THE COPPER TRANSPORTING ATPASES IN HUMAN DISEASE

Jonathan D. Gitlin

Edward Mallinckrodt Department of Pediatrics
Washington University School of Medicine
St. Louis, Missouri

Copper is an essential transition element which plays a fundamental role in biochemistry, permitting the fascile transfer of electrons in critical metabolic pathways. Menkes disease and Wilson disease are hereditary disorders of copper metabolism which underscore the essential role of copper in human biology. Each disease results from the absence or dysfunction of homologous copper transporting ATPases. The Menkes ATPase transports copper across the placenta, the gastrointestinal tract and the blood brain barrier and thus the clinical features of this X-linked disorder are the result of copper deficiency. The Wilson ATPase functions to transport copper from the hepatocyte secretory into the bile and the clinical features of this disorder are the result of hepatic copper overload. Despite the striking differences in the clinical presentation of these two diseases, the copper transporting ATPases function in precisely the same fashion within the cell. The unique phenotype of each disease is therefore the result of tissue specific expression of each ATPase. Elucidation of the basic defect in these rare disorders provides the opportunity for new approaches in the diagnosis and treatment of affected patients and permits novel insights into the cellular mechanisms of copper homeostasis.

Copper is an essential trace element which plays a fundamental role in the biochemistry of all aerobic organisms. The unique electron structure of copper permits direct interaction with dioxygen, enabling this metal to serve as an essential cofactor in enzymatic reactions. In humans these include electron transport in the respiratory chain, antioxidant defense, neurotransmitter biosynthesis, connective tissue formation and iron metabolism. The unique properties which make copper biologically useful are also potentially highly toxic. For this reason, specific proteins have evolved for the

Address all correspondence to: Jonathan D. Gitlin, M.D., Washington University School of Medicine, Department of Pediatrics, One Childrenís Place, St. Louis, MO 63110; telephone: 314-454-6124; fax: 314-454-4861; email: gitlin@kids.wustl.edu

compartmentalization and trafficking of copper within mammalian cells. Our understanding of the mechanisms of intracellular copper metabolism has increased greatly over the past several years in large part due to the elucidation of the basic genetic defect in the inherited disorders of copper metabolism, Menkes disease and Wilson disease (Culotta and Gitlin, 1999).

Menkes disease is an X-linked recessive disorder which results in hypotonia, growth failure and fatal neurodegenerative disease in early childhood (Schaefer and Gitlin, 1999). Early studies revealed that affected infants were severely copper deficient accounting for the protean manifestations of abnormal hair, absence of pigmentation, laxity of the skin and joints, bony dysplasia and cerebellar degeneration. The Menkes disease gene was identified by physical mapping and cloning in affected female patients with balanced translocations (Vulpe et al., 1993; Chelly et al., 1993; Mercer et al., 1993). This analysis identified a gene encoding a predicted protein sequence highly homologous to a cation transporting P-type ATPase essential for prokaryotic copper homeostasis (Solioz and Vulpe, 1996). The Menkes ATPase transports copper across the placenta, the gastrointestinal tract and the blood brain barrier, accounting for the clinical features of profound copper deficiency in affected individuals. Milder forms of the disease in which the neurologic features are minimal or absent have been described and arise from allelic heterogeneity at the Menkes locus.

Wilson disease is an autosomal recessive disorder resulting in hepatic cirrhosis and neuronal degeneration. Following cloning and characterization of the Menkes disease gene, the Wilson locus was identified and shown to encode an homologous member of this newly described family of copper transporting ATPases (Yamaguchi et al., 1993; Bull et al., 1993; Tanzi et al., 1993). The Wilson ATPase has 55% amino acid identity to the Menkes ATPase and is expressed predominantly within the liver. This protein transports copper into the secretory pathway of hepatocytes for subsequent incorporation into ceruloplasmin and excretion of copper into the bile. As copper homeostasis in humans is maintained entirely by intestinal absorption and biliary excretion, affected individuals develop hepatic copper overload which eventually results in hepatocellular necrosis and dissemination of excess copper to extrahepatic tissues including the limbus of the cornea (Kayser-Fleischer rings) and the basal ganglia of the brain (Schilsky, 1996; Cox, 1996).

Sequence comparison and hydropathy plot analysis of the derived amino acid sequence of the copper transporting ATPases indicates the presence of a polytopic membrane protein predicted to transport copper across biological membranes in an ATP dependent fashion. Homologous proteins have been identified in a wide range of prokaryotic and eucaryotic species and where examined play an analogous role in copper transport (Solioz and Vulpe, 1996). Conserved amino acid motifs in these ATPases include the copper binding MXCXXC motif in the amino terminus as well as the invariant aspartate residue in the DKTGDT motif within the largest cytoplasmic loop. This aspartate residue is reversibly phosphorylated in the process of energy transduction consistent with the known mechanisms of all P-type ATPases. A CPC motif is located in the 6th transmembrane domain which by analogy with similar motifs in other metal transporters is likely essential for mediating copper transfer across biological membranes. A highly conserved histidine within an SEHPL motif also located in the large cytoplasmic loop is conserved throughout all copper transporting ATPases (Petrukhin et al., 1994). This histidine which is essential for Menkes and Wilson ATPase function (vide infra) is the site of the most common mutation (H1069Q) found in up to 40% of patients with Wilson disease (Cox, 1996; Petrukhin et al., 1994).