Intracellular Copper Transport in Mammals[1,2]

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ABSTRACT Copper is an essential cofactor for approximately a dozen cuproenzymes in which copper is bound to specific amino acid residues in an active site. However, free cuprous ions react readily with hydrogen peroxide to yield the deleterious hydroxyl radical. Therefore, copper homeostasis is regulated very tightly, and unbound copper is extremely low in concentration. Copper imported by the plasma membrane transport protein Ctr1 rapidly binds to intracellular copper chaperone proteins. Atox1 delivers copper to the secretory pathway and docks with either copper-transporting ATPase ATP7B in the liver or ATP7A in other cells. ATP7B directs copper to plasma ceruloplasmin or to biliary excretion in concert with a newly discovered chaperone, Murr1, the protein missing in canine copper toxicosis. ATP7A directs copper within the transgolgi network to the proteins dopamine β-monooxgenase, peptidylglycine α-amidating monooxygenase, lysyl oxidase, and tyrosinase, depending on the cell type. CCS is the copper chaperone for Cu,Zn-superoxide dismutase; it delivers copper in the cytoplasm and intermitochondrial space. Cox17 delivers copper to mitochondria to cytochrome c oxidase via the chaperones Cox11, Sco1, and Sco2. Other copper chaperones may exist and might include metallothionein and amyloid precursor protein (APP). Genetic and nutritional studies have illustrated the essential nature of these copper-binding proteins; alterations in their levels are associated with severe pathology. J. Nutr. 134: 1003–1006, 2004.

KEY WORDS: • copper • chaperones • transport

Mammals utilize a number of strategies to catalyze 1-electron-transfer reactions. The essential metal ions cobalt, copper, iron, manganese, and molybdenum, along with flavins and quinones, perform this function. The interaction of certain 1-electron donors with hydrogen peroxide rather than redox coupled partners can yield the deleterious hydroxyl radical, one of the reactive oxygen species (ROS)[4] thought to play a key role in pathophysiology. Ferrous iron is normally thought to be a major determinant of the ROS process; however, cuprous copper is perhaps even more reactive. How do cells control potentially reactive redox metals?

Copper: an essential metal cofactor. The association between the nutrient copper and normal hemoglobin metabolism, a vital physiological process, was recognized in the middle of the 19th century (1). Over the next 150 y, approximately a dozen proteins dependent on copper for their function (cuproenzymes) were discovered (2). These cuproenzymes explain why dietary copper is essential, because copper restriction in the diet can alter the activity of a cuproenzyme and impact normal physiology. For example, in melanocytes the copper redox center of tyrosinase converts tyrosine to dihydroxyphenylalanine in the first step in the synthesis of the pigment melanin. Hypopigmentation is a consequence of dietary copper deficiency due to limiting tyrosinase. Other details of the nutritional biochemistry of copper have been summarized elsewhere (3). Copper is both potentially deleterious due to its participation in ROS chemistry and essential due to its role as a redox catalyst for a number of oxidases. It is recommended that adults consume at least 0.9 mg of copper daily (RDA) and restrict their intake to <10 mg daily (UL) to prevent overt signs of copper deficiency and toxicity, respectively (4).

Copper chaperones. Copper is transported into the cell by the protein Ctr1, which was originally discovered in yeast and later characterized in mammals (5). Acting as a permease or by endocytosis, Ctr1 delivers Cu^+ within cells (6). The mechanism for cupric ion reduction prior to uptake remains unknown. If uncontrolled, this pool of cuprous ions could lead to ROS generation. However, very few, if any, free copper ions exist in the cytoplasm (7). The delivery of copper to target cuproenzymes depends on an elegant metallochaperone system. Several cytoplasmic chaperones have been described [Atox1, CCS (copper chaperone for Cu,Zn superoxide dismutase), and Cox17] as well as membrane-associated copper-transporting ATPases (ATP7A and ATP7B). Candidate chaperones include Murr1 (a recently discovered protein whose absence results in canine copper toxicosis), metallothionein (MT-1 and MT-2), and amyloid precursor protein (APP).

There has been remarkable conservation of the copper-binding sites in the metallochaperones and ATPases from bacteria to mammals (8). For example, the sequence M-X^I-C-X^{II}-X^{III}-C is highly conserved. X^I is usually S, T, or H, and X^{III} is G in metallochaperones and S or A in ATPases. More details on the chemistry of these proteins is presented in several excellent reviews (8–11).

Atox1. The oxygen toxicity of yeast mutants lacking Cu,Zn-superoxide dismutase (SOD) can be suppressed by the expression of a small antioxidant protein, Atx1, that was subsequently shown to be a copper chaperone (12). This key discovery has been thoroughly reviewed (13). Shortly after the yeast work was published, a homolog in humans and other mammals, Atox1, was discovered (14). Human Atox1 contains 68 amino acids. Once Cu^+ enters a cell, it binds to the copper-binding site of Atox1 and is transferred to its docking

[1] Manuscript received 14 March 2004.
[2] Supported in part by the National Institutes of Health, grant NIH HD-39708.
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[4] Abbreviations used: apo, apoprotein; APP, amyloid precursor protein; CCO, cytochrome c oxidase; CCS, copper chaperone for Cu,Zn-superoxide dismutase; MT, metallothionein; PAM, peptidylglycine α-amidating monoxygenase; ROS, reactive oxygen species; SOD, Cu,Zn-superoxide dismutase.

0022-3166/04 $8.00 J. Nutr. 134: 1003–1006, 2004.
Manuscript received 5 March 2004.

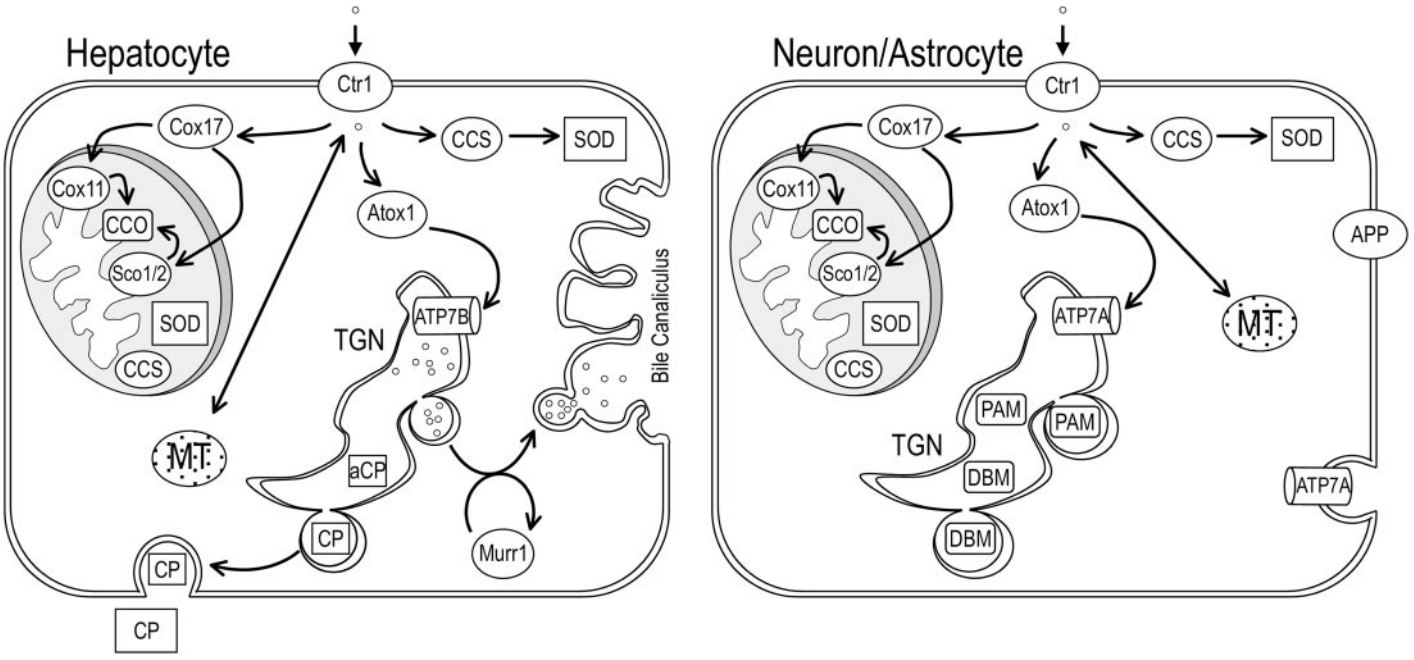

FIGURE 1 Copper transport in hepatocytes, neurons, and astrocytes. Cuproenzymes (rectangles) are dependent on copper chaperones (ellipses) and copper-transporting ATPases (ATP7A and ATP7B) for the delivery of imported copper to biosynthesis and metal-transfer sites. Chaperones and ATPases, which normally reside in the transgolgi network (TGN), are also necessary for the transport of copper to the bile (hepatocytes) or the plasma membrane for copper efflux from the cell (neurons and astrocytes).

partners in the secretory pathway (**Fig. 1**). Atox1 interacts with ATP7B in the hepatocyte and is thus required for proper biliary excretion of excess copper as well as delivery of copper for holoceruloplasmin (CP) synthesis.

Deletion of the Atox1 gene in mice causes perinatal mortality (15). Atox1-null mice exhibit hypopigmentation consistent with a role for Atox1 in copper delivery to tyrosinase. The skin distortion is likely due to reduced levels of lysyl oxidase, a cuproenzyme involved in elastic and collagen cross-linking. Copper levels in the liver and brain are decreased by 50% in mutants, consistent with an impaired efflux of copper from enterocytes. Cells lacking Atox1 have impaired movement of ATP7A in response to copper, providing a mechanistic explanation for copper retention (16). ATP7A normally resides in the transgolgi membrane but moves to the plasma membrane when cellular copper concentrations rise.

In addition to its chaperone function, other properties may be ascribed to Atox1. Atox1 is present at high levels in neurons and may protect these cells from oxidative stress (17).

Atox1 is a small copper chaperone that binds to copper-transport ATPases and is essential to the delivery of imported Cu^{+} to secretory pathways in the transgolgi network (Fig. 1). The effects of dietary copper restriction and excess on Atox1 expression have not yet been evaluated.

CCS. The second copper chaperone to be recognized, CCS, is a protein required for the delivery of copper to SOD. This chaperone was also discovered by Valerie Culotta and colleagues (18). CCS is a homodimer with 35-kDa subunits and 3 functional domains. Domain II is highly homologous to SOD, and heterodimer pairs of SOD and CCS subunits form to facilitate copper transfer (13). Domain I contains the copper-binding site but essential cysteines in domain III are involved in the transfer of copper to apoprotein (apo)-SOD.

CCS deletion greatly reduces SOD activity in mice (19). The phenotype of CCS −/− mice is similar to that of sod1 −/− mice with increased sensitivity to oxidant challenge. Lack of CCS does not alter other copper chaperone–dependent pathways, such as ceruloplasmin activation (19). A fraction of yeast CCS is located in the mitochondrial intermembrane space with SOD (20). We detected both CCS and SOD in purified rat brain mitochondria (Fig. 1). Presumably, SOD in the mitochondria can scavenge superoxide released toward the intermembrane space, whereas matrix-generated superoxide can be disposed of by manganese-dependent superoxide dismutase (sod2). In yeast, CCS is necessary for copper transfer to apo-SOD, because the free ionic copper content is extremely low (7). Work with CCS −/− mice indicates a similar dependence on CCS in mammals (19).

Last year, 2 groups independently reported elevated CCS levels in tissues of copper-deficient mice and rats (21,22). This effect was not due to enhanced synthesis of CCS, as evidenced by unchanged mRNA levels in both rats and mice (22,23). Cell culture studies indicate that elevated CCS levels in copper-limited cells are due to slower degradation by the 26S proteasome complex (23). CCS levels are not correlated with SOD levels in mammals. We found higher brain CCS levels in mice and rats even though SOD levels were unchanged (22). In some tissues, such as the liver, SOD protein levels are markedly lower following copper deficiency or deletion of CCS protein (24,25).

CCS is a 70-kDA protein that binds Cu^{+} and is required for the conversion of apo-SOD to holo-SOD. Cell CCS levels are affected by copper status. In fact, elevated CCS concentration is one of the most robust cuproprotein changes following copper deprivation. Changes in CCS concentration may be a useful way to assess copper status in humans.

Cox17. The third classical copper chaperone, Cox17, was also discovered first in yeast as a small 8-kDa protein required for cytochrome c oxidase (CCO) functioning (26). CCO is a large protein (13 subunits) found in the mitochondrial inner membrane. CCO subunits I and II (Cox1 and Cox2) contain copper centers CuB and CuA, respectively (27). Cox17 is

found both in the cytoplasm and the mitochondrial intermembrane space. It delivers copper to other CCO assembly proteins. In humans, 2 related proteins, Sco1 and Sco2, are necessary for the transfer of copper to CCO subunit II (28). In yeast, Sco2 does not seem to be essential, but in humans, mutations of either Sco1 or Sco2 cause altered CCO activity and pathology (27,28). In yeast, subunit I receives copper from another metallochaperone, Cox11. It seems likely that a similar role for Cox11 in mammals will be discovered (Fig. 1) (27,28). Thus, in mammals, 4 copper chaperones are required to transfer copper from incoming Ctr1 to CCO: Cox17, Sco1, Sco2, and Cox11.

Some genetic and nutritional experiments have been conducted on the CCO chaperones in mammals. The essentiality of Cox17 was established when null mice missing both copies of the gene were found to die between embryonic days E8.5 and E10 (29). The mortality time course is similar to that of Ctr1 −/− mice (30,31). The CCO activity of cells in culture with Sco2 mutations is very low, and levels of Cox2 are also reduced. CCO activity can be rescued by adding exogenous copper (32). These same studies reported no copper-dependent transcriptional regulation of Cox17 or Sco1. However, the stability of subunit II (Cox2), the CuA site and docking partner of Sco2, was demonstrated.

Copper-transporting ATPases. Copper chaperones are proteins that are necessary for the delivery of copper to specific cuproenzymes such as SOD and CCO. Cuproenzymes in the secretory pathway depend on ATP7A and A7P7B for their copper delivery, although apoprotein and ATPases may not directly bind to one another (Fig. 1). Newly absorbed dietary copper enters the liver and leaves as ceruloplasmin or is excreted in bile. When ATP7B is mutated, as in Wilson's disease, holoceruloplasmin levels decrease and copper accumulates in the liver, confirming an important role for ATP7B in copper homeostasis (11).

ATP7A is not expressed in liver cells, but is an important copper-transport protein in other cells, such as neurons and astrocytes (Fig. 1). When cells are exposed to increasing levels of copper, ATP7A moves from the transgolgi network to the plasma membrane for copper efflux (11). In concert with Atox1, ATP7A also plays a key role in providing copper for secretory cuproenzymes such as peptidylglycine α-amidating monoxygenase (PAM) (33). When ATP7A is nonfunctional, as in brindled mutant mice, PAM activity is altered, and selected neuropeptide maturation is impaired (34). The activity of dopamine-β-monooxygenase is also dependent on both dietary copper and ATP7A expression (35). ATP7A is likely required for the transfer of copper to other cuproenzymes, such as hephaestin, the enterocyte ferroxidase. Mechanisms for the delivery of copper to membrane-associated cuproenzymes such as extracellular superoxide dismutase and vascular adhesion protein 1, an amine oxidase (EC 1.4.3.6), are not yet known.

Putative chaperones. Other copper chaperones that dock with specific cuproenzymes and transfer metal may yet be discovered. One strong candidate is the protein Murr1, which associates with ATPB7B (36). The loss of Murr1 causes hepatic copper overload. Murr1 does not directly interact with Atox1, suggesting that Murr1 is required for the delivery of copper to the bile from ATP7B (Fig. 1).

Another possible copper chaperone is MT, a low-molecular-weight cysteine-rich protein whose function remains elusive (37). Two isoforms of MT, MT-1 and MT-2, may play a role in intracellular copper transfer and storage. In the perinatal liver, copper stores are associated with MT, perhaps because of an immature bililary secretory system and limited ceruloplasmin synthesis. Copper can induce MT synthesis, although zinc is more likely the physiological inducer (37). Pools of Cu-MT can be depleted as mammals age, providing copper for the specific chaperone systems mentioned above. When copper is limiting in liver cells, levels of biliary copper excretion and holoceruloplasmin synthesis are low. Suzuki et al. (38) suggest that MT plays an important role under these conditions as well, by acting as a copper reserve.

Another chaperone candidate is APP, a membrane protein that contains a copper-binding site. In APP-null mice, brain copper levels are markedly elevated, compared to those in wild-type mice (39). Brain zinc and iron levels are unaffected. Conversely, in transgenic mice that overexpress APP, brain copper levels are reduced (40). This suggests that APP serves as a barrier to copper import in the brain. Dietary copper supplementation can reverse both the reduction in brain copper levels and lower the SOD activity observed in APP overexpressors (41).

The elegant system of copper chaperones provides an efficient and safe mechanism for the delivery of copper that has been transported into the cell to specific docking partners and eventually to cuproenzymes. It is conceivable that metal chaperone systems will be discovered for other redox-sensitive metals.

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