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Review

Cellular multitasking: The dual role of human Cu-ATPases in cofactor delivery and intracellular copper balance ☆

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ABSTRACT

The human copper-transporting ATPases (Cu-ATPases) are essential for dietary copper uptake, normal development and function of the CNS, and regulation of copper homeostasis in the body. In a cell, Cu-ATPases maintain the intracellular concentration of copper by transporting copper into intracellular exocytic vesicles. In addition, these P-type ATPases mediate delivery of copper to copper-dependent enzymes in the secretory pathway and in specialized cell compartments such as secretory granules or melanosomes. The multiple functions of human Cu-ATPase necessitate complex regulation of these transporters that is mediated through the presence of regulatory domains in their structure, posttranslational modification and intracellular trafficking, as well as interactions with the copper chaperone Atox1 and other regulatory molecules. In this review, we summarize the current information on the function and regulatory mechanisms acting on human Cu-ATPases ATP7A and ATP7B. Brief comparison with the Cu-ATPase orthologs from other species is included.

Diverse P-type ATPases play numerous roles in human cell biology. The ion gradients generated and maintained by these transporters are utilized for nutrient uptake, propagation of electrical signals, and muscle contraction. The subfamily of the P-type ATPases involved in transport of copper have additional and unique functions, including absorption of dietary copper, transfer of copper to CNS, regulation of overall copper status in the body, and many other essential processes [1]. In the past decade, there has been a growing appreciation for the role of copper and copper transporters in human metabolism [2,3]. Copper is a micronutrient required by all organisms to maintain life [4]. It serves as a cofactor for enzymes that catalyze a diverse array of essential biochemical reactions. In mammalian cells, the biosynthesis of neuroendocrine peptides and neurotransmitters, detoxification of radicals, formation of connective tissues and blood vessels, myelination of neurons and many other important physiologic processes rely on proper supply of cells with copper. Cells have developed an intricate network of copper-binding and transporting proteins to maintain copper homeostasis [2]. It is thought that copper enters the cell in a highly reactive Cu(I) form. In this form, it can activate molecular oxygen, oxidize hydrogen peroxide producing free radicals, or disproportionate to Cu(II) and Cu(0). To avoid these wasteful and potentially harmful reactions, Cu(I) is sequestered by a set of specific proteins, which carry and transfer copper to various cell destinations [5]. The work of many research groups over the last decade has lead to a greater understanding of the copper distribution in cells (for recent reviews see [2–4]), however, the entire array of proteins responsible for the uptake, intracellular compartmentalization of copper and copper efflux is yet to be fully characterized.

It is now apparent that the copper-transporting ATPases (Cu-ATPases)[1] are central to the maintenance of copper homeostasis in mammalian cells. Human cells express two homologous Cu-ATPases, ATP7A and ATP7B. Both of these transporters couple ATP hydrolysis to the transport of copper from the cytosol across cellular membranes, thus decreasing cytosolic copper concentration. The transported copper is either released into the bloodstream for further distribution to tissues or it is exported into the bile for eventual removal from the body. In both cases, copper export across the plasma membrane is thought to be a result of exocytosis of vesicles that have been filled with copper as a result of Cu-ATPase transport activity (see Localization and trafficking of human Cu-ATPases for details).

In addition, the Cu-ATPases are essential for transporting copper from the cytosol into the lumen of such intracellular compartments as trans-Golgi network, secretory granules, or melanosomes, where copper is utilized for biosynthetic incorporation into se-

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[1] *Abbreviations used:* Cu-ATPases, copper-transporting ATPases; MNK, Menkes disease; WD, Wilson disease; TMS, transmembrane segments; ATP-BD, ATP-binding domain; TGN, *trans*-Golgi network; CHO, Chinese Hamster ovary; CSD2, copper–zinc superoxide dismutase.

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creted copper-requiring enzymes [6–8]. ATP7A is known to deliver copper to peptydyl-α-monooxygenase [9,10], tyrosinase [7], and lysyl oxidase [9,10], while the primary acceptor of copper released from ATP7B is ceruloplasmin [11,12]. The dual role of Cu-ATPase in cofactor delivery to copper-dependent enzymes and in copper export from a cell requires trafficking of Cu-ATPases between intracellular compartments (see Localization and trafficking of human Cu-ATPases for details).

The essential physiologic roles of human Cu-ATPases are evident from the existence of disease phenotypes in humans caused by inactivation of these transporters. Mutations or deletions in the gene encoding ATP7A are associated with Menkes disease, while inactivation of ATP7B leads to Wilson disease syndrome. Abnormalities in copper metabolism due to defects in Cu-ATPases affect multiple organs with the major impact on the CNS (Menkes disease, MNK) and/or liver (Wilson disease, WD) [13,14]. Most Menkes disease patients display severe neurologic symptoms, mental retardation and other developmental delays that are invariably followed by death in early childhood [15]. These physiologic abnormalities are caused by impaired absorption of dietary copper and disrupted delivery of copper to the copper-dependent proteins in the secretory pathway in other tissues. Normally, ATP7A is expressed in intestine and is required to transport copper across the basolateral membrane of enterocytes into the circulation [16–18]. In Menkes disease patients or in the animal models of Menkes disease, copper export from enterocytes is greatly diminished [19,20]. ATP7A is also highly expressed in the choroid plexus and is critical for the proper supply of copper to the brain [21–23]. Normally, the levels of ATP7A expression peak during axon extension and synapse formation in several neuronal subpopulations [22]. The up-regulation suggests the involvement of ATP7A in synaptogenesis. The specific role of ATP7A in synaptogenesis is currently unknown; the disruption of this important function of ATP7A during development could be the origin of the neurodegeneration seen in Menkes disease [22]. ATP7A is also abundant in vascular endothelial tissue, cerebrovascular endothelial and smooth muscles [24]. Thus, vascular abnormalities, lung vasculature defects, and poor muscle tone are commonly observed in Menkes disease patients. The lack of copper delivery to tyrosinase explains the loss of pigmentation, while the lack of copper delivery to the lysyl oxidase accounts for connective tissue abnormalities.

WD symptoms are caused by the abnormal accumulation of copper. The vast majority of WD patients present with either hepatic or neuro-psychiatric symptoms, reflecting the protein's primary expression in the liver and the brain [25]. The major function of ATP7B in the brain is likely at the basal ganglia, since this and surrounding regions are most affected in WD patients with neurologic symptoms [26]. About 20% of patients with WD have symptoms attributable to the involvement of other organs [25]. The liver is particularly affected by copper accumulation due to disrupted export of excess copper into the bile. In WD, copper delivery to the secretory pathway is also abolished, resulting in the excretion of apo-ceruloplasmin. In humans, the apo-cerupoplasmin is unstable and rapidly degrades when released into the serum [27]. The low levels of ceruloplasmin in a serum along with high hepatic copper content serve as diagnostic markers for WD. The presence of Kayser-Fleischer rings caused by the deposition of copper in the cornea is also a common diagnostic feature in neurologic cases of WD.

In the last decade, significant progress has been made in understanding the biochemistry and cellular regulation of Cu-ATPases [1,28]. The initial discovery of human transporters has been followed by highly successful studies on identification and characterization of their bacterial and plant homologs (for reviews see [29,30]). Recent characterization of thermophilic Cu-ATPases yielded detailed biochemical information about the catalytic activity of Cu-ATPases, the structure of several functional domains [31–35], the conformational transitions [36], and the first low resolution structure of the full-size protein [37]. The human Cu-ATPases are more complex, both in their structure and regulation, compared to their bacterial counterparts. In this review, we will summarize what we know today about the structure, mechanism, and regulation of human Cu-ATPases ATP7A and ATP7B. We will refer to the key studies of Cu-ATPase in other phyla that enhance our understanding of human transporters.

Structural features of human copper-transporting ATPases

Human Cu-ATPases are large polytopic membrane proteins (Fig. 1). Cu-ATPase ATP7A consists of 1500 residues (163,334 kDa; pI 5.94) and is glycosylated, which brings the apparent molecular weight of this protein to 175–180 kDa when it is analyzed on denaturing SDS gels. Human Cu-ATPase ATP7B is slightly smaller, consisting of 1465 residues (157,339 Kda, pI 6.29). ATP7B is not glycosylated and runs with the mobility corresponding to a protein of 165 kDa. At the protein level, ATP7A and ATP7B are 50–60% identical. Both Cu-ATPases are phosphorylated at Ser residue(s) by a yet to be characterized kinase; the extent of a kinase-mediated phosphorylation is regulated by copper ([38,39], see Localization and trafficking of human Cu-ATPases for details).

The membrane portion of ATP7A and ATP7B has eight transmembrane segments (TMS) with the N- and C-termini of the protein both oriented towards the cytosol [40]. The topology of human Cu-ATPases has not been analyzed in detail, however, recent epitope insertion studies using ATP7B indicate that most of the luminal loops connecting TMS are short and the insertion of the HA-epitope in the most predicted loops interferes with the ATP7B folding and/or activity, with the loop connecting the TMS5 and six being the exception [40]. The only data available for ATP7A indicate that the HA-epitope can be incorporated in to the first luminal loop without an apparent negative effect on either activity or trafficking [41].

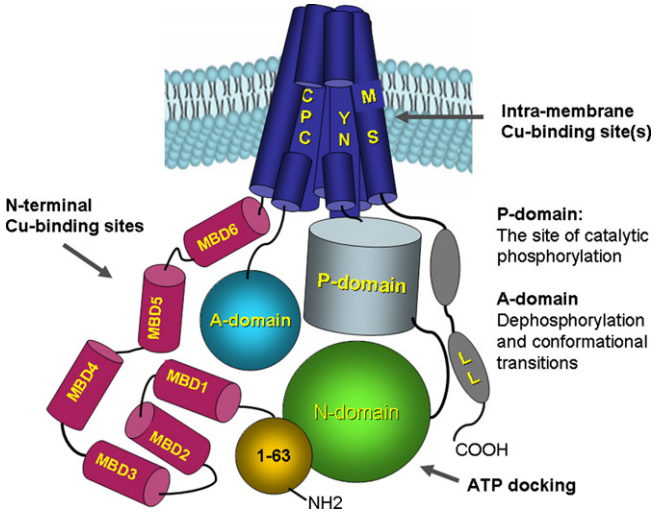

Fig. 1. The major functional domains of human Cu-ATPases. The transmembrane portion of Cu-ATPases is composed of eight transmembrane segments (dark blue) that form intra-membrane copper-binding site(s); the CPC, YN, and MxxS motifs contribute to these sites. The ATP-binding domain is composed of the P-domain (light blue) and the N-domain (green) and together with the A-domain (turquoise) is responsible for enzymatic cycle (ATP-binding, hydrolysis, phosphorylation, and dephosphorylation). The N-terminal domain has six metal (Cu)-binding subdomains (MBD1-6, red) and the very N-terminal 63 residues, that are involved in targeting of ATP7B in polarized hepatocytes. The LL letters indicate the di/tri-leucine motif.