Role of copper transporters in copper homeostasis[2,3,4]

Joseph R Prohaska
University of Minnesota Medical School, Duluth, MN

Abstract

Copper is a redox active metal that is essential for biological function. Copper is potentially toxic; thus, its homeostasis is carefully regulated through a system of protein transporters. Copper is taken up across the lumen surface of the small intestinal microvilli as cuprous ion by Ctr1. Cupric ion may also be taken up, but those processes are less well understood. Within the cell, intestinal as well as others, copper is escorted to specific compartments by metallo-chaperones. One, CCS, donates copper to superoxide dismutase. Another, COX17, delivers copper to additional chaperones within the mitochondria for synthesis of cytochrome *c* oxidase. A third chaperone, Atox1, delivers copper to the secretory pathway by docking with 2 P-type ATPases. One, ATP7A, is the protein nonfunctional in Menkes disease. This protein is required for cuproenzyme biosynthesis, and in the enterocyte it is required for copper efflux to portal blood. The second, ATP7B, predominantly expressed in liver, is required for copper metallation of ceruloplasmin and biliary copper excretion. Mutations in ATP7B lead to Wilson disease. Additional intracellular hepatic copper-binding proteins COMMD1 (copper metabolism MURR1 domain) and XIAP (X-linked inhibitor of apoptosis protein) may also be required for excretion. Other proteins involved in copper homeostasis may include metallothionein and amyloid precursor protein. Plasma protein transport of copper from the intestine to liver and in systemic circulation probably includes both albumin and α2-macroglobulin. Changes in the expression of copper "transporters" may be useful to monitor copper status of humans, provided a suitable cell type can be sampled.

INTRODUCTION

Copper, like iron and several other dietary essential metals, is carefully regulated. This is because, although essential, they are potentially toxic because of their chemical redox potential and ability to participate in free radical reactions. Sometimes primary regulation occurs at the level of the absorptive enterocyte, as for iron, and sometimes both absorption and excretion are regulated, as for copper. Because these transition metals are potentially reactive free in solution, they are predominantly associated with protein escorts as they travel across membranes, traverse intra-cellular space, and while present extracellularly (Table 1). These "transporters" could potentially serve as biomarkers if their mRNA expression or protein abundance or both changed in response to dietary metal intake.

COPPER IMPORT TRANSPORTERS

As dietary copper reaches the duodenum, it comes in contact with CTR1 (copper transporter 1) (SLC31A1). CTR1 is believed to be the primary protein responsible for import of dietary copper across the brush border microvilli. CTR1 is an integral plasma membrane protein with 3 transmembrane domains that forms a homotrimeric pore for import of Cu^+ (1). Dietary reductants such as ascorbate may provide the apropos substrate. Perhaps cupric reductases are necessary for this process. This might include Dcytb, a putative Fe^{3+} reductase, or Steap2, a newly hypothesized Fe^{3+} reductase that can also reduce Cu^{2+} (2).

On the basis of primarily cell culture work many have shown that CTR1 moves to endocytic vesicles on exposure to copper. Some believe this mechanism would thus restrict copper import when supplies are excessive. Unlike Bakers yeast ctr1, which is regulated also by transcription (Mac1), mammalian CTR1 mRNA does not respond robustly to either copper deficiency or copper excess. Thus, the cycling mechanism, although controversial and probably not in all cell types, is an appealing way to regulate intestinal copper transport. However, a seminal study with knockout of mCtr1 in the intestine has shown a more complex story (3). The CTR1−/− mouse indeed presented a phenotype of "copper deficiency," providing additional support for a role for CTR1 in transport; however, the intestine rather than becoming depleted of copper had higher than normal amounts, suggesting an intracellular role for CTR1 in copper trafficking. Those studies also suggest that copper import can occur without CTR1, at least in the suckling mouse. CTR1 expression in adult mouse intestine appears to be largely intracellular, consistent with a diet generous in copper, probably ≥5 times higher in concentration than a typical US diet of 1 mg/d. Importantly, when suckling pups consumed a diet low in copper, CTR1 was highly expressed in the apical duodenum and to a lesser extent in pups nursed by dams producing milk with adequate copper (4). It does not seem likely that CTR1 protein will be a useful marker of copper status because both copper deficiency and copper supplementation resulted in higher abundance, based on immuno-blot data (4,5).

Recent work in cell culture characterized hCTR2 a homolog of CTR1 located in lysosomes or endocytic vesicles (6). CTR2 may play a role in copper import as well as intracellular copper homeostasis. Regulation by copper of CTR2 has not been published.

The issue of other copper importers besides CTR1 is controversial. Some have suggested that DMT1 (divalent metal transporter 1), the primary Fe^{2+} importer, could transport copper. Some even suggest that DMT1 transports Cu^+. However, others have studied Cu^{2+} intestinal import in Belgrade rats, a mutant with nonfunctional DMT1, and describe a novel ATP-dependent process (7). Thus, there may be novel ways to import copper. However, it should be recalled that CTR1-null mice are not viable, reinforcing the theory that CTR1 is the primary copper importer. However, even this tenet was recently challenged in studies suggesting that CTR1 regulates embryonic stem cell differentiation in a copper-independent manner (8).

Suggestions were also proposed that other proteins may be involved in copper homeostasis in other organs, such as brain (9). Those suggestions include the prion protein PrP^c and amyloid precursor protein APP. Genetic evidence for the later is quite strong because APP-null mice have increased and APP overexpressors have decreased brain copper.

COPPER EFFLUX TRANSPORTERS

Import of copper by CTR1 does not require energy or a proton gradient, but efflux requires hydrolysis of ATP. Two well-studied ATPases are known to participate in copper homeostasis.

ATP7A is a protein coded on the X-chromosome that is missing in humans with Menkes disease. Subjects present with symptoms similar to copper deficiency because ATP7A is

required for transport of copper into the *trans*-Golgi network (TGN) for biosynthesis of several secreted cuproenzymes and for basolateral efflux of copper in the intestine and selected other cells (10,11). ATP7A has 6 copper-binding domains for Cu^+, but not all are necessary for function. There are many controversies about ATP7A and its response to copper. Those controversies include its location within the cell, its movement when presented with copper, its putative location on the cell membrane (both apical and basolateral in polarized cells), and new functions involved in release of copper for neurotransmission, or a requirement for synaptogenesis. Recent reviews elaborate on those controversies (10). There is little compelling evidence that ATP7A mRNA or protein concentrations will be useful to reflect changes in human copper status, based on extensive work with laboratory rodent models with a combination of dietary and genetic approaches.

ATP7B, a protein related to ATP7A but coded on chromosome 13, has a similar structure, including Cu^+-binding domains. ATP7B is primarily involved in hepatic copper homeostasis. Adult liver does not express ATP7A. ATP7B is also important in mammalian placenta, mammary tissue, brain, and kidney, and perhaps small intestine. Mutations in ATP7B result in copper retention by liver and brain copper toxicity. Collectively these mutations are described as Wilson disease. Like ATP7A, controversy about trafficking of ATP7B in response to copper exists (10,11). ATP7B is an efflux transporter in liver, but it is also essential in the TGN for transfer of copper for metallation of ceruloplasmin, the major plasma cuproprotein. When presented with excess copper, ATP7B translocates to a vesicular compartment to facilitate biliary copper efflux across the apical biliary canicular membrane, a key process in maintaining homeostasis. It appears that ATP7B is primarily involved in apical secretion of copper. There is little evidence that copper status affects ATP7B mRNA or protein concentrations, rendering its use as a biomarker dubious.

Efflux of copper also requires the copper chaperone Atox1, discussed later, and in liver other protein factors. One such factor originally discovered in Bedlington terriers with liver toxicosis is called MURR1. This protein is now called COMMD1 (copper metabolism MURR1 domain) and is a member of a family with ≥10 unique proteins (12). COMMD1 or MURR1 is essential in normal copper efflux from liver and interacts with ATP7B. Exciting new research suggests that the COMMD family of copper-binding proteins influences much more than copper egress and involves nuclear transcription factor κB signaling, apoptosis, and the ubiquination pathway (12). COMMD1 also interacts with another copper-binding protein XIAP (X-linked inhibitor of apoptosis). XIAP is necessary for COMMD1 degradation. Thus, the excretion of copper from liver depends on several copper-binding efflux transporters. COMMD1 expression after changes in copper status has not been evaluated. High concentrations of liver copper accelerate the catabolism of XIAP.

Iron status is reflected in its storage protein ferritin whereby higher ferritin is observed when cellular iron increases. Although, there is no known copper storage protein, some have suggested that its function is provided by MT (metallothionein), especially when copper is elevated as in the neonate when biliary excretion is immature (13). MT is a low-molecular-weight protein rich in cysteine and can bind several moles of copper in both the α and β domain of the protein. Exposure of the intestine to high concentrations of copper could result in enhanced trapping of copper by MT and loss (efflux) of copper when cells are sloughed. Copper, like certain other metals, induces transcription of MT. MT concentrations in peripheral blood monocytes were used to assess zinc intake in humans (14). Utility of MT as a copper biomarker requires further evaluation.

COPPER CHAPERONES

Approximately a dozen years ago a new concept in copper homeostasis emerged with the discovery of the first copper chaperone ATOX1. The exciting details of this discovery first in yeast and latter in mammals are described elsewhere (15,16). Atox1 is essential in mammals because perinatal lethality was described in null mice. ATOX1 binds Cu^+ and associates with ATP7A and ATP7B. Thus, ATOX1 is part of the copper metallation pathway as well as intracellular copper efflux. Expression concentrations of ATOX1 after copper deficiency or copper toxicity have not been thoroughly interrogated in mammalian models. Thus, ATOX1 is unexplored as a potential biomarker of copper.

Shortly after the discovery of ATOX1 as a copper chaperone for the secretory pathway of copper homeostasis, another chaperone was discovered. This protein forms heterodimers with copper zinc superoxide dismutase (SOD) and is called CCS (copper chaperone for superoxide dismutase). CCS has 3 domains. Domain II, which resembles SOD, binds to apo-SOD and domain III transfers copper to SOD. CCS is essential to activate mammalian SOD, but the absence of CCS similar to the absence of SOD is not lethal in the mouse. Our laboratory and the L'Abbe laboratory independently discovered that CCS protein concentrations are higher in many cells of copper-deficient mammals (9). CCS is abundant in erythrocytes; thus, it is a potential marker of copper status. Perhaps the CCS/SOD abundance will be an even better marker of copper deficiency (17). Ongoing research is aimed at finding the most appropriate blood cell to monitor changes in CCS. Recent data in humans, for example, suggest that CCS mRNA might respond to copper excess in peripheral blood mononuclear cells (16).

Copper is also escorted to mitochondria for proper homeostasis. In the intramembrane space copper is needed for SOD, and this copper transfer depends on CCS. It is generally believed that most mitochondrial copper is delivered by the chaperone COX17. COX17 is 1 of 30 assembly factors necessary for formation of active cytochrome *c* oxidase (CCO) (18). CCO itself is a complex with requirements not only for copper but also for zinc, magnesium, heme iron, and 13 polypeptide subunits. COX17 is thought to transfer copper from cytoplasm into mitochondria, but most of cellular COX17 is mitochondrial. Is there another COX chaperone?

COX17 is also essential because deletion in the mouse is embryonically lethal and temporally coincident with ctr1-null mice (9). COX17 is one of several copper-binding chaperones necessary for copper transfer to the CCO subunits COX1 and COX2. CCO is an inner membrane protein complex. The effect of altered dietary copper on COX17 expression has not been published.

The copper site in COX1 is designated as the CuB site (18). This site receives its copper by another mitochondrial chaperone COX11 (19). COX11, like most copper-binding proteins, contains critical cysteine residues needed for function. COX11 has not been evaluated as a biomarker of copper status. Its utility would be limited to white cells because erythrocytes contain no mitochondria.

COX2 contains the copper-binding site, CuA, of CCO. It receives its copper by COX17 but directly from 1 or 2 other copper chaperones SCO1 and SCO2 (19). These interesting proteins first discovered in yeast are also critical for CCO assembly and function. Mutational changes in either SCO1 or SCO2 impair CCO function and lead to pathologic outcomes. Although expression concentrations of SCO1 and SCO2 are not transcriptionally controlled by copper, these 2 proteins seem unique in that they appear to function in copper homeostasis in addition to their chaperone role (20). The copper homeostatic defect in SCO1 and SCO2 mutants results in a copper deficiency phenotype. The decrease in cellular copper appears due to enhanced efflux rather than to impaired uptake. This exciting observation, when fully integrated, will provide new insight into cellular copper homeostasis.

SUMMARY

Copper homeostasis in mammals requires complex regulation of absorption and excretion. This copper egress is accomplished by regulation of copper import, intracellular flux, and efflux across the basolateral intestinal membrane. Luminal copper is reduced and taken up by CTR1, transferred to ATOX1 and to ATP7A. Copper released by the enterocyte passes to portal blood. Copper is transported by albumin and transcuprein (recently shown to be a macroglobulin) (21). Hepatic copper is likely taken up by CTR1 and by ATOX1 delivered to the TGN and ATP7B for ceruloplasmin synthesis or to the canalicular membrane by COMMD1 en route to bile. Specific copper transporters in systemic circulation remain largely uncharacterized. CTR1, ATOX1, and either ATP7A or ATP7B or both then function in cuproenzyme biosynthesis or export across the cell. Additional copper is used by CCS or COX17 for metallation of SOD and CCO. Copper is also required for the other mammalian cuproenzymes to function properly (9).

Expression concentrations of these transporters may be useful to assess copper status (16). However, this requires further fundamental research in experimental mammals with confirmation in human samples before a robust and easy to use biomarker is formed. When copper is limiting, chaperones and transporters within cells of the blood may be useful to assess status either by enzymatic, genomic, or proteomic approaches. However, to assess copper excess will be more challenging because hepatic sensing of copper overload to a noninvasive compartment such as blood does not result in a well-known measurable and specific signal. These remain the challenges of contemporary biomarker research on copper.

References

1. Nose Y, Rees EM, Thiele DJ. Structure of the Ctr1 copper trans'PORE'ter reveals novel architecture. Trends Biochem Sci 2006;31:604–7. [PubMed: 16982196]

2. Knutson MD. Steap proteins: implications for iron and copper metabolism. Nutr Rev 2007;65:335–40. [PubMed: 17695374]

3. Nose Y, Kim BE, Thiele DJ. Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. Cell Metab 2006;4:235–44. [PubMed: 16950140]

4. Kuo YM, Gybina AA, Pyatskowit JW, Gitschier J, Prohaska JR. Copper transport protein (ctr1) levels in mice are tissue specific and dependent on copper status. J Nutr 2006;136:21–6. [PubMed: 16365053]

5. Bauerly KA, Kelleher SL, Lönnerdal B. Functional and molecular responses of suckling rat pups and human intestinal Caco-2 cells to copper treatment. J Nutr Biochem 2004;15:155–62. [PubMed: 15023397]

6. van den Berghe PV, Folmer DE, Malingre HE, et al. Human copper transporter 2 is localized in late endosomes and lysosomes and facilitates cellular copper uptake. Biochem J 2007;407:49–59. [PubMed: 17617060]

7. Knopfel M, Smith C, Solioz M. ATP-driven copper transport across the intestinal brush border membrane. Biochem Biophys Res Commun 2005;330:645–52. [PubMed: 15809046]

8. Haremaki T, Fraser ST, Kuo YM, Baron MH, Weinstein DC. Vertebrate Ctr1 coordinates morphogenesis and progenitor cell fate and regulates embryonic stem cell differentiation. Proc Natl Acad Sci U S A 2007;104:12029–34. [PubMed: 17620605]

9. Prohaska, JR. Copper. In: Bowman, BA.; Russell, RM., editors. Present knowledge in nutrition. Washington, DC: International Life Sciences Institute; 2006. p. 458-70.

10. La Fontaine S, Mercer JF. Trafficking of the copper-ATPases, ATP7A and ATP7B: role in copper homeostasis. Arch Biochem Biophys 2007;463:149–67. [PubMed: 17531189]

11. Lutsenko S, Barnes NL, Bartee MY, Dmitriev OY. Function and regulation of human copper-transporting ATPases. Physiol Rev 2007;87:1011–46. [PubMed: 17615395]

12. Maine GN, Burstein E. COMMD proteins: COMMing to the scene. Cell Mol Life Sci 2007;64:1997–2005. [PubMed: 17497243]

13. Kelly EJ, Palmiter RD. A murine model of Menkes disease reveals a physiological function of metallothionein. Nat Genet 1996;13:219–22. [PubMed: 8640230]

14. Sullivan VK, Burnett FR, Cousins RJ. Metallothionein expression is increased in monocytes and erythrocytes of young men during zinc supplementation. J Nutr 1998;128:707–13. [PubMed: 9521632]

15. Field LS, Luk E, Culotta VC. Copper chaperones: personal escorts for metal ions. J Bioenerg Biomembr 2002;34:373–9. [PubMed: 12539964]

16. Danzeisen R, Araya M, Harrison B, et al. How reliable and robust are current biomarkers for copper status? Br J Nutr 2007:1–8.

17. West EC, Prohaska JR. Cu, Zn-superoxide dismutase is lower and copper chaperone CCS is higher in erythrocytes of copper-deficient rats and mice. Exp Biol Med 2004;229:756–64.

18. Carr HS, Winge DR. Assembly of cytochrome c oxidase within the mitochondrion. Acc Chem Res 2003;36:309–16. [PubMed: 12755640]

19. Hamza I, Gitlin JD. Copper chaperones for cytochrome c oxidase and human disease. J Bioenerg Biomembr 2002;34:381–8. [PubMed: 12539965]

20. Leary SC, Cobine PA, Kaufman BA, et al. The human cytochrome c oxidase assembly factors SCO1 and SCO2 have regulatory roles in the maintenance of cellular copper homeostasis. Cell Metab 2007;5:9–20. [PubMed: 17189203]

21. Liu N, Lo LS, Askary SH, et al. Transcuprein is a macroglobulin regulated by copper and iron availability. J Nutr Biochem 2007;18:597–608. [PubMed: 17363239]

TABLE 1

Copper-binding proteins involved in copper transport[1]

Protien	Acronym	Putative copper function
Membrane		
Copper transporter 1	CTR1	Plasma membrane uptake
Copper transporter 2	CTR2	Endosomal pump
Divalent metal transporter 1	DMT1	Cu^{2+} or Cu^{1+} import
ATPase	None	Cu^{2+} import
Amyloid precusor protein	APP	Copper homeostasis
Intracellar		
Menkes disease protein	ATP7A	Enzyme biosynthesis and efflux
Wilson disease protein	ATP7B	Enzyme biosynthesis and efflux
Metallothionein	MT	Storage and chaperone
Copper metabolism MURR domain	COMMD1	Hepatic efflux
X- linked inhibitor of apoptosis	XIAP	Hepatic efflux
Chaperones		
Antioxidant	ATOX1	ATP7A, ATP7B target
Copper chaperone SOD	CCS	SOD target
Assembly factor 17 for CCO	COX17	CCO targets
Assembly factor 11 for CCO	COX11	COX2 Site B
Supressor of COX17 mutation 1	SCO1	COX1 Site A
Supressor of COX17 mutation 2	SCO2	COX1 Site A

[1] ATPase, adenosine triphosphatase; SOD copper, zinc-superoxide dismutase; CCO, cytochrome *c* oxidase.