Published in final edited form as:

Metallomics. 2018 March 01; 10(3): 378–387. doi:10.1039/c7mt00314e.

A glimpse into the regulation of the Wilson disease protein, ATP7B, sheds light on the complexity of mammalian apical trafficking pathways

Arnab Gupta^{a,*}, Santanu Das^{a,iD}, and Kunal Ray^{b,iD}

^aDepartment of Biological Sciences, Indian Institute of Science Education and Research - Kolkata (IISER K), Mohanpur 741246, West Bengal, India

^bAcademy of Scientific & Innovative Research (AcSIR), CSIR - HRDC Campus, Ghaziabad, Uttar Pradesh – 201002, India

Abstract

Wilson disease (WD), a Mendelian disorder of copper metabolism caused by mutations in the *ATP7B* gene, manifests a large spectrum of phenotypic variability. This phenomenon of extensive symptom variation is not frequently associated with a monogenic disorder. We hypothesize that the phenotypic variability in WD is primarily driven by the variations in interacting proteins that regulate the ATP7B function and localization in the cell. Based on existing literature, we delineated a potential molecular mechanism for ATP7B mediated copper transport in the milieu of its interactome, its dysfunction in WD and the resulting variability in the phenotypic manifestation. Understanding the copper-induced apical trafficking of ATP7B also significantly contributes to the appreciation of the complexities of the ligand-induced transport pathway. We believe that this holistic view of WD will pave the way for a better opportunity for rational drug design and therapeutics.

Introduction

Copper, a transition metal and essential micronutrient, participates in redox reactions in the physiological system by either donating or accepting electrons, thus acting as an electron transfer agent. Copper serves as an essential co-factor for vital enzymes and proteins (both intracellular and secretory) and participates in maintaining the overall cellular homeostasis.¹ Among the key proteins responsible for maintaining optimum copper levels in mammalian cells are the ATP-driven copper pumps (copper-ATPases, ATP7A and ATP7B).^{2–7} Mutations in ATP7B cause WD;⁸ the enzyme is primarily expressed in the liver and brain.^{9,10} WD follows an autosomal recessive pattern of inheritance and manifests with copper

iD^{Arnab Gupta}: 0000-0002-9806-6320

iD^{Santanu Das}: 0000-0002-8445-2589

iD^{Kunal Ray}: 0000-0002-9989-1760

arnab.gupta@iiserkol.ac.in, arnabatiicb@gmail.com.

Conflicts of interest

There are no conflicts to declare.

accumulation in the brain, liver and other organs, and severe hepatic and neurological symptoms.⁴ WD patients may develop signs and symptoms of chronic and long-lasting liver disease that includes jaundice and cirrhosis, nausea, lack of appetite, pain and bloating from fluid accumulating in the abdomen. Most WD patients have a low ceruloplasmin level (NIDDK, NIH¹¹). Central nervous system-related symptoms occur frequently in adults and sometimes in children. Signs and symptoms include tremors, muscle stiffness, dysarthria (problems with speech), swallowing, or muscle coordination.¹² The age of disease onset usually ranges between 8–16 years; however, the onset of WD has been reported as late as in septuagenarians.¹³ Early diagnosis can help in better management and alleviation of the disease.^{14,15}

An intriguing aspect of WD is that despite being a monogenic disorder, extensive phenotypic variations are observed among the patients. Such variations have been well exemplified by Gupta and co-workers and Yahata and co-workers, where the studies showed that siblings suffering from WD, living in the same household and sharing the same ATP7B mutations, exhibit remarkably different disease phenotypes.^{16,17} This extent of dissimilarity is conspicuous, based on the finding, for example, that an elder sister had the hepatic form of the disease while the younger showed neurological symptoms. In the present review, we attempt to delineate the molecular basis of such phenotypic heterogeneity with the regulation and function of the ATP7B protein.

To date, multiple studies have been carried out to identify modifier loci for WD, but with limited success. Two major genetic and clinical issues that have plagued the progress of such studies are as follows: (i) the scarcity of multi-generation families with broad WD phenotypic variation and (ii) unlike typical WD features, there is difficulty in establishing a formal scale for the ‘atypical WD symptom spectrum’.¹⁸ We argue that a global approach utilizing genomic, clinical as well as proteomic tools has the potential to design an ideal strategy towards laying out a mechanistic blue print for WD pathogenesis. In this article, we have attempted to decipher the phenotypic diversity of WD by exploring the functional and structural aspects of ATP7B and its interaction patterns with other proteins that regulate its copper export and trafficking pathway.

ATP7B: the Wilson disease ATPase and its functional domains

The *ATP7B* gene encodes a large membrane protein with significant sequence homology (50–60% identity) with the Menkes disease Cu-ATPase ATP7A. In the past several years, considerable progress has been made in analyzing the biochemical properties of these two proteins. In the present article we focus primarily on the ATP7B structure and function with special reference to ATP7A as needed. ATP7B belongs to the large family of P-type ATPases and several functional domains have been identified in their structures as depicted in the illustration for ATP7B (Fig. 1).

Human ATP7B is an approximately 160 kDa membrane protein with 8 transmembrane helices. Its general architecture is similar to that of other P1-type ATPases, which consists of a large N-terminal domain containing six sub-domains, each having a metal-binding site (MBD); an ATP binding domain is composed of the nucleotide-binding and phosphorylation

domains (NBD), an actuator domain (A-domain) and a long C-terminal tail (Fig. 1).^{19–22} The NH₂-terminal portion of human Cu-ATPases is large (>650 residues) and contains six repetitive sequences, each harboring the sequence motif GMT/HCxxCxxxIE (Fig. 1). Each of these repeats forms a subdomain with a single metal-binding site, *i.e.*, the total stoichiometry is six copper ions per NH₂-terminal domain. All these major domains lie on the cytosolic face of the protein, with only short loops connecting the transmembrane regions exposed to the lumen.

Similar to other ATPases, ATP7B transports ligands (*i.e.*, copper) from the cytosol across the cellular membranes using the energy derived from ATP hydrolysis. This process involves multiple steps, *i.e.*, specific recognition of copper, delivery of copper to the membrane portion of the transporter with subsequent release at the other side of the membrane (lumen) synchronized with binding and hydrolysis of ATP into ADP and Pi. The central step in the catalytic cycle is the transfer of γ -phosphate from ATP to the invariant Asp residue in the DKTG motif of the phosphorylation domain with the formation of a transient phosphorylated intermediate (shown in Fig. 1 inset). The prerequisite for this reaction is the binding of copper to the sites within the membrane portion of the enzyme, while the release of copper from these sites stimulates dephosphorylation (Fig. 1 inset). The structural organization of Cu-ATPases reflects the need to accommodate and couple these reactions.

The copper translocation pathway is located in the trans-membrane portion of Cu-ATPases, which is composed of eight transmembrane segments (TMs). The highly conserved Cys-Pro-Cys (CPC) sequence in TM6 is one of the signature motifs characterizing Cu-ATPases and other ATPases involved in the transport of Zn, Cd, Ni, Ag, and Pb, which together form the P_{1B} subfamily. Along with the Cys residues of the CxxC motif, they are likely to form copper binding site(s) within the membrane portion in human Cu-ATPases. All other functional domains of Cu-ATPases are cytosolic. We will elaborate on the structure–function relationship between the cytosolic domains with the WD phenotype in the following sections.

Trafficking cues of ATP7B lie within the protein: complex inter-domain interactions in ATP7B shed light on WD phenotypic complexity

Yeast two-hybrid screening and other protein–protein interaction studies have identified multiple proteins that bind to ATP7B and have established that the large cytosolic domains act as the major interfaces of these interactions.^{23–25}

Under normal physiological conditions, ATP7B resides in the Trans Golgi Network (TGN). It is trafficked from TGN to apically targeted vesicles in response to high copper concentration, with subsequent fusion of the vesicles with the apical surface for copper release into the lumen.^{26–28} ATP7B is a member of a small but highly specialized group of proteins that are trafficked out from the TGN towards the apical membrane in response to a cue, *i.e.*, the binding of a ligand (copper) to the protein. Most of the apically trafficking proteins belong to the family of ‘constitutive traffickers’; therefore, the mechanism of ATP7B trafficking serves as an appropriate model to study the domain conformation and domain movements that trigger the trafficking of ligand-induced apically targeted proteins.

The following are two overarching reasons for this: (i) it is a multi-spanning membrane protein with large cytosolic domains that co-interact among themselves; (ii) copper levels in the cells regulate the trafficking of ATP7B in distinct compartments that can be easily determined by high resolution microscopy.

N-terminal metal binding sites and their interaction patterns with other domains dictate anterograde trafficking

In this section, we attempt to correlate the domain movements and domain–domain interactions within the ATP7B protein with its cellular localization (*sub-step in its trafficking itinerary*) and activity. Further, we delineate the structure–function relationship of WD mutations harbored in these domains with respect to their phenotypes/disease manifestation.

Some recent interesting studies have correlated intradomain movements in ATP7B with its stability and copper dependent localization. Gupta *et al.* demonstrated that the interaction of the A-domain with the copper bound N-terminal domain stabilizes the protein, making it accessible for the ER to Golgi cargo.²⁹ A variant, Gly875Arg (in the A-domain), shows a very interesting phenotype; it has been reported in the Chinese population as a non-disease causing variant, whereas it is claimed as a disease-causing variant in the Indian population.^{30,31} Detailed mechanistic studies revealed that this mutation results in a loss of interaction between the N-terminal and the A-domain, causing ATP7B to accumulate at the endoplasmic reticulum. This leads to intracellular copper accumulation. Copper supplementation within physiological limits stabilizes the N-terminal–A-domain interaction, leading to the rescue of the protein to the Trans-Golgi Network (TGN). Once the protein attains the ‘*traffic-ready conformation*’ and resides on the TGN, its exit to vesicles in response to copper largely depends on the interaction between the N-terminal and the nucleotide binding domain (NBD). The authors suggest that intracellular copper levels dictate whether Gly875Arg will manifest as a disease or a non-disease variant.²⁹ It would be interesting to probe what might be the underlying modifying factor(s) between the two populations, including dietary habits, which influence the threshold of Cu-levels to cause different cellular fates and the disease outcome. Copper bound to the six copper-binding sites (MXCXXC) on the N-terminus releases its interaction with the NBD and allows the protein to attain the ‘*TGN-exit conformation*’. It is tempting to propose that the copper dependent interaction of the N-terminal with either the A-domain or NBD primarily dictates ATP7B localization. The role of the N-terminus in the stability of ATP7B can be further reinforced as two common WD mutations found in the multi-ethnic world populations; Gly85Val and Gly591Asp exhibit ER localization similar to Gly875Arg.³² A systematic study of the interaction of the N-terminus (wild type and mutant) with cytosolic domains (A and NBD) will help in dissecting the roles of these individual domains in the localization and stability of ATP7B.

The *in vivo* studies to date on copper binding to the N-terminus employ techniques that address this interaction as an all or none phenomenon, *i.e.*, whether all six MBDs are saturated or empty.²¹ It should be very interesting to determine whether the semi-saturated state of the N-terminus alters its interaction with other domains (NBD or A-domain), in turn triggering the ER- or TGN-exit conformations. *In silico* studies have shown that the N-

terminus of ATP7B has been altered the most, compared to other domains, as organisms have evolved from bacteria through invertebrate metazoans to higher chordates.³³ The *E. coli* copper-ATPase CopA has two N-terminal metal binding domains (MBD), each with one copper-binding site; likewise, in the amoeba, *Dictyostelium*, only two MBDs have been found. Also, in yeast (*Saccharomyces* and *Candida*) two MBDs are present. The Cu-ATPase of *C. elegans*, CUA1, has three MBDs. Further up the evolutionary ladder, *Drosophila* ATP7 has four MBDs. Among chordates (*Danio*, *Xenopus* and humans), six MBDs have been observed.³³ The first four metal binding sites (MBD1–4) are present in chordate Cu-ATPases and have been reported to have a regulatory function in mammals.²¹ *In vitro* experiments have demonstrated that deletion of the region containing these four sites has no inhibitory effect on copper-ATPases, but it accelerates the catalytic cycle instead.²¹ The deletion also does not alter the copper affinity of the intra-membrane transport sites, at least in ATP7B. This observation strongly suggests that the MBD1–4 region of the protein has a regulatory role and autoinhibits the catalytic function of the protein.²¹ Functional assays of missense mutations harbored on the N-terminus are limited. L492S, V519M, E541K, L549P, A604P, R616W are a few common mutations that have been linked to severe and early onset symptoms of WD;^{34–38} however, their unique role(s) in imparting a ‘specific symptom’ or ‘a set of symptoms’ in the patients could not be determined, primarily for two reasons. (i) Most of the mutations are present as compound heterozygotes and hence the phenotype is an outcome of the contribution from two different mutations. (ii) Most of the disease causing variants are private mutations. Establishing a cause–effect relationship with statistical significance will therefore remain challenging but some success may be accrued based on the meta-analysis of multiple well planned studies.

It is worth mentioning that most of the functional studies have been carried out in unpolarized cells; these cells do not always faithfully replicate the true physiological scenario. To gain physiological insight into the trafficking mechanism, these studies should be carried out in polarized hepatic cells, e.g., WIF-B or polarized HepG2 cell lines.

Besides insight into the mechanism of the exiting of ATP7B from various compartments with increasing copper levels in the cell, understanding the polarity of its trafficking remains a partially addressed topic. ATP7A, the homologue of ATP7B, is often used as a comparative model for the latter. ATP7A is mutated in a rare genetic disorder of systemic copper deficiency, Menkes disease. It is responsible for exporting copper in the blood that has been absorbed from food in the duodenum. Interestingly, despite their high homology (~60% identity in amino acid sequence), ATP7A and ATP7B show opposite trafficking directions in high copper (ATP7A traffics basolaterally, ATP7B apically); thus, studying the basic differences in the domain architecture between these two copper transporters is essential in understanding their copper dependent regulation.

Detailed studies on mutations and deletions in ATP7B have shown that its unique N-terminal sequence (residues 1–63) is necessary for TGN retention at low copper, and for apical targeting in high copper.^{39,40} Truncation of the ATP7B N-terminus up to MBD-5 yields an active ATPase that is insensitive to cellular copper levels and is constitutively trafficked to the opposite (basolateral) plasma membrane. Fusion of the N-terminal 1–63 AA to the truncated protein restores both its Cu responsiveness and correct intracellular targeting.

However, 1–63 AA alone or fused to the N-terminus of an unrelated membrane protein that was normally targeted to the basolateral membrane, failed to drive the reporter to the apical surface, indicating that 1–63 AA alone was not sufficient for apical targeting. N41S, which is part of the signal, is the only reported WD causing variant identified within residues 1–63 of ATP7B.³⁹ This N41 is contained within a 9 amino acid stretch (37–45) in the amino terminus, which is critical for apical targeting and Golgi retention in a copper dependent manner in polarized hepatocytes. The disease-causing mutant N41S completely abolishes its apical polarity, as is evident from its presence at the basolateral membrane and in the TGN in both copper depleted and high copper conditions.

The TGN exit of ATP7B involves multiple complex events that are highly interdependent. As discussed in the preceding sections, the exit of ATP7B in response to copper is dictated by domain reorganizations and transient domain–domain interactions. Co-purification experiments involving individual overexpressed ATP7B domains show that although these interactions are specific, they require elements of stabilization. This phenomenon adds another level of complexity in the regulation of ATP7B *via* domain–domain interactions. It is now established that kinase mediated phosphorylation at the N-terminus is a key event that stabilizes domain–domain interactions in response to copper. Hasan *et al.* illustrated that the phosphorylation of Ser340 and Ser341 residues at the N-terminal domain (NTD) are critical for the initial exit of the protein from the TGN to the vesicles.⁴¹ It has been elegantly demonstrated that phosphorylation of the N-terminus in response to copper keeps it secluded from the NBD by destabilizing their interaction and this ‘*open conformation*’ of ATP7B maintains its ‘*TGN-exit or vesicularized*’ stage.^{41,42} Phosphorylation diminishes the interaction between the NTD and the NBD (described in previous section) and the protein attains a trafficking-suitable conformation. This conformation is probably favorable for the binding of proteins to ATP7B, which dictates the TGN exit and polarity of its trafficking.

Some mutational studies on the N-terminus in polarized hepatocytes have further established it as the major domain influencing ATP7B trafficking. The common mutation described earlier, ATP7B-N41S, shows mislocalization in elevated copper, primarily localizing at the basolateral membrane. Studies on the interaction pattern between the N-terminus harboring these mutations and the NBD will be useful for better understanding the mechanism of the copper-induced TGN exit and trafficking of ATP7B.

Secretory function and excretory function of ATP7B: are they interdependent or mutually exclusive?

Mutational studies have been beneficial towards dissecting the two primary function of the ATP7B protein, *i.e.*, the secretory and the excretory functions. A small subgroup of WD patients manifested hepatic copper accumulation, but showed normal levels of ceruloplasmin as observed in a patient carrying the homozygous mutation S653Y.⁴³ Braiterman and co-workers demonstrated that the trafficking-defective phenotype of the ATP7B-S653Y was able to transport copper in the TGN to apo-ceruloplasmin for its maturation. This study emphasizes the importance of differential therapy in different forms of WD. Also, the study provides evidence that the transmembrane region of the protein harboring the residue S653, previously not thought to be involved in trafficking, may actually play a role in the sorting of

ATP7B in the TGN. The review of WD patient mutations revealed that two common mutations in ATP7B, G710S in TM2 and G943S in TM5, may behave similarly to ATP7B S653Y. Both mutants were reported to localize to the Golgi in basal medium and exhibit some Cu(I) transport activity in WD cases where serum ceruloplasmin is normal. It is tempting to argue that the region might be important in protein–lipid interaction that may in turn affect the lateral movement of ATP7B. Further studies on such TM mutants of ATP7B can be utilized to explore copper-induced sorting/clustering of the protein in the TGN preceding its copper-induced exit in vesicles.⁴³

The C-terminal: the domain critical for retrieval and trafficking regulation to and from TGN

Unlike other conserved domains, the C-terminal region of ATP7B shows less homology with its counterpart of ATP7A; thus, it is likely that it has a specialized function of ATP7B that is absent or different in ATP7A. The C-terminal of ATP7B contains a highly conserved dileucine motif (LL). Mutational studies demonstrate that this motif is important for the TGN localization of the protein in basal copper levels. Fig. 2 illustrates the localization of various ATP7B mutations in different sub-cellular compartments. Two mutations, L1373P and L1373R, causal to neurological as well as hepatic WD, reported from Japanese and Brazilian populations respectively, have been thoroughly characterized in polarized hepatic cells.³⁹ Both L1373 mutants also exhibited abnormal trafficking behavior and protein stability. L1373P, the more stable molecule, was found predominantly in the ER, as well as in vesicles, but not in the Golgi, when copper levels were depleted. These results suggest that the mutation of L1373 to either L1373R or L1373P slows the exit of the mutant proteins from the ER and perturbs their retention in the Golgi under low copper conditions. The canonical sequence of the C-terminus implicated in ATP7B localization is the DKWSLLL sequence in the COOH-domain. It is a versatile signal that is required for the correct sorting of ATP7B in the TGN, a step preceding its copper induced TGN exit; also, the signal functions in ATP7B endocytosis and the protein's recycling to the plasma membrane.⁴⁴ Among the WD mutations, there are three C-terminal mutations that are likely to interfere with the function of the DKWSLLL signal. These include c.4310A duplication leading to premature truncation of the protein removing the DKWSLLL signal, and the c.4374–c.4375 deletion that introduced a premature stop codon resulting in loss of the signal. Interestingly, the WD patient with mutation 4310A duplication also manifested G6PD deficiency.⁴⁵ In experiments conducted in our laboratory (Prof. Ann Hubbard, Johns Hopkins University), we noticed partial co-localization of ATP7B with G6PD in copper induced vesicles (unpublished data), which tempts us to hypothesize that the mis-sorting of ATP7B also caused mislocalization of the common cargo G6PD resulting in the additive/atypical phenotype of WD.

In polarized rat primary neuron, this dileucine motif mediates the TGN localization under normal physiological copper concentrations and redistributes to plasma membranes and vesicles at high copper concentrations, which is even restricted to soma and dendrites, thus mediating somatodendritic polarity. Mutating the dileucine residues L1454 and L1455 in the DKWSLLL motif caused a similar redistribution of ATP7B to the cell periphery and also a loss of polarity consistent with a function of this motif in determining TGN localization and somatodendritic polarity.⁴⁶

It is interesting to note that the ATP7B motif DKWSLLL that corresponds to [DE]XXXL[LI] has been reported to sort and retain cargo to lysosomal compartments.⁴⁷ This hints towards the fact that a certain part of the ATP7B itinerary is spent in the lysosomes as shown later by Polishchuk and colleagues.⁴⁸

Wilson disease: correlating the ATP7B trafficking and the disease phenotype

ATP7B, unlike most other apically targeted proteins, has some special trafficking attributes that makes it advantageous for use as a model to study trafficking and understand the mechanism of WD pathogenesis. Its intracellular localization, showing a specific correlation with copper levels in the cell is most interesting, *i.e.*, ATP7B localizes to the TGN in basal copper and travels to endosomal vesicles as copper increases in the cell and is recycled between the apical compartments and the apical membrane as high levels of copper are maintained in the cells.²⁶ Usually, other apically targeted proteins either follow constitutive trafficking or show a much broader or non-specific correlation between their localization and the ligand.⁴⁹ In this part of the article we shall focus on reviewing the advances in the regulation of ATP7B by other proteins and its possible implication in WD pathogenesis.

Luminal pH plays a possible role in ATP7B activity and localization

Cu-ATPases have been shown to be affected by changes in luminal pH.²⁷ The physiological maintenance of luminal pH is critical for intracellular trafficking.⁵⁰ Luminal pH acidifies from pH of ~6.0 in the Golgi to pH 5.5 in exocytotic vesicles near the cell surface. In the endocytic pathway, luminal pH forms a gradient beginning with pH of ~6.5 in early endosomes to between pH 6 and 5.5 in late endosomes and lysosomes. Organelle acidification is driven primarily by V-ATPase, the ATP-dependent proton pump.⁵¹ Nyasae *et al.* showed that although perturbants of luminal acidification using Bafilomycin had little effect on the TGN localization of ATP7B in low Cu, they blocked delivery to the apical membrane in elevated Cu; ATP7B accumulated in large endosomes in the basolateral region.²⁷ pH also affected the copper transport activity of the protein. The chloride channel CIC-4 regulates copper incorporation into ceruloplasmin by ATP7B; overexpression of CIC-4 drastically reduces the copper incorporation into ceruloplasmin. The effect of CIC-4 was most pronounced under copper-limiting conditions. Immunohistochemistry confirmed that the chloride pump co-localized with ATP7B in the same vesicles in hepatocytes.⁵² Regulation of ATP7B activity by CIC-4 is possibly *via* the maintenance of optimum pH in the lumen of TGN and vesicles by the channel rather than its direct interaction with ATP7B.

Some very interesting but conflicting views exist in the field of copper transport and the role of pH modulation in the regulation of ATP7B activity. In contrast to what Nyasae *et al.*²⁷ demonstrated, it has been claimed that low pH blocks ATP7B trafficking. Polishchuk *et al.* showed that ATP7B exudes copper *via* lysosomes and endosomal organelles with a very low luminal pH.⁴⁸ The group demonstrated that in response to elevated copper, ATP7B moves from the Golgi to lysosomes and imports it into their lumen *via* lysosomal exocytosis. ATP7B enables lysosomes to undergo exocytosis through the interaction with the p62 subunit of dynactin that allows lysosome movement toward the apical surface of polarized

HepG2 cells. Interestingly, the activation of lysosomal exocytosis stimulates copper clearance from the hepatocytes. Lysosomal exocytosis also rescues the most common caucasian WD mutation, H1069Q, from ER. Infecting HepG2 cells expressing the ATP7B-H1069Q with drugs to enhance lysosomal exocytosis triggered the mutant protein's localization to TGN and further to lysosomes with elevated copper.⁴⁸ *In vivo* experiments show the presence of ATP7B in late endosomes and lysosomes in both liver tissue⁴⁸ and primary hepatocytes.⁵³ These observations suggest that LEs/lysosomes deserve serious consideration as the intermediate to late stage in ATP7B trafficking. Drugs that elevate lysosomal exocytosis might hold promise as WD therapeutics. Studies by Pena *et al.* also supported the view that brief exposure to Cu activates lysosomal exocytosis; copper release and lysosomal exocytosis was measured in terms of the release of the lysosomal digestive enzyme β -hexosaminidase (β -hex) into the extracellular medium and by the presence of the lysosomal marker LAMP1 at the plasma membrane.⁵⁴

Lalioi and co-workers too showed that the Cu^+ -induced release of ATP7B from the TGN is pH dependent. Treatment with the acidotropic agent chloroquine and V-type H^+ ATPase inhibitor bafilomycin A1, both hampering TGN lumen pH, resulted in ATP7B vesicles remaining attached to TGN. They were swollen (chloroquine) and delayed the detachment of the cisterna carrying ATP7B from the TGN (bafilomycin A1), demonstrating that cisterna detachment is a prerequisite to the generation of transport vesicles. These findings indicate that acidity of the TGN is crucial for the release of the vesicles loaded with ATP7B.²⁸

ATP7B trafficking and the apical route: do different copper levels activate multiple trafficking routes?

The present knowledge on the entire trafficking route of ATP7B is unclear. Most of the studies have focused on subparts/sub-steps of this route. A clear understanding of the entire pathway is crucial to the design of therapeutic drugs and small molecules that will enhance or assist the entry or exit of mutant ATP7B through these multiple sub-steps.

Utilization of different cell lines to study ATP7B trafficking has led to some discord in our understanding of the same. Lalioi and co-workers demonstrated that ATP7B is transported from the TGN to the bile canaliculus by basolateral sorting and endocytosis and microtubule-mediated transcytosis through the subapical compartment in polarized hepatoma Can10 cells.²⁸ This might be attributed to cell line specific polarity issues. However, *in vivo* experiments in hepatic tissue do not show basolateral localization preceding its apical trafficking in response to copper. Further, in contrast to what Polishchuk *et al.* observed in cell lines as well as mouse liver, this study demonstrated that ATP7B was not incorporated into lysosomes, and elevated copper does not cause relocation of lysosomes and the appearance of lysosome markers in the bile canaliculus. However, the copper concentration used in this study was different from that used in the study by Polishchuk *et al.*^{28,48} Another interesting observation from this study was the presence of transferrin in ATP7B vesicles lying close to the basolateral membrane, suggesting that in the initial phase of ATP7B trafficking comes in close proximity to the basolateral membrane *via* the EEA1 (Early Endosomal Antigen 1) positive endosomes, as was also demonstrated by Nyasae and co-authors.²⁷ The Cu-dependent route of ATP7B could vary in different hepatic

cell lines because WIF-B, Can10 and HepG2 may differ from each other and from primary hepatocytes in terms of microtubule polarity, gene expression and endocytic system organization. However, why ATP7B does not traffic directly to the apical membrane, but follows a tortuous route *via* the basolateral endosomes, remains to be understood. It would be a good idea to capture and dissect the early events that suggest ATP7B trafficking towards the basolateral membrane, but not fusing with it, by using live cell imaging followed by total internal reflection fluorescence microscopy (TIRFM).

Studies on the later phase (near the apical membrane) of ATP7B trafficking are mainly focused on the motor proteins and their roles in apical delivery. Delivery of ATP7B to the apical membrane was also confirmed by electron microscopy.⁴⁸ Gupta *et al.*, utilizing confocal microscopy and cell surface biotinylation also demonstrated that in polarized hepatic cells, WIF-B, the final delivery of ATP7B in the bile canaliculus (apical surface) is mediated by the microfilament motor myosin Vb.⁵⁵ Downregulation of myosin Vb by a dominant negative mutant arrests ATP7B in a sub-apical compartment (SAC), lying very close to the apical membrane. The investigators also hypothesized that SAC is the final sorting station of ATP7B before it reaches its destination, *i.e.*, bile canaliculus (BC). In elevated copper, the protein recycles between SAC and the BC. We argue that this last step of trafficking, *i.e.*, fast recycling between SAC and BC has evolved to reduce the energy expenditure that would have been required if ATP7B had to trafficked back to the TGN at every cycle of copper export. Previously, in HepG2 and CHO cells, Cater *et al.* showed that copper triggered the trafficking ATP7B to vesicles but it failed to reach the plasma membrane.⁵⁶ We hypothesize that this discrepancy might be due to the unpolarized state of HepG2 and CHO cells as compared to polarized WIF-B cells. Gupta *et al.* also demonstrated the increasing role of MyoVb in the apical delivery of ATP7B as the cells progressed toward apicobasolateral differentiation.⁵⁵

ATP7B binding partners play a major role in ATP7B stability and trafficking

Numerous studies over the last decade have identified the molecular partners of ATP7B that regulate the stability and copper transport function of the protein. These proteins can serve as putative therapeutic targets for WD that will stabilize ATP7B and also enhance its copper export function. In this section we discuss three such well characterized proteins, COMMD1, clusterin and glutaredoxin.

WD phenotypes in humans share high similarity with another inherited form of copper accumulation disease in dogs called 'copper toxicosis'. In dogs, mutations in the gene *COMMD1* have been implicated in copper toxicosis. Similar to WD in humans, canine copper toxicosis is an autosomal recessive disorder, with a high frequency in Bedlington terriers.⁵⁷ Biliary copper excretion is markedly reduced in affected dogs,⁵⁸ and as a result copper accumulates in the lysosomes of hepatocytes, eventually leading to liver cirrhosis and chronic hepatitis. Though COMMD1 was not found to be solely causal to any disorders of copper accumulation in humans, the only COMMD1 mutation (Thr174Met) identified to-date in humans was found to aggravate symptoms in a diagnosed and confirmed WD patient with ATP7B mutations.⁵⁹

Cellular studies have shown the partial co-localization of ATP7B and COMMD1 in HEK293 cells.³² Studies have shown that COMMD1 is able to bind to the N-terminal copper binding region of ATP7B but not that of ATP7A.⁶⁰ These data suggest that COMMD1 and ATP7B cooperate in the excretion of copper from the hepatocyte and that a possible lack of interaction underlies the possible pathophysiology of canine copper toxicosis. Interestingly, in immuno-precipitation assays to study COMMD1–ATP7B interaction, it was observed that the interaction was significantly stabilized in the presence of the common mutations G591D and G85V at the N-terminus domain of ATP7B. The increased interaction of these mutants with COMMD1 indicates that COMMD1 might facilitate the degradation of ATP7B.³² Detailed domain organization studies on COMMD1 by Burkhead and colleagues revealed that the C-terminal domain binds to lipids, specifically PtdIns(4,5)P₂.⁶¹ In native conditions, endogenous COMMD1 forms large oligomeric complexes both in the cytosol and at the membrane. COMMD1 is a scaffold protein in a distinct subcompartment of the endocytic pathway.⁶¹ Burkhead suggested that the presence of COMMD1 in compartments enriched in PtdIns(4)P and PtdIns(4,5)P₂ may serve as an important signal to direct the routing of the copper transporter ATP7B when it passes through the secretory pathway toward subapical vesicles of hepatocytes, allowing the eventual fusion of copper-containing vesicles with the canalicular membrane.⁶¹ In a recent study, it was demonstrated that COMMD1 is directly linked to early endosomes through its interaction with a protein complex containing CCDC22, CCDC93, and C16orf62 that in turn binds to the WASH complex. Depletion of the CCDC complex components abrogates the copper dependent trafficking of the basolateral copper transporter ATP7A.⁶² It is highly likely that COMMD1 also regulates trafficking of ATP7B in a similar manner. This finding of localizing COMMD1 in the endosomal pathway contradicts the function of COMMD1 in regulating protein stability *via* the ubiquitin–proteasomal system⁶³ and suggests lysosomal degradation of the copper ATPases.

Another interaction that regulates ATP7B stability is its binding with the apolipoprotein J protein, ‘clusterin’. This interaction increased under conditions of oxidative stress and with mutations in ATP7B that led to its misfolding and mislocalization. Clusterin and COMMD1 facilitated the degradation of ATP7B containing the same WD-causing mutations *via* different degradation pathways – clusterin *via* the lysosomal pathway, and COMMD1 *via* the proteasomal pathway. It has been claimed that ATP7B exists in a tripartite complex with clusterin and COMMD1, but these interactions are neither competitive nor cooperative and occur independently of each other.^{64,65} The selective and topical regulation of COMMD1 and clusterin could serve as a potent therapeutic tool to treat WD patients harboring mutations that lead to the aggregation of ATP7B in inclusion bodies.

Studies on the interaction of ATP7B with its regulators suggest that the N-terminus of ATP7B serves as the most common and efficient template for interdomain communications. Biochemical studies on purified domains demonstrate that the N-terminus containing the 6 metal binding sites is prone to oxidation. Cytosolic proteins that might act as antioxidants for those copper binding sites have remained elusive for a long period. Recently, using the yeast two-hybrid technology, glutaredoxin (GRX1) was discovered to be an interacting partner of ATP7B, confirmed by co-immunoprecipitation from mammalian cells. The interaction required the presence of copper and intact metal-binding motifs. In addition, the

interaction was related to the number of metal binding domains available. GRX1 catalyzes the reduction of disulphide bridges and reverses the glutathionylation of proteins to regulate and protect protein activity. GRX1 possibly catalyzes either the reduction of intramolecular disulphide bonds between the cysteine residues in the MXCCXXC and facilitates copper binding.²⁴ This study opens-up exciting avenues for possible glutathione and antioxidant therapy in WD.

Understanding the trafficking polarity of copper transporters has always been a topic of great interest. Besides liver cells and other polarized systems, trafficking of ATP7B has been investigated in neurons. Regulation of the TGN exit and somato-dendritic polarity of ATP7B was recently studied with regard to its association with clathrin and its adaptor proteins.⁴⁶ Clathrin adaptor protein AP-1 has a direct interaction with the dileucine motif at the C-terminal cytosolic end of ATP7B in polarized rat primary neurons, revealed by co-immunoprecipitation and immunofluorescence. In the presence of physiological copper concentration, ATP7B primarily co-localised with TGN38 in the soma, whereas high copper concentration caused redistribution to the plasma membrane and vesicles. ATP7B was largely restricted to the soma and dendrite and was absent in the axon. Using the dominant negative mutant V98S to determine whether interaction with σ 1A subunit of AP-1 is required for somatodendritic sorting of ATP7B, Jain *et al.* found that expression of the mutant resulted in the nonpolarized distribution of the transporter.⁴⁶

Using co-immunoprecipitation and mutation analysis, Lalioti and colleagues also showed that interaction of ATP7B with the σ subunit of AP-2 might participate in the clathrin-mediated endocytosis of ATP7B at TGN and mediate preliminary basolateral sorting before being targeted to the apical domain in polarized hepatocyte Can10 cells.²⁸ Although the exact reason for basolateral sorting of an apical targeted protein like ATP7B is not well understood, this study supports the observation made by Nyasae and co-workers where they reported that ATP7B localizes to large basolateral endosomal compartments before being redirected towards the apical domain of WIF-B cells at high copper concentrations.²⁷

One of the earliest studies on regulators of ATP7B function and trafficking involved the copper chaperone ATOX1. It was reported that Atox1 has a direct interaction with the N terminal ATP7B *via* its metal binding domain while delivering copper, as is evident from biochemical studies.⁶⁶ To narrow down the specific ATP7B MBDs that interact with ATOX1, deletion constructs of ATP7B were utilized. It was found that the N-terminal of ATP7B containing 1–4 MBD interacted more strongly than the intact 1–6 domain fragment with ATOX1. Three disease causing missense mutations (G85V, L492S, and G591D) were identified in the corresponding copper-binding domains of ATP7B that result in WD, which abrogates the interaction of Atox1 thus impairing copper transport.⁶⁷ Table 1 highlights the interaction of regulatory partners with specific domains of ATP7B.

Discussion

It is evident from the studies discussed in the present review that the regulation of the copper induced trafficking of the P-Type ATPase ATP7B is a highly complex phenomenon and is dependent on multiple factors, *i.e.*, interdomain and domain–domain interactions, regulatory

proteins, the pH of the vesicle lumen, the rate of copper transport and the level of intracellular copper. Any one of these factors if thrown awry might result in copper imbalance in the cells, leading to WD or other disorders of copper metabolism.

Studying the structure–function correlation of ATP7B provides an in-depth understanding of the disease. ATP7B is a fascinating transporter; its transport activity is required for the normal function of many organs in the human body. We have discussed that the activity of ATP7B is tightly regulated through complex interactions with modulatory proteins and in response to signaling pathways. Studies on the ATP7B structure, mechanism, and regulation are rapidly progressing, and new discoveries will reveal the sophistication and complexity of copper transport in the human body. Gourdon and colleagues reported the structure of LpCopA—a P1B-type ATPase from the bacterium *Legionella pneumophila*, which has provided a breakthrough and a huge opportunity to study domain organization and the movement of the copper pump at a super-resolution level.²²

In an interesting recent article, for the first time in the history of copper ATPases, Jayakanthan and colleagues utilized the electron microscopy of negatively stained samples and cell based assays to demonstrate that human ATP7B can exist as a dimer in cells as well *in vitro*;⁶⁸ a deletion mutant lacking 1–4 MBD localized it in vesicles, even in copper chelated conditions. Co-transfection and expression of this truncated ATP7B with the wtATP7B construct causes them to heterodimerize and therefore the truncated form is rescued from vesicles and retained in the TGN. The protein interface in the dimer is likely to involve transmembrane segments and some cytosolic domains without the involvement of the 1–4 MBD. Though dimers have been previously reported in P-type ATPases, *e.g.*, in K⁺-translocating KdpFABC from *Escherichia coli*,⁶⁹ this is the first report where any copper ATPase or any mammalian P-type ATPase has been shown to form dimers. Though the implication of ATP7B dimerization for copper transport or trafficking is not well understood, further investigation certainly promises better insight into the functioning of this copper pump.

Recent advances in the trafficking mechanism of ATP7B along with the high resolution crystal structure of the protein promises to provide the long-required impetus to develop therapeutics for copper imbalance diseases.

Acknowledgements

The study was funded by Wellcome Trust DBT India Alliance Fellowship (IA/I/16/1/502369) and Early Career research Award (SERB-ECR) to AG (ECR/2015/000220).

References

1. Uauy R, Olivares M, Gonzalez M. Am J Clin Nutr. 1998; 67:952S–959S. [PubMed: 9587135]
2. Forbes JR, Cox DW. Am J Hum Genet. 1998; 63:1663–1674. [PubMed: 9837819]
3. Harris ED. Annu Rev Nutr. 2000; 20:291–310. [PubMed: 10940336]
4. Huster D, Lutsenko S. Mola BioSyst. 2007; 3:816–824.
5. Inesi G, Pilankatta R, Tadini-Buoninsegni F. Biochem J. 2014; 463:167–176. [PubMed: 25242165]
6. Kaler SG. Adv Pediatr. 1994; 41:263–304. [PubMed: 7992686]
7. Kaler SG. Can Assoc Radiol J. 1995; 46:480. [PubMed: 7583733]

8. Thomas GR, Forbes JR, Roberts EA, Walshe JM, Cox DW. *Nat Genet.* 1995; 9:210–217. [PubMed: 7626145]
9. Linz R, Lutsenko S. *J Bioenerg Biomembr.* 2007; 39:403–407. [PubMed: 18000748]
10. Lutsenko S, Barnes NL, Bartee MY, Dmitriev OY. *Physiol Rev.* 2007; 87:1011–1046. [PubMed: 17615395]
11. Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. *Nat Genet.* 1993; 5:327–337. [PubMed: 8298639]
12. Rosencrantz R, Schilsky M. *Semin Liver Dis.* 2011; 31:245–259. [PubMed: 21901655]
13. Ala A, Borjigin J, Rochwarger A, Schilsky M. *Hepatology.* 2005; 41:668–670. [PubMed: 15723329]
14. Das SK, Ray K. *Nat Clin Pract Neurol.* 2006; 2:482–493. [PubMed: 16932613]
15. Gupta A, Neogi R, Mukherjee M, Mukhopadhyay A, Roychoudhury S, Senapati A, Gangopadhyay PK, Ray K. *Indian J Med Res.* 2003; 118:208–214. [PubMed: 14723487]
16. Gupta A, Aikath D, Neogi R, Datta S, Basu K, Maity B, Trivedi R, Ray J, Das SK, Gangopadhyay PK, Ray K. *Hum Genet.* 2005; 118:49–57. [PubMed: 16133174]
17. Yahata S, Yung S, Mandai M, Nagahara T, Kuzume D, Sakaeda H, Wakusawa S, Kato A, Tatsumi Y, Kato K, Hayashi H, et al. *J Clin Transl Hepatol.* 2017; 5:27–30. [PubMed: 28507923]
18. Mukherjee S, Dutta S, Majumdar S, Biswas T, Jaiswal P, Sengupta M, Bhattacharya A, Gangopadhyay PK, Bavdekar A, Das SK, Ray K. *Parkinsonism Relat Disord.* 2014; 20:75–81. [PubMed: 24094725]
19. DiDonato M, Narindrasorasak S, Forbes JR, Cox DW, Sarkar B. *J Biol Chem.* 1997; 272:33279–33282. [PubMed: 9407118]
20. Dmitriev O, Tsivkovskii R, Abildgaard F, Morgan CT, Markley JL, Lutsenko S. *Proc Natl Acad Sci U S A.* 2006; 103:5302–5307. [PubMed: 16567646]
21. Huster D, Lutsenko S. *J Biol Chem.* 2003; 278:32212–32218. [PubMed: 12794172]
22. Gourdon P, Liu XY, Skjorring T, Morth JP, Moller LB, Pedersen BP, Nissen P. *Nature.* 2011; 475:59–64. [PubMed: 21716286]
23. van Dongen EM, Klomp LW, Merkx M. *Biochem Biophys Res Commun.* 2004; 323:789–795. [PubMed: 15381069]
24. Lim CM, Cater MA, Mercer JF, La Fontaine S. *Biochem Biophys Res Commun.* 2006; 348:428–436. [PubMed: 16884690]
25. Lim CM, Cater MA, Mercer JF, La Fontaine S. *J Biol Chem.* 2006; 281:14006–14014. [PubMed: 16554302]
26. Gupta A, Schell MJ, Bhattacharjee A, Lutsenko S, Hubbard AL. *J Cell Sci.* 2016; 129:1179–1189. [PubMed: 26823605]
27. Nyasae LK, Schell MJ, Hubbard AL. *Traffic.* 2014; 15:1344–1365. [PubMed: 25243755]
28. Lalioti V, Peiro R, Perez-Berlanga M, Tsuchiya Y, Munoz A, Villalba T, Sanchez C, Sandoval IV. *J Cell Sci.* 2016; 129:2190–2201. [PubMed: 27034138]
29. Gupta A, Bhattacharjee A, Dmitriev OY, Nokhrin S, Braiterman L, Hubbard AL, Lutsenko S. *Proc Natl Acad Sci U S A.* 2011; 108:5390–5395. [PubMed: 21406592]
30. Santhosh S, Shaji RV, Eapen CE, Jayanthi V, Malathi S, Finny P, Thomas N, Chandy M, Kurian G, Chandy GM. *World J Gastroenterol.* 2008; 14:4672–4676. [PubMed: 18698682]
31. Wu ZY, Wang N, Lin MT, Fang L, Murong SX, Yu L. *Arch Neurol.* 2001; 58:971–976. [PubMed: 11405812]
32. de Bie P, van de Sluis B, Burstein E, van de Berghe PV, Muller P, Berger R, Gitlin JD, Wijmenga C, Klomp LW. *Gastroenterology.* 2007; 133:1316–1326. [PubMed: 17919502]
33. Gupta A, Lutsenko S. *Curr Genomics.* 2012; 13:124–133. [PubMed: 23024604]
34. Abdelghaffar TY, Elsayed SM, Elsobky E, Bochow B, Buttner J, Schmidt H. *J Hum Genet.* 2008; 53:681–687. [PubMed: 18483695]
35. Caca K, Ferenci P, Kuhn HJ, Polli C, Willgerodt H, Kunath B, Hermann W, Mossner J, Berr F. *J Hepatol.* 2001; 35:575–581. [PubMed: 11690702]
36. Cox DW, Prat L, Walshe JM, Heathcote J, Gaffney D. *Hum Mutat.* 2005; 26:280.

37. Kroll CA, Ferber MJ, Dawson BD, Jacobson RM, Mensink KA, Lorey F, Sherwin J, Cunningham G, Rinaldo P, Matern D, Hahn SH. *Mol Genet Metab.* 2006; 89:134–138. [PubMed: 16644258]
38. Loudianos G, Dessi V, Lovicu M, Angius A, Nurchi A, Sturniolo GC, Marcellini M, Zancan L, Bragetti P, Akar N, Yagci R, et al. *Hum Mutat.* 1998; 12:89–94. [PubMed: 9671269]
39. Braiterman L, Nyasae L, Guo Y, Bustos R, Lutsenko S, Hubbard A. *Am J Physiol: Gastrointest Liver Physiol.* 2009; 296:G433–G444. [PubMed: 19033537]
40. Guo Y, Nyasae L, Braiterman LT, Hubbard AL. *Am J Physiol: Gastrointest Liver Physiol.* 2005; 289:G904–G916. [PubMed: 15994426]
41. Hasan NM, Gupta A, Polishchuk E, Yu CH, Polishchuk R, Dmitriev OY, Lutsenko S. *J Biol Chem.* 2012; 287:36041–36050. [PubMed: 22898812]
42. Braiterman LT, Gupta A, Chaerkady R, Cole RN, Hubbard AL. *J Biol Chem.* 2015; 290:8803–8819. [PubMed: 25666620]
43. Braiterman LT, Murthy A, Jayakanthan S, Nyasae L, Tzeng E, Gromadzka G, Woolf TB, Lutsenko S, Hubbard AL. *Proc Natl Acad Sci U S A.* 2014; 111:E1364–E1373. [PubMed: 24706876]
44. Lalioti V, Hernandez-Tiedra S, Sandoval IV. *Traffic.* 2014; 15:839–860. [PubMed: 24831241]
45. Prasad R, Kaur G, Kumar S, Ram Thapa B. *J Gastroenterol Hepatol.* 2005; 20:660–662. [PubMed: 15836726]
46. Jain S, Farias GG, Bonifacino JS. *Mol Biol Cell.* 2015; 26:218–228. [PubMed: 25378584]
47. Braulke T, Bonifacino JS. *Biochim Biophys Acta.* 2009; 1793:605–614. [PubMed: 19046998]
48. Polishchuk EV, Concilli M, Iacobacci S, Chesi G, Pastore N, Piccolo P, Paladino S, Baldantoni D, van IJendoorn SC, Chan J, Chang CJ, et al. *Dev Cell.* 2014; 29:686–700. [PubMed: 24909901]
49. Weisz OA, Rodriguez-Boulon E. *J Cell Sci.* 2009; 122:4253–4266. [PubMed: 19923269]
50. Demareux N. *News Physiol Sci.* 2002; 17:1–5. [PubMed: 11821527]
51. Beyenbach KW, Wiczorek H. *J Exp Biol.* 2006; 209:577–589. [PubMed: 16449553]
52. Wang T, Weinman SA. *Gastroenterology.* 2004; 126:1157–1166. [PubMed: 15057754]
53. Harada M, Sakisaka S, Terada K, Kimura R, Kawaguchi T, Koga H, Taniguchi E, Sasatomi K, Miura N, Suganuma T, Fujita H, et al. *Gastroenterology.* 2000; 118:921–928. [PubMed: 10784591]
54. Pena K, Coblenz J, Kiselyov K. *Cell Calcium.* 2015; 57:257–262. [PubMed: 25620123]
55. Gupta A, Schell MJ, Bhattacharjee A, Lutsenko S, Hubbard AL. *J Cell Sci.* 2016; 129:1179–1189. [PubMed: 26823605]
56. Cater MA, La Fontaine S, Shield K, Deal Y, Mercer JF. *Gastroenterology.* 2006; 130:493–506. [PubMed: 16472602]
57. de Bie P, van de Sluis B, Klomp L, Wijmenga C. *J Hered.* 2005; 96:803–811. [PubMed: 16267171]
58. Su LC, Ravanshad S, Owen CA Jr, McCall JT, Zollman PE, Hardy RM. *Am J Physiol.* 1982; 243:G226–G230. [PubMed: 7114265]
59. Gupta A, Chattopadhyay I, Mukherjee S, Sengupta M, Das SK, Ray K. *Behav Brain Funct.* 2010; 6:33. [PubMed: 20550661]
60. Tao TY, Liu F, Klomp L, Wijmenga C, Gitlin JD. *J Biol Chem.* 2003; 278:41593–41596. [PubMed: 12968035]
61. Burkhead JL, Morgan CT, Shinde U, Haddock G, Lutsenko S. *J Biol Chem.* 2009; 284:696–707. [PubMed: 18940794]
62. Phillips-Krawczak CA, Singla A, Starokadomskyy P, Deng Z, Osborne DG, Li H, Dick CJ, Gomez TS, Koenecke M, Zhang JS, Dai H, et al. *Mol Biol Cell.* 2015; 26:91–103. [PubMed: 25355947]
63. van de Sluis B, Groot AJ, Wijmenga C, Vooijs M, Klomp LW. *Cell Cycle.* 2007; 6:2091–2098. [PubMed: 17786049]
64. Materia S, Cater MA, Klomp LW, Mercer JF, La Fontaine S. *J Biol Chem.* 2011; 286:10073–10083. [PubMed: 21242307]
65. Materia S, Cater MA, Klomp LW, Mercer JF, La Fontaine S. *J Biol Chem.* 2012; 287:2485–2499. [PubMed: 22130675]
66. Walker JM, Tsivkovskii R, Lutsenko S. *J Biol Chem.* 2002; 277:27953–27959. [PubMed: 12029094]

67. Hamza I, Schaefer M, Klomp LW, Gitlin JD. Proc Natl Acad Sci U S A. 1999; 96:13363–13368. [PubMed: 10557326]
68. Jayakanthan S, Braiterman LT, Hasan NM, Unger VM, Lutsenko S. J Biol Chem. 2017; 292:18760–18774. [PubMed: 28842499]
69. Heitkamp T, Kalinowski R, Bottcher B, Borsch M, Altendorf K, Greie JC. Biochemistry. 2008; 47:3564–3575. [PubMed: 18298081]

Fig. 1.

The ATP7B topology showing the conserved domain and the direction of copper transport. The six metal binding domains (MBD) are denoted by purple beads. The A-domain, the nucleotide binding domain (N) and phosphorylation domain (P) with its conserved DKTG motif are illustrated. Copper is pumped from the cytosolic face of ATP7B that consists of these conserved motifs, to the luminal side. ATP7B undergoes recycling between E1 and E2 states coupled with ATP hydrolysis, and copper transport cycle is illustrated in the inset.

Fig. 2.

The apical trafficking pathway of ATP7B and the WD mutations that affect the delivery of ATP7B to the right compartments. ATP7B exits the TGN at high copper concentrations and is finally delivered to the apical surface through multiple compartments. WD causing mutations as well as synthetic mutations at different domains arrest the proteins at distinct compartments and prevent its trafficking to the next step. Alternatively, the mutations cause complete mislocalization of the protein. The mutations are shown in red and the red triangles represent their respective trafficking phenotypic defects.

Table 1
ATP7B interacting partner at physiologically elevated copper levels

ATP7B domain	Interacting partner	Functional implication	Ref.
Amino terminal Cu binding motif (MXCXXC)	COMMD1	Aids in Cu excretion by directing the apical route of ATP7B	64 and 65
	Glutaredoxin (GRX1)	Facilitates Cu binding through reduction of cysteine disulphide bonds (Cys–Cys) in the MXCXXC motif	24
	Atox1 (specifically the 1–4 metal binding domain, MBD)	Cu delivery to the MXCXXC motif	66
	p62 subunit of dynactin (specifically the 4–6 metal binding domain, MBD)	Cu induced lysosomal exocytosis	25
Carboxy terminal Di-leucine motif (DKWSLLL)	Clathrin adaptor protein 1 (AP1), σ subunit	Somatodendritic polarized redistribution of ATP7B in rat primary neuron	46
	σ subunit adaptor protein 2 (AP2)	Polarised sorting of ATP7B at the apical membrane <i>via</i> the basolateral route	28
Domain unidentified but binds strongly with the amino terminal truncated ATP7B	Apolipoprotein J protein, 'Clusterin'	Facilitates lysosomal degradation of misfolded/mutated ATP7B under oxidative stress	64
Transmembrane and c-terminus	Transmembrane and c-terminus of another ATP7B	Stable dimer	68