Annu. Rev. Nutr. 2000. 20:291–310

CELLULAR COPPER TRANSPORT AND METABOLISM

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Key Words copper metabolism, Menkes gene, Wilson gene, Cu-transporting ATPase, copper chaperones, copper absorption

■ **Abstract** The transport and cellular metabolism of Cu depends on a series of membrane proteins and smaller soluble peptides that comprise a functionally integrated system for maintaining cellular Cu homeostasis. Inward transport across the plasma membrane appears to be a function of integral membrane proteins that form the channels that select Cu ions for passage. Two membrane-bound Cu-transporting ATPase enzymes, ATP7A and ATP7B, the products of the Menkes and Wilson disease genes, respectively, catalyze an ATP-dependent transfer of Cu to intracellular compartments or expel Cu from the cell. ATP7A and ATP7B work in concert with a series of smaller peptides, the copper chaperones, that exchange Cu at the ATPase sites or incorporate the Cu directly into the structure of Cu-dependent enzymes such as cytochrome *c* oxidase and Cu, Zn superoxide dismutase. These mechanisms come into play in response to a high influx of Cu or during the course of normal Cu metabolism.

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INTRODUCTION

Recognizing Cu as an essential nutrient obligates one to know the details of its cellular metabolism. Cellular transport and metabolism implies moving Cu within and between compartments of a cell to a defined target. One may ask whether our knowledge of this phase of Cu nutrition has advanced to a point that we can give detailed information about the reactions and components in the transport scheme. The answer to that question is found in this review and previous ones on the subject (146). Today, the guiding principle is that Cu ions move between organelles and across membranes mainly in association with small target-seeking proteins working in conjunction with ATP-driven mechanisms. Moreover, cellular Cu transport components are well conserved over eons of evolution. Thus, there are no sharp boundaries distinguishing Cu transport in higher animals from that in bacteria, fungi, and plants. Indeed, the transport systems in yeast have tended to guide research in this area and have contributed mightily to conceptual understandings of Cu transport in humans (40).

HISTORICAL

Early research into Cu transport used whole animals and focused mainly on factors that influenced intestinal absorption. Little attention was paid to movement inside a cell. Amino acids (52, 128, 130), ATP (4), and soluble proteins are capable of either assisting or deterring the movement of Cu into cells (147). Cu ions that enter hepatocytes quickly become nonextractable (9); only about 40% is subject to rapid efflux (30, 103). The largest portion is secluded to cellular compartments or binding proteins. Apparent differences in kinetic constants for influx and efflux as well as between cell types (30, 103, 147) have discouraged any implication of bidirectional transport involving a single component or a single transport system applicable to all cell types (53). As an illustration, reagents that bind sulfhydryl groups (*a*) block Cu from entering fibroblasts and lymphoblasts (110), (*b*) stimulate uptake into C6 glial cells (103), and (*c*) have essentially no effect on placental cytotrophoblasts or choriocarcinoma (BeWo) cells (104, 138). The response to sulfhydryl binding reagents has been postulated to be a function of the location of -SH groups in the cell membrane or of more than one membrane Cu transport protein (104). The implication from numerous uptake studies is that cellular Cu transport mechanisms may be cell-type specific and that influx and egress follow different paths and involve separate components.

Newly absorbed Cu ions also engage ill-defined particulate components of cells (46, 118, 130). HTC rat liver hepatoma cells and primary hepatocytes, for example, accumulate Cu in the cell membrane or in organelles; only a small fraction appears in the cytosol (124). In neonatal rat intestine, the particulate components were described as sulfur-laden vesicles located adjacent to the nucleus (39). The meaning and source of the vesicles was not apparent in the early studies. Yeast

has the capacity to trap Cu in vacuoles. The vacuoles both detoxify and merge pathways of Cu with Fe metabolism (133). Davidson et al (31) put particulate Cu in mammalian cells on a stronger theoretical base by showing that Cu that enters K562 cells localizes into two density-distinct membrane compartments. One compartment had the buoyant density of a transferrin-laden endosome (31). The other was clathrin coated and showed a time-dependent movement of Cu to heavier structures identified with the lysosomes. These data, therefore, supported vesicular Cu as a dynamic transport form. Thus, we are left with the opinion that cellular Cu metabolism is comprised of both soluble and insoluble phases, i.e. a partitioning phenomenon in which Cu ions entering a cell engages either (or both) cytosolic or membrane components. Metabolically functional Cu and Cu designed for storage or excretion are the major categories defined. Today we have a much deeper understanding of what soluble and vesicular Cu ions represent to the metabolism and cellular transport of Cu.

ACCESSING THE INTRACELLULAR POOL

In a defined culture medium, cells receive Cu ions from a wide variety of suitable donors. Penetration is generally rapid and does not depend on the ATP status of the cell. Some have argued that these data reflect a passive Cu carrier system in the membrane (116, 138). Amino acids (52) and chloride and bicarbonate ions (1) have the capacity to stimulate Cu uptake, which suggests that plasma factors have a similar capability to influence cellular access in vivo. Hepatocytes exposed to inhibitors of protein biosynthesis tend to take up more Cu (125, 148). The anomaly betrays a two-sided, influx-efflux character to the transport system, the latter perhaps being more sensitive to transcription and translation inhibitors.

Facile exchange of Cu is compulsory for a working transfer mechanism. This single property has led some workers to discount albumin-bound and ceruloplasmin-bound Cu as tissue sources. Binding strengths, however, can be compromised by reducing systems in the membrane (142), by reducing compounds in the plasma (96, 97), or by amino acids that interact with the Cu-protein complex (29).

Albumin as a Copper Transport Factor

Albumin potentially can bind Cu in several sites on the protein (11). The sites at the extreme N-terminal region or certain cysteine residues within the protein have been given the strongest consideration as transport sites (70, 131). The N-terminal site employs a histidine at position 3 to bind Cu(II). The site is not in rapid equilibrium with unbound Cu, however (69). The histidine concentration in plasma (135 μM) plus a clear demonstration of the ability of this amino acid to form stable complexes with Cu(II) have given favorable consideration to histidine as a transport ligand for Cu (114). Histidine forms a ternary complex with albumin-Cu(II) (113) and induces the protein to release Cu as a histidine-Cu(II) complex. Although histidine

allows ready access to Cu, there is evidence that the histidine ligand itself never penetrates the cell (83). This would suggest that the role of histidine ends at the cell surface.

Ceruloplasmin as a Transport Factor

A large part of the Cu in serum is bound to ceruloplasmin, a multi-Cu oxidase that has six to seven bound Cu atoms present in three chemically distinct sites (76). Ceruloplasmin Cu thus becomes a candidate for a tissue source. The discovery of specific membrane receptors for ceruloplasmin, first in chick aorta and heart tissues (129), and later in erythrocyte membranes from a variety of different species (5) as well as in heart, brain, liver, kidney, and lymphocytes (38, 51, 71, 94, 112, 127), supports but does not confirm a transport function for the protein. Maximum effective delivery of Cu at the membrane site requires ascorbate (96). Moreover, the exchange is sensitive to bathocuproinesulfonate, which suggests that Cu(I) is the transport form (96). Unlike transferrin, which is endocytosed into vesicles during uptake, ceruloplasmin exchanges its bound Cu at the cell surface and does not penetrate the cell (97, 112). An exception is in the liver, where absorption of the whole ceruloplasmin molecule is part of a two-step delivery mechanism. Liver endothelium first removes the sialic acid residues from ceruloplasmin, which permits a second step in which the underlying hepatocytes absorb the modified ceruloplasmin via the asialoglycoprotein receptor (60, 136). By absorbing and then destroying ceruloplasmin, the liver maintains homeostatic control over the copper levels in the blood. Ceruloplasmin fragments have been seen in the bile, supporting an internal proteolysis of the protein (61).

Conclusions

Despite gallant efforts by numerous laboratories and investigators, no single factor has emerged as indispensable for mediating Cu passage into cells in vivo. This apparent failure allows one to question whether delivery of an essential nutrient would be expected to depend on a single component. Thus, it is more likely that cells have evolved numerous ways for absorbing Cu from their immediate surroundings. This would imply that numerous carriers, including ceruloplasmin and albumin, have the potential to behave as Cu transporters. One transporter, a high-molecular-weight protein called transcuprein, has yet to be fully characterized (149). In those few experiments that have attempted to discern whether Cu-albumin, ceruloplasmin, or Cu-histidine gives cells more efficient access to Cu, the results have tended to show no difference among the three (23, 82). An exception is in studies of Cu incorporation into cytochrome oxidase in mitochondria, where two studies suggest ceruloplasmin is more effective than albumin in providing Cu to the protein (59, 80). These results promote an understanding that the carrier that delivers Cu to cells is not a decisive determinant of what happens inside the cell.

MEMBRANE TRANSPORT OF COPPER

Over the years, the emphasis has shifted from plasma Cu transport agents to transport at the membrane surface and within the cell. Pushing Cu research in this direction has been the discovery of membrane and cellular transport proteins that are specific for Cu. Yeast has separate low- and high-affinity systems for Cu uptake that mediate Cu transport (40). The gene *CTR1* (Cu transport 1) was the first to be discovered, paradoxically not for Cu but as a gene essential for Fe transport in *Saccharomyces cerevisiae* (24, 25). The Cu was required for Fet3, a multi-Cu ferroxidase that catalyzed the oxidation Fe^{2+} to Fe^{3+} preparatory to absorption by a ferric transport protein. Ctrp1 is a membrane-spanning protein, heavily glycosylated, with a serine- and methionine-rich composition in which the structural motif M-X-X-M is repeated 11 times. Ctr1p specifically transports Cu(I), not Cu(II) or any other metals. Its discovery establishes a mechanistic link between Cu and iron uptake. Some strains of *S. cerevisiae* have a second high-affinity transporter gene, *CTR3* (65). Ctr3p restores Cu-related functions in strains lacking the *CTR1* gene. Functional expression cloning of genes in mutant yeast lacking *CTR1* and *CTR3* led to the discovery of a structurally similar transporter gene in *Arabidopsis thaliana*, *COPT1* (62), and a human Cu transporter, *hCTR1*. In HeLa cells, the *hCTR1* gene is located on chromosome 9, more specifically 9q31/32 (153). cDNA sequence data show that hCtr1 protein is considerably smaller than is yeast Ctr1 protein. Because they recognize only Cu(I), Cu transporters require a plasma membrane reductase to reduce the Cu(II) to Cu(I) concomitant with penetration into the cell. The reductase in yeast has been identified with the expression of *FRE1* and *FRE2*, two genes that perform a dual function in reducing both Cu^{2+} and Fe^{3+} for transport (47, 55). A third gene, *FRE7*, increases in expression when extracellular Cu becomes very limiting (81). A reductase system that uses NADH as an electron donor to reduce Cu has been described in liver cells (142).

INTERNAL TRANSPORT FACTORS

Glutathione

The highly reducing cytosolic environment is conducive to converting Cu(II) to Cu(I). One of the earliest intracellular components identified with Cu transport was glutathione (GSH), a ubiquitous cysteine-containing tripeptide that is present in millimolar quantities in liver, brain, kidney, and other cells (32). A rapid turnover of a GSH-Cu(I) complex in hepatoma cells was consistent with GSH as a conveyer of Cu(I) to metallothionein (45). Cu(I) reacts directly with the internal cysteine-SH of GSH. The propensity of Cu(II) to catalyze the oxidation of -SH groups (107) makes it less likely that a Cu(II) ion is bound to GSH, although a crystal structure of GSH-Cu(II) has been reported (90). Forming the Cu(I)-GSH is a spontaneous reaction apparently independent of enzyme involvement (36). Besides transferring

Cu to metallothionein (44, 45, 125, 132), GSH is required for biliary excretion of Cu in adult rats (58, 93). GSH levels are elevated 41%–117% in rat hepatocytes isolated from Cu-deficient adult rats (18) and suppressed in liver of male rats that received injections of Cd^{2+}, Zn^{2+}, and Cu^{2+} (63). When cellular GSH is low, cells are slower to take up Cu from the medium and have a lower cellular concentration at steady state (138). In erythrocytes from a number of species, GSH levels fall at a rate proportional to the rate of Cu(II) entry into the cell (119). Although human hepatocytes show a bidirectional movement of GSH across the plasma membrane, with two very sensitive and interactive binding sites for GSH (K_m 2.36 nM), there is no known connection of the sites with Cu transport into the cell (134).

In vitro studies have provided evidence for a Cu-GSH complex that can mediate stable Cu(I) binding to apo(metal-free)-cuproproteins. These reconstitution experiments have shown Cu-GSH to transfer Cu(I) into the binding sites of apo-CuZnSOD (3, 20), apo-ceruloplasmin (91), and apo-hemocyanin (12) and to charge metallothionein with Cu(I) (41, 86, 132). A decline in GSH levels in a cell impairs the subsequent binding of Cu to apo-CuZnSOD (126) or the delivery of Cu to the cytosolic enzymes (119). Thus, there is an impressive body of evidence that Cu-GSH complexes have the capacity to mediate Cu(I) transfer to a variety of binding sites on macromolecules, which extend GSH beyond being a protectorate of Cu toxicity into the role as a major player in internal Cu metabolism.

The Copper Chaperones

Cu chaperones are a family of cytosolic peptides, better known as metallochaperones, that form transient complexes with Cu(I). An invariant MXCXXC metal binding motif in the N-terminal region is a structural feature of most chaperones. Their function is to escort Cu ions in transit to specific proteins that require Cu (141). The prototype of metallochaperones is MerP, a small, soluble mercury-binding protein that transports Hg^{2+} to a membrane transporter and eventually to a reductase that reduces the Hg^{2+} to the volatile Hg^0 as part of a detoxifying mechanism (77). Chaperones for Cu follow a similar agenda in moving Cu from one location in the cell to another, often crossing membrane boundaries. Chaperones differ from nuclear activating factors by showing no capacity to enter the nucleus or engage DNA. ATX1, a copper chaperone isolated from *S. cerevisiae*, and copZ, a chaperone in *Enterococcus hirae,* both have ferredoxin-like folds with the $\beta\alpha\beta\beta\alpha\beta$ motif in the folded chains.. Cu as Cu(I) is bound to two cysteine sulfur groups that form a linear bidentate ligand. This arrangement presumably allows facile exchange of the bound Cu to a structurally similar Cu-binding site on the receiving protein (100). Other structural features allow the peptide to specify the target proteins with practically zero tendency for mismatch. Targets are cytochrome *c* oxidase in the mitochondria, ATP7B in the *trans*-Golgi, and apo-CuZnSOD in either peroxisomes or the cell cytosol. The finding of chaperones in bacteria, yeast, plants, and mammalian cells has rekindled concepts of a uniform intracellular transport system and virtually revolutionized thinking of intracellular Cu transport. An advantage of a chaperone is in being more decisive in selecting the receiving molecule. This

contrasts with Cu(I)-GSH, which has no target-specifying property. A conceptual disadvantage is that chaperones force a partitioning of Cu into multiple pools in order to replenish Cu enzymes. What constitutes a partitioning mechanism or regulates the size of each pool has not been determined.

To date, three chaperones have been described in yeast and all three are known to share structural features with mammalian and plant counterparts. The following is a brief description of known chaperones.

ATX1 (anti-oxidant 1) was discovered as an antioxidant gene that suppressed oxygen toxicity in yeast cells that lacked superoxide dismutase activity (*sod1*Δ) and were auxotrophic for lysine (74, 75). ATOX1 (formerly HAH1), the human homologue of ATX1, was screened from a human liver cDNA library using an ATX1 probe and shown to complement yeast lacking ATX1. The cDNA product encodes a peptide with 47% amino acid sequence identity to ATX1. Like ATX1, the human orthologue contains one MTCXGC Cu-binding domain (64). CCH (copper chaperone) in arabidopsis encodes a peptide with a 48–amino acid extension at the C-terminus but otherwise has a 36% sequence identity with ATX1 (56). Chaperones with the ATX1 structural domain target P-type ATPases. In yeast, the ATPase is Ccc2p, a membrane-bound protein that mediates the transfer of Cu to a late Golgi compartment (152). Borrowing the same understanding, ATOX1 in mammals is believed to target ATP7B, a P-type ATPase identified with Wilson disease. Whereas Ccc2p conveys the Cu to FET3p, a multi-Cu oxidase responsible for oxidizing Fe^{2+} to Fe^{3+} for delivery via the ferric iron transporter, ATP7B transfers Cu to apo-ceruloplasmin or forces its extrusion into the bile (see below).

COX17 is essential for the transport of Cu to cytochrome oxidase in the mitochondria of the yeast *S. cerevisiae* (6, 49). A human homologue of COX17 has also been reported (2). Best described as a mitochondrial Cu shuttle, Cox17 is the only chaperone discovered to date that violates the GMXCXXC consensus motif. The Cu binding sites on COX17 are tandem cysteine residues (Cys-14 and Cys-16) positioned near the N terminus. Another unique property is that COX17 binds Cu(I) as a binuclear cluster very similar to the Cu cluster in metallothionein, only more labile (123). Its role in feeding Cu to cytochrome *c* oxidase has not been fully clarified, although in *S. cerevisiae* the mechanism appears to involve two inner mitochondrial membrane proteins, SCO1 and SCO2, that are penultimate receivers of the Cu (48).

LYS7 is a 27-kDa protein required to deliver Cu to the ApoSOD1 (21, 22). Yeast LYS7 mutants are defective in SOD1 activity and are unable to incorporate Cu into the protein. A single MHCXXC consensus sequence is present in the N-terminal region of the protein. A human counterpart of comparable size and 28% sequence identity is designated CCS (copper chaperone for SOD).

THE COPPER-TRANSPORTING ATPases

No event has had a more profound effect on the field of cellular Cu transport than the discovery, cloning, and sequencing of the genes responsible for Menkes and Wilson diseases. The two diseases have been the paradigm of abnormal

Cu metabolism in humans, and their causes have been shrouded in mystery for decades. Menkes disease originates in utero and manifests full symptoms during the perinatal period (146). The disease is X-linked and is characterized by a failure to pass Cu ions completely across the intestinal mucosa. The entrapment of Cu within the intestinal cells leads to a Cu deficiency in peripheral organs and tissues and a severe impairment of neurological and connective tissue functions (26, 28). Menkes disease is perhaps the best-documented evidence for a Cu deficiency in humans. The disease was originally diagnosed as a degenerative disorder of the central nervous system that affected growth and hair texture (87). The sobriquet "kinky hair" is commonly used when referring to the disease. In contrast, Wilson disease, or hepatolenticular degeneration, is an autosomal recessive disorder that results from pathological accumulations of Cu, predominantly in liver and brain tissues. The dominant symptoms relate to a failure to release liver Cu into bile or to incorporate Cu into ceruloplasmin, the major Cu binding protein in the circulation. Both diseases have provided unprecedented molecular insights into genetic factors that regulate Cu transport and bioavailability to organs and tissues. Both diseases can be fatal and both portray a mismanagement of Cu as their root cause (27, 115). Skin fibroblasts from patients with Menkes disease (50, 57) or Wilson disease (17) accumulate prodigious amounts of Cu when grown in traditional growth medium, thus demonstrating that the primary defect can be seen outside the patient.

The isolation and sequencing of the Wilson (*WD*) (13, 98, 135, 137) and Menkes (*MNK*) (19, 89, 145) disease genes has revealed that both code for P-type Cu-ATPases. These are complex integral membrane proteins that are part of an evolutionary family of ion-transporting proteins akin to the Ca transport and Na/K transport proteins in cell membranes. The Wilson and the Menkes proteins transport Cu specifically (122). Moreover, the protein for Wilson disease (ATP7B) and the protein for Menkes disease (ATP7A), in addition to having a 57% sequence homology to one another (146), have remarkable parallels to Cu-binding proteins in bacteria (117). The gene for Menkes disease spans about 150 kb (34, 140). Its mRNA is 8.3–8.5 kb encompassing 23 exons that range in size from 77 to 4120 bp, with a single open reading frame and an ATG start codon in the second exon. Exon 23 contains the TAA stop codon, 274 bp that are translated, and a 3.8-kb untranslated region that has the polyadenylation site (140). When analyzed as a cDNA, ATP7A mRNA encodes a protein of exactly 1500 amino acids, but it may have additional nucleotide sequences at the 5′ end that were not reported originally (140). A 22–amino acid presequence generated by an in-frame ATG site upstream occurs is some ATP7A transcripts (54). Strong expression of *MNK* mRNA is seen in muscle, kidney, lung, and brain; placenta and pancreas are weaker, and liver shows only traces (19, 89). The Wilson transcript is 7.5 kb and encodes a protein of 1411 amino acids. In contrast to Menkes, the Wilson gene is strongly expressed in liver and kidney (13, 150).

Although their structures may be similar, their biological functions are not. The Menkes protein (ATP7A), for example, appears to regulate the release of Cu ions at the outer membrane. Overexpression of ATP7A allows Chinese hamster ovary

(CHO) cells to tolerate highly toxic amounts of Cu in their immediate environment (14). Superior tolerance is manifested by forced expulsion, thus preventing Cu accumulation (144). Although similar in overall appearance to the Menkes protein, the Wilson protein (ATP7B) resides within an internal compartment of the cell, where it functions to incorporate Cu into apo-ceruloplasmin in either the endoplasmic reticulum or a Golgi compartment, or to force the release of Cu into the bile (37, 151).

Included in the structure of the ATP7A is a comparatively large, heavy-metal binding domain (Hmb) consisting of six metal-binding cysteine clusters within the structural motif GMT/HCXSC, which comprises eight transmembrane (Tm) regions that serve to anchor and orient the protein as well as define the channel through which Cu ions pass. ATP7A is classified as a type 2 membrane protein because both the -NH2 and the -COOH termini are on the cytosolic side of the membrane.Two flexible loops, one a 135-residue chain and the second a 235-residue chain, extend into the cytosol. The smaller one is between Tm 4 and 5 and the larger is between Tm 6 and 7. The bacterial CopA protein contains a smaller Hmb with a single GMT/HCXXC motif (121). A yeast Cu-ATPase has the motif at most twice, which leads one to speculate whether the 650-residue Hmb region performs some transport-related function other than binding Cu for export (see below). Other important features include a Cys-Pro-Cys motif in Tm 6 that is believed to form the channel that allows Cu in the cytosol to be transported through the membrane by the energy-dependent shift (122). Fusion protein constructs containing the heavy-metal binding sites have confirmed a preference for Cu(I) binding in ATP7A and ATP7B (33, 79). Although Zn(II) also shows binding, there is little or no affinity of the Hmb to bind to columns charged with Fe(II), Fe(III), Ca(II), Mg(II), Mn(II), or Ni(II) relative to Cu.

Immunochemical studies have localized ATP7A to the perinuclear area within the region of the cell thought to represent the Golgi. Localization to this region has been inferred on the basis of a punctated pattern that is seen when cells are stained with a fluorescent-labeled antibody (35). The pattern is believed to represent small vesicles that gather in Cu.These vesicles are not fixed, but relocate to the plasma membrane in response to high extracellular Cu. Camakaris and coworkers (14) have postulated that ATP7A-laden vesicles are continually moving between the Golgi and plasma membrane. Agents that affect Golgi structure or transport function, such as NH_4Cl, chloroquin, brefeldin A, or bafilomycin A1, cause the more dispersed pattern of fluorescence indicative of a major interference with localization or aggregation of ATP7A within the secretory vesicles of the Golgi. High concentrations of Cu in the exterior of the cell induce movement of the fluorescent marker to the cell boundary. The Wilson ATP7B is also localized in the Golgi (151). A truncated homologue of ATP7B lacking four of the eight membrane-spanning domains appears to reside in the cytosol (151). The *CCC2* gene in yeast, a structural analog of *MNK* or *WND*, also encodes a P-type ATPase that exports cytosolic Cu to the extracytosolic domain of Fet3p, a Cu oxidase required for iron uptake (152).

In embryonic mice, RNA in situ hybridization analysis has shown that Atp7a, the mouse homologue, is expressed in all tissues but is particularly strong in the choroid plexes of the brain. The brain site, more specifically the blood brain barrier, places the ATPase in a strategic position to control the flow of Cu into the ventricles of the brain (106). The macular mutant mouse, an animal model of Menkes disease, shows a gradual erosion of cytochrome *c* oxidase activity in the brain that can be partially prevented by a single injection of Cu in an early perinatal period (84, 85). In this mutant, Cu shows a propensity to accumulate in brain blood vessels and in astrocytes, apparently stymieing its movement to neurons (66, 67). Studies with mice have further shown that unlike that of adults, embryonic mouse liver does express Atp7a mRNA (68). Early expression of Atp7b, the rat homologue of ATP7B, is limited to the central nervous system, heart, and liver and, with development, appears in intestine, thymus, and respiratory epithelia (68). Based on transfection assays in yeast, the two ATPases have similar biochemical functions (16, 95). The Atp7b in hepatocytes from long Evan cinnamon (LEC) rats, a model for Wilson disease, follows the distribution of ceruloplasmin in cotransfected cells. The data support a metabolic connection between the plasma Cu protein and the Wilson disease ATPase (92). ATP7B may also be localized on the apical surface of hepatocytes, a location that allows the protein to expel Cu ions into the bile via the biliary canaliculi (15).

How Cu-transporting ATPases are able to translocate Cu is still unknown. Vesicles prepared from the endoplasmic reticulum of rat hepatocytes (7) or the Golgi-rich fraction from CHO cells (143) or membranes of *E. hirae* (120) have shown saturation kinetics with increasing Cu and a high sensitivity to vanadate, an ATPase inhibitor. Transport of Cu into hepatocytic vesicles requires ATP and will not function with GTP, CTP, UTP, ADP, or a nonhydrolyzable analog of ATP (7). Because *E. hirae* shows facility with Cu^+ and Ag^+, one may argue that the preferred transport form of the metal is monovalent (121). That a sulfhydryl reducing agent must be present further suggests that Cu(I) as opposed to Cu(II) is the only transport form. Liver endothelium, consisting primarily of ATP7B, however, shows ATP dependence with a Cu(II) oxalate complex but not with Cu(I) (8). This raises the question as to whether both Cu(I) and Cu(II) can engage the transporter. It is important, however, to distinguish Cu ions that engage the heavy-metal domain from those that penetrate the membrane. The two are not necessarily the same. For example, an analog of ATP7B with no heavy-metal binding sites is still able to transport Cu ions (valence unknown) across membranes (10).

The analysis of mutant forms of the ATPase have been of some advantage in singling out key residues that partake in ATP binding, phosphorylation, or transduction across the membrane. Some mutations that occur in human patients and animal models, however, give rise to truncated forms of the Menkes and Wilson protein and are not suitable for a residue by residue analysis (137, 139). A sequence comparison of the primary structure of ATP7A from rats, hamsters, mice, and

humans has permitted the identification of highly conserved, inviolable residues essential for function (105). Most deviations occur in the heavy-metal binding domain of the ATPase and less frequently in other regions of the protein. Particularly preserved are residues in the internal cytoplasmic loops that govern protein-protein recognition and energy transduction functions. In a stretch of 333 amino acids, 291 were unaltered across all species examined. The human ATPase and no other ATPase has an eight–amino acid residue inclusion string in the Hmb region between Cu binding sites 4 and 5. The function of this string of charged amino acids is not known.

A response to a toxic threat is paramount to maintaining Cu homeostasis within the cell. Movement of ATP7A to the membrane is a function of the primary structure of the protein. The heavy-metal binding domain, specifically GMTCXXC motifs in these regions, appear to play a dominant role in sensing the Cu and triggering the movement (144). The Wilson disease protein ATP7B likewise shows a disparity in structure and function of its six heavy-metal binding sites. Only those closer to the transduction site appear to influence Cu movement (42).

Transcripts for both ATP7A (108) and ATP7B (99, 151) are subject to alternative splicing. Such processing appears to be important for determining the location of the ATPases in the cell. Loss of exon 10 by an A-T transversion in intron 10 permits a constitutive skipping of exon 10. The product is an ATP7A variant that remains resident in the endoplasmic reticulum or is incapable of entering the secretory pathway (43, 101). This single splicing event is postulated to be the basis of occipital horn syndrome (OHS), the milder form of Menkes disease. Other variations in the genomic DNA at the 5′ end of the mRNA have been connected with OHS as well (72). The mottled blotchy mice, considered an animal model for OHS (109), suppress lysyl oxidase activity, which leads to improper connective tissue structure (111). The blotchy mutant likewise shows a defect in the splicing of the mRNA for Atp7a (88). In some tissues, ATP7B is present in two forms, a 160-kDa parent structure and a 140 kDa-protein that is the result of a posttranslational removal of a small peptide at the N terminus. The 140-kDa product is cell-type specific and resides in the mitochondria of liver but not in CHO cells (78). A shorter form of the ATP7B that arises by alternative splicing has also been reported (151). The shorter protein is soluble and found in the cytosol. The significance of multiple transcripts of both ATPA and ATP7B is not known, although splicing as a means of controlling the expression of the full-length transcript is one possibility. For example, BeWo cells fail to express the full-length ATP7A when developmentally arrested (104). The arrested cells fail to efflux Cu ions into the medium. The appearance of a full-length ATP7A mRNA occurs when the cells move toward differentiation, developing basolateral and apical surfaces. Only then is Cu exporting activity observed (102). A bizarre example of splicing occurs with the Wilson gene. In brain, two splice variants of the Wilson disease gene codes for an ATPase (PINA) that is found in the pineal gland and retina. The transcription start site of PINA resides between

exons 8 and 9 and requires a *cis*-acting sequence that is common to two other pineal-specific enzymes (73). The PINA variants are 100 times more prevalent at night, and although lacking all six heavy-metal binding sites, the ATPase retains its ability to restore Cu transport in a mutant that is *S. cerevisiae* deficient in Ccc2p (10). Although still under investigation, PINA appears to be a night specific Cu-transporting ATPase that functions exclusively in the retinal and pineal gland.

SUMMARY AND CONCLUSIONS

The cellular transport and metabolism of Cu comprises a series of small Cu-binding proteins working in conjunction with Cu-ATPases in vesicles that communicate between the Golgi and plasma membrane. The small proteins sense and respond to intracellular Cu. It is amazing that the versatility of the system allows a cell to function in a housekeeping mode, yet be poised to respond to a toxic Cu threat. Other components of the system not discussed here are the Cu binding proteins that remove the free ion from the cytosolic milieu and sequester it from the metabolic stream. The intracellular chaperones move Cu to specific apo-enzymes. They also transport Cu to membrane compartments or expel Cu from the cell. Newer discoveries have shown that Menkes and Wilson diseases arise by mutations in structural components of the respective ATPases that govern the movement of Cu-laden vesicles to the membranes. It now appears that the motility of the vesicles depends on an intact Cu-ATPase structure. The discovery of chaperones may hold the key as to how Cu ions become prosthetic groups of enzymes. It is still not clear what is taking place at the exchange site, although the chemistry of that reaction is yielding information about an unmistakable specificity between donor and recipient. One of the biggest challenges to be overcome is to determine how intracellular Cu is prioritized to specific target enzymes. Ultimately, this understanding may lead to therapeutic approaches to Cu disorders. Adding Cu back as a chaperone complex may be the most efficient way to correct a Cu deficiency. The impact of nutrition on chaperone levels is an area virtually untapped and overflowing with a potential for significant discoveries. Lastly, we must submit to the realization that we understand little about what controls ATP7A and ATP7B expression at the transcription and posttranscription stages in the cell.

ACKNOWLEDGMENTS

The author wishes to thank colleagues and collaborators for contributing information and discussion for this chapter. Grants HD-29952 from the National Institute of Child Health and Human Development, NIH and USDA grants 94-37300-0375 and 98-35200-6055 supported the research studies in the author's laboratory. I apologize to colleagues whose work could not be included in this review because of space limitations.

LITERATURE CITED

1. Alda JO, Garay R. 1990. Chloride (or bicarbonate)-dependent copper uptake through the anion exchanger in human blood cells. *Am. J. Physiol.* 59:C570–76
2. Amaravadi R, Glerum DM, Tzagoloff A. 1997. Isolation of a cDNA encoding the human homolog of COX17, a yeast gene essential for mitochondrial copper recruitment. *Hum. Genet.* 99:329–33
3. Ascone I, Longo A, Dexpert H, Ciriolo MR, Rotilio G, Desideri A. 1993. An X-ray absorption study of the reconstitution process of bovine Cu,Zn superoxide dismutase by Cu(I)-glutathione complex. *FEBS Lett.* 322:165–67
4. Barnea A, Cho G, Katz BM. 1991. A putative role for extracellular ATP: facilitation of ^{67}copper uptake and of copper stimulation of the release of luteinizing hormone-releasing hormone from median eminence explants. *Brain Res.* 541:93–97
5. Barnes G, Frieden E. 1984. Ceruloplasmin receptors of erythrocytes. *Biochem. Biophys. Res. Commun.* 125:157–62
6. Beer J, Glerum DM, Tzagoloff A. 1997. Purification, characterization, and localization of yeast Cox17p, a mitochondrial copper shuttle. *J. Biol. Chem.* 272:33191–96
7. Bingham MJ, Burchell A, McArdle HJ. 1995. Identification of an ATP-dependent copper transport system in endoplasmic reticulum vesicles isolated from rat liver. *J. Physiol.* 482:583–87
8. Bingham MJ, Ong TJ, Ingledew WJ, McArdle HJ. 1996. ATP-dependent copper transporter, in the Golgi apparatus of rat hepatocytes, transports Cu(II), not Cu(I). *Am. J. Physiol.* 271:G741–46
9. Bingham MJ, Sargeson AM, McArdle HJ. 1997. Characterization of intracellular copper pools in rat hepatocytes using the chelator diamsar. *Am. J. Physiol.* 272:G1400–7
10. Borjigin J, Payne AS, Deng J, Li X, Wang MM, et al. 1999. A novel pineal night-specific ATPase encoded by the Wilson disease gene. *J. Neurosci.* 19:1018–26
11. Bradshaw RA, Shearer WT, Gurd FRN. 1968. Sites of binding of copper(II) ion by peptide (1–24) of bovine serum albumin. *J. Biol. Chem.* 243:3817–25
12. Brouwer M, Brouwer-Hoexum T. 1991. Interactions of copper-metallothinein from the American lobster, *Homarus americanus*, with glutathione. *Arch. Biochem. Biophys.* 290:207–13
13. Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. 1993. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat. Genet.* 5:327–36
14. Camakaris J, Petris MJ, Bailey L, Shen PY, Lockhart P, et al. 1995. Gene amplification of the Menkes (MNK.ATP7A) P-type ATPase gene of CHO cells is associated with copper resistance and enhanced copper efflux. *Hum. Mol. Genet.* 4:2117–23
15. Castillo RO, Thaler MM, O'Toole C, Packman S. 1990. Hepatic copper metabolism in a mouse model for Menkes' kinky hair syndrome. *Pediatr. Res.* 27:492–96
16. Cecchi C, Avner P. 1996. Genomic organization of the mottled gene, the mouse homolog of the human Menkes disease gene. *Genomics* 37:96–104
17. Chan W-Y, Cushing W, Coffman MA, Rennert OM. 1980. Genetic expression of Wilson's disease in cell culture: a diagnostic marker. *Science* 208:299–300
18. Chao PY, Allen KGD. 1992. Glutathione production in copper-deficient isolated rat hepatocytes. *Free Rad. Biol. Med.* 12:145–50
19. Chelly J, Tümer Z, Tonnesen T, Peterson A, Ishikawa-Brush Y, et al. 1993. Isolation of a candidate gene for Menkes disease that

encodes a potential heavy metal binding protein. *Nat. Genet.* 3:14–19
20. Ciriolo MR, Desideri A, Paci M, Rotilio G. 1990. Reconstitution of Cu,Zn-superoxide dismutase by the Cu(I)· glutathione complex. *J. Biol. Chem.* 265:11030–34
21. Culotta VC, Klomp LW, Strain J, Casareno RLB, Krems B, Gitlin JD. 1997. The copper chaperone for superoxide dismutase. *J. Biol. Chem.* 272:23469–72
22. Culotta VC, Lin S-J, Schmidt P, Klomp LW, Casareno RLB, Gitlin D. 1999. Intra cellular pathways of copper trafficking in yeast and humans. *Adv. Exp. Med. Biol.* 448:247–54
23. Dameron CT, Harris ED. 1987. Regulation of aortic Cu,Zn-superoxide dismutase with copper. Ceruloplasmin and albumin transfer copper and reactivate the enzyme in culture. *Biochem. J.* 248:669–75
24. Dancis A, Haile D, Yuan DS, Klausner RD. 1994. The *Saccharomyces cerevisiae* copper transport protein (Ctr1p). Biochemical characterization, regulation by copper, and physiologic role in copper uptake. *J. Biol. Chem.* 269:25660–67
25. Dancis A, Yuan DS, Haile D, Askwith C, Eide D, et al. 1994. Molecular characterization of a copper transport protein in *S. cerevisiae*: an unexpected role for copper in iron transport. *Cell* 76:393–402
26. Danks DM. 1988. Copper deficiency in humans. *Annu. Rev. Nutr.* 8:235–57
27. Danks DM. 1995. Disorders of copper transport. In *The Metabolic and Molecular Basis of Inherited Disease*, ed. CR Scriver, AL Baudet, WM Sly, D Valle, pp. 2211–35. New York: McGraw-Hill
28. Danks DM, Campbell PE, Walker-Smith J, Stevens BJ, Gillespie JM, et al. 1972. Menkes' kinky-hair syndrome. *Lancet* 1:1100–3
29. Darwish HM, Cheney JC, Schmitt RC, Ettinger MJ. 1984. Mobilization of copper-(II)from plasma components and mechanism of hepatic copper transport. *Am. J. Physiol.* 246:G72–79
30. Darwish HM, Schmitt RC, Cheney JC, Ettinger MJ. 1984. Copper efflux kinetics from rat hepatocytes. *Am. J. Physiol.* 246:G48–55
31. Davidson LA, McOrmond SL, Harris ED. 1994. Characterization of a particulate pathway for copper in K562 cells. *Biochim. Biophys. Acta* 1221:1–6
32. Deneke SM, Fanburg BL. 1989. Regulation of cellular glutathione. *Am. J. Physiol.* 257:L163–73
33. DiDonato M, Narindrasorasak S, Forbes JR, Cox DW, Sarkar B. 1997. Expression, purification, and metal binding properties of the N-terminal domain from the Wilson disease putative copper-transporting ATPase (ATP7B). *J. Biol. Chem.* 272:33279–82
34. Dierick H, Ambrosini L, Spencer J, Glover TW, Mercer JFB. 1995. Molecular structure of the Menkes disease gene (ATP7A). *Genomics* 28:462–69
35. Dierick HA, Adam AN, Escara-Wilke JF, Glover TW. 1997. Immunocytochemical localization of the Menkes copper transport protein (ATP7A) to the trans-Golgi network. *Hum. Mol. Genet.* 6:409–16
36. Dierickx PJ. 1986. In vitro interaction of organic copper (II) compounds with soluble glutathione S-transferases from rat liver. *Res. Commun. Chem. Pathol. Pharmacol.* 51:285–88
37. Dijkstra M, Veld GI, van den Berg GJ, Muller M, Kuipers F, Vonk RJ. 1995. In vitro modeling of liver membrane copper transport. *J. Clin. Invest.* 95:412–16
38. Dini L, Carbonaro M, Musci G, Calabrese L. 1990. The interaction of ceruloplasmin with Kupffer cells. *Eur. J. Cell Biol.* 52:207–12
39. Dinsdale D, Holt D, Webb M. 1986. Intestinal uptake and retention of copper in the suckling rat, *Rattus rattus*. II. Copper accumulation in the ileum and distal jejunum. *Comp. Biochem. Physiol.* 83C:317–23
40. Eide DJ. 1998. The molecular biology

of metal ion transport in *Saccharomyces cerevisiae*. *Annu. Rev. Nutr.* 18:441–69
41. Ferreira AM, Ciriolo MR, Marcocci L, Rotilio G. 1993. Copper(I) transfer into metallothionein mediated by glutathione. *Biochem. J.* 292:673–76
42. Forbes JR, Hsi G, Cox DR. 1999. Role of the copper-binding domain in the copper transport function of ATP7B, the P-type ATPase defective in Wilson disease. *J. Biol. Chem.* 274:12408–13
43. Francis MJ, Jones EE, Levy ER, Ponnambalam S, Chelly J, Monaco AP. 1998. A Golgi localization signal identified in the Menkes recombinant protein. *Hum. Mol. Genet.* 7:1245–52
44. Freedman JH, Ciriolo MR, Peisach J. 1989. The role of glutathione in copper metabolism and toxicity. *J. Biol. Chem.* 264:5598–605
45. Freedman JH, Peisach J. 1989. Intracellular copper transport in cultured hepatoma cells. *Biochem. Biophys. Res. Commun.* 164:134–40
46. Gallagher CH, Reeve VE. 1971. Copper deficiency in the rat. Effect on synthesis of phospholipids. *Aust. J. Exp. Biol. Med. Sci.* 49:21–31
47. Georgatsou E, Mavrogiannis LA, Fragiadakis GS, Alexandraki D. 1997. The yeast Fre1p/Fre2p cupric reductases facilitate copper uptake and are regulated by the copper-modulated Mac1p activator. *J. Biol. Chem.* 272:13786–92
48. Glerum DM, Shtanko A, Tzagoloff A. 1996b. SCO1 and SCO2 act as high copy suppressors of a mitochondrial copper recruitment defect in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 271:20531–35
49. Glerum DM, Shtanko A, Tzagoloff A. 1996a. Characterization of COX17, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. *J. Biol. Chem.* 271:14504–9
50. Goka TJ, Stevenson RE, Hefferan PM, Howell RR. 1976. Menkes disease: a biochemical abnormality in cultured human fibroblasts. *Proc. Natl. Acad. Sci. USA* 73:604–6
51. Goode CA, Dinh CT, Linder MC. 1989. Mechanism of copper transport and delivery in mammals: review and recent findings. In *Copper Bioavailability and Metabolism*, ed. C Kies, pp. 131–44. New York: Plenum
52. Harris DIM, Sass-Kortsak A. 1967. The influence of amino acids on copper uptake by rat liver slices. *J. Clin. Invest.* 46:659–77
53. Harris ED, Qian Y, Tiffany-Castiglioni E, Lacy AR, Reddy MCM. 1998. Functional analysis of copper homeostasis in cell culture models: a new perspective on internal copper transport. *Am. J. Clin. Nutr.* 67(Suppl.):988–95S
54. Harris ED, Reddy MCM, Qian Y, Tiffany-Castiglioni E, Majumdar S, Nelson J. 1999. Multiple forms of the Menkes Cu-ATPase. *Adv. Exp. Med. Biol.* 448:39–51
55. Hassett R, Kosman DJ. 1995. Evidence for Cu(II) reduction as a component of copper uptake by *Saccharomyces cerevisiae*. *J. Biol. Chem.* 270:128–34
56. Himelblau E, Mira H, Lin SJ, Culotta VC, Penarrubia L, Amasino RM. 1998. Identification of a functional homolog of the yeast copper homeostasis gene ATX1 from *Arabidopsis*. *Plant Physiol.* 117:1227–34
57. Horn N. 1976. Copper incorporation studies on cultured cells for prenatal diagnosis of Menkes' disease. *Lancet* 1:1156–58
58. Houwen R, Dijkstra M, Kuipers F, Smit EP, Havinga R, Vonk RJ. 1990. Two pathways for biliary copper excretion in the rat. The role of glutathione. *Biochem. Pharmacol.* 39:1039–44
59. Hsieh HS, Frieden E. 1975. Evidence for ceruloplasmin as a copper transport protein. *Biochem. Biophys. Res. Commun.* 67:1326–31
60. Irie S, Tavassoli M. 1986. Liver endothelium desialates ceruloplasmin. *Biochem. Biophys. Res. Commun.* 140:94–100
61. Iyengar V, Brewer GJ, Dick RD, Owyang

C. 1988. Studies of cholescystokinin-stimulated biliary secretions reveal a high molecular weight copper-binding substance in normal subjects that is absent in patients with Wilson's disease. *J. Lab. Clin. Med.* 111:267–74

62. Kampfenkel K, Kushnir S, Babiychuk E, Inzé D, Van Montagu M. 1995. Molecular characterization of a putative *Arabidopsis thaliana* copper transporter and its yeast homologue. *J. Biol. Chem.* 270:28479–86
63. Kawata M, Suzuki KT. 1983. Relation between metal and glutathione concentrations in mouse liver after cadmium, zinc or copper loading. *Toxicol. Lett.* 15:131–37
64. Klomp LW, Lin SJ, Yuan DS, Klausner RD, Culotta VC, Gitlin JD. 1997. Identification and functional expression of HAH1, a novel human gene involved in copper homeostasis. *J. Biol. Chem.* 272:9221–26
65. Knight SAB, Labbé S, Kwon LF, Kosman DJ, Thiele DJ. 1996. A widespread transposable element masks expression of a yeast copper transport gene. *Genes Dev.* 10:1917–29
66. Kodama H. 1993. Recent developments in Menkes disease. *J. Inherit. Metab. Dis.* 16:791–99
67. Kodama H, Meguro Y, Abe T, Rayner MH, Suzuki KT, et al. 1991. Genetic expression of Menkes disease in cultured astrocytes of the macular mouse. *J. Inherit. Metab. Dis.* 14:896–901
68. Kuo Y-M, Gitschier J, Packman S. 1997. Developmental expression of the mouse mottled and toxic milk genes suggests distinct functions for the Menkes and Wilson disease copper transporters. *Hum. Mol. Genet.* 6:1043–49
69. Laurie SH, Pratt DE. 1986. Copper-albumin: What is its functional role. *Biochem. Biophys. Res. Commun.* 135:1064–68
70. Laussac J-P, Sarkar B. 1984. Characterization of the copper(II)- and nickel(II)-transport site of human serum albumin. Studies of copper(II) and nickel(II) binding to peptide 1-24 of human serum albumin by ^{13}C and ^{1}H NMR spectroscopy. *Biochemistry* 23:2832–38
71. Lee SH, Lancey R, Montaser A, Madani N, Linder MC. 1993. Ceruloplasmin and copper transport during the latter part of gestation in the rat. *Proc. Soc. Exp. Biol. Med.* 203:428–39
72. Levinson B, Conant R, Schnur R, Das S, Packman S, Gitschier J. 1996. A repeated element in the regulatory region of the MNK gene and its deletion in a patient with occipital horn syndrome. *Hum. Mol. Genet.* 5:1737–42
73. Li X, Chen S, Wang Q, Zack DJ, Snyder SH, Borjigin J. 1998. A pineal regulatory element (PIRE) mediates transactivation by the pineal/retina-specific transcription factor CRX. *Proc. Natl. Acad. Sci. USA* 95:1876–81
74. Lin SJ, Culotta VC. 1995. The ATX1 gene of *Saccharomyces cerevisiae* encodes a small metal homeostasis factor that protects cells against reactive oxygen toxicity. *Proc. Natl. Acad. Sci. USA* 92:3784–88
75. Lin SJ, Pufahl RA, Dancis A, O'Halloran TV, Culotta VC. 1997. A role for the *Saccharomyces cerevisiae* ATX1 gene in copper trafficking and iron transport. *J. Biol. Chem.* 272:9215–20
76. Linder MC. 1991. *Biochemistry of Copper*. New York: Plenum
77. Lund PA, Brown NL. 1987. Role of the merT and merP gene products of transposon Tn501 in the induction and expression of resistance to mercuric ions. *Gene* 52:207–14
78. Lutsenko S, Cooper MJ. 1998. Localization of the Wilson's disease protein product to mitochondria. *Proc. Natl. Acad. Sci. USA* 95:6004–9
79. Lutsenko S, Petrukhin K, Cooper MJ, Gilliam CT, Kaplan JH. 1997. N-terminal domains of human copper-transporting adenosine triphosphatases (the Wilson's and Menkes disease proteins) bind copper

selectively in vivo and in vitro with stoichiometry of one copper per metal-binding repeat. *J. Biol. Chem.* 272:18939–44

80. Marceau N, Aspin N. 1973. The intracellular distribution of the radiocopper derived from ceruloplasmin and from albumin. *Biochim. Biophys. Acta* 293:338–50
81. Martins LJ, Jensen LT, Simon JR, Keller GL, Winge DR. 1998. Metalloregulation of FRE1 and FRE2 homologs in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 273: 23716–21
82. Mas A, Sarkar B. 1992. Uptake of ^{67}Cu by isolated human trophoblast cells. *Biochim. Biophys. Acta* 1135:123–28
83. McArdle HJ, Gross SM, Danks DM, Wedd AG. 1990. Role of albumin's copper binding site in copper uptake by mouse hepatocytes. *Am. J. Physiol.* 258:G988–91
84. Meguro Y, Kodama H, Abe T, Kobayashi S, Kodama Y, Nishimura M. 1991. Changes of copper level and cytochrome *c* oxidase activity in the macular mouse with age. *Brain Dev.* 13:184–86
85. Meguro Y, Kodama H, Abe T, Kobayashi S, Kodama Y, Nishimura N. 1991. Changes of copper level and cytochrome c oxidase activity. *Brain Dev.* 13:184–86
86. Melkonyan VZ, Mikaelyan MV, Nalbandyan RM. 1989. Reconstitution of neurocupreins by Cu-thioneins. *Neurochem. Res.* 14:589–91
87. Menkes JH, Alter M, Steigleder GK, Weakley DR, Sung JH. 1962. A sex-linked recessive disorder with retardation of growth, peculiar hair, and focal cerebral and cerebellar degeneration. *Pediatrics* 29:764–79
88. Mercer JF, Grimes A, Ambrosini L, Lockhart P, Paynter JA, et al. 1994. Mutations in the murine homologue of the Menkes gene in dappled and blotchy mice. *Nat. Genet.* 6:374–78
89. Mercer JFB, Livingston J, Hall B, Paynter JA, Begy C, et al. 1993. Isolation of a partial candidate gene for Menkes disease by positional cloning. *Nat. Genet.* 3:20–25
90. Miyoshi K, Sugiura Y, Ishizu K, Iitaka Y, Nakamura H. 1980. Crystal structure and spectroscopic properties of violet glutathione-copper(II) complex with axial sulfur coordination and two copper sites via a disulfide bridge. *J. Am. Chem. Soc.* 102:6130–36
91. Musci G, Di Marco S, Bellenchi GC, Calabrese L. 1996. Reconstitution of ceruloplasmin by the Cu(I)-glutathione complex: evidence for a role of Mg^{2+} and ATP. *J. Biol. Chem.* 271:1972–78
92. Nagano K, Nakamura K, Urakami K-I, Umyama K, Uchiyama H. et al. 1998. Intracellular distribution of the Wilson's disease gene product (ATPase7B) after in vitro and in vivo exogenous expression in hepatocytes from the LEC rat, an animal model of Wilson's disease. *Hepatology* 27:799–807
93. Nederbragt H. 1989. Effect of the glutathione-depleting agents diethylmaleate, pho rone and buthionine sulfoximine on biliary copper excretion in rats. *Biochem. Pharmacol.* 38:3399–406
94. Orena SJ, Goode CA, Linder MC. 1986. Binding and uptake of copper from ceruloplasmin. *Biochem. Biophys. Res. Commun.* 139:822–29
95. Payne AS, Gitlin JD. 1998. Functional expression of the Menkes disease protein reveals common biochemical mechanisms among the copper-transporting P-type ATPases. *J. Biol. Chem.* 273:3765–70
96. Percival SS, Harris ED. 1989. Ascorbate enhances copper transport from ceruloplasmin into human K562 cells. *J. Nutr.* 119:779–84
97. Percival SS, Harris ED. 1990. Copper transport from ceruloplasmin: characterization of the cellular uptake mechanism. *Am. J. Physiol.* 258:C140–46
98. Petrukhin K, Fischer SG, Pirastu M, Tanzi RE, Chernov I, et al. 1993. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. *Nat. Genet.* 5:338–43

99. Petrukhin K, Lutsenko S, Chernov I, Ross BM, Kaplan JH, Gilliam TC. 1994. Characterization of the Wilson disease gene encoding a P-type copper transporting ATPase: genomic organization, alternative splicing, and structure/function predictions. *Hum. Mol. Genet.* 3:1647–56
100. Pufahl RA, Singer CP, Peariso KL, Lin S-J, Schmidt PJ, et al. 1997. Metal ion chaperone function of the soluble Cu(I) receptor Atx1. *Science* 278:853–56
101. Qi M, Byers PH. 1998. Constitutive skipping of alternatively spliced exon 10 in the ATP7A gene abolishes Golgi localization of the Menkes protein and produces the occipital horn syndrome. *Hum. Mol. Genet.* 7:465–69
102. Qian Y, Majumdar S, Reddy MCM, Harris ED. 1996. Coincident expression of Menkes gene with copper efflux in human placental cells. *Am. J. Physiol.* 270: C1880–84
103. Qian Y, Tiffany-Castiglioni E, Harris ED. 1995. Copper transport and kinetics in cultured C6 rat glioma cells. *Am. J. Physiol.* 269:C892–98
104. Qian Y, Tiffany-Castiglioni E, Harris ED. 1996. Functional analysis of a genetic defect of copper transport (Menkes disease) in different cell lines. *Am. J. Physiol.* 271:C378–84
105. Qian Y, Tiffany-Castiglioni E, Harris ED. 1998. Sequence of a Menkes-type Cu-transporting ATPAse from rat C6 glioma cells: comparison of the rat protein with other mammalian Cu-transporting ATPases. *Mol. Cell. Biochem.* 181:49–61
106. Qian Y, Tiffany-Castiglioni E, Welsh J, Harris ED. 1998. Copper efflux from murine microvascular cells requires expression of the Menkes disease Cu-ATPase. *J. Nutr.* 128:1276–82
107. Rabenstein DL, Guevremont R, Evans CA. 1979. Glutathione and its metal complexes. In *Metal Ions in Biological Systems. Amino Acids and Derivatives as Ambivalent Ligands*, ed. H. Sigel, pp. 103–41. Basel: Marcel Dekker
108. Reddy MCM, Harris ED. 1998. Multiple transcripts coding for the Menkes gene: evidence for alternative splicing of Menkes mRNA. *Biochem. J.* 334:71–77
109. Reed V, Boyd Y. 1997. Mutation analysis provides additional proof that mottled is the mouse homologue of Menkes' disease. *Hum. Mol. Genet.* 6:417–23
110. Riordan JR, Jolicoeur-Paquet L. 1982. Metallothionein accumulation may account for intracellular copper retention in Menkes' disease. *J. Biol. Chem.* 257: 4639–45
111. Royce PM, Camakaris J, Mann JR, Danks DM. 1982. Copper metabolism in mottled mouse mutants. The effect of copper therapy on lysyl oxidase activity in brindled (Mo^{br}) mice. *Biochem. J.* 202:369–71
112. Saenko EL, Yaropolov AI. 1990. Studies on receptor interaction of ceruloplasmin with human red blood cells. *Biochem. Int.* 20:215–25
113. Sarkar B. 1980. Recent trends in the application of coordination chemistry in biology and medicine. In *Coordination Chemistry*, ed. D Banerjea, 20:191–200. Oxford, UK: Pergamon
114. Sarkar B, Kruck TPA. 1966. Copper-amino acid complexes in human serum. In *The Biochemistry of Copper*, ed. J Peisach, P Aisen, WE Blumberg, pp.183–96. New York: Academic
115. Schaefer M, Gitlin JD. 1999. Genetic disorders of membrane transport. IV. Wilson disease and Menkes disease. *Am. J. Physiol.* 276:G311–14
116. Schmitt RC, Darwish HM, Cheney JC, Ettinger MJ. 1983. Copper transport kinetics by isolated rat hepatocytes. *Am. J. Physiol.* 244:G183–91
117. Silver S, Nucifora G, Phung LT. 1993. Human Menkes X-chromosome disease and the staphylococcal cadmium-resistance ATPase: a remarkable similarity in protein sequences. *Mol. Microbiol.* 10:7–12
118. Sklan D, Donoghue S. 1982. Association

of acylglyceride and retinyl palmitate hydrolase activities with zinc and copper metalloproteins in a high molecular weight lipid-protein aggregate fraction from chick liver cytosol. *Biochim. Biophys. Acta* 711:532–38

119. Smith HA, Dasmahapatra AK, Caffrey JMJ, Frieden E. 1988. A comparative study of copper ion induced lysis of vertebrate red blood cells. *Comp. Biochem. Physiol.* 91C:301–5
120. Solioz M, Odermatt A. 1995. Copper and silver transport by CopB-ATPase in membrane vesicles of *Enterococcus hirae*. *J. Biol. Chem.* 270:9217–21
121. Solioz M, Odermatt A, Krapf R. 1994. Copper pumping ATPases: common concepts in bacteria and man. *FEBS Lett.* 346:44–47
122. Solioz M, Vulpe C. 1996. Cpx-type ATPases: a class of P-type ATPases that pump heavy metals. *Trends Biochem. Sci.* 21:237–41
123. Srinivasan C, Posewitz MC, George GN, Winge DR. 1998. Characterization of the copper chaperone Cox17 of *Saccharomyces cerevisiae*. *Biochemistry* 37:7572–77
124. Steinbach OM, Wolterbeek HT. 1994. Effects of copper on rat hepatoma HTC cells and primary cultured rat hepatocytes. *J. Inorg. Biochem.* 53:27–48
125. Steinbach OM, Wolterbeek HT. 1994. Role of cytosolic copper, metallothionein and glutathione in copper toxicity in rat hepatoma tissue culture cells. *Toxicology* 92:75–90
126. Steinkühler C, Sapora O, Carri MT, Nagel W, Marcocci L, et al. 1991. Increase of Cu, Zn-superoxide dismutase activity during differentiation of human K562 cells involves activation by copper of a constantly expressed copper-deficient protein. *J. Biol. Chem.* 266:24580–87
127. Stern RV, Frieden E. 1990. Detection of rat ceruloplasmin receptors using fluorescence microscopy and microdensitometry. *Anal. Biochem.* 190:48–56
128. Sternlieb I. 1980. Copper and the liver. *Gastroenterology* 78:1615–28
129. Stevens MD, DiSilvestro RA, Harris ED. 1984. Specific receptor for ceruloplasmin in membrane fragments from aortic and heart tissues. *Biochemistry* 23:261–66
130. Stockert RJ, Grushoff PS, Morell AG, Bentley GE, O'Brien HA, et al. 1986. Transport and intracellular distribution of copper in a human hepatoblastoma cell line, HepG2. *Hepatology* 6:60–64
131. Suzuki KT, Karasawa A, Yamanaka K. 1989. Binding of copper to albumin and participation of cysteine in vivo and in vitro. *Arch. Biochem. Biophys.* 273:572–77
132. Suzuki KT, Maitani T. 1981. Metal-dependent properties of metallothionein. Replacement in vitro of zinc in zinc-thionein with copper. *Biochem. J.* 199:289–95
133. Szczypka MS, Zhu Z, Silar P, Thiele DJ. 1997. *Saccharomyces cerevisiae* mutants altered in vacuole function are defective in copper detoxification and iron-responsive gene transcription. *Yeast* 13:1423–35
134. Sze G, Kaplowitz N, Ookhtens M, Lu SC. 1993. Bidirectional membrane transport of intact glutathione in Hep G2 cells. *Am. J. Physiol.* 265:G1128–34
135. Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, et al. 1993. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat. Genet.* 5:344–50
136. Tavassoli M, Kishimoto T, Kataoka M. 1986. Liver endothelium mediates the hepatocytes uptake of ceruloplasmin. *J. Cell Biol.* 102:1298–303
137. Thomas GR, Forbes JR, Roberts EA, Walshe JM, Cox DW. 1995. The Wilson disease gene: spectrum of mutations and their consequences. *Nat. Genet.* 9:210–17
138. Tong KK, McArdle HJ. 1995. Copper uptake by cultured trophoblast cells isolated from human term placenta. *Biochim. Biophys. Acta* 1269:233–36

139. Tumer Z, Lund C, Tolshave J, Vural B, Tonnesen T, Horn N. 1994. Identification of point mutations in 41 unrelated patients affected with Menkes disease. *Am. J. Hum. Genet.* 60:63–71
140. Tumer Z, Vural B, Tonnesen T, Chelly J, Monaco AP, Horn N. 1995. Characterization of the exon structure of the Menkes disease gene using vectorette PCR. *Genomics* 26:437–42
141. Valentine JS, Gralla EB. 1997. Delivering Cu inside yeast cells. *Science* 278:817–18
142. van den Berg GJ, McArdle HJ. 1994. A plasma membrane NADH oxidase is involved in copper uptake by plasma membrane vesicles isolated from rat liver. *Biochim. Biophys. Acta* 1195:276–80
143. Voskoboinik I, Brooks H, Smith S, Camakaris J. 1998. ATP-dependent copper transport by the Menkes protein in membrane vesicles isolated from cultured Chinese hamster ovary cells. *FEBS Lett.* 435:178–82
144. Voskoboinik I, Strausak D, Greenough M, Brooks H, Petris M, et al. 1999. Functional analysis of the N-terminal CXXC metal-binding motifs in the human Menkes copper-transporting P-type ATPase expressed in cultured mammalian cells. *J. Biol. Chem.* 274:22008–12
145. Vulpe C, Levinson B, Whitney S, Packman S, Gitschier J. 1993. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nat. Genet.* 3:7–13
146. Vulpe CD, Packman S. 1995. Cellular copper transport. *Annu. Rev. Nutr.* 15:293–322
147. Waldrop GL, Ettinger MJ. 1990. Effects of albumin and histidine on kinetics of copper transport by fibroblasts. *Am. J. Physiol.* 259:G212–18
148. Weiner AL, Cousins RJ. 1980. Copper accumulation and metabolism in primary monolayer cultures of rat liver parenchymal cells. *Biochim. Biophys. Acta* 629:113–25
149. Weiss KC, Linder MC. 1985. Copper transport in rats involving a new plasma protein. *Am. J. Physiol.* 249:E77–88
150. Yamaguchi Y, Heiny ME, Gitlin JD. 1993. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson's disease. *Biochem. Biophys. Res. Commun.* 197:271–77
151. Yang X-L, Miura N, Kawarda Y, Terada K, Petrukhin K, et al. 1997. Two forms of Wilson disease protein produced by alternative splicing are localized in distinct cellular compartments. *Biochem. J.* 326:897–902
152. Yuan DS, Dancis A, Klausner RD. 1997. Restriction of copper export in *Saccharomyces cerevisiae* to a late Golgi or post-Golgi compartment in the secretory pathway. *J. Biol. Chem.* 272:25787–93
153. Zhou B, Gitschier J. 1997. hCTR1: a human gene for copper uptake identified by complementation in yeast. *Proc. Natl. Acad. Sci. USA* 94:7481–86