Advances in the Understanding of Mammalian Copper Transporters[1,2]

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ABSTRACT

Copper (Cu) is an essential micronutrient. Its ability to exist in 2 oxidation states (Cu^{1+} and Cu^{2+}) allows it to function as an enzymatic cofactor in hydrolytic, electron transfer, and oxygen utilization reactions. Cu transporters CTR1, ATP7A, and ATP7B play key roles in ensuring that adequate Cu is available for Cu-requiring processes and the prevention of excess Cu accumulation within cells. Two diseases of Cu metabolism, Menkes disease and Wilson disease, which are caused by mutations in ATP7A and ATP7B, respectively, exemplify the critical importance of regulating Cu balance in humans. Herein, we review recent studies of the biochemical and cell biological characteristics of CTR1, ATP7A, and ATP7B, as well as emerging roles for Cu in new areas of physiology. *Adv. Nutr. 2: 129–137, 2011.*

Introduction

Copper (Cu) is an essential nutrient that functions as an enzymatic cofactor in a wide range of biochemical processes that include cellular respiration, free radical detoxification, pigmentation, neuropeptide processing, cross-linking of collagen and elastin, and iron transport (1–3). Recent bioinformatics analyses suggest that ~1% of the total eukaryotic proteome is composed of putative Cu binding proteins, suggesting that the list of known cuproproteins represents only a minor fraction of the total (1). Thus, there is still much to be discovered. Although adequate Cu levels are essential for normal metabolism, excess Cu can be toxic to cells. The cellular needs for Cu are met by a tightly regulated interconnected network of proteins that include the CTR1 protein, which mediates Cu uptake across the plasma membrane, and the Cu chaperones, a collection of proteins that deliver Cu to specific target enzymes (**Fig. 1**). The devastating consequences of Cu imbalance in humans is illustrated by the 2 genetically inherited disorders, Menkes disease and Wilson disease, which are caused by mutations in the Cu transporting P-type ATPases, ATP7A and ATP7B, respectively. We have learned a great deal regarding the biochemical and cell biological aspects of these Cu transporters over the past decade. The following is a brief review of mammalian Cu transporters and emerging evidence that these proteins may function in a variety of specialized physiological and pathophysiological processes.

Cu Uptake

The CTR1 protein

Cellular Cu uptake in mammals is accomplished primarily by the Cu importer, CTR1 (SLC31A1) (2). This high affinity Cu permease is composed of 3 major domains: an extracytoplasmic N-terminal domain, 3 membrane spanning domains, and a cytosolic C-terminal tail. The N-terminal domain contains multiple methionine rich motifs, which are thought to bind and facilitate Cu transfer through the channel domain (3). A similar metal binding motif (MX_3M) also exists within the second transmembrane segment, which is essential for Cu uptake via formation of Cu-S linkages (3,4). Structural studies suggest that CTR1 assembles into a homotrimer with a minimal membrane spanning pore of 9 Å, allowing for the passage of Cu ions across the lipid bilayer (4,5). The uptake of Cu by CTR1 is time, temperature, and pH dependent and specific for reduced Cu (I) (6). However, the extracellular ligand from which Cu is delivered to CTR1 and the mechanism by which it is reduced prior to transport are unknown. Candidates for this reducing activity function include the Steap protein family (7) or the Dcytb protein (Cybrd1) (8,9), both of which are reported to possess cupric reductase activity. The essential role for CTR1 in mammalian development

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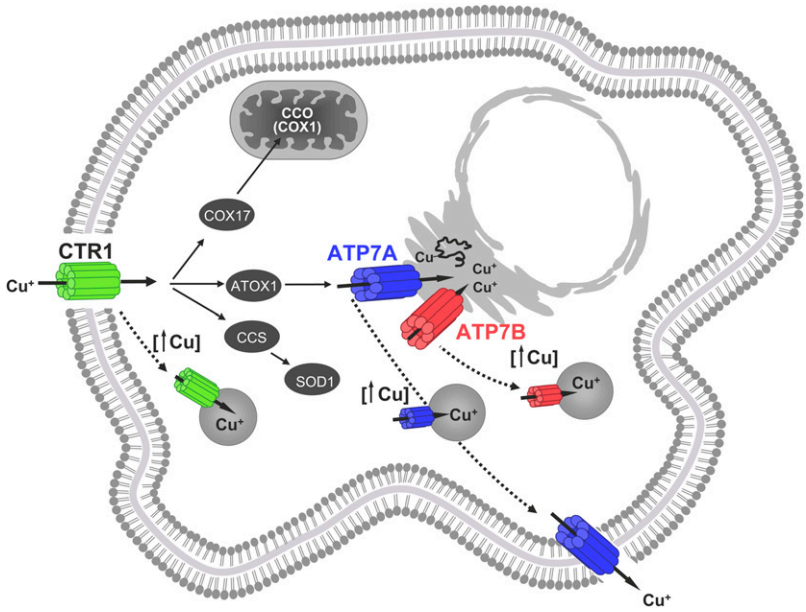

Figure 1 Cellular Cu homeostasis. The CTR1 protein functions in Cu uptake. The Cu chaperones COX17 and CCS deliver Cu to CCO and SOD1, respectively. The role of additional Cu chaperones (SCO1 and SCO2) involved in Cu incorporation into CCO is not shown. Cu is delivered to the ATP7A and ATP7B proteins by the ATOX1 Cu chaperone. Both ATP7A and ATP7B transport Cu to Cu dependent enzymes in the TGN. Elevated Cu concentrations stimulate the endocytosis and degradation of CTR1 and the exocytosis of ATP7A and ATP7B to post-Golgi vesicles or the plasma membrane.

has been demonstrated by embryonic lethality in mice lacking Ctr1 (10,11). Interestingly, embryonic fibroblasts isolated from Ctr1−/− embryos retain some Cu uptake activity, albeit with reduced apparent affinity for Cu(I) (12), suggesting that alternative pathways exist for Cu entry. Whether such Cu uptake activity is transporter mediated or occurs via endocytosis or pinocytosis is unclear.

Localization and post-translational regulation of the CTR1 protein

Despite a role for Cu uptake, the intracellular localization of CTR1 is somewhat variable in different cell lines. Immunocytochemical studies have demonstrated that CTR1 is localized at the plasma membrane in HEK293, CaCo-2, and A2780 cells, whereas, in other cells such as HeLa, it is predominantly localized to vesicular compartments (6,13,14). The underlying basis for this differential localization of CTR1 in various cell types is unclear but may relate to variability in trafficking rates between vesicular and plasma membrane compartments. Results from our laboratory demonstrated that elevated Cu concentrations stimulate the endocytosis and degradation of tagged overexpressed human CTR1 (15). Although some reports have found no evidence of Cu stimulated endocytosis or degradation of endogenous CTR1 protein in HEK293 cells (13,16), other studies have demonstrated either Cu stimulated endocytosis and/or degradation of endogenous CTR1 protein in A2780, CaCo-2, MDCK, HEK293, HepG2, and HeLa cells, and primary hepatocytes (15,17–21). The control of CTR1 abundance by Cu levels is presumably a regulatory mechanism that prevents the excessive accumulation of potentially toxic levels of Cu, which in the intestine would also serve to limit Cu entry to the body (21). In vitro mutagenesis experiments have shown that intramembranous methionines, Met-150 and Met-154, which are essential for transport activity, are also required for Cu stimulated endocytosis and degradation of CTR1 (17), suggesting that conformations associated with Cu transport might direct the protein to endocytic sorting machinery at the cytoplasmic face of the plasma membrane. However, the identity of endocytosis signal(s) in CTR1 that are presumably involved in this process have not been identified.

Adding to the complexity of post-translational regulation of CTR1 was the recent finding that O-linked glycosylation protects the protein against proteolytic cleavage of a 17-kDa fragment from the N terminus (22). The biological function of this cleaved variant, and the protease responsible, are currently unclear. CTR1 cleavage may endow novel biochemical properties to the transporter, or, alternatively, the proteolytic fragment that is released might serve a specific function in the extracellular milieu. It will be important to determine whether this peptide exists in serum and whether it might regulate Cu homeostasis when applied extracellularly.

CTR1 localization in polarized epithelial cells

Some controversy exists as to the localization of CTR1 in polarized epithelial cells, particularly in the intestine. A study by Zimnicka et al. (23) suggested that CTR1 localizes exclusively to the basolateral membrane of Caco-2 cells, a polarized intestinal human cell line, as well as epithelial cells of the mouse intestine. These findings are at odds with several other studies suggesting that CTR1 is located on the apical membrane (and vesicles) in intestinal epithelial cells of rats and mice (24–26). In a recent study, Nose et al. (21) provided compelling evidence using both immunohistochemical and biotinylation approaches that CTR1 is located at the apical membrane of enterocytes in intestinal tissue from rats, mice, and pigs. The reasons for the discrepancy in findings for CTR1 localization in the intestine are unclear, but it underscores the need to further evaluate the location of CTR1 in different polarized tissues. It is important to note that CTR1 is unlikely to reside at the apical membrane in all

polarized cell types. For example, in hepatic biliary epithelial cells, one might expect CTR1 to mediate Cu uptake across the basolateral membrane, a necessary step in Cu delivery into the bile via ATP7B mediated Cu export across the apical membrane (discussed below and **Fig. 2**).

Tissue specific functions of CTR1 and inter-organ Cu signaling

Because knockout of Ctr1 in mice results in embryonic lethality (10,11), an understanding of the physiological and tissue specific requirements of CTR1 has lagged behind studies of its biochemical function. However, this recently changed with the development of a floxed *Ctr1* mouse strain by the Thiele laboratory and the subsequent generation of *Ctr1* knockout mice in intestine, liver, and heart using Cre-Lox technology. The importance of Ctr1 in dietary Cu acquisition in the neonatal period was demonstrated by the selective knockout of *Ctr1* in intestinal epithelial cells, which resulted in severe systemic Cu deficiency, ataxia, and death prior to weaning (25). Interestingly, a single i.p. Cu injection in these knockout mice, if given within the first week of life, permitted normal growth and development to maturity. This not only demonstrated that Ctr1 mediated intestinal transport is critical to meet the Cu demands of neonatal development, but also that the dietary Cu needs in postweanling mice can be met by Ctr1 independent transport pathways across the gastrointestinal tract (25).

Knockout of *Ctr1* in hepatocytes of mice has revealed a role for Ctr1 in hepatic Cu acquisition and biliary Cu excretion, as evidenced by diminished Cu levels in the liver and reduced Cu levels in the bile (27). Interestingly, the magnitude of decreased hepatic Cu concentrations was lower in proportion to the extent of *Ctr1* deletion (27), again suggesting alternative mechanisms for Cu acquisition (28). Moreover, the existence of a higher urinary Cu concentration in hepatic Ctr1 knockout mice was suggestive of a possible inter-organ compensatory mechanism to prevent hyperaccumulation of Cu in tissues under conditions of decreased hepatobiliary Cu excretion (27). It would be interesting to explore this possibility by investigating whether altered renal expression of Cu transporters (Ctr1, Atp7a/b) might underlie the higher urinary Cu output in hepatic Ctr1 knockout mice.

Inter-organ signaling of Cu status is a new frontier in this field, revealed by a tamoxifen-inducible cardiomyocyte-specific Ctr1 knockout mouse model (29). Within days of cardiac Ctr1 deletion, mice exhibited Cu deficiency in the heart, which culminated in severe cardiomyopathy and death. Surprisingly, levels of the Atp7a Cu exporter in both intestine and liver were dramatically increased in these knockout mice, together with increased serum Cu levels. This revealed a signaling mechanism involving the inter-organ transmission of cardiac Cu status to the intestine and liver to promote Atp7A mediated Cu export from these organs (Fig. 2). It is unclear at this stage whether Atp7A expression was increased in hepatocytes (which do not normally express Atp7A) or another cell type in the liver. It was further shown that serum from the Ctr1 cardiomyocyte specific knockout mice, but not control mice, could greatly enhance Atp7A expression when applied to cultured human umbilical vein endothelial cells or CaCo-2 cells, thus confirming that this signaling factor was indeed secreted into the blood (29). The notion of a secreted inter-organ regulatory signal that promotes changes in Cu homeostasis in target organs is reminiscent of hepcidin, a peptide hormone that regulates iron export from the intestine and macrophages (29–32). There are many unknowns regarding the nature of this Atp7a regulating serum factor. Is it proteinaceous? Is it secreted from cardiomyocytes or another tissue that might respond secondarily to cardiac stress? What is being "sensed" as a result of Ctr1 deletion (Cu pools, cuproenzyme activity, cardiac enlargement, arrhythmia etc)? ATP7A expression in different cell types is known to be stimulated by various secreted factors, including INFγ, estrogen, and insulin, each of which is potentially altered in mice lacking cardiac Ctr1 (33,34). Elucidating the molecular nature of

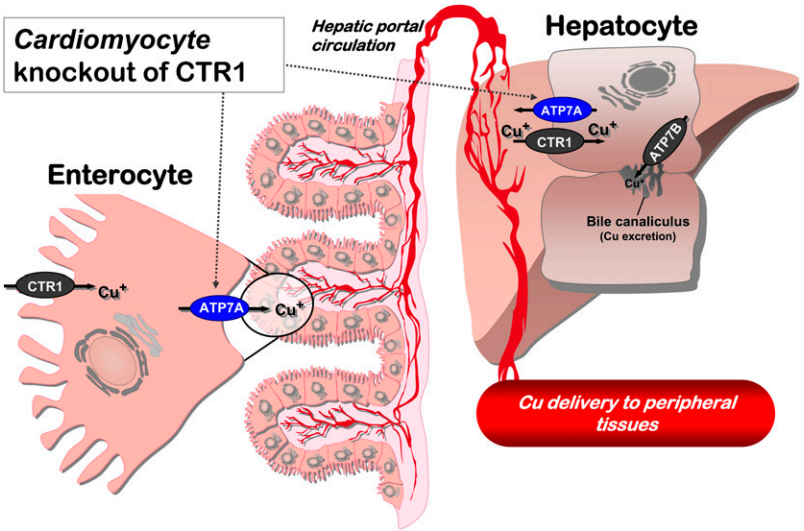

Figure 2 Effect of cardiomyocyte specific CTR1 deletion on intestinal and hepatic expression of ATP7A. A schematic model depicting the effect of cardiomyocyte deletion of CTR1 on the expression of ATP7A in intestine and liver. The release of an unknown serum factor in response to cardiac deletion of CTR1 promotes an increase in ATP7A expression in intestinal epithelial cells and hepatocytes. The resulting increase in ATP7A mediated Cu transport into the blood stream across the gastrointestinal tract and from hepatic stores is proposed to increase Cu availability to the heart and peripheral organs.

this signal and its mechanism of action will be an exciting area of future work.

The CTR2 Cu transporter

CTR2 is a second Cu permease in mammals that shares high sequence similarity to CTR1 (2). Its overall organization mirrors that of CTR1 (3 putative transmembrane domains, an intramembranous MX_3M motif) but without an extended N-terminal domain. Immunocytochemical experiments have localized CTR2 to intracellular compartments, most notably the endosome and lysosome, in multiple cell lines (HEK293T, HeLa, U20S, COS7) (35,36), and at least 1 study has demonstrated that a small percentage of CTR2 ($\sim$5%) exists at the PM (35). CTR2 mediates Cu transport into the cytoplasm based on measures of elevated Cu accumulation (35) or metal responsive reporter activation in cells in which CTR2 levels have been altered (36). It is currently unclear whether CTR2 mediates Cu uptake across the plasma membrane or mobilization of Cu from intracellular compartments, as has been demonstrated for the *Saccharomyces cerevisiae* yCtr2 homolog (37). Indeed, CTR2 mediated Cu transport to the cytoplasm from lysosomal compartments might serve as a mechanism of Cu recycling following the degradation of cuproenzymes. Biochemical studies suggest that CTR2 functions as oligomeric protein, but the level of structural detail that exists for CTR1 is currently lacking for CTR2. Another question remaining to be clarified includes whether CTR1 and CTR2 might form heteromultimers, as occurs with some fungal Ctr protein family members (38), which might provide yet another level of regulation for controlling Cu transport into the cytoplasm.

Cu Export

The ATP7A and ATP7B P-type ATPases

The bioavailability of intracellular Cu is regulated not only by the CTR family of Cu permeases but also by the Cu exporters ATP7A and ATP7B. These 2 Cu-ATPases share $\sim$60% identity and are functionally homologous, both containing 8 transmembrane domains, 6 cysteine rich metal binding motifs (MXCXXC) in the cytoplasmic N-terminal domain, a canonical transmembrane CPX metal binding motif, and ATP binding and aspartic acid phosphorylation domains. ATP7A/B couple the energy derived from ATP hydrolysis to transport Cu through a lipid bilayer away from the cytosol. Their basic functions can be viewed as 2-fold: *1*) Cu transport to newly synthesized cuproenzymes en route through the secretory pathway; and *2*) Cu export from cells.

The 2 transporters are predominantly located in the final compartment of the Golgi apparatus known as the trans-Golgi network (TGN).[7] This intracellular location allows ATP7A and ATP7B to transport Cu to newly synthesized cuproenzymes en route through the secretory pathway via a process that is mediated via Cu exchange between the cytosolic N-terminal domain of the ATPases and the Cu chaperone, ATOX1 (39,40) (Fig. 1). As mentioned above, another critical role of the ATP7A/B proteins is Cu export, which is coupled with Cu stimulated trafficking of these proteins from the TGN (39,41–44). Generally speaking, the effect of elevated Cu levels in most cell types is to promote ATP7A relocalization to vesicles and the plasma membrane, whereas ATP7B is trafficked to cytoplasmic vesicles (Fig. 1). Although both proteins mediate Cu export under these conditions, the exact mechanism by which this occurs in the context of their differential localizations is poorly understood. Post-Golgi vesicles carrying the ATP7A/B proteins may themselves serve as receptacles for Cu transport and subsequently release their Cu load upon fusion with the plasma membrane (although this has not been demonstrated). For reasons that are unknown, ATP7B may be excluded from fusion with the plasma membrane via retention within an endosomal compartment, whereas the enrichment of ATP7A at the plasma membrane may simply reflect the absence of such retention. Mutagenesis studies suggest that the Cu stimulated trafficking of ATP7A and ATP7B from the TGN is dependent on the catalytic activity of these pumps (44) as well as 1 or more of the following proteins: p62 dynactin subunit, CDC42, and ADP-ribosylation factor (45–47), although the biological bases of these interactions are poorly understood. Other post-translational modifications of ATP7A and ATP7B have been identified, including Cu stimulated phosphorylation, which may also play roles in regulating trafficking and/or catalytic activity (48–50).

In polarized epithelial cells, ATP7A and ATP7B undergo Cu stimulated trafficking toward opposing membranes: ATP7A to the basolateral membrane and ATP7B to the subapical vesicles. While this likely reflects their different physiological roles (discussed below), insights into the signals responsible for this differential trafficking in polarized cells have been gleaned from mutagenesis experiments (51–53). Basolateral targeting of ATP7A requires a di-leucine in the carboxyl-terminal region, which also serves as an endocytic signal for recycling ATP7A from the plasma membrane to the TGN (41,42,52,54). Interestingly, ATP7B contains a tri-leucine in a similar location for retrieval from endosomes to the TGN (55). The apical targeting of ATP7B in a polarized hepatic cell line has been shown to rely on a novel sequence of 9 amino acids ($_{37}$FAFDNVGYE$_{45}$) within the amino terminal region that is absent from the corresponding region of ATP7A (51). It is speculated that the interaction of this sequence within ATP7B with a protein in the Golgi is responsible for ATP7B sorting into apically targeted vesicles in response to high Cu levels. Yet another interesting development in the post-translational trafficking of ATP7B concerns the role of COMMD1, an enigmatic protein with multiple functions, including regulation of sodium transport, adaptation to hypoxia, and regulation of NF-κB (56). Recent data has shown that COMMD1 is not essential for ATP7B trafficking to post-Golgi vesicles per se but may be involved in the latter stages of hepatic Cu excretion by regulating the exocytosis of Cu loaded vesicles (50). Studies also

[7] Abbreviations used: Aβ, amyloid β; AD, Alzheimer's disease; CCO, cytochrome c oxidase; NMDA, *N*-methyl-D-aspartic acid; SOD, superoxide dismutase; TGN, trans-Golgi network.

suggest that COMMD1 may regulate biliary Cu excretion by regulating the stability of ATP7B protein (57,58).

Physiological functions of ATP7A and ATP7B

Although ATP7A and ATP7B share similar biochemical functions, their tissue and developmental expression is distinct. ATP7A is ubiquitously expressed in mammals, whereas ATP7B is more selectively expressed in the liver, kidney, mammary epithelial cells, brain, and eyes (59,60). Not surprisingly, ATP7A functions in a wide range of tissues that play critical roles in copper physiology, including copper absorption from the intestine, copper transport into the cerebral spinal fluid, and copper transport to the bulk of known cuproenzymes in the Golgi membranes of various tissues. Accordingly, loss of ATP7A function results in a lethal disorder of Cu deficiency known as Menkes disease (discussed below). In contrast, ATP7B mutation causes symptoms of hepatic and neuronal Cu toxicosis observed in Wilson disease due to its specialized roles in these tissues.

Wilson disease

Wilson disease patients present with liver disease stemming from hepatic Cu overload (hepatomegaly, acute hepatitis, cirrhosis, or liver failure) (61). This may also be accompanied by neurological (movement disorders, tremor, dystonia, dysarthria, seizures) or psychiatric symptoms (61), which may result from secondary Cu overload in the brain. The hepatic symptoms of Wilson disease can be explained by a blockage of biliary Cu excretion across the canalicular membrane, the most critical role of ATP7B in the body (62) (Fig. 2). Life-long treatment with Cu chelators or zinc is successful at eliminating excess Cu and managing its entry across the gastrointestinal tract (61,63). Current mouse models for Wilson disease include the toxic milk mouse (64) and a global *Atp7b* knockout (65), both of which serve as models of hepatic Cu overload as well as mechanisms of hepatitis and liver regeneration (65,66).

Menkes disease

A major defect in Menkes disease is the reduced transport of dietary Cu across the basolateral membrane of enterocytes to hepatic portal circulation. This results in the entrapment of Cu within the intestinal mucosa and Cu deficiency in the blood and peripheral organs (67). Cu deficiency is further compounded by a reduction in Cu transport across the blood-brain barrier into the central nervous system (67). The transfer of Cu from the mother to the affected Menkes fetus is also defective (68,69), although it is not until 2–3 mo of age that Menkes infants exhibit overt symptoms of Cu deficiency, such as neurological impairment, convulsions, pili torti, connective tissue abnormalities, skin laxity, and hypopigmentation (68). These symptoms generally can be explained by a lack of specific Cu dependent enzymes, although the mechanisms underlying neurodegeneration are unclear. Possibilities include lower activities of cuproenzymes in the brain, including cytochrome c oxidase (CCO), superoxide dismutase (SOD) 1, dopamine β-hydroxylase, and/or peptidyl-α-amidating monoxygenase. Alternatively, defective modulation of glutamatergic signaling arising from a lack of Cu may underlie excitotoxicity and neurodegeneration in Menkes disease (discussed below) (70,71). Menkes patients have severe abnormalities of the vascular system such as weakened arteries due to defects in Cu containing lysyl oxidase (72). Patients are also prone to infection, particularly of the lung and urinary tract, as well as septicemia (73–76). Cu is critical for immune function (discussed later); however, it is unclear whether immune deficiencies in Menkes patients are the result of a direct role of ATP7A in immune defense (77). Para-enteral Cu injections have been reported to improve the outcome of the disease if treatment is commenced early (78,79).

Although most Menkes patients die during early childhood, a milder form of the disease exists, known as occipital horn syndrome. Excessive calcium deposition in the occipital bone, coarse hair, and loose skin and joints characterize this disorder; however, patients are spared the neurological manifestations of classical Menkes disease such as ataxia (80). Yet another disease caused by ATP7A mutations is distal hereditary motor neuropathy in which peripheral motor neurons are impaired (81). Affected individuals exhibit progressive weakness and chronic wasting of the distal limbs without evidence of overt Cu deficiency (81). Two missense mutations in the *ATP7A* gene have been identified in patients with distal hereditary motor neuropathy that affect highly conserved amino acids (P1386S and T994I), albeit within domains of ATP7A protein with no assigned function (81). In fibroblasts derived from patients, Cu stimulated trafficking of both mutant forms of ATP7A protein was shown to be inhibited, and this was also associated with a mildly elevated Cu retention suggestive of a partial defect in ATP7A dependent Cu export (81). Although they are important advances in understanding the roles of ATP7A in disease, these discoveries raise new questions regarding how these mild ATP7A mutations manifest as motor neuron disease rather than overt symptoms of Cu deficiency that are characteristic of occipital horn syndrome or Menkes disease. It is possible that motor neurons are especially sensitive to changes in intracellular Cu pools resulting from ATP7A mutation or that the motor neurons are hypersensitive to disruption of ATP7A specific roles in the central nervous system (81). The answers to such questions will require the development of mouse models in which ATP7A expression can be specifically altered within motor neurons.

Emerging roles for ATP7A in pathophysiology

It is becoming increasingly evident that ATP7A mediated Cu transport performs highly specialized roles in specific tissues. Below is a brief description of studies identifying emerging functions of ATP7A and Cu in new areas of physiology.

Glutamatergic signaling in neurons. In primary hippocampal neurons, stimulation of the *N*-methyl-D-aspartic acid (NMDA) receptor results in the trafficking of ATP7A

from the TGN to somato-dendritic and axonal vesicles (82). Calcium influx through the NMDA receptor regulates postsynaptic neuronal activity and is thought to be a major regulator of synaptic plasticity (83). ATP7A trafficking in response to this glutamatergic stimulation results in the Ca^{2+} dependent release of Cu to the extracellular milieu, which subsequently desensitizes further stimulation of the NMDA receptor. A detailed understanding of the underlying inhibitory mechanism is unknown but may involve Cu dependent S-nitrosylation of the NMDA receptor (71). In vivo studies suggest that ATP7A mediated Cu efflux in the brain appears to play a critical role in preventing excitotoxicity following ischemic reperfusion injury (71,82). These studies not only reveal novel roles for ATP7A in the central nervous system but also provide new insights into the possible mechanisms underlying neurodegeneration and seizures in Menkes patients.

Macrophage bactericidal activity. It has been known for decades that Cu is critical for proper functioning of both the humoral and innate immune system; however, its precise mechanisms of action are unknown (77,84). Recent studies from our laboratory have demonstrated that the proinflammatory factors, lipopolysaccharide, IFNγ, and hypoxia, stimulate ATP7A expression and promote its trafficking to post-Golgi vesicles in RAW267.4 macrophage cells (34,85). These vesicles were shown to partially overlap with the phagosomal compartments (34). Importantly, ATP7A silencing was found to suppress the killing of *Escherichia coli* bacteria phagocytosed by activated macrophage cells, suggesting that ATP7A-mediated Cu transport into the phagosome may function in bactericidal activity. Consistent with this notion, a Cu sensitive mutant of *E. coli* (ΔcopA) was found to be more susceptible to bacterial killing by macrophages compared with wild-type *E. coli*, and this sensitivity was abrogated by knockdown of ATP7A (34). The mechanism of Cu mediated bacterial killing within the macrophage is unknown but may involve the formation of hydroxyl-radicals via Fenton-like chemistry within the oxidative environment of the phagosome. Moreover, Cu dependent killing of *E. coli* may be generally applicable to other pathogenic bacteria, as suggested by recent studies showing that Cu sensitive mutants of *Salmonella enterica* (ΔcopA/ΔgolT, ΔcueO) are more susceptible to macrophage mediated killing both in vitro (86) and in vivo (87). Further studies are required to determine whether ATP7A mediated Cu transport within macrophages is a major defense mechanism in vivo and whether it is active against other pathogenic organisms.

Roles in Alzheimer's disease. Alzheimer's disease (AD) is characterized by progressive neurodegeneration as well as the deposition of the secreted peptide β-amyloid (Aβ), leading to the formation of senile plaques (88). There is abundant evidence that Cu dyshomeostasis is associated with the pathogenesis of AD. Cu concentrations are markedly elevated in Aβ plaques of AD patients and mouse models of AD (89–92) and Cu can bind to the Aβ peptide with high affinity, resulting in Aβ aggregation and enhancement of Aβ neurotoxicity (93–97). Studies from this laboratory have demonstrated that Atp7a is overexpressed in microglial cells clustered around Aβ plaques in a mouse model of AD (98). Using a cultured microglial cell line, ATP7A and CTR1 expression was also found to be increased by IFNγ, resulting in enhanced cellular sequestration of Cu (98). Although these studies suggest that proinflammatory conditions associated with AD cause marked changes in microglial Cu trafficking, further studies will be required to establish whether Cu sequestration by microglia occurs in AD mouse models and, if so, whether such changes affect disease pathogenesis.

LDL cholesterol oxidation in macrophages. Macrophages are hypothesized to play key roles in heart disease, stroke, and peripheral vascular disease via their propensity to accumulate oxidized LDL, resulting in the formation of foam cells and atherosclerotic plaques within blood vessels (99). A recent study suggests a role for ATP7A in regulating levels of LDL oxidation in macrophages, implicating the possible involvement of ATP7A in atherosclerotic lesion development (100). Atp7a protein was abundant within intimal macrophages of atherosclerotic lesions in mice lacking the LDL receptor, and silencing of ATP7A in cultured macrophages was shown to decrease the accumulation of oxidized LDL. The mechanism by which this occurs is unknown but may involve a reduction in levels of cytosolic phospholipase A2α, an enzyme responsible for conversion of arachidonic acid to proinflammatory mediators (100). This previously unrecognized role for ATP7A in cholesterol homeostasis in macrophages is intriguing, however, an understanding of its pathophysiological significance in atherogenesis will require testing in macrophage (myeloid) specific ATP7A knockout mice.

Role in vascular smooth muscle cells. Several studies have demonstrated that ATP7A function in vascular smooth muscle cells may play important roles in pathogenic processes related to vascular disease (101–103). ATP7A is essential for Cu delivery to an extracellular form of superoxide dismutase (SOD3), which may function in vascular superoxide anion production and hypertension (101,102). Other studies have revealed an unexpected connection between Cu homeostasis and lamellipodia formation in cultured vascular smooth muscle cells by demonstrating that the ATP7A is recruited to the leading edge in response to platelet derived growth factor, where it regulates recruitment of Rac1 in a CTR1 dependent manner (103). Whether such processes underlie the long suspected but poorly understood role for Cu in wound healing remains to be shown.

Conclusions

The field of Cu homeostasis has undergone a dramatic expansion over the past decade. The study of Cu transporters continues to reveal new complexities in the regulatory mechanisms of Cu homeostasis. We are only beginning to identify novel interactions of Cu homeostasis with an increasingly

divergent array of physiological and pathophysiological processes. Establishing the importance of Cu in these processes and its contribution to disease is an important direction of future work that will require further development and evaluation of tissue specific knockouts of Cu transporters, together with cellular and biochemical studies in specific physiological contexts.

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