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Molecular mechanisms of copper homeostasis

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1. ABSTRACT

The transition metal copper (Cu) is an essential trace element for all biota. Its redox properties bestow Cu with capabilities that are simultaneously essential and potentially damaging to the cell. Free Cu is virtually absent in the cell. The descriptions of the structural and functional organization of the metallothioneins, Cu-chaperones and P-type ATPases as well as of the mechanisms that regulate their distribution and functioning in the cell have enormously advanced our understanding of the Cu homeostasis and metabolism in the last decade. Cu is stored by metallothioneins and distributed by specialized chaperones to specific cell targets that make use of its redox properties. Transfer of Cu to newly synthesized cuproenzymes and Cu disposal is performed by the individual or concerted actions of the P-type ATPases ATP7A and ATP7B expressed in tissues. In mammalians liver is the major captor, distributor and excreter of Cu. Mutations in the P-type ATPases that interfere with their functioning and traffic are cause of the life-threatening Wilson (ATP7B) and Menkes (ATP7A) diseases.

2. INTRODUCTION

The indispensability of Cu arises from its redox properties and specific incorporation into a diverse but limited number of enzymatic and structural proteins (1). Cu displays four oxidation states: metallic Cu, cuprous ion Cu^{+}, cupric Cu^{2+} and trivalent Cu ion Cu^{3+}. The electronic/oxidation state of Cu deeply conditions its biochemistry since no other element provides to proteins the redox properties embodied in Cu. Any Cu^{+} present in an aqueous environment is quickly oxidized by the oxidizing agents present unless it is stabilized by complex formation. Cu functions as an electron transfer intermediate in reactions involved in cellular respiration (2, 3), free radical defence (4-7), cellular iron metabolism (8, 9), synthesis of connective tissue (10), pigmentation (11), blood clotting (12, 13), peptide hormone production (14) and synthesis of neurotransmitters (15). In addition, Cu functions as a cofactor of enzymes and operates as an allosteric component that confers into proteins diverse structures (16-19) . Overall Cu is essential for normal cell growth and development. While Cu^{+} prefers sulphur

Met/Cys, Cu^{++} uses nitrogen His or oxygen Glu/Asp as ligands (20)

Excessive Cu exposure is toxic. In the cell, under normal conditions, free Cu is virtually nonexistent. Is the redox chemistry of Cu and its capacity to catalyze the transformation of a superoxide radical anion ($^{\bullet}O^{-}_{2}$) to the highly reactive hydroxyl radical ($^{\bullet}OH$) via the metal-catalyzed reaction what makes Cu a potentially toxic metal (21).

$$^{\bullet}O^{-}_{2} + Cu^{2+} \rightarrow O_2 + Cu^{+}$$

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{-} + {}^{\bullet}OH \quad \text{(Fenton reaction)}$$

In addition, the toxicity of Cu also results of its ability to displace other metal cofactors, mainly Zn, as well as to bind ectopically and damage proteins (22). The need for Cu and its potential toxicity has forced the development in cells and organisms of refined homeostatic mechanisms that conserved through evolution maintain Cu levels within a physiological range, avoiding both deficit and excess (22).

3. Ctr1 AND Cu UPTAKE

Transfer of Cu across the plasma membrane into the cell is driven by the highly specific Cu translocase Ctr1 (Km for Cu^{+}, 1 μM). Ctr1 is conserved from yeast to humans (23, 24). In yeast, Cu^{2+} is reduced by plasma membrane reductases before its transport as Cu^{+} by the yCrt1 and yCrt3 transporters (25). Two other forms, Ctr2 in *S. cerevisiae* and Ctr6 in *S. Pombe (*26, 27), serve to mobilize vacuolar Cu stores to cytosolic Cu-chaperones (28, 29). Human hCtr1 was identified in complementation studies of Cu uptake performed in yeast with a *yctr*1/ *yctr*2 phenotype (24). The 44.4 kD hCrt1 is an ubiquitous, highly glycosylated, three transmembrane domain protein. Assembly of hCtr1 into homotrimers results in the formation of membrane pores (30).

A MX_3M motif in the second transmembrane domain of Ctr1participates in the uptake of Cu^{+} ions, but is not involved in delivery of the transporter to the cell surface (31). Cu transport by Ctr1 does not require ATP, is saturable and time dependent (32). The observation in yeast that Cu uptake by Ctr1 is coupled with K^{+} efflux may indicate that is mediated by a $Cu^{+}/2K^{+}$ antiport mechanism (32). In addition, Cu^{+} uptake is further facilitated by the functioning of cellular chaperones and the absence of free Cu ions in the intracellular medium. In mammalian Ctr1, the partly protonation of the His^{139} residue close to the exit site of the pore may narrow the pore and slow down the transport process, therefore impeding the transfer of the ion (33). *In vitro* studies performed in yeast have also shown that a pair of well conserved Cys and His residues in the C-cytoplasmic domain of yCtr1 facilitate the transfer of Cu^{+} to the Atx1 chaperone for further distribution (34, 35). To note, cells with reduced glutathione (GSH) levels are slower in Cu uptake from medium and as result have lower steady-state Cu concentrations.

Transfection studies expressing epitope-tagged constructs of Ctr1 made known that the translocase constitutively recycles between the plasma membrane and endosomes and uses the $M_{4\text{-}3}XM$ cluster near the transmembrane region for endocytosis (36). Furthermore, clathrin-dependent endocytosis of Ctr1 is rapidly stimulated by Cu concentrations near the Ctr1 Km for transport, and the Cu binding to the transmembrane MX_3M motif (36, 37). The rapid lysosomal degradation of endocytosed Ctr1 in response to excess intracellular Cu provides a rapid mechanism to regulate high-affinity Cu uptake that may be significant in the uptake of dietary Cu (36, 37). The observation that Ctr1 is predominantly apical in the intestinal cells of the new born, while most of it is sequestered into endosomes in the adult, probably reflects differences in the levels of dietary Cu (38).

The ability of Ctr1-/- deficient cells to accumulate Cu (39) and the observation that Ctr1+/- mice without obvious growth or developmental abnormalities show a striking tissue-specific reduction in Cu levels, point to the functioning of Ctr1-independent Cu uptake systems in some tissues (40, 41).

4. GSH AND METALLOTHIONEINS, Cu SEQUESTRATION AND STORAGE

The results of kinetic studies of ^{67}Cu uptake showing that GSH binds ^{67}Cu before the metal is complexed by metallothioneins (MT) and that depletion of cellular GSH inhibits the incorporation of ^{67}Cu into MT, support a model in which Cu is complexed by GSH, the most abundant low-molecular-weight thiol, immediately after entering the cell and then is transferred from the intermediate to the MT (42, 43). The small 3.5-14 kD Cyst-rich MT Cu acceptors are found in virtually all biota. Their extreme versatility in the capacity to fight stress situations of different origin is proverbial and makes of them an invaluable tool in cell homeostasis (44-47). Their unique homeostatic capacity resides in the arrangement of its many Cyst into CC, CXC and CX_2C sequences with potent metal binding and redox capabilities. Whereas the 6kD class 1 metallothioneins (MT1) chelate four divalent ions within cluster A of the alpha domain and three divalent ions within cluster B of the beta domain, the 7 kD class 3 metallothioneins (MT3) bind heavy metals and contains three Zn^{2+} and three Cu^{+} atoms per polypeptide chain and a negligible amount of Cd^{2+}. The extraordinary stability (stability constant, 10^{-19}–10^{-17}) and the high binding stoichiometry of the Cu^{+}-MT complexes (>7 Cu^{+}/mole) explains the remarkable effectiveness of MT in functioning as Cu scavengers in the cell (48). Although the Cu toxicity has not been investigated to any extent in MT-/- mice, the high sensitivity of the yeast and Drosophila with disrupted MT genes to high Cu concentrations strongly suggests that MT play an important role in the detoxification of Cu in mammals (49, 50). While the high stability of the Cu^{+}-MT complexes would argue against a role of MT as temporary Cu-storage/donors, harnessing toxic Cu for biologically useful roles may have provided an advantage in evolution. *In vivo* studies have shown in Drosophila that the induction of the transcription of MT genes and the accumulation and

detoxification of high amounts of Cu in the gut, is later used to donate controlled amounts of the sequestered Cu to tissues in need of the metal during Cu scarcity (51). Furthermore, the disappearance of the Cu luminescence that marks sites of Cu accumulation in the larval midgut and fades within 10 h upon withdrawal of Cu from the food, is also consistent with the view that Cu is not irreversibly trapped in Cu-binding MT in intestinal cells. In other examples of the role of MT in Cu homeostasis, the land snail *Helix pomatia* uses the MT isoform in the mantle to presumably regulate the Cu supply to hemocyanin (52) and in a cell-free system Cu-loaded MT can donate Cu to the Cu/Zn superoxide dismutase (53). From the inwards rotation of the cysteinyl sulfurs exposed at the surface of the metal domains upon the metal binding, it follows that modifications that induce the outwards rotation may play a role in regulating the metal release (54). On the other hand, the Cu accumulation in mice intestinal epithelial cells with the Ctr1 selectively silenced is followed by a rise in the levels of Cu-chaperones and a reduction in the Cu-ferroxidase acceptor hephaestin. This cause-effect relationship points that the Cu taken up by Ctr1-alternative mechanisms and transferred to MT may not be always bioavailable (55). Furthermore, the ability of zinc to induce tissue MT and the formation of tight Cu^+-MT complexes appear to explain that the oral administration of Zn acetate reduces Cu absorption in the intestine and prevents the iron-cause acute hepatitis in Long-Evans Cinnamon (LEC) rats by decreasing the hephaestin levels (56).

5. CHAPERONE-BASED Cu HOMEOSTASIS AND DISTRIBUTION

Cu trafficking in the cytoplasm is mediated by a small set of specialized chaperones that serve to specific cuproproteins and simultaneously provide the cell with protection against exposure to the Cu ions in transit. The Ccs chaperone conveys Cu^+ to the Cu/Zn superoxide dismutase (57), Cox17 to the cytochrome c oxidase (COX) complex in the mitochondria (58-60), and the yAtx1/mAtox1chaperone to the P-type ATPases Ccc2p/ ATP7A/ATP7B inserted into the TGN and plasma membrane (61-63) (Figure 1).

Cu-chaperones bind the metal as Cu^+ and with the exception of Cox17, which uses a C_2XC motif to bind three Cu^+ atoms arranged in a polyCu cluster (64), the akin-metal chaperones use a $MXCX_2C$ domain to bind Cu^+ (65). Proton nuclear magnetic resonance studies show that the majority of the Cu-chaperones have an "open-faced beta-sandwich" global fold with a conserved $MXCX_2C$ metal-binding motif (65, 66). Two different models propose that Cu^+ is bound digonally o trigonally to the $MXCX_2C$ motif through the Cys residues, using in the trigonal coordination GSH or a His/Cys residue as the third Cu ligand (67). Is remarkable the different metal specificity of the same $MXCX_2C$ within different metal chaperones, probably the result of variations in the local environment and disparities in their tertiary structure (68, 69).

5.1. Via to the Cu/Zn superoxidase dismutase Sod1

The 27 kD yeast LYS7 and the 29 kD mammalian Ccs are soluble chaperones with the identical role of feeding the apo-Sod1 with Cu (70). They are organized in three highly conserved distinct domains. Whereas the N-Atox1 like domain, homologous to the Atox1 chaperone functioning in the P-type ATPase via, provides the $MXCX_2C$ motif that binds Cu^+, the intermediate Sod-like domain interacts with Sod1, and the short C-terminal domain uses a CXC motif to transfer the Cu^+ ion to Sod1. Transfer occurs previous formation of a Cu^+ cluster within a Ccs dimer/tetramer and the physical contact between the two proteins (71-73). The failure of yeast with normal levels of Sod1 and a *lys7*D null mutation to incorporate ^{64}Cu into Sod1, and the capacity of LYS7 to complement the defect *in vivo*, demonstrate that LYS7 is essential for Cu incorporation into Sod1 (57). The reduced incorporation of ^{64}Cu into Sod1 in Ccs (-/-) mice and the development of phenotypes characteristic of So1 (sensitivity to paraquat, reduced fertility), in the absence of abnormalities in Cu uptake and incorporation into other cuproproteins, demonstrate that Ccs is essential in the specific transfer of Cu to Cu/Zn Sod1 (74). Yet, there is evidence indicative of the functioning of a Ccs-independent mechanisms for Cu loading onto Sod1 in the human and *C. Elegans*. The Ccs-independent pathway appears to involve GSH as Cu donor and forms of Sod1 lacking two conserved Pro residues that may preclude the docking of the Cu loaded GSH to Sod1 (75-77).

In some mammalian cells the 32 kD homodimeric Sod1 that is expressed in all eukaryotes and certain prokaryotes, makes 1-2 % of the total protein (78, 79). The localization of Sod1 to the cytosol, nucleus and the intermembrane space of mitochondria, reflects the importance and amplitude of its protective function in the cell. The Sod1 loaded with two Cu^+ ions catalyses the dismutation of $^{\bullet}O^-_2$ by two successive encounters of the anion with the redox-active Cu^+ site (80).

$$Cu^+ + {}^{\bullet}O^-_2 + 2H^+ \rightarrow Cu^{2+} + H_2O_2$$

The superoxide anion radical ($^{\bullet}O^-_2$) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly (~10^5 M^{-1} s^{-1} at pH 7), but the catalytic activity of the Sods is biologically essential because $^{\bullet}O^-_2$ reacts even faster with certain targets and this may damage their biological activity. The benefit of the superoxide dismutation reaction is therefore to lower the steady-state concentration of the free $^{\bullet}O^-_2$ and moderate its damaging potential in the cell. It is remarkable, that while the absence of Cu increases the rate of degradation of other Cu-chaperones, in the cell one-third of the total Sod1 exists as stable apoprotein and the transfer of Cu by Ccs plays a main role in controlling the activity of the enzyme. This explains that in the Ccs (-/-) mouse the levels of Sod1 are normal and the enzymatic activity is markedly reduced (81).

5.2. Via to mitochondrial cytochrome c oxidase (Cox)

Genetic studies of yeast mutants unable to assemble the mitochondrial COX complex have been decisive to better understand the mechanism of Cu delivery

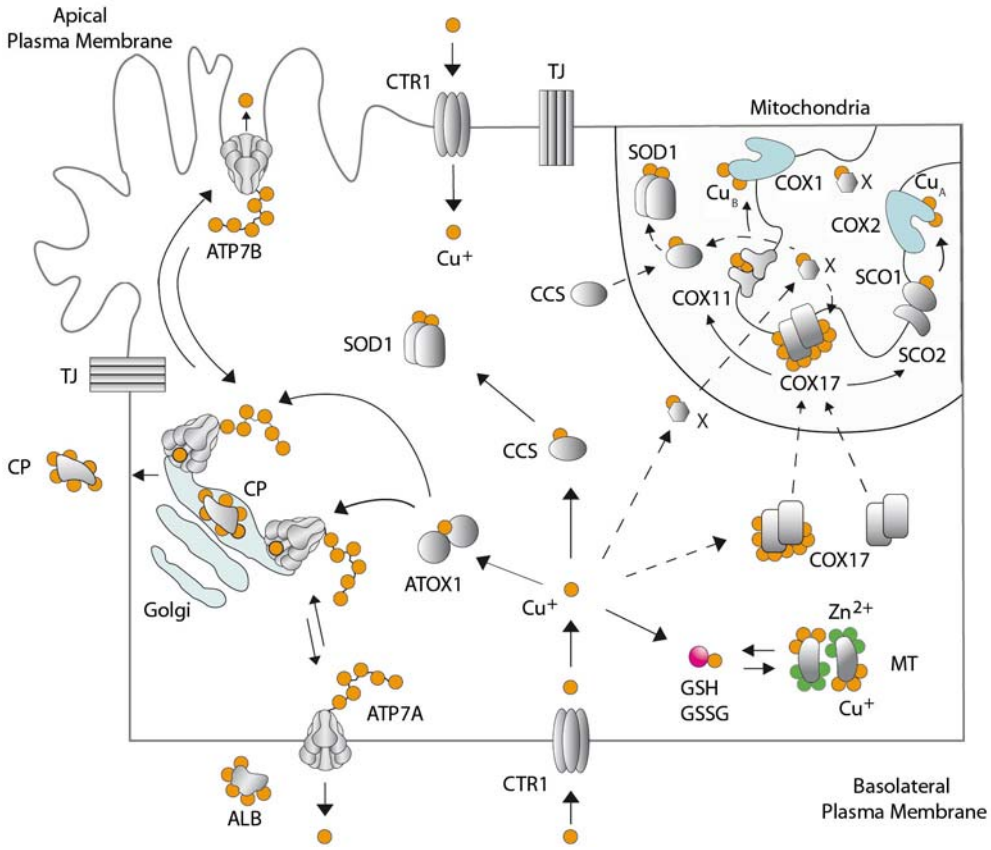

Figure 1. Cellular Cu distribution by chaperones. Dual role of Cu-P-type ATPases in the biosynthesis of cuproenzymes and Cu excretion. The cartoon depicts the ingress, distribution and egress of Cu in an epithelial cell with the attributes of both an intestinal mucosal cell and the hepatocyte. Proteins are referred in the cartoon by their gene symbols The apical and basolateral plasma membrane domains are separated by the tight junctions (TJ). The transporter Ctr1 facilitates the uptake of Cu^+ in the apical (intestinal epithelial cell) and basolateral (hepatocyte) plasma membrane. In the cytoplasm the Cu^+ ion is either stored in a complex with metallothioneins (MT), in a reaction probably mediated by GSH, or is captured and distributed by specific chaperones. The Ccs chaperone conveys the Cu^+ to the Cu/Zn superoxide dismutase Sod1/2; Cox17 to the Cu_A and Cu_B sites of mitochondrial COX, via Sco1-2, and Cox11, respectively; and, Atox1 to the P-type ATPases ATP7A/ATP7B trafficking between the TGN and plasma membrane, where they perform their biosynthetic and secretory roles. Cu ions used in the metallation of COX and Sod1 appear to bind either Cox17 and Ccs in the cytoplasm, or ingress into a novel Cu pool within the mitochondrial matrix via an unidentified factor (X) before their transport to the Cox17 and Ccs targets in the intermembrane space. Whereas ATP7B and the cuproferroxidase ceruloplasmin (CP) are targeted to the apical and basolateral membrane, respectively, ATP7A is targeted to the basolateral membrane where it supplies Cu to the albumin (ALB) and transcupreine in the circulation. Established and alternative Cu pathways are marked with solid and discontinuous arrows, respectively.

to the mitochondria. COX is a large inner integral mitochondrial membrane protein composed of 13 protein multisubunits. COX functions as a heme-Cu terminal oxidase and in the electron transport system of the respiratory chain is crucial in creating the electrochemical gradient that generates the driving force required for ATP synthesis. COX has two Cu redox centers, Cu_A in subunit I and Cu_B in subunit II. The Cu_A and Cu_B sites bind four and two Cus, respectively. The proper assembly of COX complex requires the import of Cu from the cytoplasm. Four proteins, Cox17 and the downstream Sco1-2 (58, 59, 82) and Cox11 (60) participate in the Cu insertion into the two Cu redox centers. Cox17 functions as a Cu donor of the Sco1 and Cox11 proteins and then Sco1 and Cox11 deliver the Cu to the Cu_A and Cu_b sites, respectively (83, 84). In yeast strains with *Cox17, Sco1/2 and Cox 11* defects and in human cells from *SCO1/2*-defficient patients the assembly of COX complex is aborted (58, 85, 86).

5.2.1. Cox17

The 8kD small Cox17 is a cysteine-rich cytoplasmic protein that *in vitro* exists in a dimer-tetramer equilibrium. Sequence and genetic studies as well as spectroscopic analyses have shown that Cox17 uses a C_2XC motif to coordinate Cu^+ ions. Cox17 binds Cu with great affinity. Recent studies indicate that metallation of Cox17 occurs in the intermembrane space of the mitochondria, but is unclear how Cu makes its way from the cytosol to Cox17 (87). The pathway of Cu in the mitochondria is prolonged into the matrix that retains 90%

of the Cu in the mitochondria. The matrix Cu pool is dynamic, non-proteinaceous and is unclear if it is used as a reservoir, supplies Cox17 and Sod 1 with the metal or buffers the Cu changes in the cytosol.Cox17 becomes functional after its insertion into the inner mitochondrial membrane through its C-domain (88). Though the results of ESI and ESI-Ms of yeast and mammalian Cox17 are different (89, 90), it appears that the Cys residues of the C_2XC motif localized to its N-domain coordinate with the help of three other Cys residues 4 Cu^+ ions and form a Cu4S6-type cluster/molecule (89). The $4Cu^+$ cluster appears to facilitate the metal exchange with the Sco1 acceptor, but is not required for the Cox17 target to the mitochondria. Cox17 molecules with a double Cys/Ser mutation and no Cu^+ binding are still target to the intermembrane mitochondrial space (91), but the single substitution of the Cys residues aborts the COX complex assembly and results in loss of its activity (91).

5.2.2. Sco1, Sco2 and Cu_A

The rescue in a yeast *Cox17* mutant of the COX complex assembly and respiration defects by addition of Cu to the growth medium, suggested the existing of Cu-chaperones that function downstream Cox17 to handle the Cu^+ imported by Cox17 into the COX II subunit (58). The Sco1 and Sco2 Cu-chaperone paralogs functioning downstream Cox17 were isolated as high-copy number suppressors of the Cox17-induced defects in yeast (82). The 33 kD Sco1 and 35 kD Sco2 are inserted into the inner mitochondrial membrane with the N-end facing the matrix and the C-domain retained in the intermembrane space. They can bind a single Cu^+ or Cu^{2+} ions. While the Cu^+ ion is trigonally conjugated by a separate His and the two Cys residues in the conserved CXXXC motif, the Cu^{2+} ion binds to a type II Cu^{2+} site with a higher coordination number (92, 93).

Mutations in Sco1 result in COX deficiency, but no discernible phenotype has been associated with the SCO2 deletion, therefore suggesting that they play distinct roles in the pathway of Cu delivery (58). The direct Cu transfer from Cox17 to Sco1 in an *in vitro* purified protein system and the Cox17-dependent cuprenation of the soluble Sco1 domain expressed in the yeast cytoplasm, demonstrates that Cox17 transfers Cu to Sco1 (94). Furthermore, the failure of the COX17 overexpresion and of the Cu addition to the medium to rescue growth of yeast strains containing Sco1 mutations on a non-fermentable carbon source, firmly points that Sco1 functions downstream of Cox17 (82). Confirming these observations in yeast, the COX deficiency is not rescue by COX17 overexpresion in cells from *SCO1* patients (95). This failure and the recovery of the COX deficiency by the COX17 overexpresion in cells of *SCO2* patients, point that in human cells Sco1 and Sco2 proteins have non-overlapping functions in mitochondrial Cu delivery (95). Furthermore, the dominant-negative phenotype that results from the overexpression of either wild-type SCO protein in the reciprocal *SCO1/SCO2* patient background, suggests a physical interaction between Sco1 and Sco2. The Sco1 and Sco2 chaperones may therefore function cooperatively in the insertion of Cu into the Cu_A center of the COX II subunit (95).

The organization of the Cu_A center requires the binding of two Cu ions to two His and two Cys residues of the $Hx_nCXEXCGX_2$-HX_2M consensus motif (3, 96). Is interesting that Sco1/2 contain a thioredoxin fold characteristic of thiol:disulfide oxidoreductases (97), enzymes that catalyze the formation of disulfide bonds into unfolded reduced proteins with concomitant reduction of oxygen to hydrogen peroxide. The thioredoxin fold and the redox chemistry undergone by the conserved Cys148 and Cys152 of the CXXXC motif in ySco1 crystals (90), suggests that the Cys reduction of the COX II subunit by Sco1 may mediate the Cu insertion into the Cu_A center (97). Also interesting is the proposal that Cox19, a Cu^{+}-binding protein structurally similar to Cox17, which resides in the intermembrane space and is required for the assembly of cytochrome c oxidase, may contribute to Cu_A site formation as Cu donor (98).

A study of the completely sequenced prokaryotic genomes has shown that 18% of these prokaryotes have Sco but not Cu_A-containing proteins. This discrepancy, strongly suggests the Sco1/2 proteins may have in prokaryotes other functions (99). Recent studies in fibroblasts from Sco1 and Sco2-deficient patients show that the deficiency in COX exhibited by the fibroblasts of these patients can be rescue by the sobreexpression of a Sco2 E140K mutant, but not the remarkable reduction in total cellular Cu. This dissociation strongly points to a new role of Sco proteins in Cu hoemostasis (100). If Sco2 catalyzes the oxidation of Sco1 through its intrinsic oxidoreductase activity (see below) and facilitates the transfer of Cu to COX, in Sco2-defficient fibroblasts the proportion of Cu-loaded Sco1 would be expected to increase and signal a false state of Cu overload in the mitochondria that would turn on the mechanisms that facilitate cellular Cu excretion.

5.2.3. Cox11 and Cu_B

Whereas the Cox17/Sco1, Sco2 via feeds Cu into the Cu_A nucleus, the supply of Cu to the Cu_B nucleus in the COX I subunit is mediated by the separate Cox17/Cox11pathway (60). Structurally different of Cu_A, in the resting state Cu_B is formed by a Cu^{2+} ligated by three His residues in the binding motif $Hx_3Yx_{44}HH$ and the haeme $a3^{3+}$, and therefore forms a binuclear Cu/iron center (3). Evidence that Cox11 supplies Cu to Cu_B, is indicated by the absence of COX as result of defective heme A biosynthesis and COX assembly in a *cox11*Δ yeast strain (101, 102), and the lack of a functional Cu_B nucleus in the COX of these mutants (103). Furthermore, Cox11 is unable to functionally complement the COX deficiency in either *SCO1* or *SCO2*-deficient patient cells (95).

The 34 kD Cu-chaperone donor Cox11 is inserted to the inner mitochondrial membrane, and chromatography and sedimentation equilibrium studies of a TrxCox11 fusion suggest that Cox11 self-assembles into a homodimer (85). Membrane inserted Cox11 is arranged with the N-domain projected into the mitochondrial matrix and the C-domain exposed within the intermembrane space as shown by domain mapping studies (85, 104). In the dimer, the two

trigonally bound Cu^+ ions, in transit from Cox11 to Cox, are hold at the interface between the monomers by three conserved Cys, including the two Cys of the conserved CXC motif in the C-domain. As expected, mutation of any of the three Cys implicated in the coordination of Cu reduces Cu^+ binding and confers respiratory incompetence. Remarkably, the Cu^+-binding domain from human Cox11 cannot functionally replace the yeast sequence, probably because the interactions that the yeast domain establishes with Cox17 and/or COX (104). The importance of the supply of Cu for proper assembly of Cox1 is indicated by the specific reduction in the levels of the COX I subunit among the respiration deficiency-encoding alleles generated by random Cox11 mutagenesis, a reduction that can be explained by the misfolding of subunit I and its degradation in the absence of functional Cox11 (103). The transfer of Cu to the Cu_A and Cu_B centers through the Cox17-Sco1/2 and Cox17-Cox11 pathways appears therefore to facilitate a folding path that includes the complete organization of the catalytic core of COX, organization that conditions the final assembly of the 13 subunits mature COX holoenzyme.

The three redox-active Cu ions inserted into the two Cu_A and Cu_B centers of the mature holoenzyme play an essential role in the conversion of redox energy into a transmembrane proton motive force as the cooperative steps of H^+ pumping coupled to electron transfer at the low potential Cu_A-heme *a* site and proton consumption in the reduction of the O_2 reduction intermediates at the high potential heme a_3-Cu_B binuclear center, contribute to the chemiosmotic mechanism essential in ATP synthesis at the mitochondria (105).

5.3. Via to Cu-transporting ATPases

The function of the Atx1, Atox1/ Cu-P-type ATPases pathway is to supply Cu to newly synthesized cuproenzyes and at excess Cu to pump the metal out of the cell. The two proteins pathway is highly conserved from yeast to human.

5.3.1. Atx1, Atox1

In the first study of Atx1 in yeast, the 8 kD small Cu-chaperone was classified as an antioxidant molecule with ability to suppress the oxygen–dependent growth defects produced in cells with a sod1Δ null mutation (106). Its physiological role in the export of Cu from the cytosol into an extracytosolic compartment was subsequently clarified by the observation that cells with a null mutation in *Atx1* showed impeded iron uptake-linked to Fet3, a Cu oxidase which acquires the metal in the lumen of the trans-Golgi via the Cu pump Ccc2 inserted in its membrane. Atx1 and its human homologue Atox1 (also called HAH1) have the ability to shuttle the Cu^+ taken up through Ctr1 to Ccc2 and the mammalian Cu-P-type ATPases, Cu pumps that use the energy of ATP hydrolysis to move Cu^+ through cellular membranes.

Atx1 and Atox1 are soluble proteins that in the cytoplasm are organized as homodimers and bind one atom of Cu^+ with a linear, two coordinated geometry to the Cys residues of a conserved N-domain $MXCX_2C$ motif. Nevertheless, according to recent data Atx1, Cu (I) and GSH form a high-affinity complex that involves the Atx1 dimer and its two cysteines (107). It appears, as modelling based in X-ray absorption studies of Atox1 suggest, that after the docking of the chaperone to the acceptor Ccc2/P-type ATPase, an event that involves ionic interactions between oppositely charged faces of the proteins (108), the ion is transferred to the Cu^+-binding domain (MBD) of the Cu-P-type ATPase through a three coordinated intermediate (109). The Cu-dependent physical interaction between the C-end of Atox1 and mammalian P-type ATPases has been directly demonstrated using a yeast and mammalian two-hybrid systems in combination with an *in vitro* assay, studies that have proven the involvement of the two Cys residues of the MTCXXC motif and at least 15 of the C-terminal residues of Atox1 (62). The possibility that the transfer of Cu is regulated has been raised by evidence that FKBP52, an immunophillin, stabilizes Atox1in a conformation that stimulates Cu efflux and favours the metal transfer (110). The collaborative effort between Atox1 and the P-type-ATPase ATP7A implicated in the translocation of Cu into the circulation, explains that the phenotype of the Atox1-deficient mice is the one produced by the inability to traffic Cu across the placenta in embryos, and in runted surviving mice across the intestinal mucosa (111).

6. Cu-TRANSPORTING ATPASES

Ccc2 and mammalian P-type ATPases have two and six MBDs, respectively, and while they are functionally equivalent, Ccc2 is exclusively localized at the *trans*-Golgi network (TGN) and serves to deliver Cu to the secretory pathway, while the two mammalian Cu-P-type ATPases, ATP7A and ATP7B, add the function of pumping out excess Cu through the plasma membrane (112-114) for a review see (115).

The Cu-P-type ATPases, ATP7A and ATP7B, belong to the heavy metal transport P_{1B}- ATPases, a subfamily of the P-type ATPases with a 6-8 transmembrane topology and metal binding sites as well as characteristic sequence motifs at the N- and C-cytoplasmic domains (Figure 2). The 180 kD ATP7A and 160 kD ATP7B share 67% protein identity and are large proteins structurally and functionally organized in separate modules (116). ATP7A is glycosylated, but ATP7B is not. The long N-cytoplasmic domain of the two translocases contains six Cu^+ binding domains (MBDs 1-6) and plays a mayor role in the acceptance of Cu^+ through its interaction with Atox1 (61, 62, 109, 117) as well as in controlling their phosphorylation, traffic and activity (118-120). Downstream, the clustering of eight transmembrane helixes forms the Cu^+ pore, and at the connecting loops in the cytoplasmic side of the transmembranes are localized the nucleotide binding (N)-, phosphorylation (P)- and phosphatase domains implicated in the cyclic phosphorylation of the translocase (Figure 2).

Critical to the functioning and cellular distribution of the Cu-P-type ATPases is the coordinated

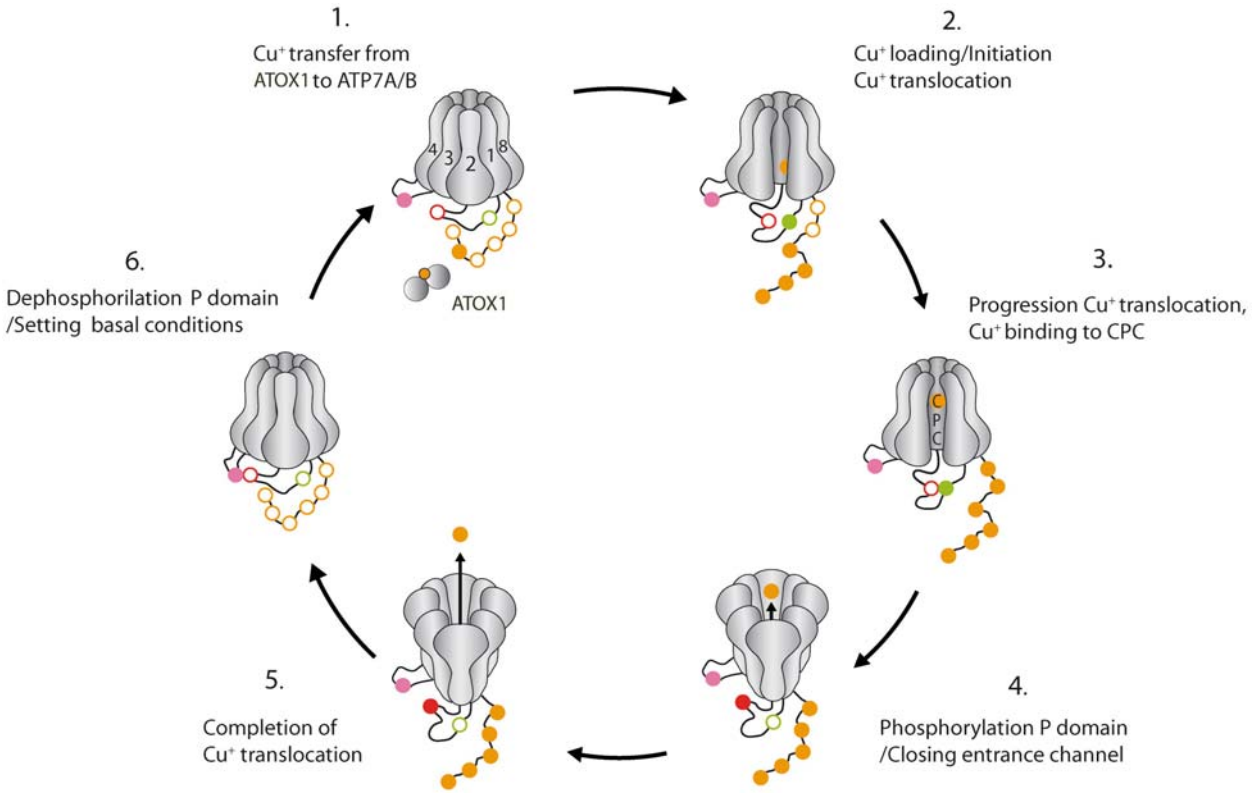

Figure 2. Cyclic phosphorylation-dephosphorylation of P-type ATPases and vectorial transport of Cu through the ion channel. Contact of the P-type ATPase with the Cu donor chaperone Atox1 and transfer of Cu^+ (●) involves at least MBD 2 (○) (1). Filling of MBDs 1-4 with Cu^+ (●) and the resulting changes in structure facilitate the binding of ATP to the N-site (○), brings the bound ATP (●) in close proximity to the phosphorylation P-site (○) and results in coordination of a Cu^+ atom by two juxtaposed Cys in the plane of TM1 and TM2 (2). Filling of MBDs 5, 6 with Cu favours the transfer of a Cu^+ atom to the CPC motif in TM6 (3). Phosphorylation of the Asp residue in the P-site (●) closes the access to the channel from the cytoplasm and may accommodate the exofacial vestibule to the reception of the translocated Cu^+ (4). Release of Cu^+ at the opposite side of the membrane (5) and the activation of the phosphatase in the A-site (●) completes the phospho-dephosphorylation cycle (○) and resets the channel to basal conditions. According to data compiled from (68, 108, 121, 123, 124,126, 123).

interaction between their separate modules. Each of the six MBDs lined up in their N-domain can bind one atom of Cu^+ (68, 121), but genetic and biochemical studies indicate that the MBDs are not functionally equivalent. Site-directed mutagenesis and cysteine protection studies indicate that among the six MBD, the MBDs 2 and 6 play a critical role in the Cu-dependent interaction of ATP7B with Atox1 and consequently in the transfer of Cu^+ to the translocase (108) (61). Given the greater affinity of isolated MBD 4 for the ion is probably that the MBD 2 preference is established by the greater accessibility of MBD 2 or its higher ion affinity within the intact protein. Furthermore, the N-extension of mammalian Cu P-type ATPases with four extra MBDs 1-4 and the stimulation of the ATP binding by either their loading with Cu^+ or truncation, suggest that the N-extension may regulate negatively the translocase activity (122, 123) (Figure 2). Equally interesting is that the binding of Cu^+ to MBDs 5 and 6 stabilizes a conformation that favours the Cu^+ binding to the CPC sequence in the 6 transmembrane segment, a critical event in the translocation of Cu through membranes (123, 124) (Figure 2). Nevertheless, some functional redundancy among the six amino terminal MBSs appears to exist since the number of missense mutations within the amino terminal tails of ATP7A and ATP7B that are cause of Menkes and Wilson disease is relatively low (125).

With regard to the Cu transfer through the membrane, there is evidence that in the first transmembrane hairpin (TMS1,2), which is unique for the P_{1B}-subfamily of ATPases, two Cys residues juxtaposed in the plane of the membrane appear to play a role in guiding Cu to the CPC site (126) (Figure 2).

The Cu binding events and the formation of the Cu^+-CPC complex initiate the phosphorylation/ dephosphorylation cycle that is characteristic of all the P-type ATPases and couples Cu-transfer and translocase traffic (127-129) (Figure 2). The conformational changes resulting from the ATP binding to the N-site appear to bring this in close proximity to the P-domain facilitating the transfer of ATP and the phosphorylation of the P-domain (130) (Figure 2).

Using purified recombinant protein fragments, ATP binding to both the N- and P-domain of ATP7B has been demonstrated and though the N-domain of ATP7B retains the common core structure of P-type ATPases, the nucleotide coordination environment within this fold is different and a role of the residues H1069, G1099, G1101, I1102, G1149, and N1150 in ATP binding has been established (131). Among the invariant ATP7B residues, mutations in E1064A and H1069Q drastically reduce nucleotide affinities (122, 131).

The phosphorylation of the Asp residue in the DKTG motif of the P domain is a critical event since it closes the access to the Cu-CPC complex within the membrane from the cytoplasmic side, and the closing is the first step that drives the vectorial transport of Cu through the membrane (132-134) (Fig.2). Consistent with this, mutation of the CPC sequence in ATP7B results in a non-functional protein (124) and mutation of the Asp residue in either ATP7A or ATP7B completely prevents formation of the translocase-acylphosphate intermediate and results in total loss of Cu translocation activity (123, 127) (128).

The Cu translocation and the phosphorylation-dephosphorylation cycle end after hydrolysis of the acyl-phosphate intermediate by the phosphatase A domain (Figure 2). Though the phosphatase mechanism is unclear, mutagenesis studies have shown that mutation of the conserved phosphatase TGE motif results in hyperphosphorylation of ATP7A and abolishes the Cu translocation (127). The released energy is used to transfer the Cu^+ ion trapped in the membrane channel to the other side of the membrane (for a review see (134). In agreement with *in vivo* observations, the conformational changes and the *in vitro* Cu translocation activities of ATP7A and ATP7B, are ATP-dependent (135); (136, 137). Measurements of the kinetics of catalytic phosphorylation and dephosphorylation show that ATP7A complete each of these steps six fold faster than ATP7B, which would allow ATP7A to transfer and remove Cu from the cell more effectively than ATP7B (138).

The release and transfer of Cu^+ on the other side of the membrane is likely to involve acceptor Met residues strategically positioned in the luminal side of the pore and/or the TMS1,2 loop whose structure is similar to the MBDs (126). It is thought that the most significant difference between ATP7A and ATP7B in their luminal portions, the 17 residues long Met-rich insert in the extracellular loop connecting the TMS1 and TMS2 of ATP7A, may accelerate the release and transfer of Cu^+ to the acceptor proteins in the lumen, and contributes further to the faster Cu transport by ATP7A. (126). Is believed that functioning of those Met residues as sensors of the Cu levels in the lumen of the TGN may provide a second layer in the control of the activity and relocation of the transporters, and that a redox environment created by helper molecules and propitiated by the lipid environment may also facilitate the structural changes in the transporter associated with the ion transfer and oxidization.

The results of genetic and biochemical studies point that metallation of cuproproteins in the lumen of the TGN does not require the participation of Cu-chaperones, a lack of input that anticipates the physical interaction between Cu acceptor and donor recently confirmed by the coimmunoprecipitation of ATP7A with the TGN-resident Sod3, an interaction that is Cu dependent (139). With regard to the mechanism of transfer it is believed that the Met residues in the exofacial loops of the P-type ATPases may accelerate the transfer of the Cu^+ to the cuproproteins acceptors (126).

Also interesting is the dramatic decrease in Sod3 specific activity in cultured ATP7A mutant fibroblasts with impeded Cu transport, a phenomenon that indicates the existence of some coordination between the biosynthetic rates of the ultimate Cu acceptors and the activity of the intermediaries. In this respect, is interesting that in ATP-deficient fibroblast Atox1 is up-regulated and functions as a positive regulator of Sod3 transcription (139, 140).

There is firm evidence that the levels of total cellular Cu regulate the traffic of ATP7A and ATP7B and, specifically, that while at normal Cu concentrations the translocases remain in the TGN to supply Cu to the newly synthesized cuproproteins transported through the secretory pathway, they are transferred to post-Golgi compartments to function in the dissipation of Cu concentrations that exceed the physiological limits (141-143). Nevertheless, the basic of this Cu-regulated traffic is only beginning to be understood. In addition, it has been observed that glutamate receptor activation results in the rapid and reversible trafficking of Menkes ATPase to neuronal processes in an intracellular Cu-independent manner (144).

Retention of transmembrane proteins in the Golgi is known to involve a dynamic process that is in part dependent upon cytoplasmic retrieval motifs, a variety of transmembrane sequences that may operate through kin recognition and formation of large size aggregates, and posttranslational modifications or bilayer-mediated sorting produced by the shorter size of the transmembrane segments (TMS) (145). One or more of these factors may function in the retention of the P-type ATPases in the Golgi as a non-Golgi reporter molecule fused to the 38 amino acid sequence containing the third transmembrane domain (TMS3) of ATP7A is retained in the Golgi (146). Furthermore, mutants affected in the capacity to bind Cu and in its related cyclic phosphorylation show clear changes in distribution. With regard to this, it has been advanced the possibility that the Cu^+-binding to the TMS1,2 loop might alter the structure of the adjacent TMS3 and decrease the translocase retention in the TGN (126).

Postranlational modifications and interactions with other protein factors often affect the functioning of the traffic motifs. Is remarkable that whereas mutations that block the phosphorylation of the DKTG motif and the Cu^+-acceptor CPC motif (DKTG/EKTG; CPC/RPC;) inhibit the Cu-induced relocation of the translocases to the plasma membrane, the same relocation is stimulated by mutations that stabilize the phosphorylated intermediate (MBDs 1-4

deletion; TGE/AAA) (127). Interestingly, of the six cytoplasmic MBDs only one of the two in the proximity of the membrane channel (MBDs 5, 6) appears to be necessary and sufficient for the Cu-induced redistribution of the ATP7A translocase (147, 148). This observation may explain why the G591D mutation in MBD 6, which also disrupts the interaction with Atox1, is cause of Wilson disease (61). Nevertheless, though Cu binding is required for trafficking of ATP7B it appears not to be strictly essential (129).

With regard to the exact location of the Cu-translocases retained in the Golgi, is known that ATP7B is retained in a syntaxin 6-rich TGN compartment and we have recently found that the insulin-regulated glucose transporter GLUT4 is also retained in the same syntaxin-rich compartment. The retention of ATP7B and GLUT4 in the same Golgi compartment suggests that plasma membrane proteins with homeostatic functions that traffic between the TGN and the cell surface in a manner-regulated by changes in the physiological constants under their control, may use similar mechanisms of distribution and move through the same pathways. This is also supported by the observation that ATP7B and GLUT4 and ATP7B are released from the TGN upon truncation of their acidic C-ends (our unpublished results). Virtually nothing is known of the TGN-based protein machinery implicated in the packing of ATP7B/ATP7A into the vesicles that transport the translocases to the cell surface. Since the packing of GLUT4 into the vesicles that fuse with the plasma membrane involves the physical interaction with sortilin (149), it would be interesting to know if sortilin, which resides in the TGN and possesses a GGA binding motif, binds to ATP7B in the TGN and if the complex is recruited to clathrin-coated transport vesicles by the GGAs adaptors.

Whereas studies of ATP7A distribution in MDCK cells loaded with Cu have implicated a diLeu motif proximal to the C-end in its targeting from the TGN to the basolateral membrane (150), the NH_2-terminal 63 aas of ATP7B, a sequence which is absent from the homologous ATP7A, restores the Cu responsiveness and correct apical targeting of an N-truncated ATP7B construct lacking the first four MBDs (151). These observations suggest that in polarized epithelial cells ATP7B might be first transported to the basolateral membrane and then to the apical domain.

With regard to the retention of the P-type-ATPases in the surface of the cells loaded with Cu, recent studies of the ATP7A distribution in MDCK cells, an *in vitro* model that reproduces the distribution of ATP7A in the duodenum, indicate that a C-cytoplasmic PDZ-binding domain may play an important role in its basolateral retention (150). The protein factors recognized by the PDZ domain remain unknown.

Since P-type ATPases are recycled to the TGN after the return of Cu to physiological levels, the mechanisms of recycling are of considerable importance. Nevertheless, except for the role of the C-diLeu motif in the clathrin mediated surface retrieval of ATP7A, and possibly of ATP7B, (150) (152), is uncertain if other clathrin-independent mechanisms of internalization operate independently, as suggests the internalization of ATP7A in HeLa cells transiently expressing dominant negative dynamin and Eps15 mutants (153). In addition, nothing is known of the mechanisms implicated in the targeting of the endocytosed translocases to the TGN.

7. DIETARY Cu UPTAKE, STORE, DISTRIBUTION AND EXPORT IN THE INTESTINE

As the body cannot synthesize Cu, the metal supplied in the diet becomes critical. Foods contribute virtually all of the Cu consumed, the contribution of water being almost insignificant. The mean daily dietary intake of Cu in human adults ranges between 1 and 2.5 mg (22). Weak organic Cu complexes dissociate in the acid environment of the stomach, and amino acids and substances in gastrointestinal secretions maintain the metal in solution and facilitate its absorption in the alkaline milieu of the duodenum, the major site of Cu absorption (154-156). Regulated intestinal absorption is critical in the regulation of total body Cu (Figure 3).

The accumulation of Ctr1 in the apical membrane of intestinal cells of suckling mice is consistent with a cause-effect relationship between the presence of Cu in the diet and the role of Ctr1 in its absorption (38). The defects in peripheral Cu accumulation, the decrease in the activities of cuproenzymes and the severe accumulation of reduced iron in peripheral tissues of mice with a specific excision of the Ctr1 locus in intestinal cells, are all explained by the ensuing severe intestinal Cu absorption block, and point to a main role of Ctr1 in dietary Cu absorption. This role is stressed by the correction of the above string of defects with a single Cu dose to the periphery (33, 39, 157).

In the intestinal brush border Ctr1 binds Cu^{+} and the co-ordinated reduction of Cu^{++} by reductases is therefore essential for dietary Cu absorption. Nevertheless, the mechanisms of Cu^{++} reduction into Cu^{+} in the intestine remain obscure. Among the candidates to catalyze the Cu^{++} reduction in the intestinal mucosal are a b-type cytochrome reductase (dCytB) isolated from the brush border (158), and the duodenal Steap 1 and 2, two members of the Steap family of Fe^{+++}/Cu^{++} reductases homologous to the yeast FRE family, that when transiently expressed in HEK-293T cells facilitate Cu uptake (159, 160) (Figure 3).

With regard to the mechanisms that may control Ctr1 and Cu absorption in the intestine, is important that in response to excess Cu, Ctr1 is rapidly endocytosed by a mechanism that involves the second transmembrane MX_3M motif and rapidly degraded in the lysosomes of transfected HEK293 cells (37) . This degradation may provide a rapid mechanism to regulate high-affinity Cu uptake in the intestine as suggested by the more efficient Cu absorption when dietary Cu levels are low (161). Is intriguing the dissimilar distribution of Ctr1 in the surface of intestinal cells (apical) and hepatocytes (basolateral) and the recent report that Ctr1 mediates basolateral uptake of Cu into enterocytes (162). In any case, it appears that not all the Cu

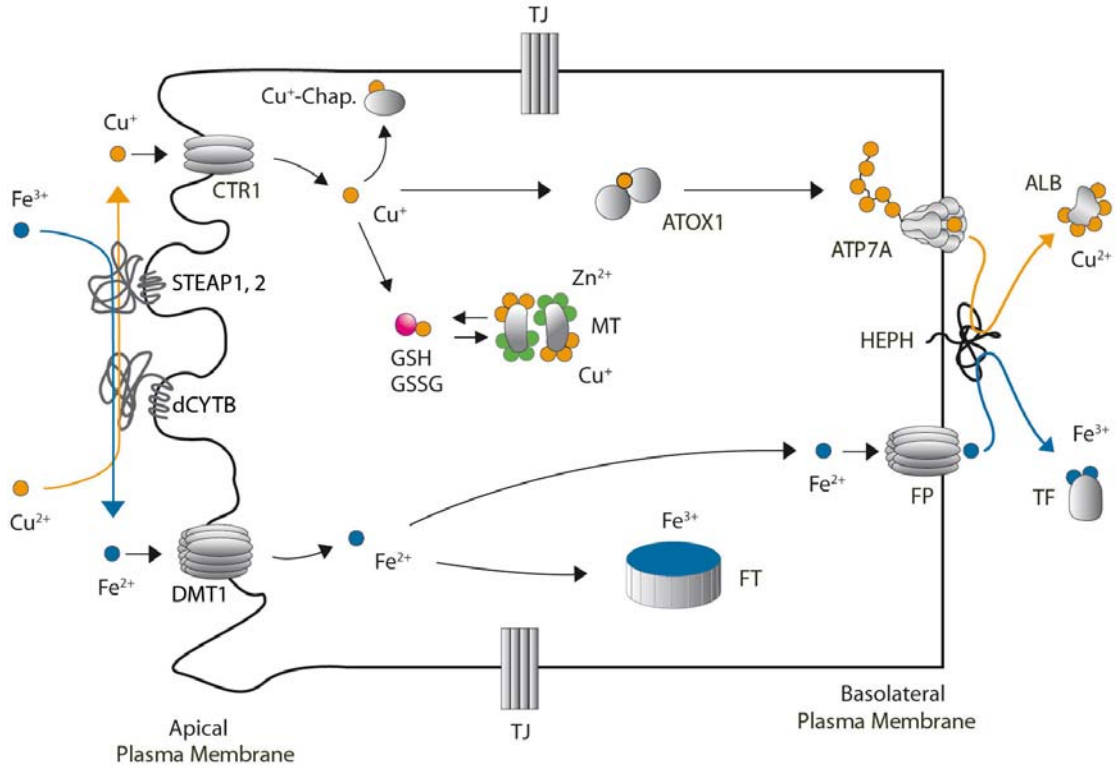

Figure 3. Coordinated Cu and iron absorption in the intestine. The apical and basolateral domains of the plasma membrane, separated by the tight junctions (TJ), face the lumen of the intestine and the circulation, respectively. Cu^{2+} (●) and Fe^{3+} (●) are reduced on the surface of the intestinal mucosal by the Cu-ferroreductases dCytp and Steap. Absorption of Cu^{+} is facilitated by the Ctr1 and/or Dmt1 transporters, while Fe^{++} is exclusively transported by Dmt1. In the cytoplasm Cu^{+} is captured and distributed by specific chaperones (Cu^{+}-Chap., see Figure 1), or stored bound to Zn (●)-metallothioneins (MT). Fe^{++} is incorporated to ferritin (FT) and stored as Fe^{+++}. Atox1 transfers the captured Cu^{+} to basolateral ATP7A. ATP7A and ferroportin (FP) facilitate the transfer of Cu^{+} and Fe^{++}, respectively, through the basolateral membrane. Upon their reduction in the basolateral surface by hepaestin (HEPH), Cu^{2+} is incorporated into serum albumin (ALB) and Fe^{3+} into transferrin (TF) for further transport by the circulation.

absorption in the intestine is mediated by Ctr1. There is evidence that part of the Cu^{+} absorbed is linked to iron absorption by the divalent-metal transporter DMT1, a mechanism that becomes fully functional in iron deficient rats (163) and whose importance is stressed by the decrease in iron and Cu uptake by Caco-2 cells made deficient in DMT1 (164) (Figure 3). The intimate relationship between the Cu and iron absorption in the intestine is further emphasized by the remarkable elevation of the ATP7A levels in the duodenum of iron deficient animals, an increase probably directed to transport into the circulation the Cu accumulated at low iron levels (165, 166). Other candidates that may also participate in the absorption of dietary Cu are the ATP-driven high-affinity Cu transport system found at the brush-border of the enterocytes (167), and the uptake by the aqueous-phase pinocytosis (168, 169).

In vertebrates the Cu^{+} taken into the intestinal epithelial cells is immediately reduced and complexed by GSH prior binding to MT, a transfer that together with the released of the Cu^{+} already bound to MT may play a important role in controlling the download of Cu in the blood (Figure 3).

In the mammalian intestine as in other tissues, ATP7A retains the dual function of supplying Cu to cuproenzymes and pumping the metal out of the cell. Among the cuproenzymes supplied with Cu in the intestinal epithelium stands out hephaestin, a multiCu-ferroxidase homologous to ceruloplasmin (CP) that oxidises Fe^{++} into the Fe^{+++} ion for further transport by ferroportin across the basolateral membrane into the circulation (170) (Figure 3). Hephaestin is mutated in sex-linked anemia (SLA) mice and the defective basolateral export of iron from enterocytes results in iron deficiency and microcytic hypochromic anemia (170). As in yeast, therefore, where the Ctr1/Ccc2 pathway is coupled to the Cu-ferroxidase FET3 (112), in intestine the pathway from Ctr1/ATP7A is also coupled to the Cu-ferroxidase, hephaestin. In a manner opposite to that of Ctr1, a decrease in the intracellular Cu concentration provoked a marked decreased in the levels of hepaestin via degradation by the proteasome (171).

With regard to its second function, in the mammalian intestine the activity of ATP7A is crucial in the transfer of Cu into the circulation (Figure 3). Studies of the ATP7A distribution in MDCK and CaCo cells of intestinal origin and in rodent jejunum with elevated Cu levels have shown that ATP7A leaves the TGN and accumulates in the basolateral plasma membrane (150, 172, 173). Nevertheless, the results from different laboratories differ in the amount of ATP7A that is detected in the basolateral membrane and in intracellular vesicles, and although a role of ATP7A in the download of Cu from the intestinal epithelium into the blood seems clear, is controversial if the translocase mediates the transfer of Cu through the basolateral membrane or if Cu is excreted by exocytosis, as result of the fusion of Cu loaded vesicles with the basolateral membrane. The functional properties of ATP7B explain why the translocation of Cu to the blood is rate-limiting, saturable and energy-dependent.

Patients harbouring a non-functional ATP7A gene or mutations that retain the protein in the TGN, inherit Menkes, a childhood disease secondary to the block in intestinal Cu absorption that results in Cu overload of intestinal epithelial cells and deficiency in the periphery, (174, 175).

After Cu is transferred to the other side of the intestinal mucosa and in a manner similar to iron, the delivery of the metal into the portal circulation is preceded by the oxidation of Cu^{+} into Cu^{++}, a step required for its conjugation to albumin and transcuprein, the main Cu vehicles in the blood.

8. Cu CIRCULATION IN THE BLOOD

Size-exclusion chromatography studies of ^{67}Cu-labeled rat plasma indicates that the bulk of Cu is complex to albumin and transcuprein, and in a small portion to aminoacids, in particular to histidine (176-178). Albumin has been shown to inhibit uptake of Cu^{++} by hepatocytes (179), and since there are no albumin receptors in the hepatocyte cell membrane that might mediate uptake of Cu, the metal is probably transferred from albumin to the hepatocyte surface by a mediator. Though there is data consistent with histidine mobilizing Cu^{++} from albumin by competing for Cu^{++}, and this could be followed by the interaction of the His_2Cu^{++} complex with the Ctr1 transporter and transport of Cu as free ionic Cu (179), the extremely high turnover of the Cu^{++} bound to amino acids and the amino acids inhibition of the Cu^{++} uptake by hepatocytes make unlikely that His mediates the transfer of Cu^{++} from albumin to the hepatocyte. This role is more likely to be played by transcuprein, which rapidly exchanges with Cu^{++} on albumin and does not inhibit Cu^{++} uptake by hepatocytes (180). The normal rate of Cu uptake and tissue distribution in analbuminemic rats intravenously injected with ^{64}Cu points to the existence of some redundancy in the mechanisms of Cu transport in the blood. Transcupreine and aminoacids are the most firm candidates to substitute albumin as the main Cu^{++} vehicle (181). Though holoceruloplasmin moves approximately 80-90% of the plasma Cu, kinetic studies of the turnover of protein and Cu moieties suggest that ceruloplasmin is not an essential Cu-transport protein (182). The normal Cu metabolism and elevated iron stores in patients with aceruloplasminemia, agree with this conclusion and emphasize the ferroxidase function of ceruloplasmin and its role in mobilizing iron from tissue stores (183).

9. Cu CAPTURE, STORAGE AND DISTRIBUTION IN THE LIVER, EXCRETION THROUGH THE BILE

Liver is not only the major captor of the Cu absorbed in the digestive tract, but is also the major reservoir and distributor of Cu to other tissues and organs. Cu may enter the hepatocyte by means of Ctr1, the transporter responsible for the uptake of dietary Cu in the intestine, but as said before the mechanisms that operate in the reduction of the oxidized Cu^{++} bound to albumin in the liver remain unknown. A role of DMT1 in the uptake of Cu by the hepatocyte can not be discarded. Approximately 80% of the Cu entering the liver is not retained in and does not enter the protein fractions. The amount of Cu captured by the natural scavengers and transferred to ceruloplasmin is small. Nonetheless, liver is the major organ storage for Cu in vertebrates.

As in other tissues Cu-scavenging in the hepatocyte involves MT and the resulting large stored Cu pool is presumably regulated by the tandem GSH and MT (184). In the hepatocyte the distribution of Cu is not different from that functioning in other tissues and involves the same set of specific chaperones and specific targets. Yet, ATP7A is not expressed in the hepatocyte and ATP7B plays the exclusive dual role of conveying Cu to newly synthesized cuproenzymes in the TGN and of excreting Cu into the bile. The hepatocyte is the mayor source of ceruloplasmin in the organism and the ferroxidase is the major Cu acceptor in the TGN. As for the ubiquitous ATP7A and other ion transporters with homeostatic roles, the function of ATP7B is controlled through the regulation of its traffic. ATP7B is not exception to the known timely target of homeostatic ion transporters to the membranes where they function and its regulation by changes in the physiological constants under their control.

With the levels of Cu in the liver under control, the bulk of ATP7B in the hepatocyte appears to be retained in the TGN with a small amount cycling constitutively through the bile canaliculi. In the TGN, the function of ATP7B is devoted to the cuprenation of the newly synthesized enzymes transported through the secretory pathway, in particular the large amounts of ceruloplasmin synthesized in the hepatocyte. The route Ctr1/Atox1/ ATP7B in liver is the functional replica of the Ccc2/Fet3p pathway in yeast and the Ctr1/Atox1/ATP7A via in intestine (Figure 3). Ceruloplasmin and hephaestin are structurally homologous (170) and their functional similarity is such that while wild-type ATP7B complements the iron-deficient phenotype of a yeast ccc2 strain (185), hephaestin complements the low-iron growth phenotype of Δfet3 mutant cells (186).

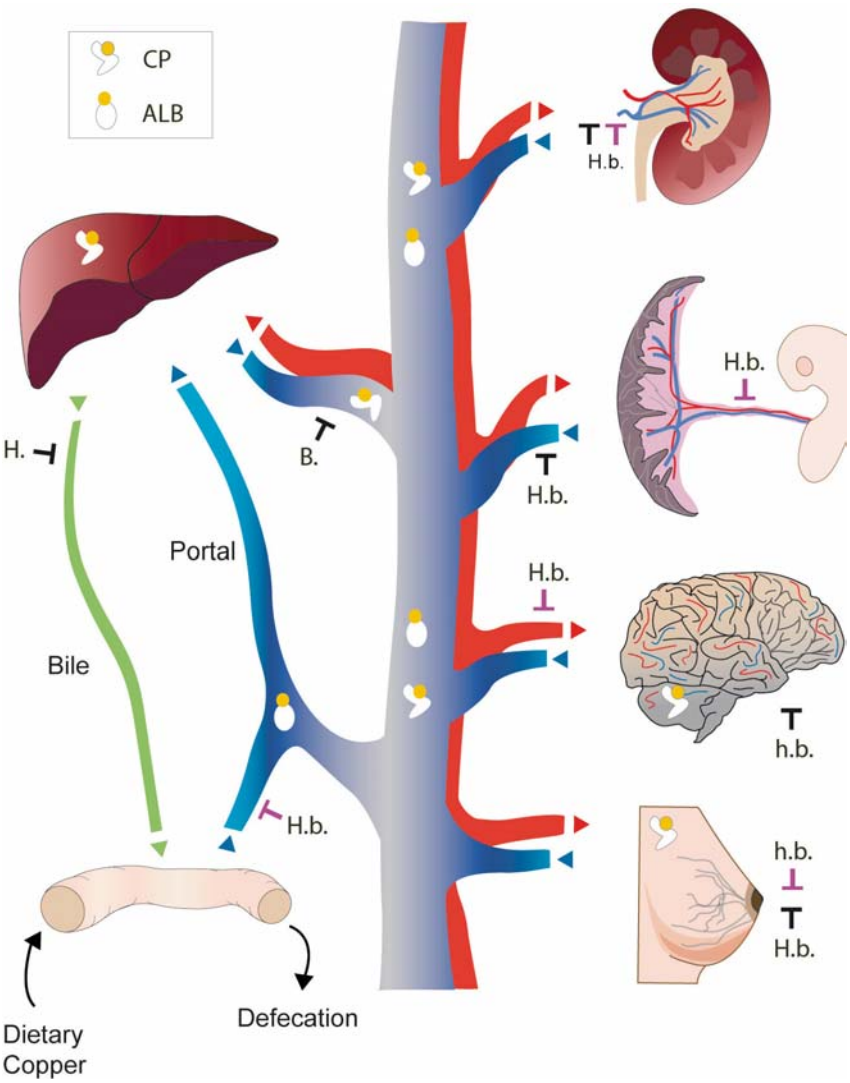

Figure 4. ATP7A and ATP7B expression in mammalian tissue: biosynthetic and homeostatic functions. Most tissues and organs express simultaneously ATP7A and ATP7B, though often in separate cells. In the cell the P-type ATPases perform the dual function of supplying Cu to cuproenzymes (biosynthesis) and pumping the Cu out of the cell (Cu homeostasis). The differential targeting of ATP7A and ATP7B to the basolateral and apical membrane in epithelia cooperates in the extracellular routing of cooper and explains the different abnormalities of Cu distribution and homeostasis in Menkes and Wilson diseases. Functionning of ATP7A in the basolateral membrane of the mucosal epithelial cells of intestine and choroids plexus correlates with its role in the download of dietary Cu to the blood and brain. In kidney ATP7A functions at the glomeruli in Cu reabsorption. In the basolateral membrane of the syncytiotrophoblasts in placenta, ATP7A works in delivering Cu to the foetal blood. Consequently, dysfunction of ATP7A in classic MD (⊥) results in retention of large amounts of Cu in the intestinal mucosa, placenta and kidney, while brain and other tissues have unusually low Cu levels. Newborns may have large Cu deficit and brain damage. The role of ATP7B is critical for the elimination of Cu via bile into the stool, and in the biosynthesis of holoceruloplasmin (CP). Synthesis of holoceruloplasmin is essential for mobilization of iron deposits and iron homeostasis. Albumin (ALB) is the mayor carrier of Cu in the blood. In WD (⊥), the malfunctioning of ATP7B in the hepatocyte impedes Cu elimination via bile and the mobilization of iron stores, and results in serious hepatic and central nervous system toxicosis. The role of ATB7B in Cu reabsorption in placenta and kidney explains the high levels of Cu in placenta, renal cortex and urine. Organ inflow (▶) and outflow (▶). Excretion into bile and duodene lumen (▶). Biosynthetic (B, b) and homeostatic (H, h) functions, the letter size is proportional to the relative importance of the function.

Ceruloplasmin is the mayor acceptor of Cu in liver, and liver is its major producer (Figure 4). The biosynthesis of ceruloplasmin is unaffected by Cu accessibility, but the turnover of apoceruloplasmin is much faster (4-5 h) than the turnover of holoceruloplasmin (4-5 d) (187). Little information is available on the mechanism of Cu transfer from ATP7B to ceruloplasmin. Yet, the physical interaction between ATP7A and Sod3 (139) suggests that the transfer

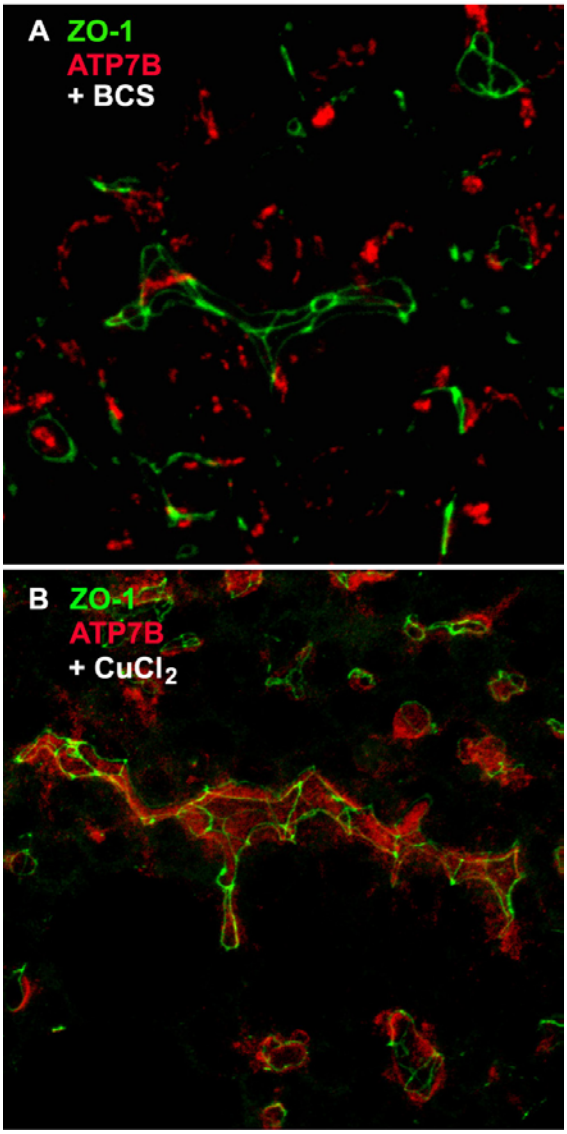

Figure 5. Cu-dependent ATP7B trafficking between Golgi and bile canalicular membrane. Polarized hepatoma Can 20 cells cultured in DMEM/10% fetal calf serum were incubated for 4h with 50 μM bathocuproine (BCS, panel A) or 200 μM $CuCl_2$ (panel B) and simultaneously stained for the tight junctions flanking the bile canaliculi using an anti ZO-1 antibody (FITC), and for ATP7B using an antibody developed against the N-cytoplasmic domain of the protein (Texas red). Observe the retention of ATP7B in the Golgi of the cells treated with the Cu chelator BCS and the translocation of ATP7B to the bile canaliculi of the cells loaded with Cu.

of Cu to ceruloplasmin in the lumen of the Golgi is not mediated by chaperones. Newly synthesized ceruloplasmin binds tightly six Cu ions in the TGN before its secretion into the blood (188). Three of these atoms are organized in a mononuclear center that participates in the capture of electrons from oxygen, and the other three form a trinuclear cluster at the interface between the N- and C-domains of the protein that is essential for ferroxidase activity (188-190). Ceruloplasmin is exported in large amounts from the liver and by catalyzing the oxidation of Fe^{++} to Fe^{+++} facilitates the loading of iron onto transferrin for delivery to peripheral organs, via transferrin receptor-mediated endocytosis. The role of ceruloplasmin in iron oxidation is essential in determining the rate of iron efflux from cells and tissues with movable iron stores and therefore for iron metabolism (189). Humans and rodents with mutations of the ceruloplasmin gene and thus suffering aceruloplasminemia show no large defects in Cu metabolism , but its absence leads to iron accumulation in various organs including liver and central nervous system (191, 192). Nevertheless, since ceruloplasmin binds 95% of the serum Cu, oxidizes Cu and may bind reversibly additional Cu, it may play a secondary role in Cu homeostasis. This role would explain the small accumulation (x 2) of Cu in the liver of $CP^{-/-}$ mice (193).

The excretion of Cu into the bile is an attribute exclusive of the hepatocyte and bile is the major route of Cu elimination and the most important mechanism in Cu homoeostasis in mammals (Figure 4). Cooper excretion into the bile is the second major function of ATP7B in the hepatocyte. Increase in Cu levels in the hepatocyte results in massive release of the ATP7B retained in the TGN (142). The correlation between the release and the role of ATP7B in excretion of Cu into the bile is firmly established. Yet, though the bile canalicular membrane seemed the most natural destiny for the released translocase, studies of the Cu-induced relocation have resulted in identification of different membrane targets. The results of studies in polarized hepatoma OUMS29 and CHO cells, and in rat liver, postulate that ATP7B is targeted to endosomes and lysosomes, and that the loading of lysosomes with Cu and the subsequent recycling of ATP7B to the TGN are followed by the fusion of the ATP7B-free/Cu-loaded lysosomes with the bile canalicular membrane and the download of Cu into the bile (142, 194-196). The results sustaining the lysosomal model are in contrast with the demonstration in polarized hepatoma HepG2, Wif-B9 and Can10 cells that ATP7B is inserted in the bile canalicular membrane and with the accompanying conclusion that ATP7B transfers the Cu from the cytoplasm to the bile (143, 151, 197) (Figure 5). Differences in the cells and tissues used in these studies as well as the different ATP7B constructions and antibodies employed may explain the different results. Polarized hepatoma cells loaded with Cu or treated with BFA retain a significant amount of ATP7B in the apical recycling compartment (ARE; also called subapical compartment, SAC) (143, 151). In the ARE converge the transcytotic and apical endocytic pathways and whereas basolateral membrane proteins bound to the apical domain stopover in the ARE, endocytosed apical membrane proteins are recycled back to the apical membrane (198-200). The detection of ATP7B in the ARE and the exclusion of this compartment from the biosynthetic and endocytic pathways that lead to lysosomes, strongly suggest that lysosomes may not be the natural target of the ATP7B released from the TGN in Cu excess conditions. Is unclear if the presence of ATP7B in the ARE and the redirection of an N-truncated ATP7B

construct to the basolateral membrane of polarized WIF cells (151) reflect the transport of ATP7B trough the basolateral surface by transcytosis. The recent report of the Cu-regulated physical interaction between the MBDs 4-6 of ATP7B and the p62 subunit of dynactin (201), is also consistent with the apical destiny of ATP7B since only the apical transport of membrane proteins requires microtubules. The lack of interaction between dynactin and ATP7A (201) agrees with the basolateral destiny of ATP7A in polarized epithelial cells. The precision of ion transporters with homeostatic functions and regulated traffic in hitting different targets is often determined by the option to use alternative pathways of transport and its regulation by the physiological constants to which they respond. It would therefore seem reasonable to study if as in the distribution of GLUT4 (149) the presence of ATP7B in lysosomes reveals a degradation pathway that is only activated by specific physiological or pathological conditions.

The recent observation that a significant population of ATP7B in the cell surface of hepatoma cells is associated with the tight junctions and is less responsive to changes in the levels of Cu, indicates that ATP7B may have separate functions in the surface of the hepatocyte (197). Is not known, but it seems unlikely that the association of ATP7B with the tight junctions facilitates the paracellular transport of Cu into the bile by perfused rat livers (197).

Finally, though ATP7A and ATP7B basically use the same distribution mechanisms in the majority of the cells (127, 147, 148), the role of MBD 6 in targeting ATP7B from the TGN to vesicles (120) and of the first 16 aminoacids and dynactin in directing ATP7B to the apical membrane (151, 201) are good reminders of the the existing differences.

By transferring Cu^{+} to ceruloplasmin and eliminating excess Cu through the bile, ATP7B mediates the two major contributions of liver to iron metabolism and Cu homeostasis (Figure 3).

10. ATP7B DYSFUNCTION AND Cu TOXICOSIS

Wilson disease (WD), idiopathic Cu toxicosis (ICT), Indian childhood cirrhosis (ICC) and the endemic Tyrolean infantile cirrhosis (ETIC) are human disorders associated with Cu excess, a feature resulting from unbalance between Cu absorption and excretion (for a review see (125).

WD is a rare autosomal recessive disorder Mendelian-linked to ATP7B (202). Clinical symptoms of the WD include cirrhosis and chronic hepatitis that end in liver failure, neurological defects that course with parkinsonian symptoms and seizures, and psychiatric features as depression, personality changes and psychosis (203). The Kayser–Fleischer ring, a deposition of Cu visible as a golden ring in the periphery of the cornea (204), the low serum levels of ceruloplasmin and high levels of Cu in the urine are often helpful in the diagnosis of the disease (205) (206). The age of presentation of the WD syndrome, the predominance of hepatic versus neurological symptoms and their severity are highly variable (125, 207, 208)

The accumulation of Cu and of the iron produced by deficient ferroxidase associated with low holoceruloplasmin levels (187, 190, 209), primarily explain the hepatic and brain intoxication (Figure 4). Nevertheless, the WD does not show the progressive neurodegeneration of the retina and the primary diabetes characteristic of aceruloplasminemia. This paradox is probably explained by the extrahepatic production of holoceruloplasmin and the supply to the serum of the concentration required to oxidize and mobilize the Fe^{++} retained in tissue stores (210). Excessive Cu-derived oxidants produced by free Cu^{2+}-catalyzed Fenton reactions and reduced Sods and GSH activities, also appear to contribute decisively to development and progression of liver abnormalities in WD (211).

More than 300 mutations in the ATP7B gene have been associated with WD, the majority missense mutations (60%) (212). Mutations affecting to different extent the binding of Cu, the cyclic phosphorylation, traffic and postranslational modifications of ATP7B as well as its physiological interaction with other proteins, may potentially interfere with the dual role of ATP7B in the biosynthesis of the ceruloplasmin and in excreting excess Cu into the bile.

Genotype variations in the mutations may partly explain the variability behind the clinical symptoms (125, 213), but is also likely that this also results from the activity of modifier genes and gene-environment interactions (214, 215) .

Mutations completely disrupting the ATP7B structure, traffic and function are thought to be behind the most serious clinical phenotypes and the early presentation of the disease. The most prevalent mutations that explain the majority of the WD cases are H1069Q/G in Europe and North America, and R778L in southeast Asia (216). The H1069 residue plays a critical role in the ATP binding to the N-domain of ATP7B and its substitution by Q/G drastically inhibits the affinity of the translocase for ATP (217). This inhibition may potentially hamper the phosphorylation of ATP7B and as result disrupt the activation of its apical trafficking and the Cu translocation, two phenomenons required for Cu elimination into the bile. Yet, the demonstration that the H1069Q mutant is retained and quickly degraded in the ER suggests that these are the primary cause of the WD. The same traffic defect has been found in studies of the R778L mutant, the second most common mutation causing WD, and of the G85v, G591D, D765N and L776V mutants (218, 219).

The association of WD with more than 40 different mutations in the N-site of ATP7B and the fact that only four of these mutations affect residues implicated in ATP binding (H1069, G1099, G1101 and I1102) (131, 212) point, together with the inhibition of the ATP binding in

the E1064A mutant (217), to the importance of cooperation between a large number of residues in the construction of a functional N-domain.

Mutations disrupting the ATP7B traffic between the TGN and post-Golgi compartments, including the plasma membrane, have been also implicated in WD development. Patients with G943S and M769V mutations that inhibit the apical trafficking of ATP7B without interfering with its retention in the TGN, and whose DNA rescues the ΔCcc2 phenotype in the yeast Ccc2 complementation assay (124, 219), are expected to coexist with normal ceruloplasmin production and may not develop severe CNS intoxication. Whereas the substitution of AAA for the conserved TGE motif in the A-domain is known to inhibit the phosphatase activity and thus result in hiperphosphorylation and displacement of the translocase to the cell periphery *in vitro* (127), no TGE mutations have been associated with WD. Nevertheless in the Menkes disease-caused by the L873R mutation in ATP7A, the mutation affects a residue localized two positions upstream the TGE motif and the traffic defects expected for the TGA/AAA mutation are displayed in association with the disease (127).

There is no example of a cause-relationship between a defect in ATP7B–Atox1 interaction since the G85V and G591D mutations produced in the vicinity of the MBD2 and 6, which inhibit the ATP7B interaction with Atox1 and cause WD, also provoke the retention of the translocase in the ER and its rapid degradation, two phenomenons that are probably the principal cause of the disease (218). Similarly the retention of the G591D mutant in the ER appears to be cause of WD and not the lack of Cu-induced phosphorylation of ATP7B (218).

The aetiology of the ICT, ICC and ETIC diseases is unclear. Yet, the Cu toxicosis (CT) in Bendlington terriers and North Ronaldsay sheep is biochemically similar to the severe ICC, ICT or ETIC which develop without defects in ceruloplasmin, iron deposits or neurological symptoms (220, 221). Interestingly, CT is produced by deletion in COMMD1 (also called MURR1) (222) and probably by defects of other unidentified modifier genes as Cu accumulation is also detected in the CT of Bedlington terriers without homozygous COMMD1 deletions (223, 224) . The demonstrated interaction of COMMD1 with ATP7B (225) and its ability to blunt the activation of NF-κB by binding to IκB-α and the Cul1 component of the E3 ligase (226) points to the possibility that COMMD1 may regulate the degradation of ATP7B by the ubiquitin/proteasomal system and, consequently, that its deletion may result in inadequate degradation of ATP7B and thus causing CT (218).

Known the similarities between the pathophysiological features of Cu-associated diseases in humans and animals, model disease-animals are being used in the identification of the molecular defects underlying CT of undetermined origin and in resolving such perplexing problems as the variability in the relationship age-onset of the disease, the prevalence of hepatic versus neurological symptoms and the sometimes fulminating liver failure. It is known that the syndromes developed by the Long-Evans cinnamon rat (5’ deletion) and the 'toxic milk' mouse (G712D) result in toxic accumulation of Cu and development of hepatic abnormalities without neurological symptoms, mimicking the disease in a group of WD patients. It would be interesting to study the cause for this absence of neurological symptoms, since the ATP7B knockout mouse in addition to hepatic abnormalities developed neurological symptoms that resemble those found in another population of WD patients (227). Also remarkable is the observation that the livers of ELC rats victims of fulminant hepatitis have elevated levels of non-heme iron, an observation that may be important in the prognosis of the hepatitis associated with CTs (228). The use of animal models should be also useful to advance in the use of liver cell transplant and gene therapy as alternative cures for Cu disorders.

The capacity of Zn to induce MT and the formation of tight MT-Cu complexes in the intestinal mucose explains the effectiveness of oral administration of Zn acetate in reducing dietary Cu absorption and the levels of intestinal haefestin, two responses that ameliorate the clinical manifestations associated with Cu and iron accumulation in WD patients (229). In addition, WD patients can be medically treated either by other chelating agents (penicillamine, trientine and tetrathiomolibdate) or by liver transplantation when medical therapy fails or in case of acute liver failure (230).

11. ATP7A DYSFUNCTION AND Cu DEFFICIENCY

Menkes disease (MD) is an ATP7A defective X-linked recessive disorder causing entrapment of Cu within the intestinal mucosa and general Cu deficiency (231). The incidence of 1:300 000[10–12] among humans places MD as an exceptionally rare disease (232). The phenotype disparities between MD and WD, Cu deficiency versus surplus, are mainly determined by differences in tissue and cellular distribution between ATP7A and ATP7B. Whereas the basolateral ATP7A transports Cu across the duodene, blood-brain barrier and placenta, in liver the apical ATP7B plays a crucial role in the elimination of Cu through the bile.

While the malfunctioning of ATP7A in the cell surface severely disturbs the distribution of Cu through the organism, the forfeit in the TGN hinders the acquisition of Cu by a group of cuproenzymes that play diverse, yet essential, roles in various aspects of cellular function. As result, the direct effect of the malfunctioning in almost all the extrahepatic tissues of lysyl oxidase, dopamine β-hydroxylase, peptidylglycine α-amidating mono-oxygenase, cytochrome C oxidase, superoxide dismutase and tyrosinase are the characteristic traits of MD.

Over 200 MD-causing mutations have been identified in ATP7A, including small insertions/deletions, nonsense, missense and splice-site mutations that are represented with comparable frequency. Whereas small insertions/deletions and nonsense MD mutations are found throughout the

whole gene, the sequence linking the last metal binding site (MBD 6) and the first transmembrane domain hoards the highest percentage of MD mutations (233). Also remarkable is the relative lack of effect of missense mutations within the six MBDs lining the N-cytoplasmic domain, a phenomenon that is probably explained by the existence of some functional redundancy among the MBDs (125).

A correlation between the extent to which mutations impair the function of ATP7A and the severity of the MD phenotype is firmly established. Based on the symptoms, two forms of MD have been described; the severe classic MD and a less severe form. A third form OHS (occipital horn syndrome) is allelic to MD.

The classic syndrome affects more than 90% of the MD patients and is manifested not later than 2 months of age. Death usually occurs in the early childhood. Clinical features comprise premature delivery and low birth weight, delay of development, severe neurological defects (neurodegeneration, mental retardation, seizures), vascular abnormalities, laxity of skin and joints, characteristic facial appearance, ''kinky'' hair, skeletal abnormalities and hypotonia, bladder diverticuli, hypothermia and hypopigmentation. Classic MD is often produced by nonsense and frameshift mutations that severely disrupt ATP7B synthesis or by missense mutations that result in ER mislocalisation and proteasomal degradation of the protein (i. e. R884H, G860V, G876R, A1325V and G1369R). In addition, missense mutations that disrupt the interdependent mechanisms of Cu transport activity, cyclic phosphorylation and trafficking of the ATPase, are also cause of classic MD. Examples of these are, the C1000R mutation that disrupts the Cu-binding CPC motif and causes the retention of the translocase in the TGN (127), and the L873A mutation that affects a residue localized two positions upstream the TGE motif and displays the traffic defects expected for the TGA/AAA mutation in association with the disease (234).

Mild MD is associated with mutations compatible with some residual ATP7A activity and as result patients affected by the disease have a longer lifespan and the neurological defects are less intense (subnormal intelligence) (235, 236).

The frequent association of the OHS with splice site mutations between exons 21 and 22 and the residual synthesis of small amounts of normally spliced protein (233, 237, 238), explains the relative benignity of a syndrome that includes connective tissue abnormalities and less severe or absent neurological abnormalities (subnormal intelligence).

As in humans affected by MD, in the series of mottled mice (*Mo*), dappled (Mo^{dp}), brindled (Mo^{br}) and blotchy (Mo^{blo}) the severity of the phenotypes correlates with the extent to which the mutations impair the function of the ATP7A translocase (239, 240).

Whereas the quite different presentation and outcome of the clinical variants of MD strongly conditions the results of the Cu-histidine injections, the treatment of choice in Menkes disease, the effectiveness of the therapy is often dependent of the undeclared CNS-dysfunction in the early stages of the disease and the corresponding delayed onset of therapeutic intervention, delay that is often responsible for the failure of the therapy in relative benign forms of MD.

12. PERSPECTIVES

Coordination between Cu uptake, distribution, storage and elimination is essential to keep the metal levels within the physiological limits to satisfy the demands and avoid its toxic effects. Cu homeostasis is particularly complex in metazoan and elucidating its physiology has been an arduous task that has conceited the efforts of different experimental approaches including the use of different cell and animal models.

Studies aimed at elucidating the Cu pathway through cells and organisms indicate that both Cu absorption and excretion in metazoan are highly dependent on the asymmetric distribution of Cu transporters and helper reductases/oxidases in polarized epithelia. However, both of these phenomena are complex processes and our knowledge of them is yet incomplete. Whereas it is clear that the high-affinity Cu Ctr1 transporter plays a major role in the uptake of Cu by eukaryote cells, its involvement in the absorption of Cu in the intestine of metazoan raises a few questions, including the recently suggested participation in the basolateral Cu uptake and its coordination with other transporters. The function of the ATP-driven high affinity Cu transporter found in enterocytes is unclear and is unknown if the iron-linked Cu absorption by DMT1 is exclusively limited to ensuring the proper Cu-mediated absorption of iron in the intestine. While the functional overlapping between the pathways of Cu uptake is clear, our knowledge of their distribution and function in different tissues is still incomplete. Moreover, adding to our incomplete knowledge of the specificities of the various reductases and oxidases, our knowledge of their interaction with the Cu carriers, transfer mediators and transporters implicated in Cu uptake is at best incomplete.

Within the cell chaperones are widely thought to transport Cu to intracellular sites and users and store proteins to act as scavengers for free cellular Cu. Yet, their cellular roles may be more sophisticated than currently appreciated. For example, there is evidence for a higher order of regulation of the function of chaperones and a signalling role for some in the sensing of Cu levels that lead to the modulation of cellular mechanisms for the uptake and elimination of Cu. Furthermore, the ability of chaperones to discriminate between metals despite the use of a universal MXCXXC motif to chelate Cu, remains a paradox that has yet to be resolved and probably reflects subtle differences in their molecular structures or variations in the local environment. It is clear that the discovery of new chaperones and transporters will be forthcoming to complete the road map of Cu in the cell and in particular in mitochondria. The description of a dynamic Cu pool in the mitochondria and the demonstration that Cox17 does not need to leave the intermembrane space to function in

delivery of Cu to COX, both suggest the involvement of new chaperones and transporters in the transport and distribution of Cu in the mitochondria.

With regard to Cu storage, there is data suggesting that metallothioneins can be regulated and used as temporal stores with an important role in Cu homeostasis, but almost nothing is known about the complexity of the different layers of regulation.

Most intriguing, however, is the non proteinaceous nature of the highly dynamic soluble matrix Cu pool in the mitochondria, pool that probably supplies the Cu needed by the Sod1 and Cox 17 residing in the intermembrane space. The role of Ctr2 and probably P-type ATPases in the creation of endosomal and lysosomal Cu pools that could be important in the maintenance of cellular Cu homoeostasis waits further description.

The number of outstanding questions about the mechanisms involved in the elimination of excess Cu from the cell is equally large. Do P-type ATPases function on the cell surface to directly pump out the Cu, or they function through exocytosis? Does the distinct spatial distribution of P-type ATPases at the cell surface create separate pathways of Cu elimination? The latter question is raised by the intriguing recent studies that demonstrate the localization of a stable ATP7B pool to the tight junctions in liver. Although a direct role of ATP7B in the paracellular transport of Cu seems unlikely due to steric constraints resulting from the geometry of the transporter insertion into the membrane and of the tight junction pores, its possible role in the secretion of Cu into the bile deserves further examination.

Genetic failure to control Cu homeostasis is associated with Cu overload and deficit, but the responsible genes are not always known and the clinical symptoms are not always understood. The Cu overload of liver in patients suffering idiopathic Cu toxicosis with a clear genetic component, and the fact that the causing genes remain unidentified, reflects our imperfect knowledge of how ATP7B functions in liver.

Similarly, we have no explanation for the predominance of neurological or hepatic symptoms in patients suffering the Wilson disease, an issue that points to our imperfect understanding of Cu homeostasis in humans.

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14. REFERENCES

1. Harris, E. D.: Cellular copper transport and metabolism. *Annu Rev Nutr*, 20, 291-310 (2000)

2. Keilin, D. & E. F. Hartree. *Proc. Roy. Soc.* , B127, 167 (1939)

3. Tsukihara, T., H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono & S. Yoshikawa: Structures of metal sites of oxidized bovine heart cytochrome c oxidase at 2.8 A. *Science*, 269, 1069-74 (1995)

4. Staveley, B. E., J. P. Phillips & A. J. Hilliker: Phenotypic consequences of copper-zinc superoxide dismutase overexpression in Drosophila melanogaster. *Genome*, 33, 867-72 (1990)

5. Paynter, D. I., R. J. Moir & E. J. Underwood: Changes in activity of the Cu-Zn superoxide dismutase enzyme in tissues of the rat with changes in dietary copper. *J Nutr*, 109, 1570-6 (1979)

6. De Freitas, J. M., A. Liba, R. Meneghini, J. S. Valentine & E. B. Gralla: Yeast lacking Cu-Zn superoxide dismutase show altered iron homeostasis. Role of oxidative stress in iron metabolism. *J Biol Chem*, 275, 11645-9 (2000)

7. Clement, A. M., M. D. Nguyen, E. A. Roberts, M. L. Garcia, S. Boillee, M. Rule, A. P. McMahon, W. Doucette, D. Siwek, R. J. Ferrante, R. H. Brown, Jr., J. P. Julien, L. S. Goldstein & D. W. Cleveland: Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science*, 302, 113-7 (2003)

8. Chan, W. Y. & O. M. Rennert: The role of copper in iron metabolism. *Ann Clin Lab Sci*, 10, 338-44 (1980)

9. Fox, P. L.: The copper-iron chronicles: the story of an intimate relationship. *Biometals*, 16, 9-40 (2003)

10. Siegel, R. C.: Lysyl oxidase. *Int Rev Connect Tissue Res*, 8, 73-118 (1979)

11. Petris, M. J., D. Strausak & J. F. Mercer: The Menkes copper transporter is required for the activation of tyrosinase. *Hum Mol Genet*, 9, 2845-51 (2000)

12. Schuschke, D. A., J. T. Saari, J. W. Nuss & F. N. Miller: Platelet thrombus formation and hemostasis are delayed in the microcirculation of copper-deficient rats. *J Nutr*, 124, 1258-64 (1994)

13. Jablonska-Kaszewska, I., E. Dabrowska & A. Ozieblowski: [Wilson's disease in personal material--disturbances in hemostasis]. *Pol Tyg Lek*, 50, 79-81 (1995)

14. Owen, T. C. & D. J. Merkler: A new proposal for the mechanism of glycine hydroxylation as catalyzed by peptidylglycine alpha-hydroxylating monooxygenase (PHM). *Med Hypotheses*, 62, 392-400 (2004)

15. Gerbasi, V., S. Lutsenko & E. J. Lewis: A mutation in the ATP7B copper transporter causes reduced dopamine beta-hydroxylase and norepinephrine in mouse adrenal. *Neurochem Res*, 28, 867-73 (2003)

16. Zhou, P. & D. J. Thiele: Rapid transcriptional autoregulation of a yeast metalloregulatory transcription factor is essential for high-level copper detoxification. *Genes Dev*, 7, 1824-35 (1993)

17. Thiele, D. J.: ACE1 regulates expression of the Saccharomyces cerevisiae metallothionein gene. *Mol Cell Biol*, 8, 2745-52 (1988)

18. Trombley, P. Q. & G. M. Shepherd: Differential modulation by zinc and copper of amino acid receptors from rat olfactory bulb neurons. *J Neurophysiol*, 76, 2536-46 (1996)

19. Virginio, C., D. Church, R. A. North & A. Surprenant: Effects of divalent cations, protons and calmidazolium at the rat P2X7 receptor. *Neuropharmacology*, 36, 1285-94 (1997)

20. Bertini, I., Gray, H. B., Stiefel, E. and valentine, J. S.: Biological Inorganic Chemistry. Sausalito, CA (2007)

21. McCord, J. M. & E. D. Day, Jr.: Superoxide-dependent production of hydroxyl radical catalyzed by iron-EDTA complex. *FEBS Lett*, 86, 139-42 (1978)

22. Dameron, C. & P. D. Howe: ENVIRONMENTAL HEALTH CRITERIA FOR COPPER. *International Programme on Chemical Safety*, (1998).

23. Dancis, A., D. Haile, D. S. Yuan & R. D. Klausner: The Saccharomyces cerevisiae copper transport protein (Ctr1p). Biochemical characterization, regulation by copper, and physiologic role in copper uptake. *J Biol Chem*, 269, 25660-7 (1994)

24. Zhou, B. & J. Gitschier: hCTR1: a human gene for copper uptake identified by complementation in yeast. *Proc Natl Acad Sci U S A*, 94, 7481-6 (1997)

25. Knight, S. A., S. Labbe, L. F. Kwon, D. J. Kosman & D. J. Thiele: A widespread transposable element masks expression of a yeast copper transport gene. *Genes Dev*, 10, 1917-29 (1996)

26. Kampfenkel, K., S. Kushnir, E. Babiychuk, D. Inze & M. Van Montagu: Molecular characterization of a putative Arabidopsis thaliana copper transporter and its yeast homologue. *J Biol Chem*, 270, 28479-86 (1995)

27. Bellemare, D. R., L. Shaner, K. A. Morano, J. Beaudoin, R. Langlois & S. Labbe: Ctr6, a vacuolar membrane copper transporter in Schizosaccharomyces pombe. *J Biol Chem*, 277, 46676-86 (2002)

28. Rees, E. M., J. Lee & D. J. Thiele: Mobilization of intracellular copper stores by the ctr2 vacuolar copper transporter. *J Biol Chem*, 279, 54221-9 (2004)

29. Portnoy, M. E., P. J. Schmidt, R. S. Rogers & V. C. Culotta: Metal transporters that contribute copper to metallochaperones in Saccharomyces cerevisiae. *Mol Genet Genomics*, 265, 873-82 (2001)

30. Guo, Y., K. Smith & M. J. Petris: Cisplatin stabilizes a multimeric complex of the human Ctr1 copper transporter: requirement for the extracellular methionine-rich clusters. *J Biol Chem*, 279, 46393-9 (2004)

31. Puig, S., J. Lee, M. Lau & D. J. Thiele: Biochemical and genetic analyses of yeast and human high affinity copper transporters suggest a conserved mechanism for copper uptake. *J Biol Chem*, 277, 26021-30 (2002)

32. Lee, J., M. M. Pena, Y. Nose & D. J. Thiele: Biochemical characterization of the human copper transporter Ctr1. *J Biol Chem*, 277, 4380-7 (2002)

33. Eisses, J. F. & J. H. Kaplan: The mechanism of copper uptake mediated by human CTR1: a mutational analysis. *J Biol Chem*, 280, 37159-68 (2005)

34. Xiao, Z., F. Loughlin, G. N. George, G. J. Howlett & A. G. Wedd: C-terminal domain of the membrane copper transporter Ctr1 from Saccharomyces cerevisiae binds four Cu(I) ions as a cuprous-thiolate polynuclear cluster: sub-femtomolar Cu(I) affinity of three proteins involved in copper trafficking. *J Am Chem Soc*, 126, 3081-90 (2004)

35. Xiao, Z. & A. G. Wedd: A C-terminal domain of the membrane copper pump Ctr1 exchanges copper(I) with the copper chaperone Atx1. *Chem Commun (Camb)*588-9 (2002)

36. Guo, Y., K. Smith, J. Lee, D. J. Thiele & M. J. Petris: Identification of methionine-rich clusters that regulate copper-stimulated endocytosis of the human Ctr1 copper transporter. *J Biol Chem*, 279, 17428-33 (2004)

37. Petris, M. J., K. Smith, J. Lee & D. J. Thiele: Copper-stimulated endocytosis and degradation of the human copper transporter, hCtr1. *J Biol Chem*, 278, 9639-46 (2003)

38. Kuo, Y. M., A. A. Gybina, J. W. Pyatskowit, J. Gitschier & J. R. Prohaska: Copper transport protein (Ctr1) levels in mice are tissue specific and dependent on copper status. *J Nutr*, 136, 21-6 (2006)

39. Lee, J., M. J. Petris & D. J. Thiele: Characterization of mouse embryonic cells deficient in the ctr1 high affinity copper transporter. Identification of a Ctr1-independent copper transport system. *J Biol Chem*, 277, 40253-9 (2002)

40. Lee, J., J. R. Prohaska & D. J. Thiele: Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. *Proc Natl Acad Sci U S A*, 98, 6842-7 (2001)

41. Kuo, Y. M., B. Zhou, D. Cosco & J. Gitschier: The copper transporter CTR1 provides an essential function in mammalian embryonic development. *Proc Natl Acad Sci U S A*, 98, 6836-41 (2001)

42. Freedman, J. H., M. R. Ciriolo & J. Peisach: The role of glutathione in copper metabolism and toxicity. *J Biol Chem*, 264, 5598-605 (1989)

43. Steinebach, O. M. & H. T. Wolterbeek: Role of cytosolic copper, metallothionein and glutathione in copper toxicity in rat hepatoma tissue culture cells. *Toxicology*, 92, 75-90 (1994)

44. Coyle, P., J. C. Philcox, L. C. Carey & A. M. Rofe: Metallothionein: the multipurpose protein. *Cell Mol Life Sci*, 59, 627-47 (2002)

45. Theocharis, S. E., A. P. Margeli & A. Koutselinis: Metallothionein: a multifunctional protein from toxicity to cancer. *Int J Biol Markers*, 18, 162-9 (2003)

46. Balamurugan, K. & W. Schaffner: Copper homeostasis in eukaryotes: teetering on a tightrope. *Biochim Biophys Acta*, 1763, 737-46 (2006)

47. Hamer, D. H.: Metallothionein. *Annu Rev Biochem*, 55, 913-51 (1986)

48. Kagi, J. H. & Y. Kojima: Chemistry and biochemistry of metallothionein. *Experientia Suppl*, 52, 25-61 (1987)

49. Egli, D., H. Yepiskoposyan, A. Selvaraj, K. Balamurugan, R. Rajaram, A. Simons, G. Multhaup, S. Mettler, A. Vardanyan, O. Georgiev & W. Schaffner: A family knockout of all four Drosophila metallothioneins reveals a central role in copper homeostasis and detoxification. *Mol Cell Biol*, 26, 2286-96 (2006)

50. Ecker, D. J., T. R. Butt, E. J. Sternberg, M. P. Neeper, C. Debouck, J. A. Gorman & S. T. Crooke: Yeast metallothionein function in metal ion detoxification. *J Biol Chem*, 261, 16895-900 (1986)

51. Balamurugan, K., D. Egli, H. Hua, R. Rajaram, G. Seisenbacher, O. Georgiev & W. Schaffner: Copper homeostasis in Drosophila by complex interplay of import, storage and behavioral avoidance. *Embo J*, 26, 1035-44 (2007)

52. Berger, B., R. Dallinger, P. Gehrig & P. E. Hunziker: Primary structure of a copper-binding metallothionein from mantle tissue of the terrestrial gastropod Helix pomatia L. *Biochem J*, 328 (Pt 1), 219-24 (1997)

53. Liu, S. X., J. P. Fabisiak, V. A. Tyurin, G. G. Borisenko, B. R. Pitt, J. S. Lazo & V. E. Kagan: Reconstitution of apo-superoxide dismutase by nitric oxide-induced copper transfer from metallothioneins. *Chem Res Toxicol*, 13, 922-31 (2000)

54. Rigby Duncan, K. E. & M. J. Stillman: Metal-dependent protein folding: metallation of metallothionein. *J Inorg Biochem*, 100, 2101-7 (2006)

55. Nose, Y., B. E. Kim & D. J. Thiele: Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. *Cell Metab*, 4, 235-44 (2006)

56. Medici, V., A. Santon, G. C. Sturniolo, R. D'Inca, S. Giannetto, V. Albergoni & P. Irato: Metallothionein and antioxidant enzymes in Long-Evans Cinnamon rats treated with zinc. *Arch Toxicol*, 76, 509-16 (2002)

57. Culotta, V. C., L. W. Klomp, J. Strain, R. L. Casareno, B. Krems & J. D. Gitlin: The copper chaperone for superoxide dismutase. *J Biol Chem*, 272, 23469-72 (1997)

58. Glerum, D. M., A. Shtanko & A. Tzagoloff: Characterization of COX17, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. *J Biol Chem*, 271, 14504-9 (1996)

59. Amaravadi, R., D. M. Glerum & A. Tzagoloff: Isolation of a cDNA encoding the human homolog of COX17, a yeast gene essential for mitochondrial copper recruitment. *Hum Genet*, 99, 329-33 (1997)

60. Hiser, L., M. Di Valentin, A. G. Hamer & J. P. Hosler: Cox11p is required for stable formation of the Cu(B) and magnesium centers of cytochrome c oxidase. *J Biol Chem*, 275, 619-23 (2000)

61. Hamza, I., M. Schaefer, L. W. Klomp & J. D. Gitlin: Interaction of the copper chaperone HAH1 with the Wilson disease protein is essential for copper homeostasis. *Proc Natl Acad Sci U S A*, 96, 13363-8 (1999)

62. Larin, D., C. Mekios, K. Das, B. Ross, A. S. Yang & T. C. Gilliam: Characterization of the interaction between the Wilson and Menkes disease proteins and the cytoplasmic copper chaperone, HAH1p. *J Biol Chem*, 274, 28497-
504 (1999)

63. Walker, J. M., R. Tsivkovskii & S. Lutsenko: Metallochaperone Atox1 transfers copper to the NH2-terminal domain of the Wilson's disease protein and regulates its catalytic activity. *J Biol Chem*, 277, 27953-9 (2002)

64. Heaton, D. N., G. N. George, G. Garrison & D. R. Winge: The mitochondrial copper metallochaperone Cox17 exists as an oligomeric, polycopper complex. *Biochemistry*, 40, 743-51 (2001)

65. Harrison, M. D., C. E. Jones & C. T. Dameron: Copper chaperones: function, structure and copper-binding properties. *J Biol Inorg Chem*, 4, 145-53 (1999)

66. Dameron, C. T. & M. D. Harrison: Mechanisms for protection against copper toxicity. *Am J Clin Nutr*, 67, 1091S-1097S (1998)

67. Halliwell, B., O. I. Aruoma, M. Wasil & J. M. Gutteridge: The resistance of transferrin, lactoferrin and caeruloplasmin to oxidative damage. *Biochem J*, 256, 311-2 (1988)

68. Lutsenko, S., K. Petrukhin, M. J. Cooper, C. T. Gilliam & J. H. Kaplan: N-terminal domains of human copper-transporting adenosine triphosphatases (the Wilson's and Menkes disease proteins) bind copper selectively *in vivo* and *in vitro* with stoichiometry of one copper per metal-binding repeat. *J Biol Chem*, 272, 18939-44 (1997)

69. Harrison, M. D., S. Meier & C. T. Dameron: Characterisation of copper-binding to the second sub-domain of the Menkes protein ATPase (MNKr2). *Biochim Biophys Acta*, 1453, 254-60 (1999)

70. Rae, T. D., P. J. Schmidt, R. A. Pufahl, V. C. Culotta & T. V. O'Halloran: Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. *Science*, 284, 805-8 (1999)

71. Caruano-Yzermans, A. L., T. B. Bartnikas & J. D. Gitlin: Mechanisms of the copper-dependent turnover of the copper chaperone for superoxide dismutase. *J Biol Chem*, 281, 13581-7 (2006)

72. Lamb, A. L., A. K. Wernimont, R. A. Pufahl, V. C. Culotta, T. V. O'Halloran & A. C. Rosenzweig: Crystal structure of the copper chaperone for superoxide dismutase. *Nat Struct Biol*, 6, 724-9 (1999)

73. Casareno, R. L., D. Waggoner & J. D. Gitlin: The copper chaperone CCS directly interacts with copper/zinc superoxide dismutase. *J Biol Chem*, 273, 23625-8 (1998)

74. Wong, P. C., D. Waggoner, J. R. Subramaniam, L. Tessarollo, T. B. Bartnikas, V. C. Culotta, D. L. Price, J. Rothstein & J. D. Gitlin: Copper chaperone for superoxide dismutase is essential to activate mammalian Cu/Zn superoxide dismutase. *Proc Natl Acad Sci U S A*, 97, 2886-91 (2000)

75. Jensen, L. T. & V. C. Culotta: Activation of CuZn superoxide dismutases from Caenorhabditis elegans does not require the copper chaperone CCS. *J Biol Chem*, 280, 41373-9 (2005)

76. Carroll, M. C., J. B. Girouard, J. L. Ulloa, J. R. Subramaniam, P. C. Wong, J. S. Valentine & V. C. Culotta: Mechanisms for activating Cu- and Zn-containing superoxide dismutase in the absence of the CCS Cu chaperone. *Proc Natl Acad Sci U S A*, 101, 5964-9 (2004)

77. Carroll, M. C., C. E. Outten, J. B. Proescher, L. Rosenfeld, W. H. Watson, L. J. Whitson, P. J. Hart, L. T. Jensen & V. Cizewski Culotta: The effects of glutaredoxin and copper activation pathways on the disulfide and stability of Cu,Zn superoxide dismutase. *J Biol Chem*, 281, 28648-56 (2006)

78. Pardo, C. A., Z. Xu, D. R. Borchelt, D. L. Price, S. S. Sisodia & D. W. Cleveland: Superoxide dismutase is an abundant component in cell bodies, dendrites, and axons of motor neurons and in a subset of other neurons. *Proc Natl Acad Sci U S A*, 92, 954-8 (1995)

79. McCord, J. M. & I. Fridovich: Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem*, 244, 6049-55 (1969)

80. Falconi, M., M. Iovino & A. Desideri: A model for the incorporation of metal from the copper chaperone CCS into Cu,Zn superoxide dismutase. *Structure*, 7, 903-8 (1999)

81. Bartnikas, T. B. & J. D. Gitlin: Mechanisms of biosynthesis of mammalian copper/zinc superoxide dismutase. *J Biol Chem*, 278, 33602-8 (2003)

82. Glerum, D. M., A. Shtanko & A. Tzagoloff: SCO1 and SCO2 act as high copy suppressors of a mitochondrial copper recruitment defect in Saccharomyces cerevisiae. *J Biol Chem*, 271, 20531-5 (1996)

83. Ferguson-Miller, S.: Mammalian cytochrome c oxidase, a molecular monster subdued. *Science*, 272, 1125 (1996)

84. Ferguson-Miller, S. & G. T. Babcock: Heme/Copper Terminal Oxidases. *Chem Rev*, 96, 2889-2908 (1996)

85. Carr, H. S., G. N. George & D. R. Winge: Yeast Cox11, a protein essential for cytochrome c oxidase assembly, is a Cu(I)-binding protein. *J Biol Chem*, 277, 31237-42 (2002)

86. Buchwald, P., G. Krummeck & G. Rodel: Immunological identification of yeast SCO1 protein as a component of the inner mitochondrial membrane. *Mol Gen Genet*, 229, 413-20 (1991)

87. Cobine, P. A., L. D. Ojeda, K. M. Rigby & D. R. Winge: Yeast contain a non-proteinaceous pool of copper in the mitochondrial matrix. *J Biol Chem*, 279, 14447-55 (2004)

88. Maxfield, A. B., D. N. Heaton & D. R. Winge: Cox17 is functional when tethered to the mitochondrial inner membrane. *J Biol Chem*, 279, 5072-80 (2004)

89. Palumaa, P., L. Kangur, A. Voronova & R. Sillard: Metal-binding mechanism of Cox17, a copper chaperone for cytochrome c oxidase. *Biochem J*, 382, 307-14 (2004)

90. Abajian, C. & A. C. Rosenzweig: Crystal structure of yeast Sco1. *J Biol Inorg Chem*, 11, 459-66 (2006)

91. Heaton, D., T. Nittis, C. Srinivasan & D. R. Winge: Mutational analysis of the mitochondrial copper metallochaperone Cox17. *J Biol Chem*, 275, 37582-7 (2000)

92. Banci, L., I. Bertini, S. Ciofi-Baffoni, I. P. Gerothanassis, I. Leontari, M. Martinelli & S. Wang: A structural characterization of human SCO2. *Structure*, 15, 1132-40 (2007)

93. Horng, Y. C., S. C. Leary, P. A. Cobine, F. B. Young, G. N. George, E. A. Shoubridge & D. R. Winge: Human Sco1 and Sco2 function as copper-binding proteins. *J Biol Chem*, 280, 34113-22 (2005)

94. Horng, Y. C., P. A. Cobine, A. B. Maxfield, H. S. Carr & D. R. Winge: Specific copper transfer from the Cox17 metallochaperone to both Sco1 and Cox11 in the assembly of yeast cytochrome C oxidase. *J Biol Chem*, 279, 35334-40 (2004)

95. Leary, S. C., B. A. Kaufman, G. Pellecchia, G. H. Guercin, A. Mattman, M. Jaksch & E. A. Shoubridge: Human SCO1 and SCO2 have independent, cooperative functions in copper delivery to cytochrome c oxidase. *Hum Mol Genet*, 13, 1839-48 (2004)

96. Tsukihara, T., H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono & S. Yoshikawa: The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. *Science*, 272, 1136-44 (1996)

97. Chinenov, Y. V.: Cytochrome c oxidase assembly factors with a thioredoxin fold are conserved among prokaryotes and eukaryotes. *J Mol Med*, 78, 239-42 (2000)

98. Rigby, K., L. Zhang, P. A. Cobine, G. N. George & D. R. Winge: characterization of the cytochrome c oxidase assembly factor Cox19 of Saccharomyces cerevisiae. *J Biol Chem*, 282, 10233-42 (2007)

99. Arnesano, F., L. Banci, I. Bertini & M. Martinelli: Ortholog search of proteins involved in copper delivery to cytochrome C oxidase and functional analysis of paralogs and gene neighbors by genomic context. *J Proteome Res*, 4, 63-70 (2005)

100. Leary, S. C., P. A. Cobine, B. A. Kaufman, G. H. Guercin, A. Mattman, J. Palaty, G. Lockitch, D. R. Winge, P. Rustin, R. Horvath & E. A. Shoubridge: The human cytochrome c oxidase assembly factors SCO1 and SCO2 have regulatory roles in the maintenance of cellular copper homeostasis. *Cell Metab*, 5, 9-20 (2007)

101. Tzagoloff, A., N. Capitanio, M. P. Nobrega & D. Gatti: Cytochrome oxidase assembly in yeast requires the product of COX11, a homolog of the P. denitrificans protein encoded by ORF3. *Embo J*, 9, 2759-64 (1990)

102. Tzagoloff, A., M. Nobrega, N. Gorman & P. Sinclair: On the functions of the yeast COX10 and COX11 gene products. *Biochem Mol Biol Int*, 31, 593-8 (1993)

103. Banting, G. S. & D. M. Glerum: Mutational analysis of the Saccharomyces cerevisiae cytochrome c oxidase assembly protein Cox11p. *Eukaryot Cell*, 5, 568-78 (2006)

104. Carr, H. S., A. B. Maxfield, Y. C. Horng & D. R. Winge: Functional analysis of the domains in Cox11. *J Biol Chem*, 280, 22664-9 (2005)

105. Capitanio, N., G. Capitanio, E. De Nitto, D. Boffoli & S. Papa: Proton transfer reactions associated with the reaction of the fully reduced, purified cytochrome C oxidase with molecular oxygen and ferricyanide. *Biochemistry*, 42, 4607-12 (2003)

106. Lin, S. J. & V. C. Culotta: The ATX1 gene of Saccharomyces cerevisiae encodes a small metal homeostasis factor that protects cells against reactive oxygen toxicity. *Proc Natl Acad Sci U S A*, 92, 3784-8 (1995)

107. Miras, R., I. Morin, O. Jacquin, M. Cuillel, F. Guillain & E. Mintz: Interplay between glutathione, Atx1 and copper. 1. Copper(I) glutathionate induced dimerization of Atx1. *J Biol Inorg Chem*, 13, 195-205 (2008)

108. Walker, J. M., D. Huster, M. Ralle, C. T. Morgan, N. J. Blackburn & S. Lutsenko: The N-terminal metal-binding site 2 of the Wilson's Disease Protein plays a key role in the transfer of copper from Atox1. *J Biol Chem*, 279, 15376-84 (2004)

109. Wernimont, A. K., D. L. Huffman, A. L. Lamb, T. V. O'Halloran & A. C. Rosenzweig: Structural basis for copper transfer by the metallochaperone for the Menkes/Wilson disease proteins. *Nat Struct Biol*, 7, 766-71 (2000)

110. Davies, T. H. & E. R. Sanchez: Fkbp52. *Int J Biochem Cell Biol*, 37, 42-7 (2005)

111. Hamza, I., J. Prohaska & J. D. Gitlin: Essential role for Atox1 in the copper-mediated intracellular trafficking of the Menkes ATPase. *Proc Natl Acad Sci U S A*, 100, 1215-20 (2003)

112. Yuan, D. S., R. Stearman, A. Dancis, T. Dunn, T. Beeler & R. D. Klausner: The Menkes/Wilson disease gene homologue in yeast provides copper to a ceruloplasmin-like oxidase required for iron uptake. *Proc Natl Acad Sci U S A*, 92, 2632-6 (1995)

113. Dierick, H. A., A. N. Adam, J. F. Escara-Wilke & T. W. Glover: Immunocytochemical localization of the Menkes copper transport protein (ATP7A) to the trans-Golgi network. *Hum Mol Genet*, 6, 409-16 (1997)

114. Bingham, M. J., T. J. Ong, W. J. Ingledew & H. J. McArdle: ATP-dependent copper transporter, in the Golgi apparatus of rat hepatocytes, transports Cu(II) not Cu(I). *Am J Physiol*, 271, G741-6 (1996)

115. La Fontaine, S. & J. F. Mercer: Trafficking of the copper-ATPases, ATP7A and ATP7B: role in copper homeostasis. *Arch Biochem Biophys*, 463, 149-67 (2007)

116. Arguello, J. M., E. Eren & M. Gonzalez-Guerrero: The structure and function of heavy metal transport P1B-ATPases. *Biometals*, 20, 233-48 (2007)

117. Banci, L., I. Bertini, F. Cantini, I. C. Felli, L. Gonnelli, N. Hadjiliadis, R. Pierattelli, A. Rosato & P. Voulgaris: The Atx1-Ccc2 complex is a metal-mediated protein-protein interaction. *Nat Chem Biol*, 2, 367-8 (2006)

118. Forbes, J. R., G. Hsi & D. W. Cox: Role of the copper-binding domain in the copper transport function of

ATP7B, the P-type ATPase defective in Wilson disease. *J Biol Chem*, 274, 12408-13 (1999)

119. DiDonato, M., H. F. Hsu, S. Narindrasorasak, L. Que, Jr. & B. Sarkar: Copper-induced conformational changes in the N-terminal domain of the Wilson disease copper-transporting ATPase. *Biochemistry*, 39, 1890-6 (2000)

120. Mercer, J. F., N. Barnes, J. Stevenson, D. Strausak & R. M. Llanos: Copper-induced trafficking of the cU-ATPases: a key mechanism for copper homeostasis. *Biometals*, 16, 175-84 (2003)

121. Jensen, P. Y., N. Bonander, L. B. Moller & O. Farver: Cooperative binding of copper(I) to the metal binding domains in Menkes disease protein. *Biochim Biophys Acta*, 1434, 103-13 (1999)

122. Tsivkovskii, R., B. C. MacArthur & S. Lutsenko: The Lys1010-Lys1325 fragment of the Wilson's disease protein binds nucleotides and interacts with the N-terminal domain of this protein in a copper-dependent manner. *J Biol Chem*, 276, 2234-42 (2001)

123. Huster, D. & S. Lutsenko: The distinct roles of the N-terminal copper-binding sites in regulation of catalytic activity of the Wilson's disease protein. *J Biol Chem*, 278, 32212-8 (2003)

124. Forbes, J. R. & D. W. Cox: Functional characterization of missense mutations in ATP7B: Wilson disease mutation or normal variant? *Am J Hum Genet*, 63, 1663-74 (1998)

125. de Bie, P., P. Muller, C. Wijmenga & L. W. Klomp: Molecular pathogenesis of Wilson and Menkes disease: correlation of mutations with molecular defects and disease phenotypes. *J Med Genet*, 44, 673-88 (2007)

126. Lutsenko, S., E. S. LeShane & U. Shinde: Biochemical basis of regulation of human copper-transporting ATPases. *Arch Biochem Biophys*, 463, 134-48 (2007)

127. Petris, M. J., I. Voskoboinik, M. Cater, K. Smith, B. E. Kim, R. M. Llanos, D. Strausak, J. Camakaris & J. F. Mercer: Copper-regulated trafficking of the Menkes disease copper ATPase is associated with formation of a phosphorylated catalytic intermediate. *J Biol Chem*, 277, 46736-42 (2002)

128. Voskoboinik, I., J. Mar, D. Strausak & J. Camakaris: The regulation of catalytic activity of the menkes copper-translocating P-type ATPase. Role of high affinity copper-binding sites. *J Biol Chem*, 276, 28620-7 (2001)

129. Cater, M. A., S. La Fontaine & J. F. Mercer: 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, 143-53 (2007)

130. Efremov, R. G., Y. A. Kosinsky, D. E. Nolde, R. Tsivkovskii, A. S. Arseniev & S. Lutsenko: Molecular modelling of the nucleotide-binding domain of Wilson's disease protein: location of the ATP-binding site, domain dynamics and potential effects of the major disease mutations. *Biochem J*, 382, 293-305 (2004)

131. Dmitriev, O., R. Tsivkovskii, F. Abildgaard, C. T. Morgan, J. L. Markley & S. Lutsenko: Solution structure of the N-domain of Wilson disease protein: distinct nucleotide-binding environment and effects of disease mutations. *Proc Natl Acad Sci U S A*, 103, 5302-7 (2006)

132. Tsivkovskii, R., J. F. Eisses, J. H. Kaplan & S. Lutsenko: Functional properties of the copper-transporting ATPase ATP7B (the Wilson's disease protein) expressed in insect cells. *J Biol Chem*, 277, 976-83 (2002)

133. Lutsenko, S., N. L. Barnes, M. Y. Bartee & O. Y. Dmitriev: Function and regulation of human copper-transporting ATPases. *Physiol Rev*, 87, 1011-46 (2007)

134. Moller, J. V., C. Olesen, A. M. Jensen & P. Nissen: The structural basis for coupling of Ca2+ transport to ATP hydrolysis by the sarcoplasmic reticulum Ca2+-ATPase. *J Bioenerg Biomembr*, 37, 359-64 (2005)

135. Dijkstra, M., G. In 't Veld, G. J. van den Berg, M. Muller, F. Kuipers & R. J. Vonk: Adenosine triphosphate-dependent copper transport in isolated rat liver plasma membranes. *J Clin Invest*, 95, 412-6 (1995)

136. Bingham, M. J., A. Burchell & H. J. McArdle: Identification of an ATP-dependent copper transport system in endoplasmic reticulum vesicles isolated from rat liver. *J Physiol*, 482 (Pt 3), 583-7 (1995)

137. Hung, Y. H., M. J. Layton, I. Voskoboinik, J. F. Mercer & J. Camakaris: Purification and membrane reconstitution of catalytically active Menkes copper-transporting P-type ATPase (MNK; ATP7A). *Biochem J*, 401, 569-79 (2007)

138. Barnes, N., R. Tsivkovskii, N. Tsivkovskaia & S. Lutsenko: The copper-transporting ATPases, menkes and wilson disease proteins, have distinct roles in adult and developing cerebellum. *J Biol Chem*, 280, 9640-5 (2005)

139. Qin, Z., S. Itoh, V. Jeney, M. Ushio-Fukai & T. Fukai: Essential role for the Menkes ATPase in activation of extracellular superoxide dismutase: implication for vascular oxidative stress. *Faseb J*, 20, 334-6 (2006)

140. Jeney, V., S. Itoh, M. Wendt, Q. Gradek, M. Ushio-Fukai, D. G. Harrison & T. Fukai: Role of antioxidant-1 in extracellular superoxide dismutase function and expression. *Circ Res*, 96, 723-9 (2005)

141. Petris, M. J., J. F. Mercer, J. G. Culvenor, P. Lockhart, P. A. Gleeson & J. Camakaris: 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, 6084-95 (1996)

142. Schaefer, M., R. G. Hopkins, M. L. Failla & J. D. Gitlin: Hepatocyte-specific localization and copper-

dependent trafficking of the Wilson's disease protein in the liver. *Am J Physiol*, 276, G639-46 (1999)

143. Roelofsen, H., H. Wolters, M. J. Van Luyn, N. Miura, F. Kuipers & R. J. Vonk: Copper-induced apical trafficking of ATP7B in polarized hepatoma cells provides a mechanism for biliary copper excretion. *Gastroenterology*, 119, 782-93 (2000)

144. Schlief, M. L., A. M. Craig & J. D. Gitlin: NMDA receptor activation mediates copper homeostasis in hippocampal neurons. *J Neurosci*, 25, 239-46 (2005)

145. Derby, M. C. & P. A. Gleeson: New insights into membrane trafficking and protein sorting. *Int Rev Cytol*, 261, 47-116 (2007)

146. Francis, M. J., E. E. Jones, E. R. Levy, S. Ponnambalam, J. Chelly & A. P. Monaco: A Golgi localization signal identified in the Menkes recombinant protein. *Hum Mol Genet*, 7, 1245-52 (1998)

147. Goodyer, I. D., E. E. Jones, A. P. Monaco & M. J. Francis: Characterization of the Menkes protein copper-binding domains and their role in copper-induced protein relocalization. *Hum Mol Genet*, 8, 1473-8 (1999)

148. Strausak, D., S. La Fontaine, J. Hill, S. D. Firth, P. J. Lockhart & J. F. Mercer: The role of GMXCXXC metal binding sites in the copper-induced redistribution of the Menkes protein. *J Biol Chem*, 274, 11170-7 (1999)

149. Shi, J. & K. V. Kandror: Sortilin is essential and sufficient for the formation of Glut4 storage vesicles in 3T3-L1 adipocytes. *Dev Cell*, 9, 99-108 (2005)

150. Greenough, M., L. Pase, I. Voskoboinik, M. J. Petris, A. W. O'Brien & J. Camakaris: Signals regulating trafficking of Menkes (MNK; ATP7A) copper-translocating P-type ATPase in polarized MDCK cells. *Am J Physiol Cell Physiol*, 287, C1463-71 (2004)

151. Guo, Y., L. Nyasae, L. T. Braiterman & A. L. Hubbard: NH2-terminal signals in ATP7B Cu-ATPase mediate its Cu-dependent anterograde traffic in polarized hepatic cells. *Am J Physiol Gastrointest Liver Physiol*, 289, G904-16 (2005)

152. Petris, M. J., J. Camakaris, M. Greenough, S. LaFontaine & J. F. Mercer: A C-terminal di-leucine is required for localization of the Menkes protein in the trans-Golgi network. *Hum Mol Genet*, 7, 2063-71 (1998)

153. Lane, C., M. J. Petris, A. Benmerah, M. Greenough & J. Camakaris: Studies on endocytic mechanisms of the Menkes copper-translocating P-type ATPase (ATP7A; MNK). Endocytosis of the Menkes protein. *Biometals*, 17, 87-98 (2004)

154. Cikrt, M.: The uptake of Hg203, Cu64, Mn52, and Pb212 by the intestinal wall of the duodenal and ileal segment *in vitro*. *Int Z Klin Pharmakol Ther Toxikol*, 4, 351-7 (1970)

155. Gollan, G. L.: Studies on the nature of complexes formed by copper with human alimentary secretions and their influence on copper absorption in the rat. *Clin Sci Mol Med*, 49, 237-45 (1975)

156. Wapnir, R. A.: Copper absorption and bioavailability. *Am J Clin Nutr*, 67, 1054S-1060S (1998)

157. Puig, S. & D. J. Thiele: Molecular mechanisms of copper uptake and distribution. *Curr Opin Chem Biol*, 6, 171-80 (2002)

158. McKie, A. T., P. Marciani, A. Rolfs, K. Brennan, K. Wehr, D. Barrow, S. Miret, A. Bomford, T. J. Peters, F. Farzaneh, M. A. Hediger, M. W. Hentze & R. J. Simpson: A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Mol Cell*, 5, 299-309 (2000)

159. Ohgami, R. S., D. R. Campagna, A. McDonald & M. D. Fleming: The Steap proteins are metalloreductases. *Blood*, 108, 1388-94 (2006)

160. Ohgami, R. S., D. R. Campagna, E. L. Greer, B. Antiochos, A. McDonald, J. Chen, J. J. Sharp, Y. Fujiwara, J. E. Barker & M. D. Fleming: Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. *Nat Genet*, 37, 1264-9 (2005)

161. Turnlund, J. R., W. R. Keyes, G. L. Peiffer & K. C. Scott: Copper absorption, excretion, and retention by young men consuming low dietary copper determined by using the stable isotope 65Cu. *Am J Clin Nutr*, 67, 1219-25 (1998)

162. Zimnicka, A. M., E. B. Maryon & J. H. Kaplan: Human copper transporter hCTR1 mediates basolateral uptake of copper into enterocytes: implications for copper homeostasis. *J Biol Chem*, 282, 26471-80 (2007)

163. Ferruzza, S., Y. Sambuy, M. R. Ciriolo, A. De Martino, P. Santaroni, G. Rotilio & M. L. Scarino: Copper uptake and intracellular distribution in the human intestinal Caco-2 cell line. *Biometals*, 13, 179-85 (2000)

164. Arredondo, M., P. Munoz, C. V. Mura & M. T. Nunez: DMT1, a physiologically relevant apical Cu1+ transporter of intestinal cells. *Am J Physiol Cell Physiol*, 284, C1525-30 (2003)

165. Collins, J. F.: Gene chip analyses reveal differential genetic responses to iron deficiency in rat duodenum and jejunum. *Biol Res*, 39, 25-37 (2006)

166. Collins, J. F., C. A. Franck, K. V. Kowdley & F. K. Ghishan: Identification of differentially expressed genes in response to dietary iron deprivation in rat duodenum. *Am J Physiol Gastrointest Liver Physiol*, 288, G964-71 (2005)

167. Knopfel, M., C. Smith & M. Solioz: ATP-driven copper transport across the intestinal brush border membrane. *Biochem Biophys Res Commun*, 330, 645-52 (2005)

168. Mistilis, S. P. & P. T. Mearrick: The absorption of ionic, biliary, and plasma radiocopper in neonatal rats. *Scand J Gastroenterol*, 4, 691-6 (1969)

169. Mann, J. R., J. Camakaris & D. M. Danks: Copper metabolism in mottled mouse mutants: distribution of 64Cu in brindled (Mobr) mice. *Biochem J*, 180, 613-9 (1979)

170. Vulpe, C. D., Y. M. Kuo, T. L. Murphy, L. Cowley, C. Askwith, N. Libina, J. Gitschier & G. J. Anderson: Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat Genet*, 21, 195-9 (1999)

171. Nittis, T. & J. D. Gitlin: Role of copper in the proteosome-mediated degradation of the multicopper oxidase hephaestin. *J Biol Chem*, 279, 25696-702 (2004)

172. Ravia, J. J., R. M. Stephen, F. K. Ghishan & J. F. Collins: Menkes Copper ATPase (Atp7a) is a novel metal-responsive gene in rat duodenum, and immunoreactive protein is present on brush-border and basolateral membrane domains. *J Biol Chem*, 280, 36221-7 (2005)

173. Nyasae, L., R. Bustos, L. Braiterman, B. Eipper & A. Hubbard: Dynamics of endogenous ATP7A (Menkes protein) in intestinal epithelial cells: copper-dependent redistribution between two intracellular sites. *Am J Physiol Gastrointest Liver Physiol*, 292, G1181-94 (2007)

174. Prins, H. W. & C. J. Van den Hamer: Primary biochemical defect in copper metabolism in mice with a recessive X-linked mutation analogous to Menkes' disease in man. *J Inorg Biochem*, 10, 19-27 (1979)

175. Bull, P. C., G. R. Thomas, J. M. Rommens, J. R. Forbes & D. W. Cox: The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat Genet*, 5, 327-37 (1993)

176. Bearn, A. G. & H. G. Kunkel: Localization of Cu64 in serum fractions following oral administration: an alteration in Wilson's disease. *Proc Soc Exp Biol Med*, 85, 44-8 (1954)

177. Neumann, P. Z. & A. Sass-Kortsak: The state of copper in human serum: evidence for an amino acid-bound fraction. *J Clin Invest*, 46, 646-58 (1967)

178. Bligh, S. W., H. A. Boyle, A. B. McEwen, P. J. Sadler & R. H. Woodham: 1H NMR studies of reactions of copper complexes with human blood plasma and urine. *Biochem Pharmacol*, 43, 137-45 (1992)

179. Darwish, H. M., J. C. Cheney, R. C. Schmitt & M. J. Ettinger: Mobilization of copper(II) from plasma components and mechanisms of hepatic copper transport. *Am J Physiol*, 246, G72-9 (1984)

180. Weiss, K. C. & M. C. Linder: Copper transport in rats involving a new plasma protein. *Am J Physiol*, 249, E77-88 (1985)

181. Vargas, E. J., A. R. Shoho & M. C. Linder: Copper transport in the Nagase analbuminemic rat. *Am J Physiol*, 267, G259-69 (1994)

182. Gitlin, D. & C. A. Janeway: Turnover of the copper and protein moieties of ceruloplasmin. *Nature*, 185, 693 (1960)

183. Harris, Z. L. & J. D. Gitlin: Genetic and molecular basis for copper toxicity. *Am J Clin Nutr*, 63, 836S-41S (1996)

184. Cousins, R. J.: Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol Rev*, 65, 238-309 (1985)

185. Hsi, G., L. M. Cullen, D. Moira Glerum & D. W. Cox: Functional assessment of the carboxy-terminus of the Wilson disease copper-transporting ATPase, ATP7B. *Genomics*, 83, 473-81 (2004)

186. Li, L., C. D. Vulpe & J. Kaplan: Functional studies of hephaestin in yeast: evidence for multicopper oxidase activity in the endocytic pathway. *Biochem J*, 375, 793-8 (2003)

187. Harris, Z. L., H. Morita & J. D. Gitlin: The bilogy of human ceruloplasmin. . *In: Multicopper-oxidases (A. Messerschmidt, ed.) World Scientific Pub Co., Singapore*285-305 (1997)

188. Zaitsev, V. N., I. Zaitseva, M. Papiz & P. F. Lindley: An X-ray crystallographic study of the binding sites of the azide inhibitor and organic substrates to ceruloplasmin, a multi-copper oxidase in the plasma. *J Biol Inorg Chem*, 4, 579-87 (1999)

189. Osaki, S., D. A. Johnson & E. Frieden: The mobilization of iron from the perfused mammalian liver by a serum copper enzyme, ferroxidase I. *J Biol Chem*, 246, 3018-23 (1971)

190. Roeser, H. P., G. R. Lee, S. Nacht & G. E. Cartwright: The role of ceruloplasmin in iron metabolism. *J Clin Invest*, 49, 2408-17 (1970)

191. Jeong, S. Y. & S. David: Age-related changes in iron homeostasis and cell death in the cerebellum of ceruloplasmin-deficient mice. *J Neurosci*, 26, 9810-9 (2006)

192. Meyer, L. A., A. P. Durley, J. R. Prohaska & Z. L. Harris: Copper transport and metabolism are normal in aceruloplasminemic mice. *J Biol Chem*, 276, 36857-61 (2001)

193. Shim, H. & Z. L. Harris: Genetic defects in copper metabolism. *J Nutr*, 133, 1527S-31S (2003)

194. Harada, M., T. Kawaguchi, H. Kumemura, K. Terada, H. Ninomiya, E. Taniguchi, S. Hanada, S. Baba, M.

Maeyama, H. Koga, T. Ueno, K. Furuta, T. Suganuma, T. Sugiyama & M. Sata: The Wilson disease protein ATP7B resides in the late endosomes with Rab7 and the Niemann-Pick C1 protein. *Am J Pathol*, 166, 499-510 (2005)

195. La Fontaine, S., M. B. Theophilos, S. D. Firth, R. Gould, R. G. Parton & J. F. Mercer: Effect of the toxic milk mutation (tx) on the function and intracellular localization of Wnd, the murine homologue of the Wilson copper ATPase. *Hum Mol Genet*, 10, 361-70 (2001)

196. Harada, M., H. Kumemura, S. Sakisaka, S. Shishido, E. Taniguchi, T. Kawaguchi, S. Hanada, H. Koga, R. Kumashiro, T. Ueno, T. Suganuma, K. Furuta, M. Namba, T. Sugiyama & M. Sata: Wilson disease protein ATP7B is localized in the late endosomes in a polarized human hepatocyte cell line. *Int J Mol Med*, 11, 293-8 (2003)

197. Hernandez, S., Y. Tsuchiya, J. P. García-Ruiz, V. Lalioti, S. Nielsen, D. Cassio & I. V. Sandoval: ATP7B copper-regulated traffic and association with the tight junctions: copper excretion into the bile *Gastroenterology* (2007)

198. Hoekstra, D., D. Tyteca & I. S. C. van: The subapical compartment: a traffic center in membrane polarity development. *J Cell Sci*, 117, 2183-92 (2004)

199. Barroso, M. & E. S. Sztul: Basolateral to apical transcytosis in polarized cells is indirect and involves BFA and trimeric G protein sensitive passage through the apical endosome. *J Cell Biol*, 124, 83-100 (1994)

200. Ihrke, G., E. B. Neufeld, T. Meads, M. R. Shanks, D. Cassio, M. Laurent, T. A. Schroer, R. E. Pagano & A. L. Hubbard: WIF-B cells: an *in vitro* model for studies of hepatocyte polarity. *J Cell Biol*, 123, 1761-75 (1993)

201. Lim, C. M., M. A. Cater, J. F. Mercer & S. La Fontaine: Copper-dependent interaction of dynactin subunit p62 with the N terminus of ATP7B but not ATP7A. *J Biol Chem*, 281, 14006-14 (2006)

202. Wilson, S. A. K.: Progressive lenticular degeneration:a familial nervous disease associated with cirrhosis of the liver. *Brain*, 34, 295 (1912)

203. Ala, A., A. P. Walker, K. Ashkan, J. S. Dooley & M. L. Schilsky: Wilson's disease. *Lancet*, 369, 397-408 (2007)

204. Schnabel, R. & G. Nisch: [A histochemical contribution to metal deposition in the Kayser--Fleischer corneal ring.]. *Graefes Arch Clin Exp Ophthalmol*, 164, 220-30 (1961)

205. Bearn, A. G. & H. G. Kunkel: Biochemical abnormalities in Wilson's
disease. *J Clin Invest*, ;31, 616

206. Scheinberg, I. H. & D. Gitlin: Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease). *Science*, 116, 484-5 (1952)

207. Riordan, S. M. & R. Williams: The Wilson's disease gene and phenotypic diversity. *J Hepatol*, 34, 165-71 (2001)

208. Cuthbert, J. A.: Wilson's disease: a new gene and an animal model for an old disease. *J Investig Med*, 43, 323-36 (1995)

209. Shiono, Y., S. Wakusawa, H. Hayashi, T. Takikawa, M. Yano, T. Okada, H. Mabuchi, S. Kono & H. Miyajima: Iron accumulation in the liver of male patients with Wilson's disease. *Am J Gastroenterol*, 96, 3147-51 (2001)

210. Osaki, S. & D. A. Johnson: Mobilization of liver iron by ferroxidase (ceruloplasmin). *J Biol Chem*, 244, 5757-8 (1969)

211. Nagasaka, H., I. Inoue, A. Inui, H. Komatsu, T. Sogo, K. Murayama, T. Murakami, T. Yorifuji, K. Asayama, S. Katayama, S. Uemoto, K. Kobayashi, M. Takayanagi, T. Fujisawa & H. Tsukahara: Relationship between oxidative stress and antioxidant systems in the liver of patients with Wilson disease: hepatic manifestation in Wilson disease as a consequence of augmented oxidative stress. *Pediatr Res*, 60, 472-7 (2006)

212. Kenney, S. M. & D. W. Cox: Sequence variation database for the Wilson disease copper transporter, ATP7B. *Hum Mutat* (2007)

213. Panagiotakaki, E., M. Tzetis, N. Manolaki, G. Loudianos, A. Papatheodorou, E. Manesis, S. Nousia-Arvanitakis, V. Syriopoulou & E. Kanavakis: Genotype-phenotype correlations for a wide spectrum of mutations in the Wilson disease gene (ATP7B). *Am J Med Genet A*, 131, 168-73 (2004)

214. Kalra, V., D. Khurana & R. Mittal: Wilson's disease--early onset and lessons from a pediatric cohort in India. *Indian Pediatr*, 37, 595-601 (2000)

215. Nadeau, J. H.: Modifier genes and protective alleles in humans and mice. *Curr Opin Genet Dev*, 13, 290-5 (2003)

216. Ferenci, P.: Regional distribution of mutations of the ATP7B gene in patients with Wilson disease: impact on genetic testing. *Hum Genet*, 120, 151-9 (2006)

217. Morgan, C. T., R. Tsivkovskii, Y. A. Kosinsky, R. G. Efremov & S. Lutsenko: The distinct functional properties of the nucleotide-binding domain of ATP7B, the human copper-transporting ATPase: analysis of the Wilson disease mutations E1064A, H1069Q, R1151H, and C1104F. *J Biol Chem*, 279, 36363-71 (2004)

218. de Bie, P., B. van de Sluis, E. Burstein, P. V. van de Berghe, P. Muller, R. Berger, J. D. Gitlin, C. Wijmenga & L. W. Klomp: Distinct Wilson's Disease Mutations in ATP7B Are Associated With Enhanced Binding to COMMD1 and Reduced Stability of ATP7B. *Gastroenterology*, 133, 1316-26 (2007)

219. Forbes, J. R. & D. W. Cox: Copper-dependent trafficking of Wilson disease mutant ATP7B proteins. *Hum Mol Genet*, 9, 1927-35 (2000)

220. Dagenais, S. L., M. Guevara-Fujita, R. Loechel, A. C. Burgess, D. E. Miller, V. Yuzbasiyan-Gurkan, G. J. Brewer & T. W. Glover: The canine copper toxicosis locus is not syntenic with ATP7B or ATX1 and maps to a region showing homology to human 2p21. *Mamm Genome*, 10, 753-6 (1999)

221. van de Sluis, B. J., M. Breen, M. Nanji, M. van Wolferen, P. de Jong, M. M. Binns, P. L. Pearson, J. Kuipers, J. Rothuizen, D. W. Cox, C. Wijmenga & B. A. van Oost: Genetic mapping of the copper toxicosis locus in Bedlington terriers to dog chromosome 10, in a region syntenic to human chromosome region 2p13-p16. *Hum Mol Genet*, 8, 501-7 (1999)

222. Klomp, A. E., B. van de Sluis, L. W. Klomp & C. Wijmenga: The ubiquitously expressed MURR1 protein is absent in canine copper toxicosis. *J Hepatol*, 39, 703-9 (2003)

223. Hyun, C., L. T. Lavulo & L. J. Filippich: Evaluation of haplotypes associated with copper toxicosis in Bedlington Terriers in Australia. *Am J Vet Res*, 65, 1573-9 (2004)

224. Coronado, V. A., D. Damaraju, R. Kohijoki & D. W. Cox: New haplotypes in the Bedlington terrier indicate complexity in copper toxicosis. *Mamm Genome*, 14, 483-91 (2003)

225. Tao, T. Y., F. Liu, L. Klomp, C. Wijmenga & J. D. Gitlin: The copper toxicosis gene product Murr1 directly interacts with the Wilson disease protein. *J Biol Chem*, 278, 41593-6 (2003)

226. Ganesh, L., E. Burstein, A. Guha-Niyogi, M. K. Louder, J. R. Mascola, L. W. Klomp, C. Wijmenga, C. S. Duckett & G. J. Nabel: The gene product Murr1 restricts HIV-1 replication in resting CD4+ lymphocytes. *Nature*, 426, 853-7 (2003)

227. Buiakova, O. I., J. Xu, S. Lutsenko, S. Zeitlin, K. Das, S. Das, B. M. Ross, C. Mekios, I. H. Scheinberg & T. C. Gilliam: Null mutation of the murine ATP7B (Wilson disease) gene results in intracellular copper accumulation and late-onset hepatic nodular transformation. *Hum Mol Genet*, 8, 1665-71 (1999)

228. Hayashi, H., S. Wakusawa & M. Yano: Iron removal by phlebotomy for the prophylaxis of fulminant hepatitis in a Wilson disease model of Long-Evans Cinnamon Rats. *Hepatol Res*, 35, 276-80 (2006)

229. Sinha, S. & A. B. Taly: Withdrawal of penicillamine from zinc sulphate-penicillamine maintenance therapy in Wilson's disease: Promising, safe and cheap. *J Neurol Sci* (2007)

230. Medici, V., L. Rossaro & G. C. Sturniolo: Wilson disease--a practical approach to diagnosis, treatment and follow-up. *Dig Liver Dis*, 39, 601-9 (2007)

231. Menkes, J. H.: Kinky hair disease. *Pediatrics*, 50, 181-3 (1972)

232. Tonnesen, T., W. J. Kleijer & N. Horn: Incidence of Menkes disease. *Hum Genet*, 86, 408-10 (1991)

233. Hsi, G. & D. W. Cox: A comparison of the mutation spectra of Menkes disease and Wilson disease. *Hum Genet*, 114, 165-72 (2004)

234. Ogawa, A., S. Yamamoto, M. Takayanagi, T. Kogo, M. Kanazawa & Y. Kohno: Identification of three novel mutations in the MNK gene in three unrelated Japanese patients with classical Menkes disease. *J Hum Genet*, 44, 206-9 (1999)

235. Procopis, P., J. Camakaris & D. M. Danks: A mild form of Menkes steely hair syndrome. *J Pediatr*, 98, 97-9 (1981)

236. Kim, B. E., K. Smith & M. J. Petris: A copper treatable Menkes disease mutation associated with defective trafficking of a functional Menkes copper ATPase. *J Med Genet*, 40, 290-5 (2003)

237. Kaler, S. G., L. K. Gallo, V. K. Proud, A. K. Percy, Y. Mark, N. A. Segal, D. S. Goldstein, C. S. Holmes & W. A. Gahl: Occipital horn syndrome and a mild Menkes phenotype associated with splice site mutations at the MNK locus. *Nat Genet*, 8, 195-202 (1994)

238. Tumer, Z., L. B. Moller & N. Horn: Mutation spectrum of ATP7A, the gene defective in Menkes disease. *Adv Exp Med Biol*, 448, 83-95 (1999)

239. Mercer, J. F., A. Grimes, L. Ambrosini, P. Lockhart, J. A. Paynter, H. Dierick & T. W. Glover: Mutations in the murine homologue of the Menkes gene in dappled and blotchy mice. *Nat Genet*, 6, 374-8 (1994)

240. Grimes, A., C. J. Hearn, P. Lockhart, D. F. Newgreen & J. F. Mercer: Molecular basis of the brindled mouse mutant (Mo(br)): a murine model of Menkes disease. *Hum Mol Genet*, 6, 1037-42 (1997)

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