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Mammalian copper-transporting P-Type ATPases, ATP7A and ATP7B: Emerging roles

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Abstract

Copper (Cu) has a role in a diverse and increasing number of pathways, physiological and disease processes. These roles are testament to the fundamental importance of Cu in biology and the need to understand the mechanisms that regulate Cu homeostasis. The mammalian Cu-transporting P-type ATPases ATP7A and ATP7B are two key proteins that regulate the Cu status of the body. They transport Cu across cellular membranes for biosynthetic and protective functions, enabling Cu to fulfill its role as a structural cofactor for many essential enzymes, and to prevent a toxic build-up of Cu inside cells. A variety of regulatory mechanisms operate at transcriptional and post-translational levels to ensure adequate Cu supplies for both physiological and pathophysiological processes. This review summarizes the recent literature that is revealing the emerging roles of the Cu-ATPases in health and disease.

Keywords

copper; copper homeostasis; ATP7A; ATP7B

1. Introduction

ATP7A and ATP7B are members of the P_{1B}-subfamily of the P-type ATPases and catalyse the ATP-dependent translocation of Cu across cellular membranes (Lutsenko et al., 2007). The genes that encode these proteins are mutated in the inherited disorders of Cu metabolism, Menkes (MD) and Wilson diseases (WD), and were identified by positional cloning in 1993 (OMIM 309400; OMIM 277900). MD is a fatal X-linked Cu deficiency syndrome that arises from defective ATP7A-driven transport of Cu from the intestine into the portal circulation, and aberrant distribution of Cu in the body. WD is an autosomal recessive Cu toxicity disorder that results from disruption of ATP7B-mediated Cu export from liver hepatocytes into bile, and consequently liver and brain Cu accumulation. Since their discovery a variety of roles for ATP7A/7B in mammalian physiology have emerged. Due to reference limitations, relevant articles published to 2005 can be found within the select number of recent comprehensive reviews used throughout this report.

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2. Structure

ATP7A is located on Xq13.2-13.3, spans ~150kb and has 23 exons (http://www.ensembl.org/Homo_sapiens/geneview?gene=ENSG00000165240). *ATP7B* is located on chromosome 13, spans ~80kb and contains 21 exons (http://www.ensembl.org/Homo_sapiens/geneview?gene=ENSG00000123191). Both genes produce transcripts of approximately 7.5 to 8.5kb with coding regions that comprise 4.5kb and are translated to produce proteins of 180kDa and 165kDa, respectively. *ATP7A* and *ATP7B* are related in structure and function (~60% amino acid identity). They have eight transmembrane domains that form a path through cell membranes for Cu translocation; and a large N terminus with six metal-binding domains (MBD), each comprising ~70 amino acids and the highly conserved metal binding site GMxCxxC (where x is any amino acid) (Figure 1). Cu-binding together with other N- and C-terminal signals regulate their activity, intracellular location and Cu-induced intracellular trafficking (La Fontaine and Mercer, 2007; Lutsenko et al., 2007).

3. Expression, Activation and Turnover

ATP7A and *ATP7B* expression patterns are somewhat complementary. *ATP7A* is expressed in the majority of tissues except for the liver. *ATP7B* expression is more restricted, with highest expression in the liver (Lutsenko et al., 2007), and increasingly, co-expression with *ATP7A* in many cell types and tissues is becoming evident (see below). The intracellular localization and Cu-regulated trafficking of *ATP7A/7B* reflects their biosynthetic and protective roles in cellular Cu homeostasis. Both proteins normally reside at the trans-Golgi network (TGN) of the cell for metallation of Cu-dependent enzymes of the secretory pathway. With elevated intracellular Cu levels, their steady state location shifts to vesicular compartments in close proximity to the cell periphery, where their primary role is in the excretion of the excess Cu from the cell (La Fontaine and Mercer, 2007).

4. Biological Function

4.1 Catalytic Cu transport

During the catalytic cycle of *ATP7A/7B*, cytosolic Cu is bound to an intramembrane CPC motif. The γ -phosphate of ATP is transferred to the invariant aspartate residue of the conserved DKTG motif, with formation of a transient acylphosphate intermediate (Figure 1). Cu is released on the other side of the membrane with concomitant dephosphorylation of aspartate, and the Cu-ATPase returns to its initial state (Lutsenko et al., 2007). The catalytic cycle of *ATP7A/7B* is coupled to their intracellular trafficking, and these activities endow them with the biosynthetic and homeostatic functions that underlie the diverse range of physiological processes that depend upon their activities.

4.2 Emerging Roles of the Cu-ATPases

The widespread expression of *ATP7A* and the systemic defects caused by its absence in MD have pointed to a house-keeping role for *ATP7A*. In contrast, the more restricted expression of *ATP7B* suggests more specialized functions in regulating Cu physiology, such as biliary excretion of Cu. Many intracellular signaling pathways are activated or influenced by Cu (Veldhuis et al., 2009), and accordingly, emerging data reveals a variety of physiological and pathophysiological processes that require *ATP7A/7B* (Figure 2). Evidence for their regulation at the level of gene transcription, post-translational modifications, and trafficking activity, by physiological stimuli in addition to Cu, such as hormones, hypoxia, and retinoids, indicates that potentially there is a plethora of unappreciated roles for *ATP7A/7B*. Furthermore, analysis of their co-expression in several tissues is providing deeper insights into their distinct but complementary roles in Cu homeostasis, where one may mediate Cu absorption or secretion

to support physiological requirements or protect against Cu overload, while the other may fine-tune intracellular Cu balance. Both pro and anti-cancer activities of the Cu-ATPases also have been identified.

(i) Biosynthetic Role—ATP7A has been identified at the TGN, melanosomes, and secretory granules. At these locations its role is in the metallation of enzymes such as peptidylglycine alpha-amidating monooxygenase, tyrosinase and lysyl oxidase, as well as in mediating Cu secretion into saliva (De et al., 2007; Lutsenko et al., 2007; Setty et al., 2008). Cu delivery to apo-ceruloplasmin is mediated by Atp7b in rat hepatocytes and mouse cerebellum (Lutsenko et al., 2007), and by ATP7A in macrophages in response to hypoxia-mediated increased Cu uptake (White et al., 2009a). ATP7A-mediated Cu delivery to, and activation of extracellular SOD3 is important in blood pressure regulation and endothelial NO signaling through scavenging of extracellular O₂⁻, which is increased during hypertension (Qin et al., 2008).

(ii) Role in development—In the developing zebrafish embryo, Cu and Atp7a are essential for notochord formation that was preferentially preserved during Cu limitation, potentially explaining some of the vascular and neurologic abnormalities seen in MD (Mendelsohn et al., 2006).

In the placenta and mammary gland, ATP7A and ATP7B expression and activity are under hormonal control to regulate Cu delivery to the foetus and neonate, respectively (La Fontaine and Mercer, 2007; Lutsenko et al., 2007). ATP7A, whose expression and distribution responds to insulin and oestrogen, mediates Cu delivery across the basolateral membrane of placental syncytiotrophoblasts to the foetus. ATP7B may serve a protective role during early pregnancy, returning excess Cu across the apical surface back to the maternal circulation. Later in gestation its levels decrease and its relocation away from the apical surface possibly ensures adequate Cu supplies to the foetus (Hardman et al., 2007). In the mammary gland, ATP7A and ATP7B are expressed in luminal epithelial cells and their levels and/or distribution respond to lactational hormones that stimulate an increased flux of Cu through the mammary gland (La Fontaine and Mercer, 2007; Michalczyk et al., 2008). Here ATP7B transports Cu across the apical surface implicating it as the major means of Cu secretion into milk during lactation; whereas the basolateral location of Atp7a in transgenic mice suggests that ATP7A may serve a protective role to export excess Cu from the mammary gland back into the circulation (Llanos et al., 2008).

(iii) Role in the central nervous system (CNS)—ATP7A is widely expressed throughout the CNS and the elevation of Cu in vascular endothelial cells of the brains of MD patients indicates a role for this ATPase in Cu transport across the blood brain barrier. ATP7A is also implicated in axon extension, synaptogenesis, synaptic plasticity and myelination. In rat hippocampal neurons, NMDA receptor activation caused Atp7a to traffic to cytoplasmic vesicles and was associated with the rapid release of Cu for modulation of, and protection against, NMDA receptor-mediated excitotoxicity (Lutsenko et al., 2007; Veldhuis et al., 2009).

ATP7B expression, localization and function in the brain are less well characterized than that of ATP7A. However, both are expressed in mouse cerebellum, where Atp7a may have a homeostatic role maintaining intracellular Cu levels, while Atp7b has a biosynthetic role delivering Cu to enzymes such as ceruloplasmin (Lutsenko et al., 2007). Both Cu-ATPases are also expressed within retinal pigment epithelium (RPE) where they may control Cu transport to the outer retina, as well as Cu delivery to tyrosinase for melanogenesis within the RPE, and to ceruloplasmin and hephaestin to maintain iron homeostasis (Veldhuis et al., 2009).

(iv) Role in the intestine and kidney—Both Cu-ATPases are expressed in the intestine and kidney and appear to have distinct but complementary functional roles. ATP7A is essential for dietary Cu absorption and mediates Cu transfer across the basolateral membrane of intestinal enterocytes into the portal circulation (Nyasae et al., 2007). ATP7B may fine-tune intestinal Cu absorption, either via Cu excretion from the apical surface of enterocytes, and/or through vesicular sequestration of excess Cu in enterocytes that are regularly lost by shedding (Weiss et al., 2008). In the kidney, ATP7A/7B are co-expressed in proximal and distal epithelial cells where ATP7A mediates Cu transport across the basolateral membrane for reabsorption into the blood and protection against Cu overload. Hence, ATP7A has a house-keeping role maintaining renal Cu homeostasis. ATP7B, which did not redistribute in response to Cu, more likely has a role in fine-tuning intracellular Cu balance through Cu storage in intracellular compartments (Linz et al., 2008; Barnes et al., 2009).

(v) Role in pathophysiological processes—Cu plays a key role in angiogenesis, neovascularization and cancer metastasis (Veldhuis et al., 2009), and ATP7A involvement is mediated through regulation of vascular endothelial growth factor receptor (VEGFR), and through phorbol-12-myristate-13-acetate (PMA)-mediated up-regulation of ATP7A expression with associated increase in Cu efflux (Afton et al., 2009). Hypoxia, which is associated with pathophysiological conditions including injury and solid tumours, stimulated an increased flux of Cu in macrophages with associated increases in ATP7A levels and activity. This together with similar effects seen in tumour-associated macrophages *in vivo*, and following stimulation with pro-inflammatory agents, further implicates ATP7A in promoting, angiogenesis, tumour growth and inflammation, as well as the bactericidal activity of macrophages (White et al., 2009a; White et al., 2009b).

ATP7A and ATP7B mediate resistance to anticancer drugs such as cis-diaminedichloroplatinum (II) (cisplatin), by sequestration within intracellular compartments and potentially within the MBDs (Furukawa et al., 2008; Mangala et al., 2009). Correlations were evident between increased ATP7A/7B expression in a variety of clinical cancers and poorer survival rates following cisplatin-based chemotherapy, and between ATP7A upregulation and cancer metastasis (Furukawa et al., 2008).

ATP7A also confers a therapeutic effect in cancer, demonstrated by its coordinate upregulation with retinoic acid receptor $\beta 2$ (RAR $\beta 2$) in neuroblastoma cells that was associated with reduced Cu accumulation, enhanced efflux and a growth inhibitory effect (Bohlken et al., 2009). Hence malignant cells requiring higher intracellular Cu for viability and proliferation, are depleted of Cu by retinoid/RAR $\beta 2$ -induced ATP7A upregulation, implicating ATP7A in the anti-proliferative and therapeutic effect of retinoids (Bohlken et al., 2009).

5. Cu-ATPases as therapeutic targets?

Cu is involved in a complex network of signaling pathways that regulate a myriad of physiological processes such as development, neurological processes, and angiogenesis, as well as pathophysiological processes including tumour growth, cancer resistance, and oxidative stress that contributes to inflammation, cardiovascular and neurodegenerative diseases. As key Cu regulators, the number of ATP7A/7B-dependent processes so far identified accounts for the severity of the diseases that result from their absence or malfunction. Their ability to transport Cu within and out of cells underlies all of these critical roles. These essential functions make them unlikely to represent effective therapeutic targets. However, silencing of ATP7B reversed the cisplatin resistance of ovarian cancer cells and showed anti-tumour efficacy in nude mice bearing ovarian tumours (Mangala et al., 2009). The more widespread effects of ATP7B silencing in this model remain to be established. Understanding and

elucidating the full complement of pathways affected by Cu and those that regulate Cu-ATPase function may lead to the further identification of new therapeutic targets.

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Figure 1.

Schematic diagram of the structure of the Cu-ATPases, ATP7A and ATP7B. The eight transmembrane domains that form the channel for Cu translocation are shown in blue. The N and C termini on the cytoplasmic side of the membrane are indicated, as are the conserved Cu-binding motifs (CxxC) of the six metal binding domains (MBD) within the N terminus. The intramembrane Cys-Pro-Cys motif that is required for Cu translocation through the membrane is also shown. The key conserved sequences of the N-domain (containing the ATP binding site), the P-domain (containing the conserved aspartic acid residue) and the A-domain (comprising the phosphatase domain) (Lutsenko et al., 2007) are shown.

Figure 2.

Emerging roles of the mammalian Cu-ATPases. Schematic representation of the physiological and pathophysiological processes, so far known to depend upon the activities of ATP7A and ATP7B. Ovals without shading represent pathophysiological processes in which the activity of the Cu-ATPases may be beneficial or protective, whereas the dark grey shaded ovals represent disease processes that are exacerbated by Cu-ATPase activity.