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Visions & Reflections (Minireview)

Menkes disease

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Abstract. Menkes disease is caused by mutations in the copper-transporting P_{1B}-type ATPase ATP7A. ATP7A has a dual function: it serves to incorporate copper into copper-dependent enzymes, and it maintains intracellular copper levels by removing excess copper from the cytosol. To accomplish both functions, the protein traffics between different cellular locations depending on copper levels. The mechanism for sensing the concentration of copper, for trafficking, as well as the details of the mechanism of copper translocation across the membrane are unknown.

Keywords. Menkes disease, ATP7A, copper trafficking and translocation, mutation.

Menkes disease (MD) is a fatal X-linked human disorder that is caused by various mutations in the gene encoding ATP7A, a copper-transporting P_{1B}-type ATPase [1, 2]. Estimates of the incidence of MD range from 1 in 100 000 live births to 1 in 250 000 [3]. The loss of ATP7A activity results in a phenotype characterized by copper deficiency, as a consequence of impaired export of copper from enterocytes. The disease-causing mutations identified so far include nonsense and missense mutations as well as small insertions and small and gross deletions [4, 5] (see www.hgmd.org). A closely related disease is Wilson disease (WD), which is caused by mutations in a protein highly similar in sequence to ATP7A, namely ATP7B [6, 7].

ATP7A (often called the Menkes protein, MNK) uses the energy of ATP hydrolysis to transport copper(I) from the cytosol either into the secretory pathway or into vesicles [1, 2, 8]. In the former case, the copper(I) ions are subsequently incorporated into a variety of copper-dependent enzymes, such as lysyl oxidase or tyrosinase. In the latter case, the copper(I) ions pumped into the vesicles are released into the extracellular environment after fusion of the vesicles themselves with the membrane. In this way, the copper ions ingested with the diet can cross the basolateral membrane of enterocytes and be further used in blood and other tissues [9]. In hepatocytes, ATP7B, through a similar mechanism, either assists the incorporation of copper into ceruloplasmin or loads copper in bile caniculi from the liver, thus leading to removal of the metal from the organism [9]. In individuals affected by MD, copper cannot leave the enterocytes therefore leading to copper deficiency. Many symptoms of the disease can be ascribed to the lack of activity of copper-dependent enzymes [10]. The proper balance between the two roles described above is ensured by the control of the intracellular localization of ATP7A, which depends on intracellular copper(I) concentration [11]. In fact, the biosynthetic activity requires ATP7A to be localized in the trans-Golgi network (TGN), whereas the homeostatic activity requires ATP7A to traffic from the TGN to vesicles (and partly

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also to the plasma membrane). Depending on the localization of the protein, the same catalytic cycle leads to copper finally being incorporated into the appropriate enzymes or removed from the cell. Unsurprisingly, the amount of copper ingested with the diet affects the levels of ATP7A in the intestine [12].

Human ATP7A is a monomeric protein of 1500 amino acids. It can be divided in several large portions: a 630-amino acid N-terminal cytosolic domain, a transmembrane portion composed of eight transmembrane helices, a so-called A domain, which is cytosolic and probably has a regulatory function, the ATP-binding domain, and the C-terminal soluble region [13]. The N-terminal cytosolic domain can be further divided into six individually folded metal-binding domains, whereas the ATP-binding domain can be sub-divided in two domains, the nucleotide-binding domain (N-domain) and the phosphorylation domain (P-domain). A small cytosolic soluble protein, called HAH1 (also called Atox1), is the best characterized physiological partner of ATP7A. HAH1 is able to deliver copper(I) ion(s) to ATP7A for subsequent translocation [14]. HAH1 can bind a single copper(I) ion per protein molecule, whereas ATP7A can in principle bind as many as six metal ions with its N-terminal domain. Further metal-binding sites are located in the transmembrane region and possibly in some other parts of the protein, *e.g.*, the luminal loops in between transmembrane helices [15]. The number of copper(I) ions transported across the membrane upon hydrolysis of one ATP molecule is unknown.

The mutations giving rise to MD can abrogate ATP7A function or can affect protein localization, or both [16]. Clearly, a non-functional ATP7A would lead to disease because of its inability to transport copper from the cytosol. This can be the result of polypeptide synthesis being terminated too early due to a nonsense mutation, so that the protein chain cannot fold properly. Alternatively, it can be the result of mutations that, for example, prevent binding of ATP, so that the enzyme cannot carry out its catalytic cycle. The role in MD of mutations that affect protein localization is more difficult to analyze, mainly because the mechanism underlying ATP7A trafficking is not understood. Trafficking is a process that is triggered by a rise in copper levels in the environment (and thus, presumably, intracellularly) [11]. Indeed, experimental studies by confocal microscopy aimed at the observation of ATP7A relocalization events *in vivo* exploit media supplemented with or deprived of copper to stimulate trafficking from or to the TGN, respectively. It has been shown that trafficking depends on the presence of the copper(I)-binding sites in the cytosolic N-terminal region as well as in the transmembrane region, on the integrity of the phosphorylation site (D1044 in the invariant DKTG signature) [17, 18] and also on the expression of HAH1 [19].

The above findings indicate that the long N-terminal tail of ATP7A is quite important for protein trafficking, both through copper binding and through the interaction with HAH1. Most likely, this role involves interactions of the tail with other domains of ATP7A. Notably, a variant of ATP7A lacking four out of the six metal-binding domains in the N-terminal region gives rise to a mild MD phenotype, although it is able to transport copper and to traffic [20]. However, it appears to localize at the plasma membrane under conditions where wild-type ATP7A would still be localized at the TGN. *In vitro* studies have shown that ATP7A has preferential binding sites for copper(I)-HAH1 within the first four metal-binding domains, potentially leading to the formation of metal-bridged ATP7A:HAH1 adducts at high intracellular copper(I) concentration [21, 22]. The interaction of these adducts with other ATP7A domains can contribute to defining the threshold for copper(I) concentration triggering protein trafficking. The only missense mutation in the N terminus leading to MD, where Ala629 is replaced by Pro, can also be examined within the above frame. A629P could exert its effect chiefly by altering the domain-domain interactions involving the N-terminal tail. In fact, this mutation minimally alters the structure and copper(I)-binding affinity of the metal-binding domain containing the mutation [23], and has a copper-transporting activity that sustains growth of a *ccc2*Δ yeast strain as much as 47 % of the wild-type enzyme [24].

A truly thorough understanding of the catalytic mechanism of ATP7A needs a characterization of the transmembrane binding site and of its affinity for copper(I) *versus* that of the cytosolic metal-binding domains also as a function of the phosphorylation state of the enzyme. The six cytosolic metal-binding domains have similar affinities for copper(I), which are larger than that of HAH1 by less than one order of magnitude [25, 26]. Therefore, a relatively shallow thermodynamic gradient exists for metal transfer from HAH1 to ATP7A. Whether the transmembrane site has a significantly higher affinity for the metal than the cytosolic sites in at least the phosphorylated state, as it is postulated on the basis of the catalytic mechanism of the calcium ATPase SERCA1, is still a completely open question. Indeed, SERCA does not possess any domain that can resemble the N-terminal tail of ATP7A, and thus a direct comparison is actually not possible.

Finally, the major challenge, once the catalytic mechanism and trafficking mechanism of ATP7A are

understood, will be to disentangle how ATP7A and ATP7B carry out their specific roles in the tissues where both proteins are expressed (brain, placenta, …), notwithstanding the many similarities in their molecular features! Copper transport in the human body is an extremely complex subject that is still poorly understood, as are the many intriguing subtleties of the mechanisms of action of ATP7A (and ATP7B).

1 Chelly, J., Tumer, Z., Tonnesen, T., Petterson, A., Ishikawa-Brush, Y., Tommerup, N., Monaco, A. P. and Horn, N. (1993) Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein. Nat. Genet. 3, 14–19.
2 Mercer, J. F., Livingston, J., Hall, B., Paynter, J. A., Begy, C., Chandrasekharappa, S., Lockhart, P., Grimes, A., Bhave, M. and Siemieniak, D. (1993) Isolation of a partial candidate gene for Menkes disease by positional cloning. Nat. Genet. 3, 20–25.
3 Kaler, S. G. (1994) Menkes disease. Adv. Pediatr. 41, 263–304.
4 Moller, L. B., Bukrinsky, J. T., Molgaard, A., Paulsen, M., Lund, C., Tumer, Z., Larsen, S. and Horn, N. (2005) Identification and analysis of 21 novel disease-causing amino acid substitutions in the conserved part of ATP7A. Hum. Mutat. 26, 84–93.
5 Tumer, Z., Birk Moller, L. and Horn, N. (2003) Screening of 383 unrelated patients affected with Menkes disease and finding of 57 gross deletions in ATP7A. Hum. Mutat. 22, 457–464.
6 Bull, P. C., Thomas, G. R., Rommens, J. M., Forbes, J. R. and Cox, D. W. (1993) The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat. Genet. 5, 327–337.
7 Tanzi, R. E., Petrukhin, K., Chernov, I., Pellequer, J. L., Wasco, W., Ross, B., Romano, D. M., Parano, E., Pavone, L., Brzustowicz, L. M., Devoto, M., Peppercorn, J. et al. (1993) The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. Nat. Genet. 5, 344–350.
8 Vulpe, C. D., Levinson, B., Whitney, S., Packman, S. and Gitschier, J. (1993) Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. Nat. Genet. 3, 7–13.
9 Linder, M. C. (1991) Biochemistry of Copper, pp. 1–13, Plenum Press, New York.
10 Menkes, J. H. (1988) Kinky hair disease: twenty five years later. Brain Dev. 10, 77–79.
11 Petris, M. J., Mercer, J. F., Culvenor, J. G., Lockhart, P. and Camakaris, J. (1996) Ligand-regulated transport of the Menkes copper P-type ATPase efflux pump from the Golgi apparatus to the plasma membrane: A novel mechanism of regulated trafficking. EMBO J. 15, 6084–6095.
12 Bauerly, K. A., Kelleher, S. L. and Lonnerdal, B. (2005) Effects of copper supplementation on copper absorption, tissue distribution, and copper transporter expression in an infant rat model. Am. J. Physiol Gastrointest. Liver Physiol. 288, G1007-G1014.
13 Lutsenko, S. and Kaplan, J. H. (1995) Organization of P-type ATPases: Significance of structural diversity. Biochemistry 34, 15607–15613.
14 Klomp, L. W., Lin, S. J., Yuan, D., Klausner, R. D., Culotta, V. C. and Gitlin, J. D. (1997) Identification and functional expression of HAH1, a novel human gene involved in copper homeostasis. J. Biol. Chem. 272, 9221–9226.
15 Lutsenko, S., LeShane, E. S. and Shinde, U. (2007) Biochemical basis of regulation of human copper-transporting ATPases. Arch. Biochem. Biophys. 463, 134–148.
16 Kim, B. E., Smith, K. and Petris, M. J. (2003) A copper treatable Menkes disease mutation associated with defective trafficking of a functional Menkes copper ATPase. J. Med. Genet. 40, 290–295.
17 Cater, M. A., La Fontaine, S. and Mercer, J. F. (2007) Copper binding to the N-terminal metal binding sites or the CPC motif is not essential for copper-induced trafficking of the human Wilson protein (ATP7B). Biochem. J. 401, 143–153.
18 Mercer, J. F., Barnes, N., Stevenson, J., Strausak, D. and Llanos, R. M. (2003) Copper-induced trafficking of the cU-ATPases: A key mechanism for copper homeostasis. Biometals 16, 175–184.
19 Hamza, I., Prohaska, J. and Gitlin, J. D. (2003) Essential role for Atox1 in the copper-mediated intracellular trafficking of the Menkes ATPase. Proc. Natl. Acad. Sci. USA 100, 1215–1220.
20 Paulsen, M., Lund, C., Akram, Z., Winther, J. R., Horn, N. and Moller, L. B. (2006) Evidence that translation reinitiation leads to a partially functional Menkes protein containing two copper-binding sites. Am. J. Hum. Genet. 79, 214–229.
21 Banci, L., Bertini, I., Cantini, F., Chasapis, C., Hadjiliadis, N. and Rosato, A. (2005) A NMR study of the interaction of a three-domain construct of ATP7A with copper(I) and copper(I)-HAH1: The interplay of domains. J. Biol. Chem. 280, 38259–38263.
22 Banci, L., Bertini, I., Cantini, F., Della Malva, N., Migliardi, M. and Rosato, A. (2007) The different intermolecular interactions of the soluble copper-binding domains of the Menkes protein, ATP7A. J. Biol. Chem. 282, 23140–23146.
23 Banci, L., Bertini, I., Cantini, F., Migliardi, M., Rosato, A. and Wang, S. (2005) An atomic level investigation of the disease-causing A629P mutant of the Menkes protein ATP7A. J. Mol. Biol. 352, 409–417.
24 Payne, A. S. and Gitlin, J. D. (1998) Functional expression of the Menkes disease protein reveals common biochemical mechanisms among the copper-transporting P-type ATPase. J. Biol. Chem. 273, 3765–3770.
25 Banci, L., Bertini, I., Chasapis, C., Ciofi-Baffoni, S., Hadjiliadis, N. and Rosato, A. (2005) An NMR study of the interaction between the human copper(I) chaperone and the second and fifth metal-binding domains of the Menkes protein. FEBS J. 272, 865–871.
26 Yatsunyk, L. A. and Rosenzweig, A. C. (2007) Copper(I) binding and transfer by the N-terminus of the Wilson disease protein. J. Biol. Chem. 282, 8622–8631.