Theme: The cutting edge of mineral nutrition: physiological and molecular perspectives

Mg, Zn and Cu transport proteins: A brief overview from physiological and molecular perspectives

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Summary

Essential major and trace elements, including magnesium (Mg), zinc (Zn) and copper (Cu), are involved in numerous physiological processes. These elements are important components for maintaining proper protein structure and function. They are also used as catalytic cofactors for enzymes and as mediators in signaling cascades. Thus, systemic homeostasis of these metals is sophisticatedly regulated at a molecular level. A balance between absorption and excretion of these metals is critical, and transport proteins play a key role in this balance. In particular, transport proteins in intestinal epithelial cells are indispensable and ensure adequate metal absorption. Regulation of the expression and activity of these proteins is complicated. Thus, dysfunction of these proteins causes an imbalance in the systemic homeostasis of corresponding metals, and thus likely links to disease pathogenesis. In this review, we briefly describe the importance of mammalian metal transport proteins, including Mg channels, and Zn and Cu transporters, focusing on their roles in the absorption process in intestinal epithelial cells. Specifically, TRPM6 channels in Mg absorption, ZIP4 and ZnT1 transporters for Zn absorption, and CTR1 and ATP7A for Cu absorption are overviewed. Furthermore, the regulation of cell surface ZIP4 expression, which is dynamically changed in response to Zn status, is extensively discussed.

Keywords: Mineral, Zinc, Copper, Magnesium, Transporters and Channels

マグネシウム、亜鉛、銅の輸送タンパク質；生理的・分子的視点からの考察

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要約

　マグネシウム（Mg）、亜鉛（Zn）、銅（Cu）などの多量元素や微量元素は、タンパク質の構造や酵素活性、シグナル調節に重要な因子として様々な生理機能を果たす必須栄養素である。そのため、これらの元素の全身の恒常性は、分子レベルで極めて複雑に制御される。輸送タンパク質は、恒常性維持にとって鍵となる吸収と排泄のバランスを制御するための重要な役割を担う。特に腸管上皮細胞における輸送タンパク質は、その発現や輸送活性による複雑な調節のもと、ミネラルの吸収にはたらく。従って、これらの輸送タンパク質の機能の破綻は、全身の恒常性の不均衡をもたらし、疾病の病因となる。本稿では、腸管上皮細胞において、これら元素の吸収に機能する Mg チャネル（TRPM6）、Zn トランスポーター（ZIP4 と ZnT1）、Cu トランスポーター（CTR1 と ATP7A）の重要性を概説する。特に亜鉛の吸収については、アピカル膜に局在し、亜鉛状態によりその発現量が大きく変化する ZIP4 の発現制御機構について詳しく論じる。

Physiological functions of Magnesium, Copper and Zinc

The adult human body contains about 25g of magnesium (Mg), which is the fourth-most abundant mineral in the body. Approximately 50% of Mg is found in bone, about 1% is found in the blood, and the remaining is distributed in other tissues and organs. Mg is an essential cofactor in numerous enzymatic reactions including, DNA, RNA, and protein synthesis, and in metabolism. Mg also plays a role in the stability of nucleic acids. Mg is essential for many physiological functions, such as maintenance of normal nerve and muscle functions, cardiac excitability, neuromuscular conduction, vasomotor tone, normal blood pressure, bone integrity, and glucose and insulin metabolism (1,2).

Zinc (Zn) is the second most abundant essential trace element next to iron in the body. The adult human body contains 2–3g of Zn. About 60% is localized in skeletal muscle and 30% is found in bone (3). Zn is a crucial structural, catalytic, and regulatory component within proteins, such as transcription factors, enzymes, transporters, and receptors. Thus, Zn has a diverse array of physiological functions in numerous biological processes including cell division, growth, differentiation and death (4).

The adult human body contains about 100mg of copper (Cu), most of which is stored in the liver. Although the amount of Cu stored in the body is relatively low, Cu serves as a catalytic and structural cofactor for numerous enzymes, such as hydrolytic, electron transfer, and oxygen utilization enzymes. Thus, Cu is required for important physiological processes such as energy generation, iron acquisition, peptide hormone maturation, blood clotting, and signal transduction. Hence, Cu is indispensable for normal development and growth (5).

Given the essential role of Mg, Zn and Cu, these metals are indispensable for proper functioning of the human body. In this review, we briefly describe their functions and specifically focus on their importance in intestinal absorption.

Mg absorption

In vertebrates, members of the melastatin-related subfamily of transient receptor potential (TRP) ion channels, specifically TRPM6 and TRPM7, play pivotal roles in Mg transport. In addition, solute carrier family 41 members SLC41A1-A3 are involved in Mg transport across either the plasma membrane or organellar membranes (6). Dietary Mg enters the body via both paracellular and transcellular pathways (7). TRPM6 plays a primary role in Mg uptake across the apical surface of membranes into enterocytes from the intestinal lumen. The transport proteins involved in exporting Mg across the basolateral membrane have not been identified.

TRPM6

TRPM6 is important for systemic Mg homeostasis because it is highly expressed along the brush-border membrane of the intestine and the apical membrane of the renal distal convoluted tubule. TRPM6 plays a key role in the absorption of Mg. (Fig.1) (8,9). TRPM6 expression in the intestine is regulated by dietary Mg. This suggests that TRPM6 functions as a gatekeeper of Mg absorption (9). Loss-of-function mutations in the *TRPM6* gene were identified in patients with the rare autosomal-recessive disease, hypomagnesemia (HSH) (10). Very low serum Mg levels and secondary hypocalcemia due to defective Mg absorption characterize HSH. Mg supplementation is required to maintain serum Mg concentration in HSH patients.

Studies have shown that TRPM7 has high sequence homology to TRPM6. Furthermore, TRPM7 and TRPM6 have been shown to form heterodimers and transport Mg across membranes. TRPM7 is expressed ubiquitously and thus, is expressed in the small intestine (7). Therefore, TRPM7 may play a role in intestinal Mg absorption in coordination with TRPM6 (8,11).

Zn absorption

Zn transporters have been divided into two SLC families, Zn transporter (ZnT)/SLC30A and Zrt, Irt-like protein/solute carrier family 39 (ZIP/SLC39A). ZnT effluxes Zn into extracellular space or intracellular organelles from the cytosol, while ZIP mobilizes Zn in the opposite direction (12). In the human genome, nine ZnT and 14 ZIP transporters are encoded. Thus, unlike Mg and Cu, over 20 transporters regulate Zn homeostasis and metabolism. In Zn absorption, ZIP4 and ZnT1 play a primary role, but other Zn transporters are also employed. Since Zn is a divalent cation and redox inactive, unlike Cu (see below), the cell surface expression levels of ZIP4 and ZnT1 are crucial in defining net Zn absorption.

ZIP4

ZIP4 is involved in Zn uptake into enterocytes at the apical surface and is the most important protein in intestinal Zn absorption. Mutations in the *ZIP4* gene cause acrodermatitis enteropathica (AE), a rare autosomal recessive, and lethal genetic disorder characterized by alopecia, diarrhea and skin lesions (13,14). AE patients require daily oral Zn supplement to alleviate Zn deficiency (3). ZIP4 is required in Zn absorption. Thus, mice deficient in intestine specific *Zip4* die in the absence of excess Zn (15). Zip4 is also critical for early development because homozygous *Zip4* knockout mice die during morphogenesis (16).

Studies have shown how ZIP4 expression is regulated. The gene and protein are dynamically regulated by multiple post-transcriptional modifications in response to Zn availability. In mice, dietary Zn deficiency causes increases in *Zip4* mRNA expression and Zip4 accumulation at the apical surface of enterocytes. Administration of Zn by oral gavage causes Zip4 internalization and degradation in enterocytes (17). When Zn is deficient, ZIP4 protein accumulates on the cell surface via increased *ZIP4* mRNA stability and reduced ZIP4 protein endocytosis from the cell surface (17). Moreover ZIP4 relocalizes on the apical surface during prolonged Zn deficiency after the long amino-terminal ectodomain of ZIP4 is removed (18). In contrast, when Zn is replete, ZIP4 is immediately endocytosed and degraded by both ubiquitin-proteasomal and lysosomal pathways (19). Some ZIP4 mutations identified in AE patients have shown impaired Zn responsive trafficking to the plasma membrane, the decreased Zn uptake (18,20). The processing of ZIP4 is thought to be physiologically important but further studies are needed to clarify its importance.

ZnT1

ZnT1 exports Zn from enterocytes into blood (Fig.1). In general, ZnT1 is ubiquitously expressed and primarily localized to the plasma membrane. Enterocyte ZnT1 is predominantly distributed on the basolateral membrane. *ZnT1* transcription is under the control of the metal response element (MRE) binding transcription factor-1 (MTF1), whose transcriptional activity is enhanced by excess Zn binding to MREs (21). Thus, dietary Zn supplementation induces both *ZnT1* mRNA and protein expression in the small intestine (22), which contributes to facilitating Zn export. ZnT1 is thought to play a pivotal role in Zn absorption but direct evidence is lacking in mammals. However, intestine-specific knockout of ZnT1 in Drosophila shows accumulate of Zn in the midgut and with a decrease in Zn in peripheral tissues (23).

Cu absorption

Two importers, CTR1 (SLC31A1) and CTR2 (SLC31A2), and two exporters, P-type ATPase, ATP7A (Menkes protein) and ATP7B (Wilson protein), maintain systemic and cellular Cu homeostasis. All Cu transport proteins recognize and mobilize Cu, and CTR1 and ATP7A are crucial in intestinal Cu absorption. Cu can exist in either the oxidized (Cu^{2+}) or reduced (Cu^{+}) states and dietary copper is likely to be in the oxidized form. Therefore, there is an obligatory metalloreduction event that occurs at the apical membrane before or concomitant with Cu^{+} transport into enterocytes from intestinal lumen. The reductase remains to be identified. (Fig.1)

CTR1 (SLC31A)

CTR1 is an integral membrane protein with three transmembrane domains that form a homotrimeric pore and import Cu^{+} (24). CTR1 is essential in embryonic development and *Ctr1* knockout mice are embryonic lethal (25). Intestinal epithelial cell-specific *Ctr1* knockout mice exhibit striking neonatal defects in Cu accumulation in peripheral tissues and have severe defects in growth and viability, thus demonstrating the *Ctr1's* importance in Cu absorption (26). A single administration of Cu partially rescues the growth and viability defects, indicating a critical neonatal metabolic requirement for Cu by intestinal Ctr1 (26).

In vitro and *in vivo* studies have shown CTR1 to be localized to both the apical surface (27) and the basolateral surface of the plasma membrane in polarized cells (28). CTR1 is also localized to intracellular vesicles in different cell lines (26,29). In intestinal epithelial cells in suckling mice under limited Cu conditions, Ctr1 is predominantly localized on the apical membrane (30), which may indicate that Cu is needed for growth and development. It is likely that Cu induces rapid endocytosis and degradation of CTR1 to prevent excess Cu absorption (29). Cu absorption mediated by CTR1 into the cytosol is delivered to the *trans*-Golgi network, cytochrome *c* oxidase in mitochondria, or superoxide dismutase 1 by the Cu chaperones, Atox1, COX17 or CCS, respectively (31).

ATP7A (Menkes protein)

ATP7A is expressed in most cells but is not expressed in hepatocytes. Conversely, ATP7B is exclusively expressed in hepatocytes (31). ATP7A plays a role in the export of Cu delivered by Atox1 from the basolateral membrane into the peripheral circulation. Mutations in the *ATP7A* gene cause Menkes disease (MD), an X-linked lethal disorder that is characterized by hyperaccumulation of Cu in the intestine and severe Cu deficiency in peripheral tissues (32). MD patients exhibit neurologic symptoms, connective tissue abnormalities, skin laxity, and hypopigmentation (32). Parenteral Cu-histidine administration is a standard treatment for MD. Intestinal *Atp7a* knockout mice or *Atp7a* mutant mice, such as brindled and macular mice, show

Cu deficient phenotypes, confirming the importance of ATP7A for Cu absorption and homeostasis (33,34).

Remarks

In the intestine, intracellular trafficking of Mg, Zn and Cu from the apical membrane to the basolateral membrane of enterocytes has not yet been fully defined. Furthermore, the molecular mechanisms regulating the homeostatic relationship between these essential metals are not well understood. It has long been known that high levels of dietary Zn can inhibit Cu absorption and *vice versa*. ZIP4 specifically transports Zn, while CTR1 specifically transports Cu. Therefore this suggests that the mutual antagonism between Zn and Cu likely occurs outside of membrane transport processes. Since a heterodimer forms between TRPM6 and TRPM7 allowing Zn to permeate cells (11), it can be hypothesized that Mg and Zn homeostasis and metabolism might be cross-talked beyond expectation. Further investigation into these issues will lead to a more comprehensive understanding of metal absorption and contribute to a more complete picture of human health.

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Figure legend

Fig. 1. Model of intestinal absorption of Mg, Zn and Cu.

TRPM6 localized to the apical membrane takes Mg up into the enterocytes. In this process, TRPM7 may function with TRPM6 by forming heterodimers. Transport proteins exporting Mg out of enterocyte into the blood circulation across the basolateral membrane have not been identified. ZIP4 localized to the apical membrane mobilizes Zn into the enterocytes. The mobilized Zn is exported into the circulation via ZnT1 localized to the basolateral membrane. CTR1 mobilizes Cu into enterocytes after or concomitant with reduction of Cu^{2+} to Cu^{+} by unknown metalloreductase(s) at the apical membrane. Cu taken up by CTR1 is exported into the circulation by exocytosis of Cu loaded vesicles, which is mediated by ATP7A following Cu transfer by cytosolic Cu chaperone Atox1.

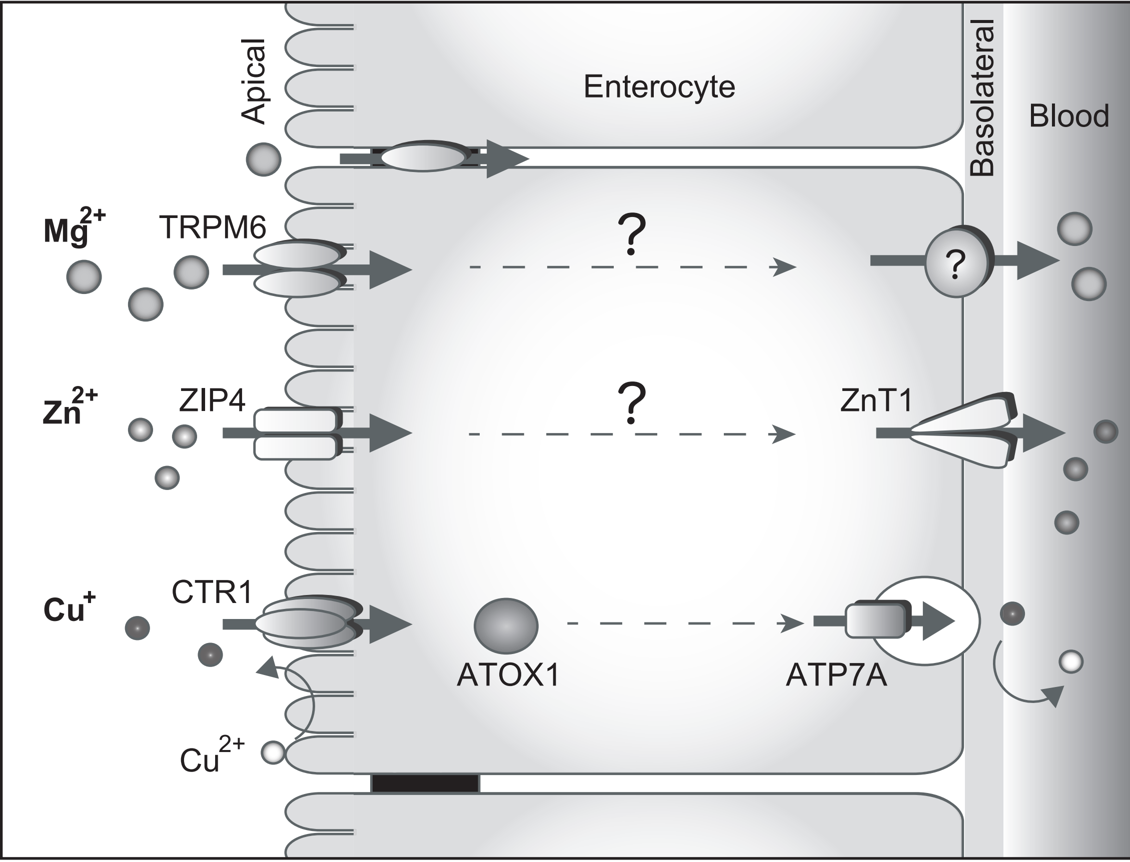

Fig.1 Hashimoto