COMMENTARY

Differential regulation of the Menkes and Wilson disease copper transporters by hormones: an integrated model of metal transport in the placenta

Des R. RICHARDSON¹ and Yohan SURYO RAHMANTO

Iron Metabolism and Chelation Program, Department of Pathology, Blackburn Building (D06), University of Sydney, Sydney, New South Wales, 2006 Australia

Copper (Cu) plays a critical role in the developing foetus, but virtually nothing is known concerning the regulation of its uptake and metabolism in the placenta. In this issue of the *Biochemical Journal*, Hardman and colleagues, using a model of placental trophoblasts in culture, identify differential hormonal regulation of two copper-transporting ATPases; namely, those responsible for Menkes disease (ATP7A; MNK) and Wilson disease (ATP7B; WND). Insulin and oestrogen, which are essential during gestation, up-regulate MNK and this leads to trafficking of the MNK protein from the Golgi to the basolateral membrane, resulting in increased Cu efflux. At the same time, insulin decreased WND levels, and this leads to intracellular sequestration of the protein to

a perinuclear region that reduces apical Cu release. As such, this results in a concerted flux of Cu from the basolateral surface of the trophoblast that would potentially be used by the developing foetus. An integrated model of vectorized Cu transport is proposed, which involves co-ordinated expression of transporters, organelle interactions and probable protein–protein interactions. The findings have wider implications for considering general models of intracellular metal transport.

Key words: copper transport, Menkes disease, metal trafficking, metal transport, Wilson disease.

Copper (Cu) plays vital roles in many metabolic processes, such as angiogenesis, respiration and the immune response [1]. This is due to its crucial role in the active sites of enzymes such as cytochrome *c* oxidase, lysyl oxidase, ceruloplasmin, tyrosinase and superoxide dismutase. Considering the importance of these functions, Cu homeostasis is tightly regulated. Once absorbed from the gut, Cu is transported in the serum, mainly co-ordinated to ceruloplasmin and bound to albumin and amino acids [1].

Uptake of Cu into cells is achieved by a closely integrated network of proteins and chaperones [1,2]. Two groups of membrane proteins are vital for maintaining mammalian Cu homeostasis. The first family is involved in the initial uptake of Cu, and includes the high-affinity Cu transporter-1 (hCTR1). This trimeric transporter complex forms a pore-like structure for Cu transport across the plasma membrane [3]. The second family consists of two ATPase enzymes, namely ATP7A and ATP7B (encoded by the genes *MNK* and *WND*, respectively), which are involved in the secretion and intracellular sequestration of Cu [1]. Despite having 56% sequence homology, MNK and WND have very different functions and tissue distributions [1].

Mutations in *MNK* lead to Menkes disease, which results in decreased intestinal absorption of Cu, defective Cu distribution in the body and decreased activity of enzymes where Cu is a cofactor [1]. On the other hand, WND is involved in Cu efflux from cells, and mutations in this molecule lead to Wilson disease that is characterized by toxic Cu accumulation in the liver and brain. Interestingly, Cu levels modulate the trafficking of these two enzymes to regulate cellular concentrations of the metal [4,5]. Both MNK and WND work in concert with a series of chaperone molecules that are involved in donation of Cu to their ATPase sites [1,2]. The Cu chaperones also donate this metal ion to other proteins, where it acts as a cofactor for enzymatic catalysis [1,2].

Despite the crucial role of Cu in proliferation, development and foetal growth, virtually nothing is known concerning its transport across the placenta or the hormonal regulation of MNK or WND. In this issue of the *Biochemical Journal*, Hardman et al. [6] take the first step in characterizing the molecular ‘players’ involved in placental Cu transport to the foetus. These authors demonstrate that MNK and WND respond to hormones that play an important role in gestation. Specifically, using polarized JEG-3 cells as a model of trophoblasts, they demonstrate that oestrogen and insulin markedly up-regulate MNK at the mRNA and protein levels [6]. At the same time, treatment with these hormones leads to increased trafficking of MNK from a perinuclear region, consistent with the *trans*-Golgi network to the basolateral surface by a process independent of intracellular Cu concentration. This leads to an increased Cu efflux from cells at their basolateral membrane that corresponds to the foetal side of the placenta. In contrast, insulin, but not oestrogen, results in decreased WND protein, but not mRNA levels. In addition, there was sequestration of WND to a perinuclear area in the cell, leading to decreased Cu efflux from the apical surface. Therefore, oestrogen and insulin lead to changes in the trafficking of intracellular Cu away from the apical surface to the basal membrane [6]. Trafficking of MNK protein to the basal surface of other polarized cell types has also been reported in epithelial Madin–Darby canine kidney cells when Cu levels are high [7]. These cells are a model for systemic Cu absorption and reabsorption in the kidney [7]. Hence, the role of MNK as an efflux mechanism at the basal cell surface of several polarized cell types represents a general system of effecting Cu release.

The study by Hardman et al. [6] characterizes the increase in Cu transport to the basal membrane, which is, in part, achieved by the co-ordinate regulation of MNK and WND (Figure 1A). However, it is likely that there are also other changes in the Cu-metabolizing

¹ To whom correspondence should be addressed (email d.richardson@med.usyd.edu.au).

Figure 1 Models of Cu and Fe transport in (A) trophoblasts and (B) reticulocytes, respectively

These models depend on molecular collaborations and also interactions between organelles. (A) The demonstrated and potential effects of insulin and oestrogen on Cu transport machinery in trophoblasts. Insulin and oestrogen were shown to up-regulate MNK and induce redistribution to the basal membrane, whereas only insulin leads to the internalization of WND, leading to net trafficking of Cu to the foetal circulation. It is still unclear whether these hormones affect the expression or distribution of Ctr1 and chaperones that are involved in Cu uptake and intracellular Cu transport respectively. However, based upon their functions, it can be speculated that there could be up-regulation and re-distribution to mediate the inward flux of Cu across the trophoblast towards the foetal circulation. (B) Although strong evidence exists for molecular interactions and organelle collaboration for the transport of Cu, there are more limited data indicating that interactions between organelles and molecules play a role in the intracellular transport of Fe. In fact, Tf-containing endosomes may associate with the mitochondrion to mediate efficient Fe transfer for the biogenesis of haem. The so-called 'kiss-and-run' hypothesis is supported by the fact that there is no clear evidence for a metabolically active pool of low-molecular-mass Fe in the cytosol, which acts as an intermediate for haem synthesis [10].

machinery to achieve the vectorized transport from the apical to basolateral surface that were not assessed. For instance, it would be of interest to determine whether insulin and oestrogen also induce changes in expression and localization of Ctr1, which is involved in Cu transport into the cell [3] (Figure 1A). Apart from Ctr1, it is likely there may also be alterations in the expression and localization of intracellular Cu chaperones that donate Cu to the active sites of MNK and WND [2] (Figure 1A). The change in expression and cellular localization of such proteins is important to assess after incubation with insulin and/or oestrogen and remains an important research goal for future studies.

The results of Hardman et al. [6] also contribute evidence to a more general model of metal ion transport in cells that involves directed metal ion trafficking, which does not appear to be widely recognized. This is of importance and underlines the significance of the work in addition to the effects of hormones on Cu uptake [6]. Classical thinking has been that the more prevalent metal ions, namely Fe and Cu, form part of a poorly characterized intracellular pool composed of low-molecular-mass metal complexes that are freely diffusible [8]. There are problems with this hypothesis that deserve discussion. First, low-molecular-mass Fe or Cu complexes are toxic and can contribute to free radical reactions leading to cellular damage. Secondly, such a freely diffusible pool would not act efficiently to deliver metals to the active sites in proteins. Thirdly, low-molecular-mass Cu or Fe complexes that act as metabolic intermediates for incorporation into enzymes or haem have not been consistently identified within cells [9,10].

The investigation by Hardman et al. [6] adds further weight to a thesis of Cu transport that includes specific trafficking mechanisms involving protein expression, organelle interactions (e.g. between the Golgi and plasma membrane) and protein-protein collaborations that form an integrated network. The field of copper metabolism has provided evidence for such networks in a variety of different cell types [1,2]. However, fewer data are apparent or even rarely sought in studies examining the intracellular Fe processing. Nonetheless, evidence of specific intracellular trafficking of Fe is observed in developing erythroid cells. Indeed, transferrin (Tf) binds to the transferrin receptor 1 (TfR1) on the cell surface, and is then endocytosed (Figure 1B). Within the endosome, Fe is released from Tf and then transported through the endosomal membrane by the divalent metal ion transporter-1 (DMT1). It can be speculated that DMT1 may associate with the TfR1 in the endosome to enable highly efficient Fe uptake (Figure 1B). After transport of Fe through the endosomal membrane, its immediate fate remains unclear. However, there is no evidence for a low-molecular-mass pool of Fe complexes that acts as an intermediate for haem synthesis [10]. In these latter studies, Fe was shown to be rapidly directed to the mitochondrion for haem synthesis, with little incorporation of Fe into ferritin or other cytosolic compartments [10]. This could indicate that, as found for the metabolism of Cu, specific Fe-binding chaperone proteins and/or organelle interactions may exist that prevent the deleterious effects of low-molecular-mass Fe.

There is some evidence for trafficking of Tf-containing endosomes towards the mitochondria of erythroid cells that efficiently mediates the transport of Fe for haem synthesis. This sequence of events is known as the 'kiss-and-run' hypothesis (Figure 1B) [11]. In haemoglobin-deficient (hbd) mice there is a mutation in the *Sec15l1* gene, which encodes a protein involved in the mammalian exocyst complex, and it has been suggested to dock endosomal vesicles with mitochondria [11]. Hence understanding the coupling of molecular pathways via organelle interactions is essential for full comprehension of metal ion transport, and this is underlined by the study by Hardman et al. [6].

In summary, the work of Hardman et al. [6] provides the first step in the molecular characterization of the uptake of Cu into the placenta and the hormonal control of MNK and WND. Further studies *in vitro* and *in vivo* are now required to extend the current findings, particularly with regard to understanding the effects of insulin and oestrogen on the other players in the Cu transport network. This work also adds significantly to the concept that intracellular metal ion transport is specifically mediated through co-ordinated changes in gene expression, organelle interactions and a system of closely collaborating proteins.

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