Genetic Disorders of Membrane Transport
IV. Wilson’s disease and Menkes disease*

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Schaefer, Mark, and Jonathan D. Gitlin. Genetic Disorders of Membrane Transport. IV. Wilson’s disease and Menkes disease. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G311–G314, 1999.—Copper is an essential transition metal that permits the facile transfer of electrons in a series of critical biochemical pathways. Menkes disease and Wilson’s disease are inherited disorders of copper metabolism resulting from the absence or dysfunction of homologous copper-transporting ATPases that reside in the trans-Golgi network of all cells. Despite striking differences in the clinical presentation of these two diseases, the respective ATPases function in precisely the same manner within the cell and the unique clinical features of each disease are entirely the result of the tissue-specific expression of each protein. Elucidation of the basic defect in these rare genetic disorders has provided a valuable heuristic paradigm for understanding the mechanisms of cellular copper homeostasis.

Wilson’s disease; Menkes disease; adenosine triphosphatase

GENETIC DISORDERS OF MEMBRANE TRANSPORT

MENKES DISEASE IS AN X-linked disorder of copper metabolism resulting in growth failure and severe neurodegenerative disease in early childhood. The Menkes disease gene was physically mapped by analysis of a balanced translocation in an affected female infant, and subsequent cloning of the gene revealed a predicted protein sequence of 90% identity to the Menkes ATPase. The Wilson’s disease protein is expressed in the liver and transports copper into the hepatocyte secretory pathway for subsequent incorporation into ceruloplasmin and excretion into bile. Affected individuals present with signs and symptoms arising from impaired biliary copper excretion. Because this route represents the only physiological mechanism of copper excretion, excessive accumulation of copper in the hepatocyte cytoplasm eventually results in cellular necrosis with leakage of copper into the plasma and deposition of copper in extrahepatic tissues, including the limbus of the cornea and the basal ganglia of the brain (1).

STRUCTURE OF COPPER TRANSPORTERS

Sequence comparison and hydropathy plot analysis of the derived amino acid sequence of the Menkes and Wilson’s ATPases reveal a polytopic membrane protein predicted to transport copper across biological membranes in an ATP-dependent manner (Fig. 1). Homologous proteins have now been identified in a wide range of prokaryotic and eukaryotic species and shown to play an analogous role in copper transport in these organisms. Conserved amino acid motifs in such proteins include the MXXCXC copper-binding sequences in the amino terminus, an IGTEA phosphatase domain, a conserved DKTGT sequence that includes the invariant aspartyl residue, which is reversibly phosphorylated in the process of energy transduction, and a GDGVND ATP binding domain (Fig. 1). In addition to these functionally defined regions, a highly conserved SEHPL sequence is present within the large cytoplasmic loop containing the ATP binding domain. The histidine residue in this motif is conserved in all known copper-transporting P-type ATPases and is the site of the most common mutation (H1069Q) in patients with Wilson’s disease (21).

Biosynthetic studies of the Wilson’s and Menkes proteins indicate that each ATPase is synthesized as a single-chain polypeptide, localized to the trans-Golgi network of the cell (3, 8, 17, 20, 22). In this location, these ATPases transport copper into the secretory pathway of the cell for incorporation into specific copper-enzymes and export from the cell. Support for this model has come from studies in Saccharomyces cerevisiae deficient in the homologous copper-transporting ATPase CCC2 (23, 24). Expression of the Wilson’s or...
Menkes proteins in ccc2Δ yeast restores copper incorporation into the ceruloplasmin homologue FET3, providing direct evidence of copper transport by these ATPases into the secretory pathway of the cell (8, 14).

Mutation of a conserved histidine to glutamine (H1086Q) in the Menkes protein, homologous to the common H1069Q mutation in Wilson's disease, abrogates copper transport by this protein in ccc2Δ yeast (14). The finding that the most commonly occurring mutation in Wilson's disease similarly compromises function of the Menkes protein provides evidence of a commonality of transporter function. Studies demonstrating that the human Wilson's protein can rescue the phenotype of Menkes disease protein-deficient cells also provide compelling evidence that these ATPases work through common biochemical mechanisms (15).

These data support the concept that the differences in clinical presentation of these diseases are the result of tissue-specific differences in expression and raise the possibility that interventions to induce expression of the Wilson's protein early in development in cells normally expressing the Menkes protein might provide a novel therapeutic approach in this otherwise fatal disease.

MECHANISMS OF CELLULAR COPPER HOMEOSTASIS

Although the Menkes and Wilson's ATPases are localized to the trans-Golgi network under steady-state conditions, an increase in the copper concentration results in trafficking of these proteins to a cytoplasmic vesicular compartment visible by electron microscopy (8, 10, 17). In the polarized hepatocytes of the liver these vesicles are localized adjacent to the canalicular membrane (20). As copper is transported into this compartment, the intracellular copper concentration falls and these proteins are recycled back to the trans-Golgi network while copper is exported from the cell. This copper-dependent trafficking of the Menkes and Wilson's proteins is rapid, occurring within minutes of exposure to increased copper, and represents a novel posttranslational mechanism allowing for restoration of cellular copper homeostasis (8, 17, 20).

The mechanisms determining the intracellular location of these ATPases are not well understood. In a patient with a mild form of Menkes disease, in which
mutation of the splice donor site of exon 10 results in an in-frame deletion of the third and fourth transmembrane domains of the Menkes protein. Immunofluorescence studies indicate that this transmembrane region is essential for the trans-Golgi network localization (4, 19). Studies of the H1069Q mutant Wilson’s protein suggest that this conserved histidine residue is also essential for localization to the trans-Golgi network and copper-dependent recycling (15). Recent studies (16) utilizing site-directed mutagenesis of the Menkes protein have revealed that a dileucine motif in the carboxy terminus is necessary for this protein to respond to intracellular copper, suggesting that trafficking signals common to the cytoplasmic domain of many intracellular cargo proteins may be utilized by these ATPases in the physiological response to copper.

THE COPPER CHAPERONE HAH1

A series of genetic studies in Saccharomyces cerevisiae have revealed that the delivery of copper to specific proteins within the cell is mediated by a group of intracellular proteins termed copper chaperones. ATX1 encodes a small cytosolic copper-binding protein in Saccharomyces originally identified as a multicopy suppressor of sod1Δ mutants (11). This protein delivers copper to CCC2 for subsequent transport into the secretory pathway and incorporation into the multicopper oxidase FET3, which is required for high-affinity iron uptake (12). The identification of a homologous protein in humans termed HAH1 has defined a role for this chaperone in mammalian cells and reveals a remarkable evolutionary conservation of the mechanisms of copper trafficking and compartmentalization (9).

HAH1 contains a single copy of the amino acid sequence MTCGGC in the amino terminus. A homologous sequence is present in the amino terminus of the Menkes and Wilson’s proteins and has been shown to bind copper in vitro and in vivo (2, 13). Solution structure analysis of a single domain containing this motif from the Menkes protein and structure-function studies on ATX1 and HAH1 reveal a novel linear bicordinate copper ligand capable of binding and rapidly transferring this metal (6, 7, 18) (Fig. 2). Consistent with this, in vitro and in vivo studies have revealed the essential role of these cysteine residues in HAH1 in copper trafficking to the secretory pathway (7). The homologous MXCXXC motifs on the yeast copper transporter CCC2 are the site of interaction with ATX1 and play an essential role in copper transfer between these proteins (18).

FUTURE DIRECTIONS

The last several years have witnessed significant progress in our understanding of the molecular mechanisms of cellular copper transport. As the balance of copper in the body is determined entirely by gastrointestinal absorption and biliary excretion, understanding these mechanisms is of direct relevance to gastrointestinal physiology. Most interestingly, recent data from yeast suggest that the function of a CLC chloride channel homologue is essential for copper transport by CCC2 into a post-Golgi vacuolar compartment (5). These findings suggest that the physiological principles derived for the movement of other cations across biological membranes may also prove relevant for the function of the copper-transporting ATPases and imply that the spectrum of diseases involving abnormal copper homeostasis may extend to abnormalities in associated anion transport proteins. Elucidation of the molecular basis of the genetic disorders of copper transport has thus provided useful direction for the future of investigation in this field.

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