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Archives of Biochemistry and Biophysics 463 (2007) 134–148
ABB
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Minireview

Biochemical basis of regulation of human copper-transporting ATPases ☆

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Received 19 February 2007, and in revised form 10 April 2007
Available online 2 May 2007

Abstract

Copper is essential for cell metabolism as a cofactor of key metabolic enzymes. The biosynthetic incorporation of copper into secreted and plasma membrane-bound proteins requires activity of the copper-transporting ATPases (Cu-ATPases) ATP7A and ATP7B. The Cu-ATPases also export excess copper from the cell and thus critically contribute to the homeostatic control of copper. The trafficking of Cu-ATPases from the *trans*-Golgi network to endocytic vesicles in response to various signals allows for the balance between the biosynthetic and copper exporting functions of these transporters. Although significant progress has been made towards understanding the biochemical characteristics of human Cu-ATPase, the mechanisms that control their function and intracellular localization remain poorly understood. In this review, we summarize current information on structural features and functional properties of ATP7A and ATP7B. We also describe sequence motifs unique for each Cu-ATPase and speculate about their role in regulating ATP7A and ATP7B activity and trafficking.

Keywords: ATP7A; ATP7B; Copper; P-Type ATPase; Atox1

Introduction

The human Cu-ATPases ATP7A and ATP7B are essential for intracellular copper homeostasis. The Cu-ATPases use the energy of ATP hydrolysis to transport copper from the cytosol into the secretory pathway and thus supply the metal for subsequent biosynthetic incorporation into various copper-dependent enzymes. ATP7A is required for formation of functional tyrosinase [1], peptidyl-α-monooxygenase [2], lysyl oxidase [3], and possibly some other enzymes [4], while ATP7B is essential for the biosynthesis of holo-ceruloplasmin, a copper-dependent ferroxidase [5]. In addition to their biosynthetic role, human Cu-ATPases participate in the export of excess copper from the cells. Over-expression of ATP7A in transgenic animals is associated with a decrease of copper content in tissues, which is particularly apparent in the heart and the brain [6]. The essential role of ATP7A in copper export from intestinal epithelium is best illustrated by the phenotype of Menkes disease. In this lethal human disorder, the functional ATP7A is lost due to various mutations in the corresponding gene, resulting in greatly impaired export of copper from the enterocytes [7–9].

In hepatocytes, a copper exporting role belongs to another copper-transporting ATPase, ATP7B [10]. Liver is the major organ of copper homeostasis in the body and is involved in removal of excess copper [11]. Copper is exported from the liver into the bile and then to the feces in a process that requires the activity of ATP7B. Genetic inactivation of ATP7B results in accumulation of copper in the liver and a severe human disorder, Wilson disease. The disease is characterized by a spectrum of liver pathologies ranging from hepatitis and cirrhosis to liver failure [12]. In both Menkes disease and Wilson disease, the lack

☆ This work has been supported by the National Institute of Health Grants R01 DK071865 to SL; ESL is supported by the NIH training Grant 5-T32-HL007781.
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doi:10.1016/j.abb.2007.04.013

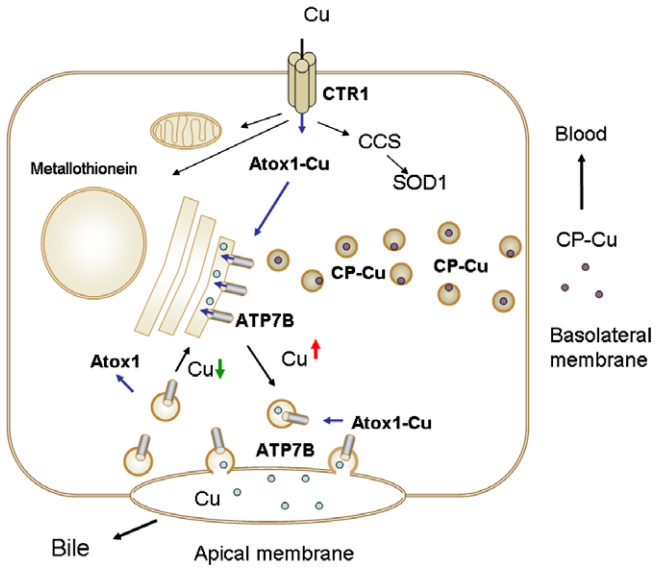

Fig. 1. The dual role of copper-transporting ATPase ATP7B in hepatocyte. Copper enters the cell from the basolateral membrane via high-affinity copper transporter Ctr1 and is delivered to various cell targets with the help of copper chaperones. Atox1 transfers copper to ATP7B located in the TGN. ATP7B transports copper into the lumen of TGN, where copper is incorporated into ceruloplasmin (CP), which is subsequently excreted into the blood. When copper is elevated (red arrow), ATP7B traffics to vesicles. Vesicles filled with copper fuse with the apical (canalicular) membrane, copper is exported, and ATP7B is rapidly endocytosed. When copper is decreased ATP7B returns back to the TGN. It is possible that Atox1 regulates both copper delivery to ATP7B when copper is high and copper removal from ATP7B when copper is low.

of functional Cu-ATPase is also associated with the disrupted delivery of copper to the secretory pathway. The lack of copper incorporation into ceruloplasmin in Wilson disease is utilized as a biochemical marker for diagnosing the disease. In Menkes disease, the deficiency of active copper-dependent enzymes, for example lysyl oxidase, greatly contributes to the severity of the disease phenotype [13].

Two functions of human copper-transporting ATPases can be described as biosynthetic (the delivery of copper to the secretory pathway for metallation of cuproenzymes) and homeostatic (the export of excess copper from the cell). These two functions are associated with the distinct intracellular targeting of the transporters (Fig. 1). The localization in the *trans*-Golgi network (TGN)[1], which is observed for both ATP7A and ATP7B under low copper conditions, reflects their role in the delivery of copper to copper-dependent enzymes. Such enzymes as tyrosinase, peptidyl-α-monooxygenase, and ceruloplasmin have been shown to co-localize with Cu-ATPases in the TGN and require the ATPase-mediated copper transport for formation of holo-enzyme [1].

It is not known whether the metallation of cuproenzymes occurs only in the TGN or if small quantities of Cu-ATPases are also present along the secretory pathway for re-metallation of secreted enzymes, if the latter looses copper. Such a scenario is possible in the case of ATP7A, since this Cu-ATPase (unlike ATP7B) can migrate towards the basolateral membrane in the same direction as secreted proteins (see below). In fact, in the rat parotid acinar cell ATP7A is found not only in the TGN (predominant localization), but also in immature and mature secretory granules [14], where it may participate in copper delivery to peptidyl-α-monooxygenase and/or other copper-binding proteins.

The second function of Cu-ATPases—the export of copper from the cell for further utilization in the blood, milk, or for removal into the bile—requires trafficking of Cu-ATPases from the TGN to vesicles (Fig. 1). This re-localization occurs in response to copper elevation, hormone release, or other signaling and developmental events [15–18]. It is thought that in response to these signals the Cu-ATPases sequester copper into the vesicles. The vesicles then fuse with the membrane releasing copper into the extracellular milieu [18–20]. Therefore, the regulation of intracellular localization of Cu-ATPases represents the key mechanism that determines whether the Cu-ATPases perform their homeostatic or biosynthetic function at a given moment.

Another level of regulation of copper transport must exist in cells where both Cu-ATPases are simultaneously co-expressed. While certain cells have only one Cu-ATPase (for example, ATP7B in hepatocytes), a number of cells and tissues (such as brain, mammary gland, and placenta) express both ATP7A and ATP7B. In this latter case, it is not known whether a preference exists in the distribution of copper between ATP7A and ATP7B and whether or not the same mechanisms regulate the Cu-ATPases function. Recent data from several laboratories suggest that two human Cu-ATPases differ in their enzymatic characteristics, trafficking properties, interacting partners, and regulation (see below for details). It is also clear that unique sequence elements are present in the structure of two human copper pumps that may contribute to their distinct properties. In this review, we summarize what is currently known about structure, function, and regulation of ATP7A and ATP7B, and speculate about possible contribution of unique sequence elements in the Cu-ATPase to their activity and regulation.

ATP7A and ATP7B are representatives of the P_{1B}-family of ion-transporting ATPases

At the biochemical level, the function of Cu-ATPases is to translocate copper across the membrane from the cytosol into the lumen of appropriate intracellular compartment (either TGN or vesicles). The vectorial copper translocation across the membranes is driven by the hydrolysis of ATP; the number of copper ions transported per

[1] *Abbreviations used:* TGN, *trans*-Golgi network; TMS, transmembrane segments; BCS, bathocuproine disulphonate; MBDs, metal-binding domains; N-domain, nucleotide-binding domain; P-domain, phosphorylation domain; CP, ceruloplasmin.