Calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) minerals are essential for many physiologic processes.\(^1\) Ca\(^{2+}\) plays a pathologic role in osteoporosis, nephrolithiasis, vascular calcification, nephrocalcinosis, and chronic kidney disease, and disturbances in Mg\(^{2+}\) contribute to muscle cramps, paraesthesia, convulsions, arrhythmias, and cardiac arrest. Their overall mineral balance is regulated by the concerted actions of kidneys, intestine, and bone.\(^2\) The kidneys determine the final excretion of these cations and fulfill, and therefore, an important step in homeostatic control. This role was recognized by Homer Smith, who wrote “...Little is known concerning Ca\(^{2+}\) excretion except that the total excretion can be increased or decreased by a variety of circumstances. Much that has been said about Ca\(^{2+}\) applies to Mg\(^{2+}\). The mechanism by which renal excretion of Mg\(^{2+}\) is controlled is unknown...”\(^3\)

In the last decade, considerable progress has been made in elucidating the molecular mechanisms underlying the reabsorption of these minerals by the kidney. Instrumental in this respect are studies of rare monogenic diseases related to defective renal Mg\(^{2+}\) handling and genetically modified mice with deleted Ca\(^{2+}\) transport proteins.\(^4,5\) These studies identified new transport proteins and have led to the development of new concepts for the renal handling of minerals.

**IMPORTANT OF THE DISTAL PART OF THE NEPHRON**

Active reabsorption of Mg\(^{2+}\) and Ca\(^{2+}\) takes place in the distal part of the nephron only. More precisely, this part of the nephron is comprised of the distal convoluted tubule (DCT) and the connecting tubule (CNT) leading to the collecting duct.\(^6\) The former can be further subdivided into early (DCT1) and late (DCT2) segments. Based on micropuncture experiments and the conspicuous localization of transport proteins, active Mg\(^{2+}\) transport is confined to the DCT1 and DCT2 segments, whereas active Ca\(^{2+}\) reabsorption mainly occurs along the DCT2 and CNT segments (Figure 1).\(^1\) Thus, DCT2 functions as a transition area between Mg\(^{2+}\) and Ca\(^{2+}\) reabsorption.

**Mg\(^{2+}\) REABSORPTION IN DCT1 AND DCT2 SEGMENTS**

The DCT is famous for the presence of the thiazide-sensitive NaCl co-transporter (NCC) along the luminal membrane, which is energized by a Na\(^{+}\)-K\(^{+}\)-ATPase.\(^7,8\) Active transcellular Mg\(^{2+}\) transport along the DCT is envisaged by the following sequential steps (Figure 2).\(^4\) Driven by a favorable membrane potential, Mg\(^{2+}\) enters the DCT cell through an apical epithelial Mg\(^{2+}\) channel. The chemical driving force for Mg\(^{2+}\) is limited because the extra- and intracellular Mg\(^{2+}\) concentrations are in the same millimolar range. Importantly, Mg\(^{2+}\) entry into the cells seems to be the rate-limiting
step and thus the site of regulation. Subsequently, Mg$^{2+}$ diffuses through the cytosol to be extruded actively against an electrochemical gradient across the basolateral membrane. For the Mg$^{2+}$ extrusion, unidentified can-

didates could be a Na$^+$-dependent exchange mechanism or an ATP-dependent Mg$^{2+}$ pump. Some of the salient features are described below.

**Transient Receptor Potential Melastatin, Subtype 6**

The apical epithelial Mg$^{2+}$ channel is known as the transient receptor potential melastatin, subtype 6 (TRPM6). TRPM6 is a cation channel composed of six transmembrane-spanning domains and a conserved pore-forming region that assembles in a tetrameric configuration. Studies of families with autosomal recessive hypomagnesemia with secondary hypocalcemia identified mutations in TRPM6.$^{10,11}$ TRPM6 is one of eight members of the identified TRPM cation channel subfamily and is composed of 2022 amino acids encoded by a large gene containing 39 exons.$^{10–12}$ TRPM6 displays a restricted expression pattern and is predominantly present in reabsorbing epithelia.$^{10,11,13}$ In the kidney, TRPM6 localizes along the apical membrane of the DCT.$^{13}$ This channel is a unique bifunctional protein consisting of an Mg$^{2+}$ permeable cation channel with protein kinase activity and is occasionally referred to as chanzymes.$^{14}$ Electrophysiologic characterization of TRPM6 shows that TRPM6-transfected human embryonic kidney 293 cells exhibit outwardly rectifying currents. Mg$^{2+}$ itself has a profound effect on the activity of TRPM6. For instance, intracellular Mg$^{2+}$ levels tightly regulate TRPM6 activity with an apparent $K_i$ of 0.5 mM that is comparable to physiologic intracellular Mg$^{2+}$ concentrations.$^{13}$ Furthermore, extracellular Mg$^{2+}$ also affects TRPM6, because Mg$^{2+}$ restriction significantly up-regulates levels of mRNA encoding renal TRPM6.$^{15}$

**Kv1.1**

The voltage-gated K$^+$ channel, Kv1.1, is a new protein thought to regulate Mg$^{2+}$ influx through TRPM6. Recently, there has been evidence that a mutation in KCNA1 encoding Kv1.1 causes autosomal dominant hypomagnesemia.$^{16}$ The phenotype detectable from infancy consists of recurrent muscle cramps, tetany, tremor, muscle weakness, cerebellar at-

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**Figure 1.** Overview of Mg$^{2+}$ and Ca$^{2+}$ handling in the distal nephron. The active reabsorption of the minerals Mg$^{2+}$ and Ca$^{2+}$ takes place in the distal part of the nephron only. More precisely, this part of the nephron is comprised of the DCT and the CNT to the collecting duct. The former can be further subdivided into an early (DCT1) and late (DCT2) portion. Active Mg$^{2+}$ transport is confined to the DCT1 and DCT2, whereas active Ca$^{2+}$ reabsorption mainly occurs in the DCT2 and CNT segments. Thus, DCT2 functions as a transition area between Mg$^{2+}$ and Ca$^{2+}$ reabsorption.

**Figure 2.** Mechanism of active Mg$^{2+}$ reabsorption in DCT1 and DCT2 segments. Apical membrane TRPM6 channels are located in the apical membrane, which facilitates transport of Mg$^{2+}$ from the tubular fluid into the cell. Mg$^{2+}$ reabsorption is primarily driven by the luminal membrane potential established by the voltage-gated K$^+$ channel, Kv1.1. The Na$^+-$K$^+$-ATPase, situated in the basolateral membrane, provides a sodium (Na$^+$) gradient that is used by the thiazide-sensitive NCC to facilitate transport of Na$^+$ from the tubular fluid into the cytoplasm and a K$^+$ gradient to generate local membrane potential. K$^+$ is supplied to the Na$^+-$K$^+$-ATPase through recycling through Kir4.1. The $\gamma$-subunit of the Na$^+-$K$^+$-ATPase regulates the function of Na$^+$ pump. Transcription factor HNF1B (hepatocyte nuclear factor 1 homeobox B) regulates the expression of the $\gamma$-subunit of the Na$^+-$K$^+$-ATPase. EGF is the first magnesiotropic hormone to regulate active Mg$^{2+}$ reabsorption through the TRPM6 channel.
rophy, and myokymia. The K⁺ channel co-localizes with TRPM6 along the luminal membrane of the DCT. The identified mutation results in a nonfunctional channel with a dominant-negative effect on wild-type channel function. Thus, Kv1.1 is a new luminal K⁺ channel in the DCT that establishes favorable luminal membrane potential controlling TRPM6-mediated Mg²⁺ reabsorption. 

γ-Subunit of the Na⁺-K⁺-ATPase 

We also identified FXYD2 as being involved in hypomagnesemia. FXYD2 encodes the γ-subunit of the basolateral Na⁺-K⁺-ATPase and is mutated in patients with autosomal dominant renal hypomagnesemia associated with hypocalciuria. Currently, the exact molecular mechanism by which the γ-subunit controls Mg²⁺ handling in the DCT remains elusive. It is postulated this transmembrane protein facilitates the basolateral extrusion of Mg²⁺ in renal epithelial cells. Others suggest the γ-subunit regulates additional transport mechanisms that localize to the basolateral membrane such as the Na⁺-K⁺-ATPase, Kir4.1/5.1, or the unidentified basolateral Mg²⁺ extrusion mechanism (Figure 2).1,5

Hepatocyte Nuclear Factor 1B 

Further support for an active role of the γ-subunit in Mg²⁺ reabsorption is suggested by the observation that a transcription factor, hepatocyte nuclear factor 1B (HNF1B), modulates the FXYD2 gene. Hypomagnesemia, hypermagnesemia, and hypocalciuria are observed in one half of the HNF1B mutation carriers. Analyses of the FXYD2 promoter region identify two highly conserved HNF1B recognition sites. Future studies should confirm the role of HNF1B in the regulation of FXYD2 and possibly other components of the molecular machinery involved in renal Mg²⁺ handling.

Kir4.1 

Two independent studies recently described a mutation within the KCNJ10 gene as the underlying cause of a hypomagnesemia syndrome. The first study described two nonrelated consanguineous families with a disorder characterized by epilepsy, ataxia, sensorineural deafness, and tubulopathy (also referred to as SeSAME), whereas the other study described four kindreds with similar clinical findings. The KCNJ10 gene encodes a K⁺ channel called Kir4.1, expressed in brain, ear, and kidney, in keeping with the phenotype observed in these patients. The renal phenotype of EAST syndrome (a syndrome characterized by epilepsy, ataxia, sensorineural deafness, and tubulopathy) is similar to the Gitelman’s syndrome phenotype and consists of polyuria, hypokalemic metabolic alkalosis, hypomagnesemia, and hypocalciuria. In kidney, Kir4.1 is expressed along the basolateral membrane of DCT cells with the Na⁺-K⁺-ATPase. Kir4.1 is thought to recycle K⁺ into the interstitium to allow a sufficient supply of K⁺ for optimal Na⁺-K⁺-ATPase activity.

EGF 

We identified EGF as the first magnesiotropic hormone directly stimulating TRPM6 activity. Genetic analyses showed that a point mutation in the pro-EGF gene causes a rare inherited autosomal recessive form of renal hypomagnesemia. EGF acts as an autocrine/paracrine magnesiotropic hormone, specifically increasing TRPM6 activity by engagement of its receptor along the basolateral membrane of DCT cells. This activation relies on both the Src family of tyrosine kinases and the downstream effector, Rac1. Activation of Rac1 increases the mobility of TRPM6, assessed by fluorescence recovery after photobleaching, and a constitutively active mutant of Rac1 mimics the stimulatory effect of EGF on TRPM6 mobility and activity. Ultimately, TRPM6 activation results from increased cell surface abundance. These findings provide the first insight into the molecular regulation of TRPM6 by extracellular EGF. Moreover, it shows the molecular basis for the hypomagnesemia after treatment with cetuximab, an EGF receptor blocking antibody used in the treatment of colorectal cancer, and indicates TRPM6 is a potential pharmacologic target during cetuximab therapy.

Ca²⁺ Reabsorption in DCT2 and CNT Segments 

In DCT2 and CNT segments, Ca²⁺ reabsorption takes place against its chemical gradient, indicating that the transport is active. In addition to the ubiquitously expressed Na⁺-K⁺-ATPase, the Na⁺/Ca²⁺ exchanger (NCX1) and the plasma...
membrane ATPase type 1b (PMCA1b) are also found along the basolateral site of the DCT2 and CNT segments. DCT2 shares similarities with the CNT segment, because both segments express the transient receptor potential vanilloid subtype 5 (TRPV5) channel and the Ca\(^{2+}\)-binding protein, calbindin-D28K. Transepithelial transport of Ca\(^{2+}\) is a three-step procedure and is outlined in more detail below (Figure 3).

Ca\(^{2+}\) influx across the apical membrane is mediated by TRPV5. Subsequently, the specialized intracellular carrier protein, calbindin-D28K, sequesters Ca\(^{2+}\) entering the cell, and this complex diffuses toward the basolateral membrane. Finally, transporter proteins, such as NCX1 and PMCA1b, extrude Ca\(^{2+}\) from the epithelial cell back into the circulation.

**Apical Entry of Ca\(^{2+}\)**

To identify the apical Ca\(^{2+}\) influx channel involved in transcellular Ca\(^{2+}\) reabsorption, we performed functional expression cloning using a cDNA library from rabbit primary CNT and the corticomedullary junction. Expression cloning using a cDNA library from rabbit primary CNT and the corticomedullary junction yielded a single transcript was isolated that encodes a novel epithelial Ca\(^{2+}\)-channel protein, calbindin (CaBP) buffers Ca\(^{2+}\) in the basolateral membrane, Ca\(^{2+}\) is extruded by PMCA1b and NCX1.

**Figure 3.** Mechanism of active Ca\(^{2+}\) reabsorption in DCT2 and CNT. A three-step process facilitates active and transcellular Ca\(^{2+}\) transport. The first step is entry of luminal Ca\(^{2+}\) at the apical side of the cell through the TRPV5 channel. Subsequently, calbindin (CaBP) buffers Ca\(^{2+}\), and the Ca\(^{2+}\) diffuses to the basolateral membrane. At the basolateral membrane, Ca\(^{2+}\) is extruded by PMCA1b and NCX1. This process is controlled by calciotropic hormones including parathyroid hormone and 1,25(OH)\(_2\)D\(_3\).

**Thiazide Diuretics**

Thiazide diuretics, in contrast to loop diuretics, have the unique characteristic of decreasing Na\(^+\) reabsorption while increasing Ca\(^{2+}\) reabsorption. In addition, mutations in the NCC gene encoding the NaCl co-transporter cause Gitelman’s syndrome. Patients with Gitelman’s syndrome exhibit hypovolemia, hypokalemia, alkalosis, hypomagnesemia, and hypocalciuria. Intriguingly, the molecular mechanisms responsible for the hypocalciuria and hypomagnesemia with thiazide administration or in Gitelman’s syndrome remain elusive. Two hypotheses exist with respect to the Ca\(^{2+}\)-sparing effect of thia-
First, renal salt and water loss caused by thiazide treatment results in contraction of the extracellular volume (ECV), which triggers a compensatory increase of proximal Na⁺ reabsorption. This in turn enhances the electrochemical gradient driving passive Ca²⁺ transport in proximal tubular segments. Second, thiazide treatment stimulates Ca²⁺ reabsorption in DCT, possibly through the TRPV5 channel, that could explain the Ca²⁺-sparing effect. We showed in rats that hydrochlorothiazide-induced hypocaliuria is accompanied by a significant decrease in body weight compared with controls, confirming ECV contraction. Because sodium depletion results in a similar hypocaliuria, it is likely that the ECV contraction by itself is responsible for the thiazide-induced hypocaliuria. Further evidence supporting this notion is the finding that sodium repletion during thiazide treatment, thereby preventing the ECV contraction, normalizes the calciresis. A direct role for TRPV5 in the thiazide-induced hypocaliuria seems unlikely, because thiazides also have a hypocaliuric effect in TRPV5⁻/⁻ mice, and the overlap in the expression of NCC and TRPV5 in the distal part of the nephron is restricted to DCT2. Taken together, enhanced proximal tubular Na⁺ transport as a consequence of ECV contraction stimulates paracellular Ca²⁺ transport and best explains the tubular mechanism for thiazide-induced hypocaliuria.

**Activation of the Ca²⁺-Sensing Receptor Prevents Nephrolithiasis**

TRPV5⁻/⁻ mice display hypercalciuria from impaired active Ca²⁺ reabsorption but also hyperphosphaturia, polyuria, and increased urinary acidification. The latter two adaptations seem highly beneficial because there are no renal calcium precipitates. Polyuria also diminishes the risk of renal stone formation by reducing urinary Ca²⁺ concentration. In mice, calciresis linearly correlates with urinary volume because an increase in Ca²⁺ excretion leads to an enhanced urinary volume. The consistent polyuria in hypercalciuric TRPV5⁻/⁻ mice, noted by a substantial decrease in urinary osmolality, is caused by downregulation of renal AQP2 water channels, possibly a result of activating the Ca²⁺-sensing receptor along the luminal membrane of the collecting duct. Furthermore, gene ablation of the collecting duct-specific B1 subunit of H⁺-ATPases in TRPV5⁻/⁻ mice abolishes enhanced urinary acidification, which resulted in severe tubular precipitations of Ca²⁺-phosphate in the renal medulla. Thus, in TRPV5⁻/⁻ mice, activation of the renal Ca²⁺-sensing receptor promotes H⁺-ATPase–mediated H⁺ excretion and downregulation of AQP2, leading to urinary acidification and polyuria, respectively (Figure 4).

**FUTURE PERSPECTIVES ON RENAL Ca²⁺ HANDLING**

Ca²⁺ reabsorption in the kidney, and particularly in the distal DCT2 and CNT segments, is crucial for the maintenance of the Ca²⁺ balance. The identification and characterization of the proteins mediating this active Ca²⁺ transport provides novel insight and means to study molecular relationships. In these segments, TRPV5 facilitates the gatekeeper function of Ca²⁺ entry, and therefore, a tight control of its activity enables the organism to adjust Ca²⁺ reabsorption according to the demands of Ca²⁺ load. The molecular mechanism of Ca²⁺ shuttling between calbindin-D₂₈K on one site and NCX1 and PMCA1b on the other site is not clear. Another interesting and unaddressed question is the regulation of NCX1 and PMCA1b in DCT2 and CNT cells. Whether there is a crosstalk between apical Ca²⁺ entry and basolateral Ca²⁺ extrusion regulatory systems is not known. The next step is to investigate how these Ca²⁺ transport proteins communicate with each other to facilitate optimal and regulated Ca²⁺ reabsorption under conditions of disturbed Ca²⁺ homeostasis. Finally, the role of TRPV5 in Ca²⁺-related disorders needs further study.
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