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The Genetics of Essential Metal Homeostasis During Development

Taiho Kambe, Benjamin P. Weaver, and Glen K. Andrews*

Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas

Summary

The essential metals copper, zinc, and iron play key roles in embryonic, fetal, and postnatal development in higher eukaryotes. Recent advances in our understanding of the molecules involved in the intricate control of the homeostasis of these metals and the availability of natural mutations and targeted mutations in many of the genes involved have allowed for elucidation of the diverse roles of these metals during development. Evidence suggests that the ability of the embryo to control the homeostasis of these metals becomes essential at the blastocyst stage and during early morphogenesis. However, these metals play unique roles throughout development and exert pleiotropic, metalspecific, and often cell-specific effects on morphogenesis, growth, and differentiation. Herein, we briefly review the major players known to be involved in the homeostasis of each of these essential metals and their known roles in development.

Keywords

copper; development; genetics; homeostasis; iron; knockout mice; review; zinc

REGULATION OF COPPER HOMEOSTASIS

Copper plays a critical role in metabolism as a cofactor for several cupric enzymes, including cytochrome *c* oxidase (CCO), lysyl oxidase, tyrosinase, copper/zinc superoxide dismutase (SOD1), and ferroxidases (Pena *et al.*, 1999; Prohaska and Gybina, 2004; Shim and Harris, 2003). However, copper is a potentially toxic metal because of its ability to redox cycle and support Fenton chemistry leading to the production of free radicals (Halliwell and Gutteridge, 1984). Therefore, copper homeostasis is very tightly regulated (see Fig. 1). Copper deficiency stimulates the absorption of copper by the intestine (Kuo *et al.*, 2001), but the mechanisms of uptake of dietary copper are not understood (Maryon *et al.*, 2007). CTR1 (Slc31a1), a high affinity, trimeric copper transport protein, is essential for acquisition of dietary copper (Nose *et al.*, 2006). CTR1 has been localized to basolateral and intracellular membranes of enterocytes in the adult (Kuo *et al.*, 2006; Zimmicka *et al.*, 2007), and thus does not appear to play a role in the uptake of dietary copper in the adult, although it has been localized to the apical surface of the enterocyte in the suckling pup (Kuo *et al.*, 2006). Intestine-specific *Ctrl*-knockout mice accumulate copper in the intestine (Nose *et al.*, 2006). The subcellular localization and

* Correspondence to: Dr. G.K. Andrews, Department of Biochemistry and Molecular Biology, Mail Stop 3030, University of Kansas Medical Center, 39th and Rainbow Blvd., Kansas City, KS 66160-7421. E-mail: gandrews@kumc.edu.

NOTE ADDED IN PROOF

A recent report (Galy *et al.*, 2008) demonstrated that mice lacking only intestinal IRP expression develop malabsorption and dehydration and die before weaning. Those studies also revealed that IRPs control the expression of DMT1 and ferroportin in the duodenum. (Galy B, Ferring-Appel D, Kaden S, Grone HJ, and Hentze MW. 2008. Iron regulatory proteins are essential for intestinal function and control key iron absorption molecules in the duodenum. *Cell Metab* 7: 79–85).

abundance of CTR1 are cell-type specific (Klomp *et al.*, 2002; Kuo *et al.*, 2006). Copper is exported from enterocytes into the blood stream via the ATP7A P-type ATPase transporter which is localized to the basolateral membrane and secretory pathway (Hamza *et al.*, 2003; Nyasae *et al.*, 2007). The importance of ATP7A in copper homeostasis is revealed by the fact that copper export from enterocytes is impaired in patients with Menkes disease (MNK) and in mice with mutations in the *Atp7a* gene (*mottled/brindled* mouse), resulting in a severe copper deficiency phenotype (Mercer, 1998). Copper homeostasis is further regulated by the liver which sequesters copper absorbed from the diet (Lutsenko *et al.*, 2007). Hepatocytes are important for copper loading into ceruloplasmin (CP), a ferroxidase, which is the primary copper-binding protein in serum, and excess copper is excreted into the bile (Lutsenko *et al.*, 2007; Shim and Harris, 2003). Both of these processes are regulated by ATP7B P-type ATPase transporter. Patients with Wilson's disease (WND) have mutations in *Atp7b* resulting in the hyperaccumulation of copper in the liver because of impaired excretion of copper into the bile and secretory pathway (where copper is loaded into CP).

Copper deficiency in neonatal mice leads to increase levels of CTR1 in several organs (Kuo *et al.*, 2006) whereas highly elevated copper stimulates its rapid endocytosis and degradation (Petris *et al.*, 2003). In the mammary gland, prolactin increases the abundance and plasma membrane localization of CTR1 and ATP7A (Kelleher and Lonnerdal, 2006a). Copper stimulates the trafficking of ATP7A and ATP7B proteins, which are localized to the *trans*-Golgi network (TGN) when copper concentrations are normal, and are trafficked to cytosolic vesicles as copper increases. ATP7A is further trafficked to the plasma membrane in response to extreme concentrations of copper whereas ATP7B apparently is not (Kim and Petris, 2007; Lutsenko *et al.*, 2007).

Copper inside the cell is distributed to three major destinations: SOD1 in the cytosol, CCO in mitochondria, and cupric enzymes in the TGN (Prohaska and Gybina, 2004). Specific copper chaperones have essential functions in the distribution of intracellular copper (Fig 1, see GENERIC CELL). CCS, the copper chaperone for SOD delivers copper specifically to SOD1 and the copper chaperone ATOX1 interacts with ATP7A and ATP7B and delivers copper into the TGN. The assembly of CCO is a complex process that involves several copper chaperones (Cobine *et al.*, 2006; Hamza and Gitlin, 2002; Horng *et al.*, 2005; Sacconi *et al.*, 2005). It is thought that COX17, and perhaps the structurally related COX19 and COX23/MTCP1 (Barros *et al.*, 2004; Sacconi *et al.*, 2005; Schoenfeld *et al.*, 2005), transfer copper to the mitochondrial inner membrane. COX17 transfers copper to both COX11 and SCO1 which participate in the assembly of CCO (Cobine *et al.*, 2006; Horng *et al.*, 2005). Studies of Bedlington Terriers with copper toxicosis resembling WND lead to the identification of COMMD1 (MURR1) (Van de Sluis *et al.*, 2002) which directly interacts with ATP7B and regulates its stability (de Bie *et al.*, 2007; Tao *et al.*, 2003). Regulation of the ubiquitin pathway may account for many functions of the COMMD protein family (Maine and Burstein, 2007). It was recently discovered that X-linked Inhibitor of Apoptosis (XIAP) regulates COMMD1 protein levels via its ubiquitin E3 ligase activity, and therefore functions as a modulator of copper homeostasis (Burstein *et al.*, 2004). XIAP directly binds copper leading to a conformational change and causing decreased stability of the protein and loss of XIAP's caspase blocking activity (Mufti *et al.*, 2007). This feedback regulation may be important for copper homeostasis.

COPPER HOMEOSTASIS AND DEVELOPMENT

Copper is required for normal development (Keen *et al.*, 1998; Madsen and Gitlin, 2007) and dietary copper deficiency during embryonic development can be teratogenic and embryotoxic. Mouse embryos with swollen hind brains and offspring with severe neurological impairment and organogenesis defects in multiple tissues (severe connective tissue abnormalities, skeletal defects, lung abnormalities, etc.) are observed in offspring from copper deficient dams (Keen

et al., 1998). The extent and timing of copper deficiency dictate the severity and tissue-specificity of the effects on the embryo, fetus, and newborn.

Mutations in genes that control copper homeostasis accentuate the multiple roles of copper during embryogenesis and early development (Table 1). This topic has been the subject of recent reviews (Madsen and Gitlin, 2007; Shim and Harris, 2003). *CTR1* and *Cox17* homozygous knock-out mice both die around E8.5–E10.5 suggesting that the acquisition of copper by the embryo becomes critical during gastrulation and mesoderm formation (Kuo *et al.*, 2001; Lee *et al.*, 2001; Takahashi *et al.*, 2002). As discussed later, the acquisition of zinc and iron by the embryo also becomes essential at about this stage of mouse development. It is interesting to note, however, that *CTR1* has recently been shown to play roles in coordinating morphogenesis and progenitor cell fate, and in regulating embryonic stem cell differentiation (Haremaki *et al.*, 2007). In *Xenopus laevis*, *CTR1* both promotes differentiation and inhibits the morphogenesis of mesoderm and neurectoderm, perhaps by activating the Ras-MAP kinase cascade (Haremaki *et al.*, 2007). Similar concepts regarding roles of zinc in regulating signal transduction cascades have recently emerged (Beyersmann and Haase, 2001; Haase and Maret, 2005; Haase and Rink, 2007; Klein *et al.*, 2002; Yamasaki *et al.*, 2007).

Mutations in *Atp7a* in *mottled/brindled* mouse strains lead to three distinct phenotypes which reflect differences in copper transport and trafficking functions of the ATP7A protein (Kim and Petris, 2007). An early embryonic lethal phenotype of *Atp7a* (mo11H) reflects a lack of copper delivery to the secretory pathway, whereas perinatal lethality of *Atp7a* (moMacular) reflects decreased copper delivery to the secretory pathway and constitutive localization to the plasma membrane (Kim and Petris, 2007). ATP7A plays a role in brain development. It is abundant in axons of the neonatal mouse brain but absent from adult axons (El Meskini *et al.*, 2005), and in the *mottled/brindled* mouse ATP7A deficiency in Purkinje cells and olfactory sensory neurons is associated with impaired synaptogenesis and axonal targeting (El Meskini *et al.*, 2007; Niciu *et al.*, 2007). In *Drosophila*, the orthologous copper transporter DmATP7 is essential for early larval development and adult pigmentation (Norgate *et al.*, 2006). In zebrafish, copper plays a key role in development of the notochord. Mutations in the orthologue of *Atp7a* (*calamity*) result in copper deficiency phenotypes, and *Atp7a* gene dosage determines the sensitivity to copper deficiency consistent with the concept that suboptimal copper can contribute to birth defects (Mendelsohn *et al.*, 2006).

The functions of the newly identified COMMD1 and XIAP in copper homeostasis during development remain to be clarified, but current information suggests links between the control of apoptosis, hypoxia, copper homeostasis, and the ubiquitin pathway. Although *Xiap* knockout mice display no overt phenotype (Harlin *et al.*, 2001), cells from these mice contain reduced copper levels and modestly elevated levels of COMMD1 (Burstein *et al.*, 2004). In contrast, *Commd1* knockout embryos die on E9.5–E10.5, similar to the timing of lethality of *Ctrl* and *Cox17* knockout embryos, as mentioned earlier (Van de Sluis *et al.*, 2007), but *Commd1* knockout embryos display heightened expression of hypoxia-inducible factor-1 (HIF-1) target genes, because of increased stability of HIF-1 and an absence of placental vascularization (Van de Sluis *et al.*, 2007). The role of copper homeostasis in these processes remains obscure.

The proper transfer of copper from the mother to the developing embryo and neonate is also of critical importance. *Toxic milk* mice and mice with targeted mutations in *Atp7b* produce copper deficient breast milk, leading to neurological abnormalities, growth retardation, and death of the suckling pup (Buiakova *et al.*, 1999). Similarly, the conditional knockout of *Ctrl* in the neonatal intestine is lethal before weaning, but this phenotype can be ameliorated by copper administration (Nose *et al.*, 2006).

The copper chaperone ATOX1 plays a critical role in perinatal copper homeostasis (Hamza *et al.*, 2001), and about half (45%) of homozygous *Atox1* knockout offspring die before weaning while surviving pups fail to grow, have loose skin, hypopigmentation, and seizures. Maternal *Atox1* deficiency exacerbates the severity of the copper deficiency phenotype of homozygous offspring (Hamza *et al.*, 2001). *Atox1* knockout cells accumulate excess intracellular copper because of impaired efflux, and ATOX1 plays an essential role in the copper-mediated trafficking of ATP7A (Hamza *et al.*, 2003).

Mutations in *Sco1/Sco2* copper chaperone genes have been documented in humans. Mutations in *Sco2* may cause fatal infantile cardioencephalomyopathy and CCO deficiency (Papadopoulou *et al.*, 1999), whereas mutations in *Sco1* are associated with neonatal-onset liver failure and encephalopathy (Valnot *et al.*, 2000). It is interesting to note that these phenotypes differ from patients with other mutations that affect CCO assembly. This suggests that SCO1 and SCO2 may have functions in addition to CCO assembly.

Almost all of the copper in blood is bound to CP but mutations in the *cp* gene are not associated with defective copper metabolism, but rather have been associated with defective iron metabolism. In humans CP deficiency has been associated with late-onset retinal and basal ganglia degeneration (Harris *et al.*, 1995) and increased iron accumulation in the liver and other tissues. This role for CP in iron metabolism reflects its function as a copper-containing ferroxidase, and mice with CP deficiency have impaired intestinal uptake of iron (Cherukuri *et al.*, 2005).

REGULATION OF ZINC HOMEOSTASIS

Zinc serves structural and/or catalytic roles in hundreds of peptides (Berg and Shi, 1996; Krishna *et al.*, 2003; Ravasi *et al.*, 2003; Vallee and Auld, 1990) and is the second most abundant essential metal. Therefore, when zinc is deficient numerous cellular processes are affected. Zinc deficiency during development is highly teratogenic and embryotoxic, and the outcomes are highly variable depending on the extent and timing of the zinc deficiency. Zinc is also toxic when in excess. Therefore, the maintenance of zinc homeostasis is critical and multiple genes have evolved to modulate the storage (4 *Metallothionein* genes in mice), efflux (10 *Znt* genes), and uptake (14 *Zip* genes) of this metal in response to zinc availability (see Fig. 2). In addition, recent studies suggest the possibility that zinc may be stored in and released from intracellular vesicular compartments (Devirgiliis *et al.*, 2004; Yamasaki *et al.*, 2007). The zinc transporters of higher eukaryotes are often expressed in a tissue-specific manner and in specific cellular localizations. In addition, they can display specific changes in cellular localization and stability in response to zinc deficiency or excess (Cousins *et al.*, 2006; Eide, 2006; Kambe *et al.*, 2004). The diverse roles of these molecules in zinc homeostasis are only beginning to be understood and much remains to be determined.

When zinc is replete in the diet, it is absorbed from the gut by enterocytes in the small intestine, and excess zinc is released through the pancreas and small intestine, as well as in small amounts from the kidney (Hambidge and Krebs, 2001; King *et al.*, 2000; McClain, 1990). However, under zinc-deficient conditions, zinc absorption by the small intestine increases, and release from the pancreas and small intestine decreases (Hambidge and Krebs, 2001). In the embryonic environment of the mouse, the visceral endoderm plays an important role in zinc homeostasis during peri-implantation development (Dufner-Beattie *et al.*, 2007). How animals and cells adapt to zinc status and the roles of these zinc transporters (uptake and efflux) in the adaptive process are active areas of investigation.

METALLOTHIONEINS AND MTF-1 IN DEVELOPMENT

A portion of the total cellular zinc pool (~ 5–15%) can be bound by the cysteine-rich metallothioneins (MT) (Coyle *et al.*, 2002). In the mouse, there are four MT family members, and in humans there are eleven (Kagi, 1991). *Mt* genes (*Mt-I* and *Mt-II* in mice) are zinc-regulated by the transcription factor MTF-1 (Laity and Andrews, 2007); in the presence of high zinc their transcription rate is increased (see Fig. 2). In contrast, under low zinc conditions, MT levels decrease because of a lower transcription rate and protein destabilization (Andrews, 1990). Although *Mt* genes are nonessential in mice, MTs appear to function as a “zinc buffer” that can provide a labile pool of zinc for use by other proteins when zinc is limiting (Krezel and Maret, 2007; Maret and Krezel, 2007). Deletion of *Mt-I* and *Mt-II* genes sensitizes mice to the teratogenic effects of zinc deficiency during pregnancy (Andrews and Geiser, 1999), whereas overexpression of *Mt-I* dramatically protects mice from these effects (Dalton *et al.*, 1996) (Table 2).

The zinc-finger transcription factor MTF-1 regulates basal and metal-induced expression of mammalian *Mt* genes (Lichtlen and Schaffner, 2001). Deletion of the *Mtf-1* gene in mice is lethal at E14 because of degeneration of the liver (Günes *et al.*, 1998). This appears to be a cell-autonomous effect (Wang *et al.*, 2004), but its relationship with zinc metabolism in the embryo is unclear. MTF-1 is also essential for basal expression and zinc regulation of *Mt* genes in the visceral endoderm of the mouse embryo (Andrews *et al.*, 2001), and this tissue appears to play a key role in the acquisition of essential metals during development, as is discussed later. Interestingly, MTF-1 regulates zinc-responsiveness of the *Znt1* (a major zinc efflux transporter) gene (Langmade *et al.*, 2000) and represses expression of the *Zip10* gene, a member of the zinc uptake transporter family whose function during development is unknown (Wimmer *et al.*, 2005). These findings suggest that mammalian MTF-1 plays an important role in zinc homeostasis.

ZnT FAMILY ZINC TRANSPORTERS (Slc30a) IN DEVELOPMENT

Two superfamilies of mammalian zinc transporters have been identified that belong to the solute carrier (Slc)30a and the Slc39a families (Guerinot, 2000; Palmiter and Huang, 2004; Taylor and Nicholson, 2003). Slc30a members, named ZnTs, function in zinc efflux and compartmentalization and are cation diffusion proteins (Palmiter and Huang, 2004). There are 10 members of this family in mammals and several are known to have important functions during development. Only those are discussed herein (Seve *et al.*, 2004) (Table 2). Targeted deletion of *Znt1* results in early embryonic lethality in homozygous knockout mice (Andrews *et al.*, 2004). High levels of *Znt1* mRNA are found in the maternal deciduum and in the visceral yolk sac surrounding the embryo, which suggests that this protein is involved in the transfer of maternal zinc into the uterine and embryonic environments (Andrews *et al.*, 2004). ZnT1 is a ubiquitous zinc efflux transporter that can protect cells *in vitro* from zinc toxicity (Palmiter, 2004). The naturally occurring *lethal milk* mouse carries a nonsense mutation in the *Znt4* gene (Huang and Gitschier, 1997). These mice produce zinc-deficient milk which, in turn, causes a lethal zinc deficiency in the nursing pups. A recent study found heterozygous mutations in the human *Znt2* gene in women with low zinc concentrations in milk leading to transient zinc deficiency and dermatitis in the nursing child (Chowanadisai *et al.*, 2006). Homozygous deletion of *Znt5* results in poor growth, osteopenia, low body fat, muscle weakness, and male-specific cardiac death (Inoue *et al.*, 2002). Complexes containing ZnT5 and 6, as well as complexes containing homo-oligomers of ZnT7 function to transport zinc into the secretory pathway which activates zinc-requiring enzymes such as alkaline phosphatase (Suzuki *et al.*, 2005a,b). Our understanding of this family of zinc transporters has advanced significantly in the past few years. In contrast, we know much less about zinc uptake transporters.

ZIP FAMILY ZINC TRANSPORTERS (Slc39a) IN DEVELOPMENT

Members of the Slc39a family, named ZIPs, function in the uptake of zinc and other metals (Eng *et al.*, 1998; Guerinot, 2000; Taylor and Nicholson, 2003), and members of this family are found in all eukaryotes. In mice and humans there are 14 members of the ZIP family. The Slc39a superfamily can be subdivided into four subfamilies based on structural homology (Taylor and Nicholson, 2003). In mammals, most ZIP proteins fall into one of two subfamilies named subfamily II (3 members) and LIV-1 (9 members).

Members of subfamily II (ZIPs1–3) were the first to be described in mammals. They are well conserved, can function to transport zinc in transfection studies, and are actively expressed in a highly cell-specific manner in mice (Dufner-Beattie *et al.*, 2003a, 2005, 2006; Gaither and Eide, 2001; Huang *et al.*, 2006; Peters *et al.*, 2007). Targeted deletion of mouse *ZIP1*, 2, or 3 genes revealed that none of these genes is essential (Dufner-Beattie *et al.*, 2006; Peters *et al.*, 2007) and deletion of the entire subfamily also does not cause an obvious phenotype (Andrews *et al.*, manuscript in preparation). However, subfamily II knockout mice are dramatically more sensitive to the teratogenic effects of dietary zinc deficiency during pregnancy (Dufner-Beattie *et al.*, 2005, 2006). The zinc deficient knockout embryos are severely growth retarded and have obvious cranio-facial abnormalities and limb defects. ZIPs1, 2, and 3 appear to function specifically during adaptation to zinc deficiency consistent with the finding that these proteins are recruited to the surface in zinc deficient cells (Wang *et al.*, 2004).

Several members of the LIV-1 subfamily have interesting roles in early development. In humans, mutations in the *Zip4* gene cause the rare and fatal genetic disorder acrodermatitis enteropathica (Kury *et al.*, 2002; Wang *et al.*, 2002) which reflects an impaired ability of the intestine to absorb dietary zinc. The symptoms of this disease often appear after weaning in humans but can be ameliorated by dietary zinc supplementation (Maverakis *et al.*, 2007). In contrast, the mouse *Zip4* gene is essential during early embryonic development (E7.5–E9) and the homozygous knockout embryos fail to undergo morphogenesis (Dufner-Beattie *et al.*, 2007). Although expression of *Zip4* can be detected in the preimplantation mouse embryo, it is actively expressed only in the visceral endoderm on E7.5 when the embryo is at the egg cylinder stage of development.

Interestingly, *Zip4* heterozygosity is teratogenic and embryotoxic in mice. These effects are highly pleiotropic and include growth retardation, exencephalia, and cranio-facial abnormalities in midgestation embryos, as well as hydrocephalia, anopia (loss of eyes), heart and skeletal defects in a significant percentage of the offspring (Dufner-Beattie *et al.*, 2007). These abnormalities are largely prevented by supplementation of dietary zinc. Later during pregnancy (E14), *Zip4* gene expression in the visceral endoderm is regulated by zinc availability (Dufner-Beattie *et al.*, 2003b). During zinc deficiency, *Zip4* mRNA and protein stability are increased and ZIP4 is trafficked to the apical membrane. Repletion of zinc causes the rapid internalization and destabilization of ZIP4 in the visceral endoderm (Weaver *et al.*, 2007). This pattern of regulation mimics that seen in the adult intestine where ZIP4 serves an essential function in the uptake of dietary zinc.

The functions of other mammalian ZIP family members in development remain to be explored, but it is interesting to note that the *fear-of-intimacy* gene in *Drosophila* encodes a LIV-1-like protein that can function as a zinc transporter and that is essential during early development of the fly (Mathews *et al.*, 2005; Moore *et al.*, 1998). This gene controls germ cell migration and gonad formation which suggests a key role for zinc in these processes. Similarly, morpholino knockdown of zebrafish *zLiv-1* revealed a function in epithelial–mesenchymal transition in the gastrula (Yamashita *et al.*, 2004). *zLiv-1* is a downstream target of STAT3, and is essential for the nuclear localization of the zinc-finger protein Snail, which regulates epithelial–

mesenchymal transition. Remarkably, human ZIP6, the founding member of the LIV-1 family, plays a role in cell invasion by targeting the ERK1/2-Snail/Slug pathway (Zhao *et al.*, 2007). In addition, ZIP10 has recently been shown to play a role in breast cancer invasive behavior (Kagara *et al.*, 2007), and aberrant *Zip4* expression in pancreatic ductal carcinoma has been reported to correlate with metastatic potential (Li *et al.*, 2007). *Zip4* expression in the pancreas is normally restricted to pancreatic β -cells (Dufner-Beattie *et al.*, 2004). Thus, several members of the LIV-1 family may have fundamental functions in regulating cell migration and differentiation during early development.

ZINC SIGNALING AND ITS POTENTIAL ROLE IN DEVELOPMENT

Zinc has been suggested to function as an intracellular second messenger as well as extracellular signaling molecule. These effects may, in turn, impact development of the embryo, although this concept has not yet been supported by direct evidence (Beyersmann and Haase, 2001; Hansson, 1996; Kim *et al.*, 2000; Yamasaki *et al.*, 2007). Zinc influences signal transduction cascades in a cell-type and zinc concentration-dependent manner. For example, low micromolar concentrations of zinc inhibit hormone- or forskolin-stimulated cAMP production in neuroblastoma cells, perhaps by directly interacting with adenylate cyclase and altering the V_{\max} of the enzyme (Klein *et al.*, 2002). Similarly, zinc treatment can inhibit cyclic nucleotide phosphodiesterase activity and expression in monocytes, resulting in increased levels of cGMP, activation of protein kinase A, and subsequent inhibition of Raf-1. This leads to altered NF- κ B signaling and suppression of TNF- α production (vonBulow *et al.*, 2007). Zinc also has insulinomimetic effects and apparently small increases in intracellular zinc can inhibit protein tyrosine phosphatase 1B activity in insulin and insulin-like growth factor signaling leading to increased phosphorylation of the receptor (Haase and Maret, 2005; Samet *et al.*, 2003). Zinc also influences the EGF pathway and the NMDA receptor (cited in Samet *et al.*, 2003). In *C. elegans*, *Cdf-1*, which encodes a protein functionally similar to vertebrate ZnT-1, was identified as a positive regulator of the Ras pathway, by promoting zinc efflux and relieving repression of Ras-mediated signaling (Bruinsma *et al.*, 2002).

The release of intracellular zinc leads to the activation of 12-lipoxygenase and p38 mitogen-activated protein kinase (MAPK), which contributes to the toxicity of zinc in neurons and oligodendrocytes (Zhang *et al.*, 2007). These authors also comment that extracellular signal-regulated kinase (ERK1/2) activation, caused by glutathione depletion during oxidative stress, is mediated by the intracellular release of zinc in HT22 cells (Zhang *et al.*, 2007). A recent study of mast cells revealed that crosslinking IgE receptors induces the release of free zinc from the perinuclear area and ER, an effect dependent on calcium influx and MAPK activation, suggesting that zinc may function as a novel intracellular second messenger (Yamasaki *et al.*, 2007). Given the roles of extracellular and intracellular zinc in regulating cell signaling, it seems likely that altering zinc homeostasis at a critical time in early development would have dramatic, pleiotropic effects on morphogenesis of the embryo.

REGULATION OF IRON HOMEOSTASIS

Iron is the most abundant transition metal and is utilized in both Fe-S cluster containing proteins and proteins that contain porphyrin conjugates, primarily heme. Iron deficiency is the most common cause of anemia and iron overload results in toxicity because of increased free radical formation. In the past decade significant advances have been made in our understanding of iron homeostasis and this subject has been recently reviewed in much greater detail than can be presented herein (Andrews and Schmidt, 2007; DeDomenico *et al.*, 2008). A brief overview is presented later (see Fig. 3).

Iron is regulated primarily at the level of absorption (Andrews and Schmidt, 2007; DeDomenico *et al.*, 2008) (see Fig. 3) and is lost from the body by bleeding and sloughing of the gut epithelium. Heme-conjugated iron is a major dietary source of iron, but a high-affinity heme transporter has not been identified (Qiu *et al.*, 2006). Nonheme iron in the gut is reduced to Fe^{2+} by a ferric reductase(s) (Miret *et al.*, 2003) and transported into the enterocyte by DMT1 (Slc11a2) (DeDomenico *et al.*, 2008). Iron is transported out of the enterocyte into the circulation by ferroportin (Slc40a1) (Donovan *et al.*, 2005) where it is oxidized to Fe^{3+} by hephaestin and perhaps CP (Miret *et al.*, 2003). DMT1 is not essential for iron uptake in the fetus (Gunshin *et al.*, 2005). In fact, alternate and as yet unidentified iron uptake mechanisms exist in hepatocytes, placenta, and most other cells. Ferroportin, in contrast, plays a key role in iron export from the visceral endoderm of the yolk sac (Donovan *et al.*, 2005). In the circulation, transferrin binds Fe^{3+} and transports it throughout the body.

Transferrin receptor-mediated endocytosis is the major path by which all cells take up iron (DeDomenico *et al.*, 2008). Receptor bound transferrin undergoes endocytosis, Fe^{3+} is released from transferrin in the acidified endosome, and apo-transferrin and its receptor are recycled back to circulation and plasma membrane, respectively (Fig. 3, see GENERIC CELL). Transferrin receptor-1 is a high-affinity, ubiquitously expressed receptor; in contrast transferrin receptor-2 is expressed predominately in the liver and its function is not known (Wallace *et al.*, 2008). The released Fe^{3+} is reduced to Fe^{2+} by a member of the STEAP metalloredutase family (STEAP3 in the erythroblast) and transported out of the endosome by DMT1 (Ohgami *et al.*, 2006). In all nonerythroid cells, any free iron is chelated by ferritin. Iron is transported into mitochondria by mitoferrin (Slc25a37) (Shaw *et al.*, 2006) where it is loaded into Fe-S clusters in a frataxin-dependent process and used for heme synthesis in red blood cells (Muhlenhoff *et al.*, 2002). Mitochondria also export a substrate essential for cytosolic Fe-S cluster assembly via the ATP-binding cassette transporter ABCB7 complex (Burke and Ardehali, 2007; Lill *et al.*, 2006). The heme in red blood cells represents the largest pool of iron in the body. Heme iron is recycled by macrophages of the reticuloendothelial system which phagocytose aged red blood cells (see Fig. 3). Iron is transported out of the lysosome by NRAMP1 (Slc11a1) (Lam-Yuk-Tseung *et al.*, 2006) and ultimately released from the macrophage by ferroportin as Fe^{2+} which is then oxidized by CP and bound again by transferrin (Andrews and Schmidt, 2007). Macrophages play an essential role in iron homeostasis.

Iron homeostasis is tightly controlled by several posttranscriptional mechanisms. In brief, the iron-regulatory proteins (IRP1 and IRP2) function as iron sensors that regulate iron metabolism genes as well as some genes with no direct link to iron metabolism. IRPs regulate the stability of transferrin receptor-1 mRNA and the translation of ferritin mRNA by binding to stem-loop structures termed iron-responsive elements (IRE) in the UTRs of these mRNAs. Transferrin receptor-2 mRNA lacks IREs and is not iron-regulated (Fleming *et al.*, 2000). When iron is limiting, IRPs bind to the 5' UTR of ferritin mRNA and block its translation and bind to the 3' UTR of transferrin receptor-1 mRNA which is stabilized (DeDomenico *et al.*, 2008). DMT1 and ferroportin mRNAs also contain IREs that are thought to be regulated by IRPs.

Ferroportin transporter activity is downregulated by the binding of the peptide hormone hepcidin which causes phosphorylation of ferroportin and its subsequent internalization, ubiquitination, and degradation (DeDomenico *et al.*, 2008; Ganz, 2007). Hepcidin therefore inhibits dietary iron absorption and the efflux of recycled iron from macrophages. Hepcidin is secreted by hepatocytes in response to several different stimuli (DeDomenico *et al.*, 2007b). Hypoxia resulting from anemia or other causes, inflammatory cytokines, and iron all regulate hepcidin secretion. Not all of the mechanisms of *hepcidin (hamp)* gene regulation are understood, but transcription of *hamp* is induced by Stat 3 in response to IL-6 (Wrighting and Andrews, 2006) and by bone morphogenic protein/Smad4 signaling in response to membrane-bound hemojuvelin (Babitt *et al.*, 2006, 2007; Silvestri *et al.*, 2008). Hemojuvelin acts as a

BMP coreceptor which facilitates BMP receptor activation (Babitt *et al.*, 2006). The transcription factor HIF-1 α represses *hamp* gene expression in response to hypoxia (Peyssonnaud *et al.*, 2007). Regulation of ferroportin is important for proper maintenance of iron homeostasis.

IRON HOMEOSTASIS AND DEVELOPMENT

Hypochromic, microcytic anemias resulting from defects in iron metabolism genes leading to impaired development of red blood cells have provided insight into the mechanisms of homeostasis of this essential metal. Several rodent strains (Table 3) have been described that have defective erythropoiesis such as hypotransferrinemic (*Tf^{hpx}*) mice, sex-linked anemia (*Heph^{sla}*) mice, *nm1054* (*Steap3*) mice, the Belgrade rat (*Slc11a2^b*), and microcytic anemia (*Slc11a2^{mk}*) mice (Andrews, 2000; Ohgami *et al.*, 2005; Touret *et al.*, 2004). Similarly, *Dmt1* (*Slc11a2*) knockout mice (Gunshin *et al.*, 2005), the zebrafish mutants *Chianti* (*Tfr1a*) (Wingert *et al.*, 2004), *Chardonnay* (*Dmt1*) (Donovan *et al.*, 2002), and *Frascati* (*mitoferrin: Mfrn*) (Shaw *et al.*, 2006) show erythroid maturation arrest. The zebrafish mutant *weisshergst* exhibits a defect in hemoglobin accumulation, because of mutations in the *Ferroportin* (*Fpn*) gene, which is also eventually lethal (Donovan *et al.*, 2000).

Mutations in iron metabolism genes can profoundly affect embryonic, fetal, and perinatal development. Mice with targeted deletions of the *Abcb7* gene, which is essential for cytosolic Fe-S cluster assembly, die soon after implantation because of a defect in extraembryonic tissues, but this gene is critical for the development of many cell types and is mutated in X-linked sideroblastic anemia with ataxia (Pondarre *et al.*, 2006). Frataxin (FXN) functions as a chaperone for mitochondrial (and extramitochondrial) Fe-S cluster biogenesis (Martelli *et al.*, 2007; Muhlenhoff *et al.*, 2002). Knockout of the mouse *Fxn* gene revealed that it is essential, and homozygous embryos also die a few days after implantation (Cossee *et al.*, 2000). The mechanism of lethality may not be associated with iron accumulation as is seen in Friedreich's ataxia. It is interesting to note that the *Fxn* gene in *Arabidopsis thaliana* is essential at the globular stage of early development when the three major tissue types emerge (Vazzola *et al.*, 2007). The *flatiron* (*ffe*) mutation in mice is due to a hypomorphic *Fpn* mutation that exerts a dominant negative function in heterozygous mice leading to iron loading in the liver, high serum ferritin, and low transferrin iron saturation, whereas homozygous *ffe* embryos die at midgestation and have severe anemia (Zohn *et al.*, 2007). In contrast, *Fpn* knockout mice revealed that this iron export protein is essential for development of the early (~ E7.5) embryo (Table 3) (Donovan *et al.*, 2005). Ferroportin is localized to the basolateral membrane of the visceral endoderm at this stage of development and conditional knockout of *Fpn* in the visceral endoderm confirms its importance in those cells. Mice heterozygous for *Fpn* exhibit smaller cell volume and lower intracellular hemoglobin in both developing reticulocytes and mature erythrocytes in adults (Donovan *et al.*, 2005).

Perturbations of intracellular iron storage and distribution can also negatively impact development. Knockout of the mouse *H-ferritin* (*Fth1*) gene is early embryonic lethal when homozygous (Ferreira *et al.*, 2000) and L-ferritin cannot complement the loss of function of *Fth1*. This suggests that these isoforms of ferritin have unique functions and that the ability to store/chelate/distribute iron is essential during early development. The expression pattern of *Fth1* suggests a prominent role in development of the heart (Ferreira *et al.*, 2000). *Fth1* expression in the E9.5 mouse embryo is restricted to heart and CNS but later becomes more ubiquitous (Ferreira *et al.*, 2000).

IRP1 and IRP2 regulate iron metabolism genes at the posttranscriptional level. Neither alone is essential for development. *IRP1* knockout mice misregulate iron metabolism in the kidney and brown fat, whereas *IRP2* knockout mice misregulate iron homeostasis in all tissues and

develop adult-onset neurodegenerative disease (Smith *et al.*, 2004). Mice with a single allele of *IRP1* and no functional *IRP2* genes develop a more severe form of neurodegeneration (Smith *et al.*, 2004), whereas a complete loss of both *IRP1* and *IRP2* genes is embryonic lethal (Smith *et al.*, 2006). These double-knockout blastocysts were discolored and morphologically abnormal perhaps because of the overexpression of ferritin and they did not survive past E6.5 in utero (Smith *et al.*, 2006).

Homozygous knockout of *transferrin receptor-1 (TfR1)* in mice revealed its essential roles in erythropoiesis and in development of the central nervous system (Levy *et al.*, 1999). Homozygous *TfR1* knockout mice die in utero and are severely anemic with increased apoptosis in the neuroepithelium. Haploin sufficiency of *TfR1* is associated with microcytic anemia and reduced iron stores. In contrast, *TfR2* knockout mice are viable but conditional knockout of this gene in the adult liver leads to iron overload (Wallace *et al.*, 2004, 2007) similar to type 3 hemochromatosis in humans, suggesting a role for TFR2 as an iron-bound transferrin sensor (Johnson and Enns, 2004; Robb and Wessling-Resnick, 2004; Wallace *et al.*, in press).

ESSENTIAL METAL INTERACTIONS

Although most of the above discussion has dealt with these essential metals individually, it should be noted that there are significant interactions between essential metals and the status of one metal can affect that of another. For example, excess zinc induces copper deficiency which may lead to diminished intestinal absorption of iron (Cherukuri *et al.*, 2005) and decreased activity of CP, the copper containing ferroxidase, which in turn may destabilize ferroportin (DeDomenico *et al.*, 2007a). Marginal zinc deficiency in pregnant rats leads to increased maternal CP and increased copper in the milk, perhaps by increasing the expression of *Ctr1*, *Atp7a*, and *Atp7b* genes in the mammary gland, which in turn can cause signs of copper toxicity in the nursing pups (Kelleher and Lonnerdal, 2003). Analyses of all of the essential metal ions in the liver and embryos of *Zip2*- knockout mice revealed unique alterations in iron but not zinc levels (Peters *et al.*, 2007). Zinc deficiency has been shown to cause an increase in iron in the liver, whereas zinc excess competes for iron uptake in the gut (Hurley *et al.*, 1983; Kelleher and Lonnerdal, 2006b; Kordas and Stoltzfus, 2004; Yamaji *et al.*, 2001), perhaps by altering the activity of iron transport in the intestine. *Zip2*-knockout mice fail to accumulate iron in the liver during zinc deficiency (Peters *et al.*, 2007), but the mechanisms underlying this effect are unknown.

Zinc and calcium metabolism are also interconnected (Maret, 2001). A functional link has been proposed between L-type calcium channels and the zinc efflux transporter ZnT1 (Ohana *et al.*, 2006; Segal *et al.*, 2004), and extracellular zinc can trigger the release of calcium from intracellular pools in HT29 cells (Hershinkel *et al.*, 2001) and inhibit calcium influx in several cultured cell lines (Gore *et al.*, 2004). A dramatic increase in calcium occurs in embryos from zinc-deficient *Zip2*-knockout mice (Peters *et al.*, 2007). However, there is no evidence that any ZIP family member can transport calcium suggesting an indirect role of ZIP2 in calcium metabolism.

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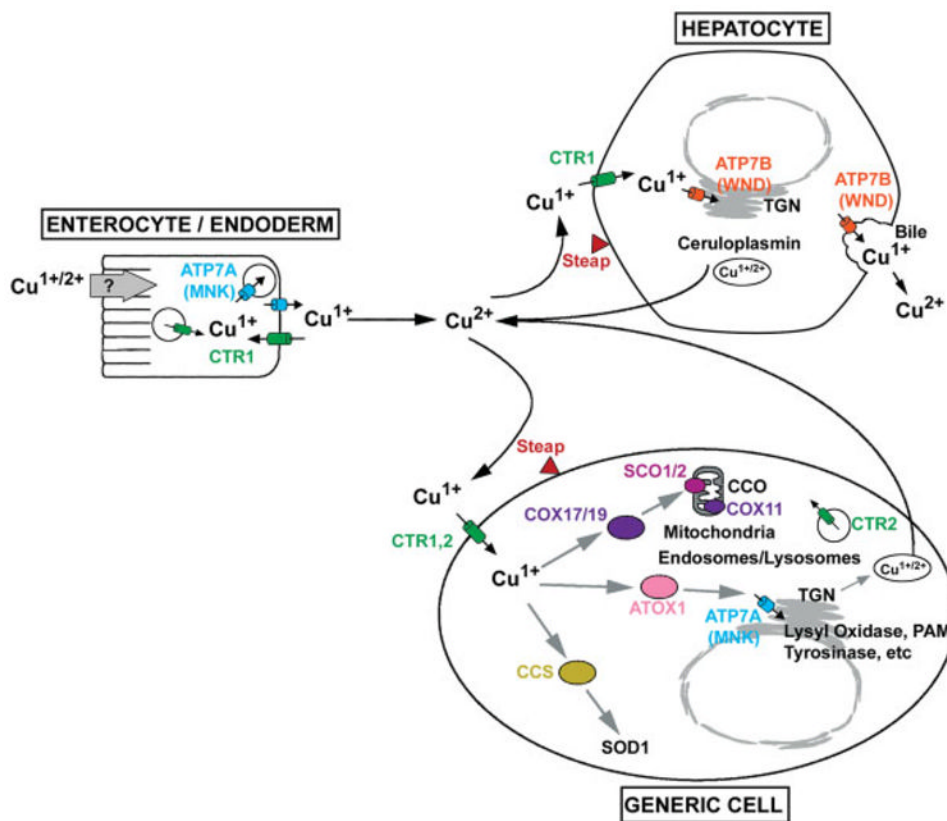


FIG. 1.

Overview of copper homeostasis. Copper is oxidized (Cu^{2+}) in the intestinal lumen and in the serum, but is reduced to Cu^{1+} before transport into cells. The Steap ferric/cupric reductases (Steaps 2, 3, and 4) are localized to endosomes/lysosomes and the plasma membrane and may be essential for the reduction of copper, as well as the reduction of iron (Ohgami *et al.*, 2006). Copper is transported into ENTEROCYTE/ENDODERM cells by unknown mechanisms (endocytosis?). CTR1 is essential for the acquisition of dietary copper but its function in the enterocyte is unknown. In many other cell types (GENERIC CELL), copper is taken up by CTR1 localized to the plasma membrane and perhaps also by CTR2 (Slc31a2). Copper taken up by ENTEROCYTE/ENDODERM is exported into portal blood/conceptus, respectively, by ATP7A (Menkes disease protein, MNK) which is localized to vesicles trafficking toward the basolateral membrane and to the basolateral membrane. Copper exported to portal blood is taken up into the liver, the primary organ that regulates copper homeostasis. In the HEPATOCYTE, ATP7B (Wilson's disease protein, WND) effluxes excess copper into the bile and puts copper into the *trans*-Golgi network (TGN) where it is loaded into ceruloplasmin, a ferroxidase that is the primary copper binding protein in serum. Inside the cell (GENERIC CELL), copper is distributed to cytoplasmic copper chaperones (COX17/19, ATOX1, CCS) which, in turn, deliver copper to mitochondrial inner membrane and ultimately cytochrome C oxidase (CCO) or to ATP7A in the TGN, and cytoplasmic SOD1, respectively. The assembly of copper into CCO is an active area of investigation. It is thought that copper in COX17/19 (and probably COX23/MTCP1) is first transferred to both COX11 and SCO1/2 and ultimately to CCO. ATP7A transports copper into the TGN and activates copper containing secretory and membrane-bound proteins [lysyl oxidase, tyrosinase, peptidylglycine α -amidating monooxygenase (PAM)]. It should be noted that the cellular localization and abundance of CTR1, ATP7A, and ATP7B are dynamically regulated by copper availability which is not reflected in this static cartoon.

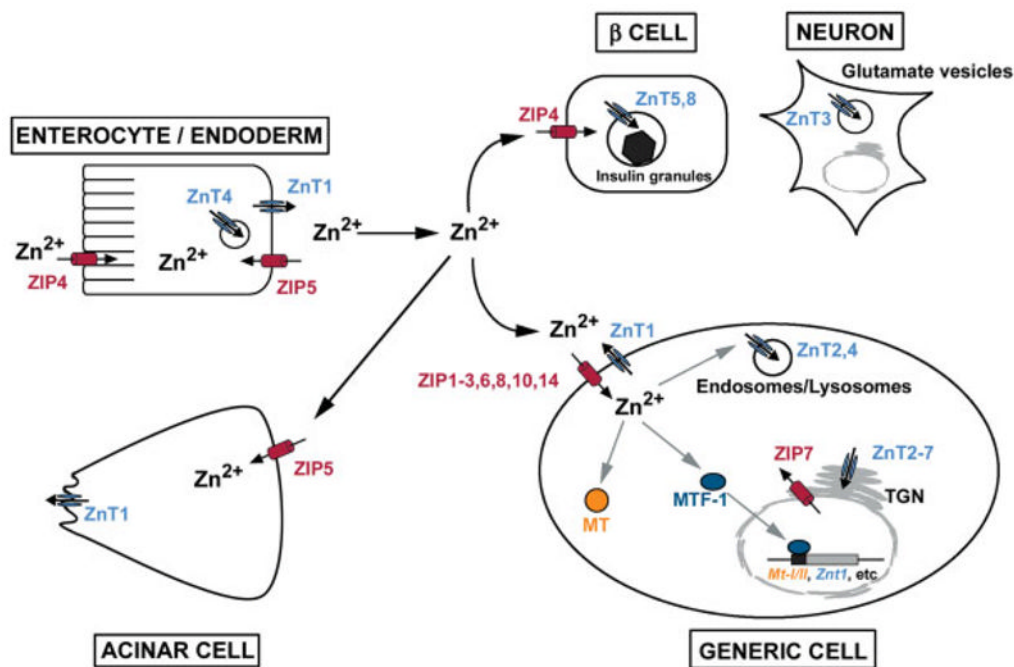


FIG. 2.

Overview of zinc homeostasis. Twenty four different genes encode proteins that may be involved in the uptake (*Zip* genes) or efflux (*ZnT* genes) of this metal in a cell-specific, developmentally regulated, and zinc-regulated manner. The functions of many of these genes remain to be determined. Therefore, this cartoon provides only a superficial and static view of zinc homeostasis. ZIP4 plays a critical role in the absorption of dietary zinc by ENTEROCYTE/ENDODERM cells when zinc is limiting, but other transporters must also play important roles. Zinc is thought to be exported into portal blood or into the conceptus by ZnT1 localized on the basolateral membrane. Other ZnT proteins (i.e. ZnT4) also likely play a role in this process. ZIP5 is localized to the basolateral membranes of enterocytes, endoderm cells and pancreatic acinar cells where it may serve to remove zinc from the blood when zinc is replete. In peripheral tissues (GENERIC CELL), zinc is probably taken up by various ZIP transporters localized on the plasma membrane. To date ZIPs 1, 2, 3, 6, 8, 10, and 14 have each been shown to have zinc transport activity in transfection or oocyte injection studies, and most show tissue-specific patterns of expression. Inside the cell, free zinc levels are kept low and zinc can be bound to MT or transported into secretory vesicles, endosomes/lysosomes or zinosomes by ZnT2 and ZnT4. Zinc activates the zinc-sensing transcription factor MTF-1 which regulates transcription of the mouse *Mt-I/II* and *Znt1* genes and represses expression of *Zip10* in an effort to control excess zinc. ZnT2–7 participate in the delivery of zinc into the secretory pathway, whereas ZIP7 may transport zinc out of the Golgi apparatus into the cytoplasm. ZnT3 transports zinc into glutamate containing vesicles in the brain whereas ZnT8 transports zinc into pancreatic β-cell insulin secretory granules. ZIP4 is expressed in β-cells, but its localization in those cells has not been reported.

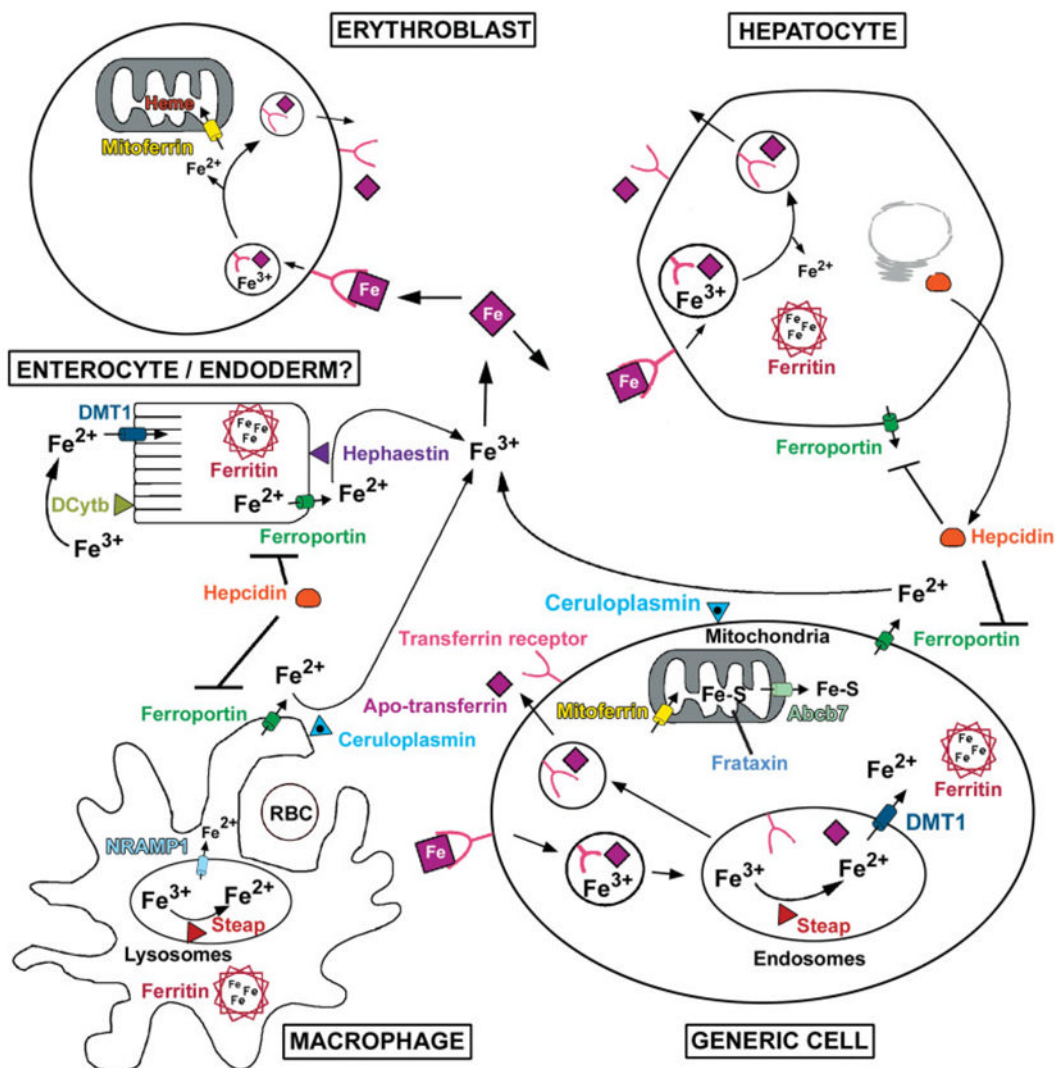


FIG. 3.

Overview of iron homeostasis. Iron is oxidized (Fe^{3+}) in the intestinal lumen and in the serum, but is reduced (Fe^{2+}) before transport into cells. Iron (Fe^{2+}) is transported into ENTEROCYTES by DMT1 after extracellular reduction perhaps by DCytb. Iron uptake by visceral ENDODERM cells is apparently not dependent on DMT1 and could require the transferrin uptake system. Iron is transported out of cells by Ferroportin and is thought to be subsequently oxidized (Fe^{3+}) by membrane bound Hephaestin and Ceruloplasmin. Iron, inflammation and hypoxia regulate Hepcidin, a polypeptide hormone made by the HEPATOCYTE. Hepcidin binds to Ferroportin resulting in degradation of the transporter. In the serum, Fe^{3+} binds to Transferrin. Developing ERYTHROBLASTS (heme is the major pool of iron) and many other cell types take up iron via the transferrin-mediated endocytic pathway (see GENERIC CELL). After binding to the transferrin receptor, the iron–transferrin complex is internalized and iron is released from transferrin in the acidified endosome, reduced by a Steap metalloreductase and then transported into the cytoplasm by DMT1. Apo-transferrin and the transferrin receptor are returned to circulation and plasma membrane, respectively, via the recycling endosome pathway. All nonerythroid cells store iron complexed with the ferritin heavy and light chains (Ferritin) which can store up to 4,000 atoms of iron per molecule. Cytoplasmic iron is transported into mitochondria by mitoferrin where it is loaded into Fe-S

clusters in a process that requires Frataxin. A substrate essential for Fe-S assembly in the cytoplasm is transported out of the mitochondria by the ATP-binding cassette transporter ABCB7 (see GENERIC CELL). In the erythroblast, mitochondrial iron is primarily utilized for heme synthesis. A major fraction of iron is recycled by the reticulo-endothelial MACROPHAGE which phagocytoses aged red blood cells (RBC). The iron is solubilized in the lysosomes, reduced by a Steap metalloreductase and exported out of the lysosome by NRAMP1. Iron is released from the macrophage by ferroportin, subsequently reoxidized by Ceruloplasmin, and conveyed again via transferrin through the systemic circulation.

Table 1
Phenotypes of Animals With Mutations in Genes Critical for Copper Homeostasis During Development

Gene	Functions	Phenotypes	Reference
<i>Atox1</i>	Copper chaperone for ATP7A/B	Perinatal lethal, severe growth retardation in null mouse	(Hamza et al., 2001)
<i>Atp7a</i>	Copper transporter into TGN	Fatal in early childhood in MNK disease Embryonic lethal, perinatal lethal or hypopigmentation in <i>mottled/brindled</i> mouse Impaired synaptogenesis and axonal targeting, cytoskeletal dysfunction in <i>mottled/brindled</i> mouse Impaired notochord development in <i>calamity</i> zebrafish	(Mercer, 1998) (Mercer, 1998) (El Meskini et al., 2007; Niciu et al., 2007) (Mendelsohn et al., 2006)
<i>Atp7b</i>	Copper transporter into TGN	Impaired early larval development and adult pigmentation in <i>Drosophila</i> Abnormal copper accumulation in the liver and CNS in WND patients Abnormal copper accumulation in the liver of Long–Evans Cinnamon rat Copper deficiency in breast milk in <i>toxic milk</i> and null mouse	(Norgate et al., 2006) (Van Hoof et al., 1992) (Muramatsu et al., 1994) (Buiakova et al., 1999)
<i>Commd1</i>	Ubiquitin pathway regulation ATP7B stability	Copper toxicosis in Bedlington terrier Embryonic lethal (E9.5–E10.5) in null mouse, no placental vascularization	(van de Sluis et al., 2002) (van de Sluis et al., 2007)
<i>Cox17</i>	Copper chaperone for CCO	Embryonic lethal (E8.5–E10) in null mouse	(Takahashi et al., 2002)
<i>Ctrl</i>	Copper importer (cell signaling?)	Embryonic lethal (E8.5–E10.5) in null mouse	(Kuo et al., 2001; Lee et al., 2001) (Turski and Thiele, 2007)
<i>Sco1/2</i>	Copper chaperone for CCO	Impaired development of neuroectoderm and mesoderm arrest at larval stage in <i>Drosophila</i> Fatal infantile cardioencephalomyopathy and CCO deficiency (<i>Sco2</i>) in humans Neonatal liver failure and encephalopathy (<i>Sco1</i>) in humans	(Papadopoulou et al., 1999) (Valnot et al., 2000)

Table 2
Phenotypes of Animals With Mutations in Genes Critical for Zinc Homeostasis During Development

Gene	Functions	Phenotypes	Reference
<i>Mt-1/II</i>	Zinc buffer-pool	Increased embryo sensitivity to zinc deficiency in null mouse and increased resistance to zinc deficiency when over-expressed	(Andrews and Geiser, 1999) (Dalton et al., 1996)
<i>Mtf-1</i>	Zinc-sensing transcription factor	Embryonic lethal (E14) due to liver degeneration in null mouse	(Wang et al., 2004)
<i>Zip1</i>	Zinc importer	Impaired embryonic adaptation to zinc deficiency in null mouse	(Dufner-Beattie et al., 2006)
<i>Zip2</i>	Zinc importer	Impaired embryonic adaptation to zinc deficiency and altered iron and calcium homeostasis in null mouse during zinc	(Peters et al., 2007)
<i>Zip3</i>	Zinc importer	Impaired embryonic adaptation to zinc deficiency (cell type-specific) in null mouse	(Dufner-Beattie et al., 2005)
<i>Zip4</i>	Zinc importer	Childhood lethal acrodermatitis enteropathica in humans Embryonic lethal (E8–E9) in homozygous null mouse and hypersensitivity to zinc deficiency in heterozygous null mice	(Maverakis et al., 2007) (Dufner-Beattie et al., 2007)
<i>Liv-1 like</i>	Zinc importer	Lethal trait A46 in bovine (<i>Zip4?</i>) Impaired epithelial–mesenchymal transition in zebrafish gastrula (zLIV-1) Impaired germ cell migration and gonad morphogenesis in <i>Drosophila</i> (<i>Fear of intimacy</i>)	(Machen et al., 1996) (Yamashita et al., 2004) (Moore et al., 1998)
<i>ZnT1</i>	Zinc exporter	Embryonic lethal (E8–E9), transporting maternal zinc into embryo	(Andrews et al., 2004)
<i>ZnT2</i>	Vesicular zinc transporter	Dermatitis due to low zinc in breast milk (in human)	(Chowanadisai et al., 2006)
<i>ZnT4</i>	Vesicular zinc transporter	<i>Lethal milk</i> mutation in mice, suckling pups die	(Huang and Gitschier, 1997)
<i>ZnT5</i>	Zinc transporter – Golgi apparatus	Growth retardation, osteopenia, male specific cardiac sudden death in null mouse	(Inoue et al., 2002)

Table 3
Phenotypes of Animals With Mutations in Genes Critical for Iron Homeostasis During Development

Gene	Functions	Phenotype	Reference
		<i>Hypochromic, microcytic anemia, defective erythropoiesis</i>	
<i>Dmt1</i>	Iron transporter (Slc11a2) in <i>Slc11a2^b</i> Belgrade rat in <i>Slc11a2^{mk}</i> microcytic anemia mouse in <i>Slc11a2^{-/-}</i> null mouse in zebrafish Chardonnay		(Touret et al., 2004) (Touret et al., 2004) (Gunshin et al., 2005) (Donovan et al., 2002)
<i>Fpn</i>	iron efflux in zebrafish Weisshergst		(Donovan et al., 2000)
<i>Heph</i>	Iron oxidase in sex-linked anemia mouse		(reviewed by Andrews, 2000)
<i>Mifrn</i>	Mitochondrial iron importer in zebrafish <i>Frascati</i> and null mouse ES cells		(Shaw et al., 2006)
<i>Steap3</i>	Endosomal ferric/cupric reductase in null mouse and <i>nm1054</i> mouse		(Ohgami et al., 2005)
<i>Tf</i>	Serum iron carrier in <i>Hpx</i> mouse		(reviewed by Andrews, 2000)
<i>Tfr1</i>	Transferrin receptor for iron uptake in zebrafish <i>Chianti</i>		(Wingert et al., 2004)
<i>Abcb7</i>	Mitochondrial efflux of Fe-S substrate	<i>Impaired embryonic development</i> Embryonic lethal ~ E6.5 in null mouse, extraembryonic defect	(Pondarre et al., 2006)
<i>Fpn</i>	Iron efflux	Embryonic lethal ~ E7.5 in null mouse, visceral endoderm defect Hypomorphic allele, midgestation lethal, neurogenesis defects in <i>Flatiron mouse</i>	(Donovan et al., 2005) (Zohn et al., 2007)
<i>Fxn</i>	Fe-S cluster assembly	Embryonic lethal 3E5–E7 in null mouse (early postimplantation)	(Cossee, et al., 2000)
<i>Fth1</i>	Iron storage	Embryonic lethal (3E3.5–E9.5) in null mouse, defective heart development	(Ferreira, et al., 2000)
<i>Irp1 + Irp2</i>	Posttranscriptional regulation	Embryonic lethal blastocyst stage in double null mouse, iron overload	(Smith et al., 2004, 2006)
<i>Tfr1</i>	Endocytic uptake of iron in mouse	Embryonic lethal 3E12.5 in null mouse, impaired CNS, and erythroid development	(Levy et al., 1999)