlling the overall Mg^{2+} balance are not generally known (Table 2). Recently, EGF has been identified as a new magnesiotropic hormone regulating specifically the renal handling of Mg^{2+} [15, 35]. In a Dutch family with isolated recessive renal hypomagnesemia (IRH), Tiel Groenestege and colleagues employed a homozygosity-based mapping strategy and revealed a mutation in the pro-EGF gene where the highly conserved proline in the cytoplasmic ^{1067}PKN*P*^{1070} motif has been substituted by a leucine. This mutation diminishes basolateral release of pro-EGF and seriously hampers the EGF-dependent activation of the basolateral EGF receptor, leading to insufficient activation of TRPM6, less Mg^{2+} influx, and IRH [15]. Accordingly, it has been proposed that the pro-EGF gene mutation may result in improper basolateral sorting, leading to an inadequate secretion of the hormone into the circulation. Logically, the next question was how EGF activates TRPM6. To answer this question, it was demonstrated that EGF stimulates the mobility of subcellular TRPM6 to increase the plasma membrane

Table 2 Regulation of TRPM6 channel activity in DCT

Regulatory Factors	TRPM6	Reference
PTH	= Transcription	[14]
Estrogen	⇑ Transcription	[14]
EGF	⇑ Trafficking/channel activity	[15, 58]
Aldosterone	= Transcription	[56]
1,25-$(OH)_2D_3$	= Transcription	[14]
Low dietary Mg^{2+}	⇑ transcription	[14]
Acidosis	⇓ Transcription	[37]
Alkalosis	⇑ Transcription	[37]
Extracellular acidic pH	⇑ Channel activity	[27]
$[Mg^{2+}]_e$	⇓ Channel activity	[66]
$[Ca^{2+}]_e$	⇓ Channel activity	[66]
RACK1	⇓ Channel activity	[6]
ATP	⇓ Channel activity	[59]
$[Mg^{2+}]_i$	⇓ Channel activity	[59, 66]
Tacrolimus	⇓ Transcription	[36]
Regulators of Na^+ transporters		
FXYD2-mutant	?	[24]
NCC-knockout	⇓ Transcription	[36]
Thiazides	⇓ Transcription	[38]
Potential MgBP		
Parvalbumin	Co-localizes in DCT1	[59, 66]
CaBP28	Co-localizes in DCT2	[59, 66]
Putative basolateral transporter		
Na^+/Mg^{2+} exchanger	?	[12]
Mg^{2+}-ATPase	?	[12]

$[Mg^{2+}]_e$, extracellular Mg^{2+} concentration, $[Mg^{2+}]_i$, intracellular Mg^{2+} concentration, $[Ca^{2+}]_e$ extracellular Ca^{2+} concentration.
? not known, = no effect, ⇑ transcription upregulation or increase of channel activity, ⇓ downregulation of transcription or inhibition of the channel activity

expression of TRPM6 [58]. In detail, using the fluorescence recovery after photobleaching technology and cell surface biotinylation experiments, Thébault et al. visualized the EGF-enhanced TRPM6 trafficking from the cytosol to the plasma membrane [58]. This process is mediated via a sarcoma inducing gene (Src) kinase and Ras-related C3 botulinum toxin substrate 1 (Rac1) [58]. In line with this study, Ikari et al. reported that, in renal epithelial cells, EGF upregulates the TRPM6 expression by increasing the phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), leading to Mg^{2+} influx and an increase in cell proliferation [23]. In agreement with this study, the blockade of the EGF receptor (EGFR) induces hypomagnesemia. This is commonly seen in the colorectal cancer patients undertaking treatment with an EGFR-targeting antibody, cetuximab, and can now be explained by the inhibition of TRPM6 through the block of EGFR by cetuximab [15, 57]. Collectively, these data provide an insight into the mechanism of EGFR activation of TRPM6 and reiterate the importance of EGF in the overall Mg^{2+} homeostasis.

Another magnesiotropic hormone is 17-β-estradiol as evidenced by Tiel Groenestege et al., who showed that 17-β-estradiol upregulates mRNA level of TRPM6 in mouse kidney [14]. In line with this observation, surgical removal of ovary in rats caused a significantly reduction of TRPM6 mRNA level in the kidney, indicating the regulatory role of estrogen in the transcription process of TRPM6. In addition, subsequent treatment with 17-β-estradiol restored the TRPM6 mRNA levels [14], further supporting the candidature of 17-β-estradiol as a magnesiotropic hormone. In fact, this is also in agreement with a previous clinical study, showing that estrogen substitution therapy significantly decreases postmenopausal hypermagnesuria [32, 49]. Therefore, it is likely that 17-β-estradiol regulates transcellular Mg^{2+} reabsorption via an enhanced renal TRPM6 expression [14].

Besides EGF and 17-β-estradiol, other hormones including calcitonin, glucagon, arginine vasopressin, mineralocorticoid hormones, prostaglandins, and insulin [12] have been indirectly implicated in the process of Mg^{2+} handling, but their effect on TRPM6 activity is unknown. It has, however, been suggested that parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) do not regulate the transcription of TRPM6 in mouse [14]. This is interesting since both hormones have been suggested to increase the Mg^{2+} reabsorption in DCTs as evidenced from in vivo microperfusion experiments and immortalized mouse DCT cells from Mg^{2+} uptake experiments [12, 42]. Hence, there must be other pathways for PTH and 1,25$(OH)_2D_3$ to enhance transcellular Mg^{2+} reabsorption. In general, detailed molecular studies are needed to further substantiate the involvement of these latter hormones in transcellular Mg^{2+} reabsorption.

Other factors that regulate TRPM6

Other conditions can also regulate TRPM6 and thus renal Mg^{2+} handling, such as metabolic acidosis and alkalosis, immunosuppressive drugs, and thiazide diuretics (Table 2). Nijenhuis et al. reported that renal TRPM6 expression in kidney is decreased by chronic metabolic acidosis but increased by chronic metabolic alkalosis [37]. Furthermore, the immunosuppressive drugs tacrolimus and cyclosporin A both downregulate TRPM6 [22, 36]. Similarly, chronic application of thiazide diuretics, mimicking Gitelman's syndrome, has been shown to reduce TRPM6 expression [31, 38]. Collectively, these data support the key role of TRPM6 in transcellular Mg^{2+} reabsorption and imply TRPM6 as a potential target for pharmacological treatment of various Mg^{2+}-deficient disorders.

TRPM6 as distinct Mg^{2+} channel

TRPM6 and TRPM7 have an overall amino acid sequence identity of 52%, which increases to more then 80% in the

region between the fifth and sixth transmembrane domains where the pore-forming loop is presumably located [66]. Moreover, both TRPM6 and TRPM7 have a C-terminal region with sequence similarities to the atypical α-kinase family. It is likely that TRPM6 and TRPM7 form distinct cation channels, but there is also experimental evidence that they can function as a heteromeric complex (TRPM6/7) [7, 28]. Importantly, there are several signature features of TRPM6 that distinguish this channel from homomeric TRPM7 and heteromeric TRPM6/7 channels. TRPM6 single-channel current is larger than TRPM7 or TRPM6/TRPM7 [28]. At micromolar concentrations, 2-aminoethoxydiphenyl borate (2-APB) enhanced TRPM6 but inhibited TRPM7 currents, whereas at millimolar concentrations, 2-APB increased TRPM7 and TRPM6/7 currents [28]. Hence, TRPM6 has different pharmacological properties from TRPM7 and TRPM6/7. Furthermore, the pH sensitivity of TRPM6 is distinctive from those of TRPM7 channels and TRPM6/7 complexes [28].

Mg^{2+}-binding proteins

Several substances present in DCT cells have the potential to bind significant amounts of Mg^{2+}, and thus could affect intracellular free Mg^{2+} levels. For instance, ATP, CaBP28, and parvalbumin are abundantly present in DCT [17, 66]. Furthermore, intracellular Mg^{2+} can also be modulated by the unidirectional transport of Mg^{2+} into subcellular organelles including nucleus, mitochondrion, endoplasmic reticulum, and ribosomes [17]. At present, evidence for a Mg^{2+}-binding protein specifically controlling the process of transcellular Mg^{2+} reabsorption is lacking. This is in contrast to transcellular Ca^{2+} transport, where calbindins (CaBP28 in kidney and CaBP9 in intestine) specifically buffer Ca^{2+} to maintain low intracellular Ca^{2+} levels during the process of transcellular Ca^{2+} (re)absorption, which is a prerequisite for the signaling function of Ca^{2+}. Additionally, the apical Ca^{2+} channel TRPV5 is strongly inhibited by intracellular Ca^{2+} in the submicromolar range [39]. Hence, without sufficient local Ca^{2+}-buffering, the Ca^{2+} influx via TRPV5 would not be feasible. In contrast, extracellular and intracellular Mg^{2+} levels are both in the submillimolar range. Thus, there is virtually no chemical gradient of Mg^{2+} across the plasma membrane, and this questions the necessity of a specific Mg^{2+}-binding protein. However, Voets and coworkers reported that the activity of TRPM6 is strongly controlled by the local Mg^{2+} concentrations with an IC_{50} of 0.5 mM, suggesting that adequate Mg^{2+} buffering could be important. Indeed, both parvalbumin and CaBP28 partly co-localize with TRPM6 in the DCT and could thus potentially function as local Mg^{2+} buffers. A recent study using parvalbumin knockout mouse failed to show a disturbance in the Mg^{2+} homeostasis, which seriously questions the candidateship of parvalbumin [4]. However, this may be due to a compensatory intestinal Mg^{2+} hyperabsorption, or increased high bone turnover could occur in these knockout mice. Similarly, this compensatory situation also happens in the $CaBP28^{-/-}$ mouse, which does not exhibit hypocalcemia [13]. Alternatively, ATP could, in addition to its direct effect on TRPM6 activity, also function as physiological and local Mg^{2+} buffer [59]. Taken together, further experimental studies are needed to fully understand the role of Mg^{2+}-binding substances in transcellular Mg^{2+} reabsorption.

Mg^{2+} extrusion systems

A Na^+/Mg^{2+} exchanger and a Mg^{2+} pump, in analogy with Ca^{2+} extrusion systems, have been postulated as candidates for Mg^{2+} extrusion system [12]. A putative Na^+/Mg^{2+} exchanger has been studied functionally in many cells including epithelial cells, but its molecular identity remains unknown [16, 26, 52]. Schweigel and colleagues showed that sheep rumen epithelial cells take up Mg^{2+} when incubated in a Mg^{2+}-containing medium and that this uptake is stimulated when the external medium contains no or small amounts (10 mM) of Na^+ [52, 53]. Moreover, this Mg^{2+} influx was accompanied by a decrease in the intracellular Na^+ concentration and reduced by quinidine and imipramine, which are both putative inhibitors of Na^+/Mg^{2+} exchange [63]. Collectively, these data suggest the existence of a Na^+/Mg^{2+} exchanger in epithelial cells, but its presence remains to be shown in the DCT. Alternatively, a Mg^{2+} pump, which molecular identity awaits experimental confirmation, has been proposed [51]. Taken together, further investigation is necessary to explore the molecular details of the Mg^{2+} extrusion across the basolateral membrane of DCT cells.

Diseases related to Mg^{2+} handling in DCT

Abnormal Mg^{2+} handling in the DCT, which is the major site of transcellular Mg^{2+} reabsorption, can cause sever hypomagnesemia [60], including HSH [50], isolated dominant hypomagnesemia (IDH) [5, 34], and Gitelman's syndrome [31, 38].

TRPM6 mutation leads to HSH

The HSH is an autosomal recessive disease characterized by hypomagnesemia and hypocalcemia. The latter is possibly secondary to parathyroid failure resulting from Mg^{2+} deficiency due to Mg^{2+} mal(re)absorption [1]. Patients show neurologic symptoms of hypomagnesemia during infancy, including seizures and muscle spasms. Oral administration of

high doses of Mg^{2+} can relieve clinical symptoms. Without treatment, it may be fatal or may result in neurological damage. In 2001, two different groups simultaneously discovered that HSH is caused by mutations in TRPM6 [50, 67]. This serendipitous discovery demonstrates that TRPM6 is the epithelial Mg^{2+} channel primarily controlling intestinal Mg^{2+} uptake and renal Mg^{2+} excretion.

FXYD2 mutant induces IDH

The FXYD2 gene encodes a single transmembrane protein that functions as the γ-subunit of Na^+/K^+-ATPase. A mutation of FXYD2 substituting glycine at position 41 to arginine (FXYD2-G41R) causes IDH, a dominant renal hypomagnesemia associated with hypocalciuria through a dominant negative mechanism [33, 34]. These disorders share the general symptoms of hypomagnesemia, tetany, and epileptiformic convulsions and often include secondary or associated disturbances in Ca^{2+} excretion. The γ-subunit is a small hydrophobic protein of 10 kDa, originally identified in purified preparations of the Na^+/K^+-ATPase. FXYD2 protein is highly expressed in the basolateral membrane of TAL and DCT [2, 41]. In kidneys of FXYD2 knockout mouse, Na^+/K^+-ATPase displayed a higher apparent affinity for Na^+ without significant change in apparent affinity for K^+ compared to wild-type animals [24]. The consequence of this alteration in affinity for the Mg^{2+} flux across the DCT is, despite intense research, not yet elucidated. Surprisingly, these knockout mice apparently do not exhibit a disturbance in overall Mg^{2+} balance as reflected by normal blood and urine Mg^{2+} values [24], indicating that the presence of wild-type FXYD2 per se is not required for the maintenance of the Mg^{2+} balance. Since a mutation (G41R) in, unlike a knockout of, FXYD2 causes renal Mg^{2+} wasting and consequently hypomagnesemia, the mutant protein possibly impairs another protein partner directly regulating the Mg^{2+} handling in DCT [34]. Now, the exact molecular consequence of FXYD2-G41R remains elusive. Indeed, it may cause malfunctioning of the Na^+/K^+-ATPase by destabilizing the association between FXYD2 and Na^+/K^+-ATPase [5] and thus directly depolarize the membrane potential [34]. Alternatively, both wild-type FXYD2 and FXYD2-G41R mutant form homomeric and heteromeric oligomers [5]. In Madin–Darby Canine Kidney (MDCK) cells, wild-type FXYD2 leads to an increase in transepithelial current, and this current is significantly reduced by co-transfection with the FXYD2-G41R mutant [54]. Hence, FXYD2-G41R mutant is dominant over wild-type FXYD2 [54]. In line with this observation, FXYD2-G41R has been shown to reduce the trafficking of wild-type FXYD2 to the plasma membrane [5]. Given that the dominant nature of the FXYD2-G41R mutation may be mediated through its association with wild-type FXYD2 [54], the oligomers of FXYD2-G41R mutant with FXYD2 wild-type seems essential for the occurrence of hypomagnesemia. Hypothetically, this small protein may directly regulate the activity of a relevant ion channel, including TRPM6, as FXYD proteins have in general been considered as regulators of ion channels [9].

NCC mutations cause Gitelman's syndrome

Gitelman's syndrome is one of the most frequently inherited renal tubular disorders [25]. It is an autosomal recessive Na^+ wasting disease, characterized by hypomagnesemia, hypocalciuria, and secondary aldosteronism [25]. This leads to hypokalemia and metabolic alkalosis. Genetic analysis revealed that this syndrome is caused by mutations in NCC [55], which has been further confirmed by studies with NCC knockout mice and the chronic application of thiazide diuretics [31, 38]. It is still unclear how abolishment of NCC function induces hypomagnesemia. Nijenhuis et al. have shown that TRPM6 mRNA and protein abundance are reduced in a mouse model of Gitelman's syndrome [38]. Furthermore, the hypomagnesemia in this mouse model is due to renal Mg^{2+} wasting [38]. Thus, reduced NCC activity seems to regulate transcellular Mg^{2+} reabsorption in DCT by controlling the expression of TRPM6. In addition, another study has revealed that aldosterone levels were significantly elevated in NCC knockout mice compared with wild-type mice [43]. Hypomagnesemia has been observed in primary aldosteronism [68]. Collectively, these data imply that aldosterone may be involved in hypomagnesemia in Gitelman's syndrome. However, surprisingly, a recent report shows that aldosterone did not affect the TRPM6 expression in mouse kidney [56]. Thus, the molecular mechanism of TRPM6 downregulation and accompanied hypomagnesemia in Gitelman's syndrome is still unclear.

Unresolved issues

The identification of TRPM6 as gatekeeper of transcellular Mg^{2+} reabsorption and of EGF and estrogen as magnesiotropic hormones acting on TRPM6 are recent breakthroughs in unraveling the molecular mechanism of transcellular Mg^{2+} reabsorption in DCT. Our picture of this process is, however, far from complete, and there are still many important questions unanswered. The activity of TRPM6 has been measured in heterogeneous expression systems like HEK293 cells. Here, the inward Mg^{2+} current is surprisingly small at physiological membrane potentials, and it is unknown how these small inward currents contribute to the overall transcellular Mg^{2+} reabsorption [66]. Furthermore, the contribution of organelles to the

regulation of intracellular Mg^{2+} levels is unknown. Given that Mg^{2+} with an IC_{50} of 0.5 mM inhibits TRPM6 activity, it is important to delineate the role of subcellular organelles, like nucleus, mitochondria, and endoplasmic reticulum [44], in this process. Additionally, it is still unspecified how epithelial cells sense the extracellular Mg^{2+} concentration in order to trigger Mg^{2+} reabsorption. It has been established that the Ca^{2+}-sensing receptor (CaSR) can also monitor extracellular Mg^{2+} concentration [20]. Additionally, CaSR activation may decrease protein kinase A activity, resulting in a decrease in phosphorylated claudin-16, the translocation of claudin-16 to lysosome, and a decrease in paracellular Mg^{2+} reabsorption [21]. Recently, our group discovered that CaSR is expressed in basolateral domain in the DCT cells (unpublished data) and regulates TRPV5 channel activity. Therefore, it would be interesting to test if this CaSR can sense extracellular Mg^{2+} concentration and modulate transcellular Mg^{2+} reabsorption. Finally, the relation between Ca^{2+} and Mg^{2+} reabsorption in the DCT needs further investigation. Hypomagnesemia is frequently accompanied by inappropriate renal Ca^{2+} wasting, but the molecular explanation of this phenomenon remains obscure. A multidisciplinary approach to explore disturbances in Mg^{2+} homeostasis will be necessary to further increase our understanding of transcellular Mg^{2+} reabsorption and ultimately develop new possibilities to treat Mg^{2+}-related syndromes.

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