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SLC31 (CTR) Family of Copper Transporters in Health and Disease

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Abstract

Copper is a vital mineral for many organisms, yet it is highly toxic as demonstrated by serious health concerns associated with its deficiency or excess accumulation. The SLC31 (CTR) family of copper transporters is a major gateway of copper acquisition in eukaryotes, ranging from yeast to humans. Characterization of the function, modes of action, and regulation of CTR and other molecular factors that functionally cooperate with CTR for copper transport, compartmentalization, incorporation into cuproproteins, and detoxification has revealed that organisms have evolved fascinating mechanisms for tight control of copper metabolism. This research progress further indicates the significance of copper in health and disease and opens avenues for therapeutic control of copper bioavailability and its metabolic pathways.

Keywords

SLC31; CTR; Copper; Transporter; Metallochaperone; Cisplatin; Menkes disease; Wilson disease

1. Copper in biology and medicine

Multiple essential biological pathways, such as energy generation, neurotransmission, and maturation of the extracellular matrix, are dependent on copper-containing enzymes (Penna et al., 1999). Serious health problems, including growth and developmental retardation, anemia, and cardiac hypertrophy, are associated with nutritional or genetic copper deficiency (Danks, 1988; Vulpe and Packman, 1995; Lutsenko et al., 2007; Leary et al., 2004). Excess copper is toxic as manifested in Wilson disease patients possessing genetic defects in copper excretion (Lutsenko et al., 2007). Identification and characterization of various molecular factors responsible for cellular copper uptake (e.g., SLC31 transporters, which are classified as “1.A.56 Copper transporter (CTR)” family (<http://www.tcdb.org/>)) followed by subcellular and body distribution (e.g., cytoplasmic copper chaperones, including ATOX1, and CCS; copper transporting P-type ATPases ATP7A and ATP7B at the secretory pathway; and copper assembly factors, such as SCO1 and SCO2 for cytochrome oxidase) have revealed that virtually all eukaryotes have evolved delicate mechanisms for copper acquisition and control of its toxicity (Culotta, 2006; Lutsenko et al., 2007; Kim et

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al., 2008; Robinson and Winge, 2010). This research progress has revealed several unique features at the interface between an inorganic element and cellular macromolecules.

Poorly characterized copper-linked human health issues, including copper deficiency induced anemia (Collins et al., 2010), immune dysfunction (Prohaska and Lukasewycz, 1990), acquired copper deficiency (Kumar, 2006), implication of copper in neurodegenerative diseases (Barnham and Bush, 2008), and copper facilitation of wound healing and angiogenesis (Brewer, 2005; Xie and Kang, 2009), are all important research topics in biology and medicine. Moreover, the implication of copper metabolic pathways in cellular accumulation and therapeutic outcomes of platinum (Pt)-based anticancer drugs, such as cisplatin, is an unanticipated but important finding (Ishida et al., 2002; Howell et al., 2010). Further studies on the mechanism of action and regulation of copper trafficking pathways would ultimately advance our ability to combat human diseases.

2. CTR copper transporters conserved in eukaryotes

Between one and six CTR family members have been identified in each eukaryote with some subcellular and/or organ specificities (Dumay et al., 2006). Yeast *S. cerevisiae* expresses three CTR members (yCTR1, yCTR2, and yCTR3). yCTR1 and yCTR3 are functionally redundant in high-affinity copper uptake (1–4 M *K_m* for copper) across the plasma membrane (Dancis et al., 1994a; Knight et al., 1996). yCTR2 appears to mobilize copper from the vacuole (Rees et al., 2004). The plant *A. thaliana* genome carries five CTR members (COPT1–5) (Peñarrubia et al., 2010; Pilon et al., 2009). Two CTR members, CTR1 and CTR2, are encoded in the human genome by the *SLC31A1* and *SLC31A2* genes respectively (Table 1). They are expressed in all organs and tissues examined, with high levels in the liver and kidney (Zhou and Gitschier, 1997; Lee et al., 2000). Excess copper accumulation in cells over-expressing human CTR1 indicates that it is a limiting factor for cellular copper acquisition (Zhou and Gitschier, 1997; Lee et al., 2000). However, distinct from CTR1, human CTR2 expression levels do not lead to obvious change in cellular copper metabolism (Moller et al., 2000; van den Berghe, 2007; Bertinato, 2008). CTR2 predominantly resides within intracellular compartment(s) (van den Berghe, 2007; Bertinato, 2008), which is similar to budding yCTR2 (Rees et al., 2004), fission yeast CTR6 (Bellemare et al., 2002) and *A. thaliana* COPT5 (Garcia-Molina, 2011). A processed gene that is highly homologous to *SLC31A1* has been identified as *SLC31A1P1*; however, over-expression of the putative encoded C-terminal truncated CTR1 (95 amino acids) does not enhance copper uptake (Moller et al., 2000). The roles for each CTR protein and the functional and physical interactions between CTR members remain to be further elucidated.

Subcellular localization of CTR1 is distinct in mammalian cell lines. The majority of CTR1 in human embryonic kidney (Hek293) cells is localized at the plasma membrane like yCTR1 in yeast (Dancis et al., 1994a; Lee et al., 2002a) (Fig. 1A and B). However, in some cell lines, including lung carcinoma cell lines A549 and H441, and hepatocellular carcinoma cell line HepG2, CTR1 is predominantly at cytoplasmic vesicular compartments (Klomp et al., 2002). Duodenal CTR1 distribution in mice is different in adult mice when compared to suckling mice (Kuo et al., 2006). The physiological significance of these distribution patterns has not been defined, but might be associated with cell-type specific dynamics of CTR1 secretion or recycling between plasma membrane and intracellular compartments.

An additional layer of complexity has been reported with regard to CTR1 subcellular localization in mammalian enterocytes. CTR1 is detected at the apical side of intestinal epithelial cells of mouse and pig (Nose et al., 2010), which is consistent to the notion that it functions as a copper importer from the diet. However, the majority of CTR1 is localized at the basolateral side of cultured Caco2 cells that are differentiated to a polarized intestinal

cell type in vitro (Zimnicka et al., 2007). The discrepancy between these in vivo and in vitro experimental data might reflect the degree of differentiation of enterocytes. CTR1 might be localized at the basolateral membrane of less differentiated Caco2 cells and cryptic epithelial cells to acquire copper from the plasma.

N- and O-glycosylation occurs at the extracellular N-terminus of human CTR1 (Eisses and Kaplan, 2002; Maryon, 2007) (Fig. 1A), which is similar to heavily O-glycosylated yeast yCTR1 (Dancis et al., 1994b). O-glycosylation defective human CTR1 manifests a cleavage of approximately 30 amino acids from the N-terminus (Maryon et al., 2007). Despite a lack of evidence indicating this event is regulated in response to bio-available copper, this post-translational modification might be important for assembly, trafficking and/or activities of CTR1.

3. Functional roles for CTR

3.1. CTR-mediated copper uptake in organs and tissues

Several lines of evidence confirmed that the CTR family of proteins plays a major role in copper translocation across the cellular membranes into the cytoplasm in eukaryotes. *Slc31a1* gene knockout mice have revealed the important roles for CTR1 in mouse embryo development. Whole body knockout of *Slc31a1* in mice leads to death of the embryos at the mid-gestation stage (Lee et al., 2001; Kuo et al., 2001), which is consistent to the severe growth defect or perinatal death observed in mice when copper delivery pathways to the mitochondria or secretory pathway are genetically ablated (Hamza et al., 2001; Takahashi et al., 2002). *Slc31a1* heterozygous knockout mice are similar to wild-type control mice in growth and reproduction; however, copper levels in the brain and spleen of the *Slc31a1* knockout mice are approximately 50% less than those of control mice (Lee et al., 2001). This indicates that both *Slc31a1* alleles are necessary for copper uptake in those organs, but the reason underlying this organ-specific haploid insufficiency of CTR1 have not been defined.

Intestine-specific *Slc31a1* knockout in mice showed its functional role in copper absorption from the diet (Nose et al., 2006). Intriguingly, these mice accumulate excess copper in the intestinal epithelial cells despite systemic copper deficiency. It appears that copper can be carried to intracellular compartment(s) without CTR1. Moreover, this observation is somewhat inconsistent with the localization of CTR1 predominantly at the apical side of the intestinal epithelial cells (Nose et al., 2010) and does not necessarily support its role in copper uptake at the apical side of enterocytes. It is worthy to note that a mammalian iron uptake system is comprised of the transferrin receptor that binds to extracellular iron-containing transferrin followed by internalization of the complex for iron transport to the cytoplasm via DMT1 (DCT1, Nramp2) (Mackenzie and Hediger, 2004). It is possible that mammalian CTR1 might traffic between cell surface and intracellular compartment(s) where copper translocation to the cytoplasm occurs. Mammalian CTR1 function might be dependent on other component(s) of the copper uptake system like the transferrin receptor for iron uptake. Alternatively, copper might be brought into intracellular CTR1-containing compartment(s) via endocytosis/pinocytosis, especially in immature mice. This mechanism of copper uptake might be true for enterocytes; however, this mechanism unlikely explains CTR1-mediated copper uptake in other organs and tissues. *Slc31a1* knockout in the liver and heart in mice results in a severe defect in copper accumulation as well as copper-dependent biochemical pathways in the organs (Kim et al., 2009; Kim et al., 2010), which is distinct from excess accumulation of non-bioavailable copper in the CTR1-ablated enterocytes. Moreover, no correlation between copper uptake in Sf9 and Hek293 cells and internalization of CTR1 was observed (Eisses et al. 2005b), suggesting that CTR1 endocytosis is not a necessary process in copper uptake at least in these cell types.

Elevated copper excretion in the urine with no significant difference in the copper levels in other organs and tissues of the liver-specific *Slc31a1* knockout mice (Kim et al., 2009) indicates that the kidneys play an important role in systemic copper homeostasis when copper uptake into the hepatocytes followed by excretion to the bile is reduced. These data also suggest that, despite a major portion of copper being routed to the liver after absorption from the diet, copper-binding molecule(s) (e.g., ceruloplasmin) that are secreted to the plasma from the liver do not play a significant role in copper delivery to other organs and tissues.

Mouse fibroblasts isolated from growth-arrested *Slc31a1* knockout embryos express copper transport system(s) that maintain approximately one third of the copper levels relative to wild-type control cells (Lee et al., 2002b). This CTR1-independent copper transporter system transports Cu(II), which is distinct from Cu(I)-transporting CTR1. DMT1 (DCT1, Nramp2)-mediated copper uptake has been proposed (Arredondo et al., 2003). Two ZIP proteins, ZIP2 and ZIP4, are shown to transport copper in *A. thaliana* (Wintz et al., 2003). Anion exchangers that might transport copper-chloride complexes may be another copper transporter in mammals (Zimnicka et al., 2011). A novel copper transporter Mcf1 is involved in meiotic differentiation of fission yeast (Beaudoin et al., 2011a). These and other unknown copper transporters might cooperate with CTR1 in copper acquisition and distribution in a manner dependent on tissue, developmental and growth stage, or copper bioavailability.

3.2. Unanticipated roles of CTR

CTR1 is also an important factor in determining cellular accumulation and toxicity of platinum-based potent and effective anti-cancer drugs, such as cisplatin (Ishida et al., 2002; Howell et al., 2010; Kuo et al., 2007). Consistently, expression levels of copper transporting P-type ATPases (ATP7A and ATP7B) that transport copper into the lumen of secretory pathway affect cellular cisplatin accumulation and resistance as well (Kuo et al., 2007; Komatsu et al., 2007). It is also shown that ATOX1, a cytoplasmic copper carrier to ATP7A/B, binds cisplatin (Palm et al., 2011). Given that development of cisplatin resistance resulting from reduced uptake is a major limitation of cisplatin (Howell et al., 2010; Kuo et al., 2007), understanding the mechanism of intrinsic and acquired resistance to cisplatin is critical for developing more effective treatment of cancer. For instance, expression levels of copper transporters might be associated with cisplatin resistance in patients, and cancer cell-specific expression control of copper transporters could be a therapeutic approach to enhance cisplatin efficacy. Nevertheless, it is uncertain how CTR1 can transport both copper and cisplatin that have distinct chemical properties. The amino acid residues and functional domains in CTR1 that are essential for copper uptake are dispensable for CTR1-mediated cellular accumulation of cisplatin, and CTR1-mediated translocation of copper is not directly affected by cisplatin (Sinani et al., 2007). These data indicate that the mechanisms underlying CTR1-dependent copper and cisplatin transport are distinct from each other. Cisplatin and related drugs appear to bind with high affinity to copper-binding residues and domains of molecules involved in copper metabolism, including CTR1, ATOX1, and ATP7A/B (Guo et al., 2004b; Crider et al., 2010). In the case of CTR1, cisplatin bound to the extracellular domains could be carried into intracellular compartment(s) via the CTR1 internalization process where it can be released to the cytoplasm by mechanism(s) not yet known. ATOX1 and ATP7A/B might sequester cisplatin to reduce its cytotoxic effects, which could explain how over-expression of these molecules confers cisplatin resistance.

Intriguingly CTR1, independent of its copper transport activities, plays a critical role in routing the fibroblast growth factor (FGF) signal during early embryogenesis (Haremak et al., 2007). Physical interaction of *Xenopus* CTR1 with an EGF receptor-associated Src-related kinase activates the Ras-MAP kinase cascade, which sorts the EGF signal toward

germ layer differentiation rather than morphogenesis. *Slc31a1* knockout mouse embryonic stem cells are unable to respond to the differentiation signal. Hence, the death of *Slc31a1*-deleted mouse embryos could be attributed at least partially to this copper transporter-independent role of CTR1. It is unknown what triggers *Xenopus* CTR1 association with the signaling cascade in a developmental stage dependent manner and whether or not a similar mode of interaction of CTR1 affects other cellular signaling pathways under certain conditions.

4. Mode of action of CTR

4.1. Channel-like architecture based on oligomerization

CTR family members are unique in their amino acid sequences, and the sequence homology among family members is low. However, they carry several common structural features, such as two to three predicted trans-membrane domains with some exceptions, and multiple potential metal-binding residues (e.g., methionine, histidine, cysteine) (Fig. 1A) (Petris, 2004; Dumay et al., 2006; Kaplan and Lutsenko, 2009; De Feo et al., 2007). Consistent with the smaller size and also with fewer transmembrane helices (TMs) (e.g., 190 amino acids with three TMs in the case of human CTR1) than many other known ion transporters, biochemical analyses indicate multimerization of CTR1 (Fig. 1C) (Lee et al., 2002a). Projection structures of CTR1 confirmed that it forms a compact homo-trimer with a novel channel-like architecture (Fig. 1C) (De Feo et al., 2007; Aller and Unger, 2006). It is uncertain yet how the channel is controlled and which residues are involved in copper relay through the channel. Monitoring of the dynamics of budding yeast yCTR1 in vivo, partial proteolysis of human CTR1, and computational modeling of TMs based on projection structures and mutagenesis data of human CTR1 showed that the multimer complex changes conformation in conjunction with copper transport (Sinani et al., 2007; Eisses and Kaplan, 2002; Schushan et al., 2010).

Despite low sequence identity at the 3rd TM like other regions in this family of proteins, there is a conserved GXXXG (GG4) motif that is shown to be involved in oligomerization of other integral membrane proteins (Fig. 1A and C) (De Feo et al., 2007). Indeed, this GG4 motif is critical for formation of a functional CTR1 multimer and the face opposite to the GG4 appears to interact with the other two TMs (De Feo et al., 2007; Aller et al., 2004).

Fission yeast CTR4 and CTR5 forms a hetero-multimeric complex, which is important for secretion of the transporter to the cell surface (Beaudoin et al., 2011b). Hetero-multimerization of CTR family of proteins may exist in other organisms, and its functional roles could be explored. Secondly, excess copper and cisplatin stabilizes the multimeric complexes (Sinani et al., 2007; Guo et al., 2004b), which may be an important mechanisms underlying CTR1-mediated copper and cisplatin uptake and/or control of CTR1 activities and/or subcellular trafficking. Hence, it appears that multimerization of CTR is implicated in its expression and regulation in addition to the formation of functional copper transporters.

4.2. Mechanism of CTR-mediated copper transport

CTR family members do not possess a domain that might be associated with ATP utilization as an energy source for copper transport. Consistently, inhibitors of ATP synthesis did not abolish human CTR1-mediated copper transport (Lee et al., 2002a). It has been suggested that the concentration and/or electrochemical gradient of copper or other ions (e.g., K⁺) across the plasma membrane may be the driving force for CTR1-mediated copper uptake. However, considering that only a small fraction of copper in both cytoplasm and plasma likely exist as free forms, the trans-membrane ionic gradient of copper itself would be minimal.

Human CTR1 expressed in Hek293 cells transports copper with an apparent K_m of ~1.7 M copper when the assays are conducted in the DMEM culture medium containing 10% fetal bovine serum (Lee et al., 2002a). The initial rate of copper uptake is over 10 times higher in the Hepes-buffered salt solution (HBSS) relative to DMEM, and albumin or histidine supplementation in the HBSS diminishes copper uptake to the levels that occur in the DMEM media (Lee et al., 2002a), indicating competition for ionic copper between CTR1 and copper-binding molecules in the DMEM medium and possibly plasma as well. However, the possibility that CTR1 transports copper bound with small molecule(s) rather than ionic forms of copper cannot be ruled out.

CTR1-mediated copper uptake is enhanced by ascorbic acid (copper reductant) and competes with silver (a metal ion that possess similar characteristics to Cu(I)) but not with divalent metals, such as zinc, and iron, suggesting that reduced Cu(I) is a substrate of CTR1 (Lee et al., 2002b). This is consistent with reports indicating roles for FRE metalloreductases in yCTR1-mediated copper transport in yeast (Hassett and Kosman, 1995); however, the identities of mammalian metalloreductases that are responsible for CTR1-mediated copper uptake have not been defined.

A significant portion of copper does not likely exist in free ionic form(s) in the plasma. Copper-containing ferroxidase ceruloplasmin (Cp) reflects more than 90% of serum copper (Hellman and Gitlin, 2002), but characterization of *Cp* knockout mice showed no significant change in copper uptake at the organs and tissues (Meyer et al. 2001). Copper bound to other plasma proteins, amino acids, or ions might be captured by CTR1 or other cell surface molecule(s), such as metalloreductases, and adaptors, to be processed for CTR1-mediated translocation. Since ionic forms of Cu(I) can be captured by not only CTR1 but also by other molecules in the cell surface and plasma, there may be a specific mechanism that protects Cu(I) for its transport by CTR1. Cu(I) may bind to a molecule that has a high affinity to the N-terminus of CTR1. Alternatively, it is possible that the metalloreductases form a complex with CTR1 for directly passing the reduced copper.

Site-directed mutagenesis and truncation of CTR1 followed by functional assays have identified several residues and motifs that are involved in its copper transport activities (Kaplan and Lutsenko, 2009; De Feo et al., 2007; Puig et al., 2002; Eisses and Kaplan, 2005a). The methionine (Met)-rich extracellular N-terminal domain of CTR1 (Fig. 1A) binds copper and enhances efficiency of copper transport (Puig et al., 2002). These motifs may interact with plasma copper carrier(s) and metallo-reductase(s) and/or control gating of the copper channel formed within the CTR1 multimer complex. Experimental data suggests that three conserved Met residues, the last Met at the extracellular N-terminus and two at the 2nd TM of both human and budding yeast CTR1 (Fig. 1A), are essential for copper translocation (Puig et al., 2002). Recent data also suggests that the first histidine-rich motif of human CTR1 plays a role in copper acquisition from the plasma followed by reduction to Cu(I) (Haas et al., 2011). Collectively, multiple residues and motifs within the CTR1 trimer complex are involved in copper transport, and more research must be performed to gain a clear picture of this process.

4.3. Interactions of CTR with other factors involved in copper metabolism

Once copper is transported across the cell membrane, it must efficiently reach its appropriate destination to be incorporated into copper-requiring proteins without participating in harmful reactions (Fig. 1B). It has been demonstrated that target-specific cytosolic copper carriers, named metallochaperones, distribute copper to different subcellular compartments. ATOX1 delivers copper via direct interaction with copper-transporting ATPase(s) at the trans-Golgi network where copper is loaded into copper-containing secretory proteins (Robinson and Winge, 2010; Pufahl et al., 1997; Huffman and O'Halloran, 2001). Several

critical players in copper incorporation into cytochrome c oxidase, including COX17, SCO1, and SCO2, have been defined (Robinson and Winge, 2010). The delivery of copper to superoxide dismutase (SOD1) is mediated largely by CCS, which physically interacts with SOD1 to transfer copper (Huffman and O'Halloran, 2001; Culotta et al., 2006).

CTR1 knockout cells exhibit defects in copper delivery through copper chaperones, indicating that CTR1 is a major copper supplier for the copper carriers and all cuproproteins (Lee et al., 2001; Lee et al., 2002b). It is reasonable to predict that the cytosolic copper chaperones take copper directly from CTR1; however, interaction between CTR1 and the copper chaperones has not been demonstrated. This process may be very transient. Alternatively, copper chaperones may interact with a component of the CTR1 copper transport system rather than directly with CTR1 and/or cytoplasmic compartment(s) or molecule(s) receiving copper from CTR1.

5. Regulation of copper acquisition and mobilization

5.1. Expression and activity control of CTR via various mechanisms

Strict regulation of uptake, distribution, and excretion of copper is necessary for maintaining optimal activities of copper-dependent cellular pathways. MAC1 and ACE1-mediated transcriptional regulation of genes encoding components of copper transport and detoxification in yeast has been characterized in detail (Labbe et al., 1997; Winge, 1998). Despite the role for Sp1-binding elements in copper-responsive CTR1 expression control being documented in a cancer cell line (Song et al., 2008), it is uncertain how cells sense copper status and convey the signal for CTR1 expression control and whether Sp1 regulates CTR1 in other cell types. Expression of some members of the COPT family in plants is regulated by the SPL7 transcription factor in a copper-dependent manner, which constitutes a negative-feedback loop (Peñarrubia et al., 2010; Yamasaki et al., 2009).

Copper-deficient animals exhibit higher CTR1 expression in a tissue-specific manner. For instance, total and apical membrane CTR1 levels are elevated in the intestine of mice fed with a copper-deficient diet by increasing protein stability (Kuo et al., 2006; Nose et al., 2010). It was also shown that elevated extracellular copper triggers endocytosis and turnover of CTR1 in mammalian cell lines (Petris et al., 2003; Guo et al., 2004a1), although contradictory data is reported as well (Klomp et al., 2002; Eisses et al., 2005b). It is also interesting to note that the C-terminal cytosolic domain of yeast yCTR1 binds with copper through Cys residues (Xiao et al., 2004; Wu et al., 2009), which is essential for rapid inactivation of CTR1 in response to excess copper, but not for high-affinity copper transport (Wu et al., 2009). Given the presence of Cys and/or other potential metal binding residues in the C-terminus of many other family members, including human CTR1, copper binding at the C-terminus and its regulatory role might be conserved in CTR copper transporters.

5.2. Regulation of intracellular and systemic distribution of copper

Multiple important pathophysiological conditions besides copper bioavailability control copper metabolism by the regulation of expression levels and subcellular localization of CTR and other molecular factors in copper metabolic pathways. Hypoxia stimulates copper uptake and increases the expression of CTR1 in a macrophage cell line, which results in increased copper delivery to the ATP7A at the secretory pathway (White et al., 2009). Interferon-gamma was found to increase expression of CTR1 to stimulate copper uptake followed by trafficking of ATP7A from the Golgi to vesicles that partially overlapped with the phagosome in macrophages (Zheng et al., 2010). This supports a significant role for cellular copper distribution in innate immunity that was first observed several decades ago. A similar regulation of the copper transporters was observed in microglial cells that are clustered around amyloid plaques in a mouse model of Alzheimer's disease and Interferon-

gamma stimulated microglial cells (Zheng et al., 2010). Copper sequestration by microglia under these conditions may be a mechanism for protection of neurons from copper toxicity and/or acquisition of copper for neurons.

It is interesting to note that *Slc31a1* knockout in the heart of mouse leads to induction of ATP7A expression at the intestine and liver (Kim et al., 2010), which indicates that mammals possess a mechanism for inter-organ communication for systemic copper homeostasis. Defects in the *ATP7A* and *ATP7B* genes that encode copper-transporting P-type ATPases are attributed to Menkes and Wilson diseases, respectively (Vulpe and Packman, 1995; Lutsenko et al., 2007). The *ATP7A* gene is strongly expressed in many organs and tissues (including intestinal mucosal cells); however, its expression in the liver is gradually decreased upon maturation of fetus (Kim et al., 2010). In contrast, ATP7B is highly abundant in the liver and brain relative to other organs. In a copper-limited medium, the human ATP7A and ATP7B are localized in the trans-Golgi network where they transport copper for its incorporation into secretory proteins (Fig. 1B) (Lutsenko et al., 2007). When copper levels are elevated, they are mobilized to vesicle-like compartments in a reversible manner (Fig. 1B) (Lutsenko et al., 2007). This trafficking event is associated with copper extrusion at the intestine as well as other organs and tissues. In particular, it is an important event for ATP7B to excrete excess copper into the bile to maintain systemic copper homeostasis. Higher expression of intestinal and hepatic ATP7A but not ATP7B in the heart-specific *Slc31a1* deletion mice (Kim et al., 2010) may reflect a mechanism for facilitating copper uptake at the intestine and mobilization from the liver in response to cardiac copper deficiency. Supplementation of the serum collected from heart-specific *Slc31a1* knockout mice to the culture media of a cell line also leads to ATP7A up regulation (Kim et al., 2010), suggesting that certain signaling molecule(s) exist in the serum. The Mechanisms underlying ATP7A regulation (e.g., transcription vs protein stability changes) in the mice, nature of the signaling molecule(s) (e.g., hormone, small molecule), and their mechanism of action (e.g., receptor, and signaling transduction pathways) remain to be studied.

6. Summary and perspective

Active research progress on identification of molecular factors involved in copper metabolism followed by characterization of their mechanism of action and regulation continues to reveal the novel layers of elegance that nature has created to control optimal acquisition and utilization of copper. This also has opened many research avenues that would elucidate the implications of copper in human diseases and lead to therapeutic control of copper metabolism. Multiple exciting questions that would facilitate a better understanding of the copper metabolic pathways and combat copper-related human diseases remain to be answered.

- Given embryonic lethality of CTR1 deficiency in mice, it is assumed that defect of CTR1 is lethal in humans and some cases of reproduction problems might be associated with non-functional CTR1. Despite no report documenting a direct association of CTR1 defects with human disease, further research on implications of genetic and epigenetic variations of CTR1 sequence or expression levels in disease is warranted. Individual difference or adaptive change in expression levels, subcellular distribution, and/or activity control of CTR1 might be important factors involved in human health concerns, such as degenerative neuronal diseases, cardiovascular disease, and intrinsic and acquired resistance to platinum-based chemotherapeutics.
- Further studies on mouse models possessing tissue and cell type-specific knockout of *Slc31a1* and *Slc31a2* would provide useful experimental systems defining the

physiological roles CTR1/2 and the communication among organs in maintaining systemic copper homeostasis as demonstrated in the intestine, heart, or liver-specific *Slc31a1* knockout mice.

- Many organisms express multiple CTR family members. Dissection of their specificities in terms of organs, growth stages as well as subcellular compartments and regulation patterns by copper bioavailability would lead to a better understanding of the functional and physical cooperation among CTR members.
- High-resolution structure of CTR1 with and without binding of copper is critical for ascertaining the mechanisms underlying copper translocation across the transmembrane channel that is formed within the multimer complex and the functional roles of the N-terminus and cytosolic domains.
- Functional and/or physical interactions of CTR1 with extracellular copper carrier, metalloreductases, cytoplasmic metallochaperones, and intracellular copper transporters remain to be elucidated.

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Fig. 1. Schematic illustration of the structure, subcellular localization, and function of human CTR1

(A) Topological model of human CTR1. Demonstrated and potential copper-binding residues and conserved Gly residues are marked using color codes. The asterisks indicates two Met residues (M150 and 154) at the 2nd trans-membrane helice (TM), a His residue (H139) at the 2nd TM, and two Gly residues (G167, 171) at the 3rd TM. N- and O-glycosylations occur at N15 and T27, respectively. (B) Subcellular distribution of copper ions (depicted as blue dots) that are transported by CTR1. CTR1 is detected at the plasma membrane and intracellular vesicular compartments. ATP7A/B are copper transporting P-type ATPases at the secretory pathway. (C) Homo-trimer formation of CTR1 and the arrangement of TMs at the extracellular face (top), lipid bilayer (middle) and cytoplasmic face (bottom). Numbers, 1, 2 and 3, indicate 1st, 2nd and 3rd TM, respectively. The Met (red dots), Gly (black dots) and His (green dots) residues that play important roles for copper transport and/or assembly of the multimer complex are indicated.

Table 1

Human SLC31 (CTR) family of copper transporters (adapted from <http://www.bioparadigms.org/slc/menu.asp>).

Human gene name	Protein name	Aliases	Pre- dominant substrates	Transport type & coupling ions	Tissue & cellular/ subcellular distribution	Human gene locus	Sequence accession ID
SLC31A1	CTR1	hCtr1, Ctr1, COPT1	Copper(I) Cisplatin	Energy in- dependent, potassium dependent	Ubiquitous mRNA expression; Highest in the liver, lowest in the brain and skeletal muscle; Protein localized to plasma membrane and intracellular vesicles of cultured cells	9q32	NM_001859.3
SLC31A1P1	pseudo- gene	SLC31A1P, CTR1psi, CTR1P				3q26.31	AF238997
SLC31A2	CTR2	hCtr2, Ctr2, COPT2	Copper Cisplatin	Unknown	Ubiquitous mRNA expression; Plasma membrane, late endosome and lysosome	9q32	NM_001860.2