Copper-induced trafficking of the Cu-ATPases: A key mechanism for copper homeostasis

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Abstract

The Menkes protein (MNK) and Wilson protein (WND) are transmembrane, CPX-type Cu-ATPases with six metal binding sites (MBSs) in the N-terminal region containing the motif GMXCXXC. In cells cultured in low copper concentration MNK and WND localize to the trans Golgi network but in high copper relocalize either to the plasma membrane (MNK) or a vesicular compartment (WND). In this paper we investigate the role of the MBSs in Cu-transport and trafficking. The copper transport activity of MBS mutants of MNK was determined by their ability to complement a strain of Saccharomyces cerevisiae deficient in CCC2 (Δccc2), the yeast MNK/WND homologue. Mutants (CXXC to SXXS) of MBS1, MBS6, and MBSs1-3 were able to complement Δccc2 while mutants of MBS4-6, MBS5-6 and all six MBS inactivated the protein. Each of the inactive mutants also failed to display Cu-induced trafficking suggesting a correlation between trafficking and transport activity. A similar correlation was found with mutants of MNK in which various MBSs were deleted, but two constructs with deletion of MBS5-6 were unable to traffic despite retaining 25% of copper transport activity. Chimeras in which the N-terminal MBSs of MNK were replaced with the corresponding MBSs of WND were used to investigate the region of the molecules that is responsible for the difference in Cu-trafficking of MNK and WND. The chimera which included the complete WND N-terminus localized to a vesicular compartment, similar to WND in elevated copper. Deletions of various MBSs of the WND N-terminus in the chimera indicate that a targeting signal in the region of MBS6 directs either WND/MNK or WND to a vesicular compartment of the cell.

Introduction

Copper homeostasis is the process by which organisms maintain adequate supplies of copper to essential cuproenzymes, and excrete or sequester excess amounts of this potentially toxic element. At a systemic level in mammals, copper supplies are maintained by a balance between rate of uptake of dietary copper in the small intestine and the rate of excretion of copper in the bile. The amount of copper absorbed from food has been found to depend on the copper content of the diet: when the dietary copper intake is very low, a high percentage is absorbed while the amount absorbed is reduced as the copper content of the diet increases (Turnlund et al. 1998). The liver is the central organ in copper homeostasis and rapidly removes most of the absorbed copper from the blood. The regulation of copper status in the hepatocyte is achieved by secretion of copper as ceruloplasmin or by excretion of excess copper in the bile. If the amount of copper in the hepatocyte is excessive, copper is excreted in the bile which is the main regulatory step in maintaining overall copper homeostasis (Linder 1991). The systemic processes involved in copper transport has been well summarized in a number of reviews (Linder 1991; Danks 1995; Linder et al. 1999). More recently, reviews incorporating the molecular advances have appeared (Vulpe & Packman 1995; Pena et al. 1999; Harris 2000).
The effects of disruption of these homeostatic mechanisms are illustrated by the genetic disorders of copper homeostasis, Menkes and Wilson diseases (Danks 1995). Menkes disease is a genetic copper deficiency, which is due to defective uptake and distribution of copper, and Wilson disease is a copper toxicosis condition, due to defective biliary excretion of copper. Identification of the gene affected in Menkes disease was reported in 1993 (Chelly et al. 1993; Mercer et al. 1993; Vulpe et al. 1993), and subsequently led to the isolation of the gene affected in Wilson disease, as the two genes encode closely related copper ATPases with approximately 67% amino acid identity (Bull et al. 1993; Tanzi et al. 1993; Yamaguchi et al. 1993). The Menkes protein is referred to as MNK or ATP7A and the Wilson protein is WND or ATP7B. Both proteins are members of the P-type ATPase family of cation transporters, which include transmembrane domains that form a channel through a cell membrane, an ATP binding site and an invariant aspartic acid that is reversibly phosphorylated during the reaction cycle (Kuhlbrandt et al. 1998). MNK and WND are the first heavy metal transporting ATPases identified in mammals. Heavy metal P-type ATPases have additional features that adapt to the transport of heavy metals: they have metal binding sites (MBSs) with a canonical sequence Cys XX Cys, where X is a variable amino acid in the N-terminal region of the protein, and a CysProCys motif in the channel. The cysteines are thought to bind the metal as it traverses the membrane. MNK and WND have the unusual feature of six CXXC motifs in the N-terminal region, which contrasts with the one or two MBSs found in the heavy metal ATPases of the bacteria and the Cu-ATPase in yeast. Figure 1 shows a diagrammatic representation of the structural features of these proteins. The function of the six metal binding sites has been of some interest, and in particular, whether they are involved in regulation of the copper transport activity of these proteins.

Despite the marked difference in clinical phenotypes of Menkes and Wilson diseases, both MNK and WND have similar copper efflux roles in the cell. The different clinical phenotypes resulting from mutation of these genes can be explained by their distinct pattern of expression, as MNK is expressed in most tissues except in the liver, and WND is expressed mainly in the liver. Both proteins deliver copper into the secretory pathway for incorporation of copper into secreted cuproenzymes such as lysyl oxidase (MNK) and ceruloplasmin (WND). In addition, they are the key cellular copper efflux molecules. Efflux of copper from cells forming various epithelial barriers such as the intestinal enterocytes, and the blood brain barrier is an important role for MNK, and efflux of copper from hepatocytes into the biliary canaliculae is a pivotal role for WND. Thus these two molecules play a central role in physiological copper homeostasis.

Both MNK and WND exhibit the phenomenon of Cu-induced trafficking, a process by which the intracellular location of the proteins is influenced by the concentration of copper in the cytoplasm. In cultured cells exposed to normal copper concentrations both proteins are located in the transGolgi network, consistent with their role in supplying copper to secreted cuproenzymes. When the cells are exposed to high copper, MNK and WND relocalize to either the plasma membrane (MNK) (Petris et al. 1996) or a vesicular compartment (WND) (Hung et al. 1997). In polarized hepatocytes, WND traffics first to the vesicular compartment and then to the apical surface of the cell (Roelofsen et al. 2000). It is likely that copper-induced trafficking underlies the regulation of copper absorption and excretion, and is a key mechanism in the physiological regulation of copper. Thus there has been considerable interest in the mechanism by which copper causes the relocation of these proteins. Our previous work demonstrated that only one MBS, number five or six in MNK was necessary for Cu-induced trafficking (Strausak et al. 1999), if both MBS5-6 are mutated, no Cu-induced trafficking occurs. Goodyer et al. reported, however, that any one of the metal binding sites was sufficient for trafficking (Goodyer et al. 1999). The copper transport activity of the mutant proteins was not determined. Studies with WND have demonstrated that MBS6 alone is sufficient for copper transport activity, but the trafficking activity of the proteins was not determined (Forbes et al. 1999). Other studies have shown that trafficking can be prevented by mutations in other regions of the proteins. A missense mutation causing mild Menkes disease results in a protein that remains in the transGolgi network even in elevated copper (Ambrosini & Mercer 1999) and the mutant MNK found in the mouse homologue of Menkes disease, the brindled mouse, also does not traffic (La Fontaine et al. 1999).

Similar results for WND were reported by Forbes et al. who found that a number of mutations blocked trafficking of WND (Forbes & Cox 2000) and in addition these mutations also reduced or abolished the copper transport activity of the protein (Forbes & Cox 1998). They considered that these mutations