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Review

Absorption Mechanisms of Iron, Copper, and Zinc: An Overview

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Summary Essential trace elements play pivotal roles in numerous structural and catalytic functions of proteins. Adequate intake of essential trace elements from the daily diet is indispensable to the maintenance of health, and their deficiency leads to a variety of conditions. However, excessive amounts of these trace elements may be highly toxic, and in some cases, may cause damage by the production of harmful reactive oxygen species. Homeostatic dysregulation of their metabolism increases the risk of developing diseases. Specific transport proteins that facilitate influx or efflux of trace elements play key roles in maintaining the homeostasis. Recent elucidation of their crucial functions significantly facilitated our understanding of the molecular mechanisms of iron (Fe), copper (Cu), and zinc (Zn) absorption in the small intestine. This paper summarizes their absorption mechanisms, with a focus on indispensable functions of the molecules involved in it, and briefly discusses the mechanisms of homeostatic control of each element at the cellular and systemic levels.

Key Words iron, copper, zinc, transporter, intestinal epithelial cells

Essential trace elements, including iron (Fe), copper (Cu), and zinc (Zn), are important for a variety of physiological functions in all living organisms. These elements play critical roles as a cofactor or a structural component for numerous enzymes and proteins involved in many biological processes. Though, they are required in minor quantities, they are important and their deficiency can lead to a variety of disorders. For example, iron deficiency leads to anemia and zinc deficiency causes dermatitis and taste disorder (*1*, *2*). However, excessive intake of these elements can lead to toxicity. For example, the iron overload disorder, hemochromatosis, is very common and is characterized by iron deposition in the liver resulting in fibrosis, while surplus amounts of copper lead to the progression of liver damage and neurological dysfunction because of the generation of hydroxyl radicals through Fenton-type reactions (*1*, *3*, *4*). Thus, it is important to tightly control the homeostasis of each metal, in particular, iron, copper, and zinc, at both systemic and cellular levels. Each metal is taken in from the apical side of intestinal epithelial cells and exported to the portal blood for its delivery to the peripheral tissues, in which specific transport proteins are equipped for its mobilization across the biological membranes (Table 1). Moreover, specific chaperones facilitate the vectorial transport of iron and copper in intestinal epithelial cells. Thus, these molecules play key regulatory roles in maintaining systemic and cellular homeostasis of the elements. This article briefly summarizes the intestinal absorption mechanisms of two redox metals, iron and copper, and one non-redox metal, zinc, focusing on the functions of molecules involved in their uptake or export. The molecular mechanisms regulating the metabolism of these three metals in generic cells and other specific cells have been described in many reports, and hence is not discussed here in detail (*5*–*11*).

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Iron Absorption

Iron is important for various cellular proteins, including the oxygen transport protein, hemoglobin, and redox enzymes involved in electron transfer. Because of these critical roles in our body, all life would cease to exist without iron. Iron deficiency results in anemia. Conversely, since free iron is very toxic when present in excess, iron overload causes severe consequences in the body, including liver damage, fibrosis, cancer, and heart failure known as a hemochromatosis (*1*). As a result, iron levels must be tightly regulated, both at the cellular level and systemically. Since mammals have no obvious physiological mechanism for excretion of excess iron (iron can be excreted by sloughing mucosal cells and by blood loss), the systemic iron homeostasis is primarily controlled by regulating the balance of iron absorption in the small intestine, and storage in the peripheral tissues (*12*, *13*).

Dietary iron exists in two forms: non-heme (inorganic) iron and heme iron. Non-heme iron is mainly found in plant foods, such as vegetables and seaweed, whereas heme iron is mainly present in animal foods, such as meat and fish (*14*). Both heme and non-heme iron are taken up on the apical brush border membrane of the small intestine by an independent pathway (Fig. 1A). Dietary non-heme iron (mostly ferric, Fe^{3+}) is taken up by the divalent metal transporter 1 (DMT1) in intestinal epithelial cells, which is a proton-coupled transporter located on the apical membrane (*15*).

Table 1. Properties of transporters involved in absorption of iron, copper, and zinc in intestinal epithelial cells.

Protein name	Gene name	Physiological substrate	Transmembrane helices[1]	Multimeric complex[2]	Length of human protein (A.A.)	Localization in the cells	Reference
DMT1	*SLC11A2*	Iron	11 or 12	Dimer	561	Apical membrane	(*15*, *89*, *90*)
FPN	*SLC40A1*	Iron	12	Dimer	571	Basolateral membrane	(*25*, *91*)
CTR1	*SLC31A1*	Copper	3	Trimer	190	Apical membrane	(*92*)
ATP7A	*ATP7A*	Copper	8	Monomer	1,500	TGN, Cytosolic vesicles, Basolateral membrane	(*45*, *46*, *93*)
ZIP4	*SLC39A4*	Zinc	8	Dimer	647	Apical membrane	(*59*, *72*, *94*)
ZNT1	*SLC30A1*	Zinc	6	Dimer	507	Basolateral membrane	(*59*, *95*)

[1,2] Putative structure, which is predicted by the 3D structure of bacterial homologues, and partial structure.

The ferric form (Fe^{3+}) has to be reduced to the ferrous form (Fe^{2+}) by a ferrireductase duodenal cytochrome B (DCYTB), before its uptake by DMT1 because DMT1 transports Fe^{2+} but not Fe^{3+} (*16*). The critical function of DMT1 in iron uptake is clearly explained by mutant and knockout (KO) animals. The G185R mutation in the *Dmt1* gene is responsible for severe anemia in the microcytic anemia (*mk*) mouse and the anemic Belgrade (*b*) rat (*17*, *18*). Moreover, the intestine-specific (conditional) *Dmt1*-KO mouse develops severe hypochromic microcytic anemia due to impaired intestinal iron absorption (*19*, *20*). The iron, taken up into the intestinal epithelial cells, is delivered to the cytosolic iron storage protein, ferritin. Ferritin consists of 24 heavy (H) and light (L) subunits, in which the ferrous iron (Fe^{2+}) is oxidized to ferric iron (Fe^{3+}) by the ferroxidase activity of the ferritin H-subunit (*8*, *9*, *21*). The delivery of iron to ferritin is mediated by an iron chaperone, poly C binding protein 2 (PCBP2) (*22*). Recent studies indicate that PCBP2 also binds to DMT1 and ferroportin (FPN), the iron exporter at the basolateral membrane (*23–25*). Thus, PCBP2 is considered to be responsible for delivering iron from the apical side to the basolateral side of the intestinal epithelial cells. Since, FPN is a major cellular iron exporter from intestinal epithelial cells to the portal vein, the conditional deletion of Fpn in the intestine causes accumulation of iron in the cells, and severe iron deficiency anemia (*26*). Ferrous iron (Fe^{2+}) exported by FPN is rapidly oxidized to ferric iron (Fe^{3+}) by hephaestin, a multicopper ferroxidase, and then Fe^{3+} is bound to transferrin for delivery to various tissues via circulation (*27*).

In contrast to the defined pathway of non-heme iron uptake, that of heme iron is obscure. Two heme transport proteins have been proposed thus far for its uptake: heme carrier protein 1 (HCP1) and heme responsive gene-1 (HRG-1). HCP1 has been identified to be involved in heme absorption; however it has been revealed that HCP1 exhibits high affinity for folate, and thus, rather functions as a folate transporter (*28*). A recent study has revealed that HRG-1 has high affinity for heme and may mediate heme transport into the cytosol via the endocytosis pathway (*29*). Then, heme is degraded by heme oxygenase and generates ferrous iron (Fe^{2+}), which is subsequently metabolized in the same pathway as that of the non-heme iron (*30*). Further investigation is needed to fully understand the heme absorption process.

To maintain iron homeostasis, strict regulations of the systemic balance of iron storage, distribution, and utilization are essential where hepcidin is a primary regulator of iron homeostasis. Hepcidin is a 25 amino-acid peptide hormone, synthesized and secreted by the liver, which controls FPN expression by mediating degradation of FPN via direct binding (*31*, *32*). Excessive increase in iron levels stimulates the expression of hepcidin, which degrades FPN in intestinal epithelial cells, leading to reduction of the plasma iron. In contrast, the hepcidin level is decreased in the iron-deficient condition, thereby sustaining FPN expression, and thus delivering iron to the plasma (*33*). Apart from intestinal epithelial cells, FPN is also highly expressed in macrophages and hepatocytes, both of which are essential for iron recycling, because after an average lifespan of 120 d, erythrocytes are phagocytosed and degraded by macrophages, and surplus iron is stored in the liver as ferritin (*34*). Hence, iron transport into plasma from dietary sources and from recycled sources is regulated by hepcidin.

Copper Absorption

Copper is a critical functional component of a number of essential enzymes such as superoxide dismutase (SOD) in the cytosol, cytochrome C oxidase (CCO) in the mitochondria, and tyrosinase and dopamine β-hydroxylase in the secretory compartments (*35*). On the other hand, like iron, excess amounts of copper, are also toxic as it is a potential generator of free radicals via Fenton chemistry (*3*, *5*). Thus, copper homeostasis must also be strictly regulated in the systemic, cellular, and subcellular levels as dysregulation causes severe consequences such as Menkes disease, characterized by copper deficiency and Wilson disease by excessive accumulation of copper (*4*).

Dietary cupric copper (Cu^{2+}) needs to be reduced to cuprous copper (Cu^{+}) before uptake across the apical membrane by copper transporter 1 (CTR1), a high-affinity copper uptake transporter (*36*) (Fig. 1B). The reduction is thought to be mediated by several reduc-

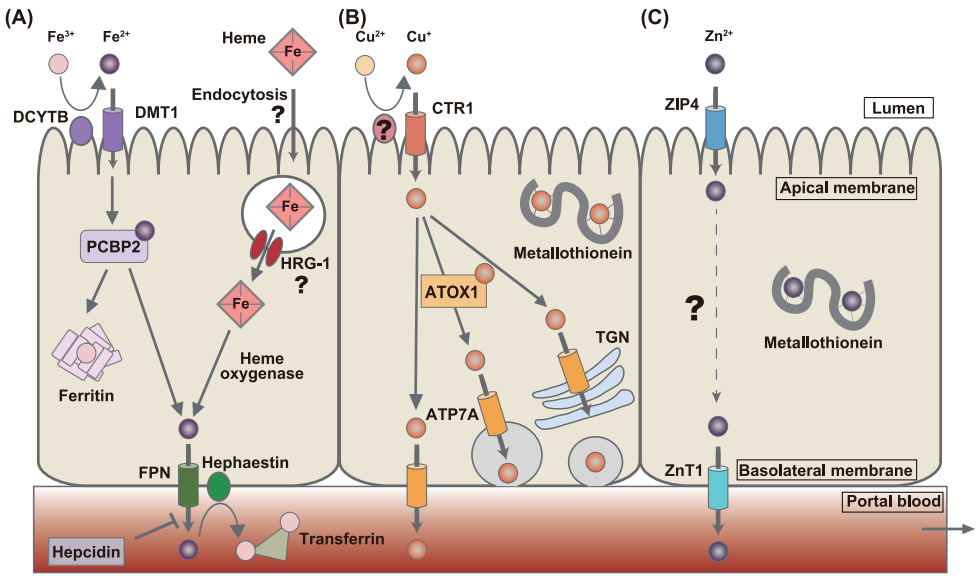

Fig. 1. Molecules involved in the absorption of iron, copper, and zinc in the intestinal epithelial cells. (A) Dietary non-heme ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}) by DCYTB and taken up by DMT1 at the apical membrane. After this uptake, iron is stored in the ferritin or conveyed to the basolateral membrane by iron chaperon PCBP2, and then is exported to the portal blood by FPN and oxidized to ferric iron (Fe^{3+}) by hephaestin. Exported iron is bound to transferrin and delivered to the various peripheral tissues. Heme may be taken up by HRG-1 via the endocytosis pathway. After the uptake, heme is degraded by heme oxygenase. The iron released from heme is transported to the portal blood by FPN in the same manner as that for the non-heme iron. When the iron level is increased, hepcidin degrades FPN, leading to reduction of the plasma iron levels. (B) Dietary copper (cupric form, Cu^{2+}) is probably reduced by several reductases and taken up by CTR1 at the plasma membrane. After the uptake, copper is transferred to ATOX1, and then delivered to ATP7A for export to the portal vein. ATP7A may transport copper to the TGN or vesicles to exocytose it to the portal blood, or may directly export copper at the basolateral membrane. Exported copper is transported to the liver. Cytosolic excess copper binds to metallothionein for reducing copper toxicity. (C) Dietary zinc is taken up by ZIP4, and is delivered to the basolateral membrane or bound to the metallothionein; the molecular mechanism behind this process has not yet been elucidated. Zinc is exported to the portal vein by ZNT1, and delivered to the peripheral tissues.

tases such as ferrireductase, DCYTB, and STEAP2 metalloreductase (*37*, *38*). Cuprous copper (Cu^{+}) is taken up by CTR1, which localizes to the apical membrane, and early endosomes in the intestinal epithelial cells (*39*). The cell surface expression of CTR1 is likely to be regulated by cellular copper levels: excess copper promotes clathrin-mediated endocytosis of CTR1, whereas copper deficiency restores the CTR1 expression on the apical membrane (*40*, *41*). The intestinal epithelial cell-specific *Ctr1*-KO mice show severe copper deficiency (*42*, *43*). These evidences demonstrate a crucial role of CTR1 for copper acquisition. In intestinal epithelial cells, the copper chaperone, antioxidant-1 (ATOX1), shuttles copper to the copper-transporting ATPase, ATP7A, which exports copper into the portal blood (*6*, *44*). Mutations in *ATP7A* gene are associated with Menkes disease, an X-linked recessive copper deficiency disorder characterized by neurological defects, growth failure, and kinky hair (*45*, *46*). ATP7A is normally located to the *trans*-Golgi network, but in response to high extracellular copper, it is known to relocate to the cytosolic vesicles and undergo trafficking to the basolateral membrane (*47*–*50*). A possible mode is that ATP7A may mobilize copper into vesicles, which then fuses with the basolateral membrane to release it for export. Moreover, ATP7A may directly export copper across the basolateral membrane (*7*). However, how copper is exported to the portal blood is not yet completely characterized.

Copper exported from the intestinal epithelial cells binds to albumin or α_2-macroglobulin in the blood and is transported to the liver, where copper loading onto ceruloplasmin occurs for systemic circulation (*51*, *52*). Ceruloplasmin binds 95% of copper in serum (*53*); this copper loading is critical and mediated by another copper-transporting ATPase, ATP7B (*49*). ATP7B is important for copper excretion from the liver, and therefore, mutations in ATP7B lead to Wilson disease, which is characterized by hepatic and neurological disorder caused by copper overload (*54*).

In cellular homeostasis, copper chaperones like ATOX1 play pivotal roles. ATOX1 delivers copper to ATP7A and ATP7B, both of which are located to the *trans*-Golgi network, and thus are functional for facilitating copper transport into the lumen of the organelles (*55*). In addition, other copper chaperones, such as CCS and COX17, are essential for copper metabolism. The former is functional for loading copper to the SOD1, while the latter is necessary for copper mobilization into the mitochondria (*56*, *57*). Excess copper in the cytosol binds to the metallothionein, thereby reducing free copper ions, which is thought to be important for avoiding the toxicity caused by free copper ions (*58*).

Zinc Absorption

Zinc is present as a divalent cation and does not require a redox reaction during the membrane transport process, as observed for iron and copper metabolism. Thus, expression of zinc transporters under strict spatiotemporal regulation is crucial for the membrane transport of zinc for maintaining systemic and cellular zinc homeostasis. Zinc influx and efflux are controlled by two zinc transporter families, Zn transporter (ZNT) and Zrt-, Irt-related protein (ZIP). To date, 9 ZNT, and 14 ZIP transporters have been identified, which is larger in number than those of iron and copper transporters (*59–61*). Among these transporters, ZIP4 is essential for uptake of dietary zinc on the apical membrane in intestinal epithelial cells (*62*, *63*) (Fig. 1C), and thus, mutations in ZIP4 result in the occurrence of acrodermatitis enteropathica (AE), a rare genetic recessive disorder associated with zinc deficiency (*64–66*). AE patients are characterized by acral dermatitis, alopecia, and diarrhea (*66*). The importance of ZIP4 in zinc homeostasis is confirmed by using intestine-specific *Zip4*-KO mice that die unless fed with a high-zinc diet (*67*). Moreover, AE patients with symptoms of zinc deficiency are treated with high-dose oral zinc supplementations (*68*). These facts raise the possibility that other transporters may contribute towards the uptake of zinc into the intestinal epithelial cells, but a secondary zinc transporter has not yet been identified.

ZIP4 expression is tightly regulated by cellular zinc at the post-translational level. Zinc deficiency causes stabilization of *ZIP4* mRNA, resulting in ZIP4 protein accumulation on the apical membrane (*69*). If excess zinc is added, the surface accumulated ZIP4 under zinc deficiency is rapidly internalized by endocytosis and degraded via the ubiquitin proteasome pathway, thereby suggesting that the ZIP4 protein escapes from its degradation when the zinc level is decreased (*70*, *71*). Importantly, ZIP4 protein accumulation on the apical membrane by zinc deficiency is rapid. For instance, Zip4 accumulation is detected in rat jejunum by immunoblotting as early as one day following a zinc-deficient diet (*63*). Under prolonged zinc deficiency, the extracellular amino-terminal domain of ZIP4 protein, which was recently shown to form homodimers (*72*), is proteolytically cleaved (processed), and consequently, the ZIP4 protein lacking an amino-terminal portion accumulates on the apical membrane (*63*, *66*, *67*).

Zinc taken up by the intestinal epithelial cells is thought to be exported to the portal blood by ZNT1, although it has not yet been directly demonstrated (*73*). This idea is supported by the evidence that ZNT1 ortholog in Drosophila (dZnt1) is localized to the basolateral membrane of the intestinal epithelial cells and plays a key role in zinc export (*74*). *ZNT1* mRNA expression is upregulated by excess zinc content. The *ZNT1* promoter has the metal-response element for the binding site of metal-response element-binding transcription factor-1 (MTF-1), which is responsible for metal-induced transcription (*75*, *76*). However, how upregulation of *ZNT1* mRNA contributes to ZNT1 protein expression on the basolateral membrane remains unknown. Zinc exported by ZNT1 from the intestinal epithelial cells into the portal vein binds to albumin and α_2-macroglobulin for delivery to the peripheral tissues. Zinc accumulated in the intestinal epithelial cells can be excreted through their sloughing (*60*, *77*).

During the zinc absorption process, the process of zinc trafficking from the apical membrane (ZIP4) to the basolateral membrane (ZNT1) in the intestinal epithelial cells is yet to be understood. The cytosolic zinc binds to metallothionein (*78*) or is mobilized into the vesicles, which may be involved in the transcellular trafficking (*79*). A zinc chaperone, like PCBP2 in iron absorption or ATOX1 in copper absorption, might be operative in zinc absorption as well. Further investigations are needed to clarify this point.

Conclusion and Perspectives

Recent progress reveals that the absorption of iron, copper, and zinc occurs by means of a sophisticated control system in which unique transport proteins are operative for each metal. Unintended imbalance in the concentrations of these metals can lead to deficiency or overload disorders as described above, which may cause some diseases. For example, excess zinc is known to induce copper deficiency, leading to reduction of iron absorption, which eventually results in anemia (*80*, *81*). Moreover, intricate interactions between iron, copper, and zinc have been found, although the molecular mechanisms underlying these interactions are not yet known (*11*). Several transporters involved in iron, copper, and zinc metabolism may be involved in the absorption of other essential trace elements. Recent studies reveal that manganese mobilization is conducted by some iron and zinc transporters (*82–85*), and thus, this non-substrate element might affect the absorption efficiency of iron and zinc. Further investigation is required to clarify the interactions and relationships between transporters and substrates in the absorption processes.

Among iron, copper, and zinc, deficiency of iron and zinc is responsible for serious and widespread nutritional disorders in the world. Thus, to increase their absorption, various strategies have extensively been explored—specifically, consumption of iron/zinc fortified cereals, reduction of inhibitors (e.g., phytic acid) for better iron/zinc absorption, and dietary factors that have positive effects on absorption (*86–88*). Complete understanding of their absorption processes at the molecular level would significantly facilitate development of strategies for preventing their deficiencies.

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