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Orchestration of dynamic copper navigation – new and missing pieces

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A general principle in all cells in the body is that an essential metal – here copper – is taken up at the plasma membrane, directed through cellular compartments for use in specific enzymes and pathways, stored in specific scavenging molecules if in surplus, and finally expelled from the cells. Here we attempt to provide a critical view on key concepts involved in copper transfer across membranes and through compartments in the human body. The focus of this review is on the influence of bioinorganic and thermodynamic rules on the flow in cellular copper networks. Transition of copper from one oxidation state to another will often lead to errant electrons that are highly reactive and prone to form radicals and reactive oxygen or nitrogen species (ROS and RNS). Strict control of potentially toxic oxidative species is an important part of understanding the edge of human copper metabolism. The present review critically covers translocation across simple and complex membranes as well as extracellular and intracellular copper routing. We discuss in depth four tissues with polarized cell barriers – the gut, liver, kidneys, and brain – to illustrate the similarities and differences in transcellular transfer. Copper chaperoning, buffering and binding dynamics to guide the metal to different sites are also covered, while individual molecular interaction kinetics are not detailed. Sorting and targeting mechanisms and principles crucial for correct localisation will also be touched upon.

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Introduction

Copper (Cu) is an essential transition metal with an electron configuration conferring high ability to accept and donate electrons. This redox property makes copper suitable for transfer of electrons in enzymatic reactions, but a high propensity to

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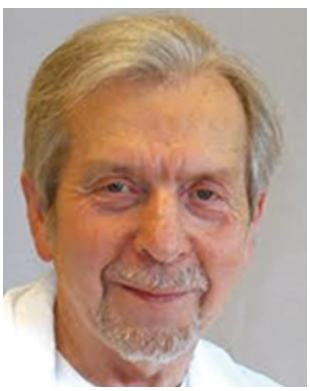
Helena Öhrvik

Dr Helena Öhrvik is a post-doctoral associate at the Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden. She did her graduate studies in Pharmacology at the Swedish University of Agricultural Sciences and received her PhD degree in 2011. The same year she joined Professor Dennis J. Thiele's laboratory at Duke University School of Medicine, Durham, for her post-doctoral training. Early during her graduate studies she was interested in how transport proteins interplay with metal homeostasis and was given the opportunity to study copper transporters in mammals, particularly Ctr1 and Ctr2. At the Uppsala University she is now focusing on the metal homeostasis in innate immune cells.



Jan Aaseth

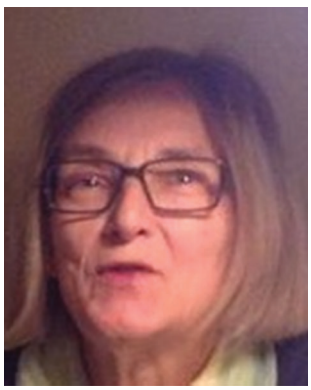
Dr Jan Aaseth is a professor in public health and internal medicine at Inland Norway University of Applied sciences and Innlandet Hospital, Norway. He has been head of the department of toxicology at the National Institute of Occupational Health and professor at the University of Tromsø, the Arctic University of Norway. He was also head of the department of Clinical chemistry at Hedmark County Hospital. His research fields include clinical and laboratory studies on the metabolism of essential and non-essential elements, with a focus on metal complexation and chelation. He has worked on disorders related to copper retention and mercury exposure, and on endogenous complexing sites and therapeutic antidotes in these disorders.

bind to all available electrons (copper is electrophilic) also makes copper reactive and potentially poisonous to handle for living organisms.[1–3] In the human body specific mechanisms have evolved to secure safe delivery to sites where copper is needed and at the same time keep the metal shielded from unwanted adverse reactions.[1] Within cells copper is carefully guided to its destination[4] (summarized in Fig. 1) and free copper ions are kept at extremely low levels.[5] Bioinorganic terms used can be found in IUPAC.[6]

Copper in the body is usually found in the biologically important oxidation states Cu(I) and Cu(II). Copper will coordinate with several electron donor ligands and is often ligated to amino acids like cysteine, methionine and histidine,[7] but also the cyclic amino acids tyrosine, phenylalanine, and tryptophan have significant copper binding capacities. To traverse a membrane the metal needs to be in its native ionic form to use binding sites in an integral transporter.

Copper traverses cellular and intracellular membranes to take part in a vast amount of functions. Copper participates in numerous enzymatic processes[1,4,8,9] as an integral part of enzymes, where the metal works as a prosthetic group to facilitate the transfer of electrons from one molecule to another. During this process it is of utmost importance that copper is delivered at the right time at the right location for correct integration into enzymes.

Intracellularly the potential toxicity is handled by channeling copper directly from one site into another site without ever being free. The driving force that fine-tunes copper delivery to sites is the resulting combination of multiple needs,[10,11] and we here attempt an overview of the guiding dynamics without going into the details of individual molecular binding kinetics. Extracellularly copper transfer is also the result of multiple binding dynamics, but the system shows a much broader flexibility.



Nina Horn

Dr Nina Horn is a previous laboratory director at the Kennedy Centre in Glostrup, Denmark where she worked on metabolic diseases and biochemical causes for mental retardation, including energy metabolism. Copper was the focus of her research. Her scientific career started by investigating active sodium coupled hexose transport in the gut, and ended by describing mutational effects on the energy dependent copper pump, ATP7A. Her first paper on Menkes disease (1973) demonstrated abnormal copper distribution in foetal tissues refuting a copper absorption defect as the underlying cause. Despite being out of active copper research for some years, she has kept a continuous interest in the field. Her proverb is: 'When you zoom in, it all becomes too important, but zooming out again makes new patterns visible.'

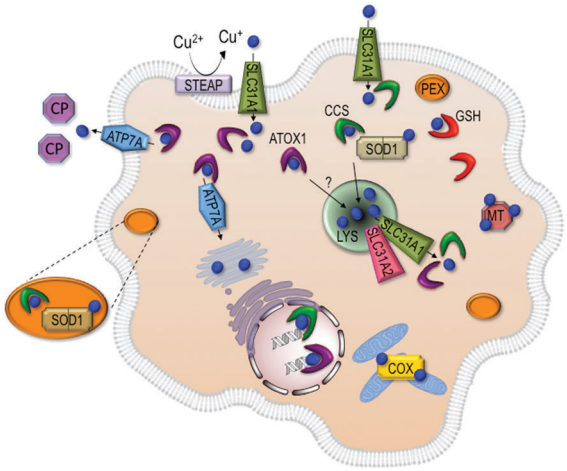


Fig. 1 Intracellular copper navigation: cells have evolved a complex regulatory network to maintain copper homeostasis. Copper (blue) is taken up at the plasma membrane as Cu(I) by the copper importer SLC31A1/CTR1. Extracellular Cu(II) is reduced by STEAP located in membrane vesicles to promote Cu(I) uptake and expression of the importer SLC31A1/CTR1 is regulated by the intracellular copper level. Intracellularly copper binds to glutathione (GSH) (red) and chaperones. Chaperones may receive copper directly from SLC31A1/CTR1 or GSH can act as an intermediary molecule. Copper is involved in many essential cellular processes and three principally different chaperone guided pathways have been identified, and the cytosolic copper chaperones (ATOX1, CCS, and COX17) compete for the Cu–GSH pool and sort copper to various destinations. Copper distribution between chaperones and other copper-binding molecules is determined by their relative abundance, and different affinities and structural dynamics shape the driving forces.[2,12,175] Excess copper is scavenged as Cu-thionein (MT) (light brown), which is not easily mobilized again. ATOX1 (dark maroon) transfers copper to the MBDs and regulates the activity of the copper transporting ATPases located in the Golgi network and secretory vesicles. Increase in cytosolic copper triggers trafficking from the TGN to exporting vesicles that will fuse with the plasma membrane to release copper. Before release the metal will undergo a shift from cupro Cu(I) to cupri Cu(II) ions. Under certain conditions ATOX1 translocates to the nucleus. CCS (dark green) guides copper loaded SOD1 to cellular compartments depending on the cellular redox state *e.g.* peroxisomes (PEX) (orange), nucleus, cytoplasmic vesicles, or minute amounts in the intra mitochondrial membrane space (IMS) (<5%). Copper is stored in a matrix located mitochondrial pool, but the copper pathway to this site is not resolved and the cytosolic role of COX17 is still unknown though possibly regulatory. COX17 donates copper to acceptor proteins within the IMS. Transfer of copper through the mitochondrial membranes is not elucidated, but GSH and its chelatin polymers may play a role in matrix copper uptake and export. Lysosomes (LYS) (green) receive copper from degraded copper proteins (denser area) and recirculate the metal by SLC31A1/CTR1 and its regulator SLC31A2/CTR2. During sorting to and from membranes copper chaperones and transporters pass lysosomes for quality control but routing determinants are not defined.

Copper homeostasis

Whole body copper homeostasis is regulated at the levels of intestinal absorption and hepatic excretion. Between these two sites copper traverses several barriers and compartments involving many copper specific molecules comprising the overall copper homeostatic network.[12]

Copper cannot traverse any lipophilic membrane without the assistance of a well-suited amphiphilic molecule containing

specific hydrophilic sites for binding of copper as well as lipophilic properties to facilitate integration in a membrane. In this review we focus on transcellular transport in the gut, liver, kidneys, and brain, emphasizing the similarities and differences. We review copper buffering and complexing in extra- and intracellular compartments and discuss cellular and subcellular transfer mechanisms. These include facilitated copper transfer across complex and single membranes and chaperoning between compartments.

Intracellular copper occurs as stable cuprous complexes, while the labile extracellular fraction is made up of less stable cupric complexes. Copper ion transition easily gives rise to errant electrons that are highly reactive and prone to form free radicals including reactive oxygen or nitrogen species (ROS and RNS) and strict control is required. Numerous enzymes have evolved to regulate these reactive species and some make use of copper's intrinsic ability to transfer and disproportionate electrons.

Dysregulation of copper homeostasis in humans is primarily found in two genetic diseases of copper transport, Menkes (OMIM 309400) and Wilson (OMIM 277900) disease, which show symptoms of copper deficiency[13] or overload,[14] respectively. Menkes disease is a multi-systemic disorder caused by mutations in ATP7A (OMIM 300011), while the homolog ATP7B (OMIM 606882) is mutated in Wilson disease, with primarily hepatic and cerebral affects. Several other diseases may also influence copper homeostasis.

Gut

Copper can be absorbed in the stomach, duodenum, and small intestine of mammals.[15] Rapid appearance of Cu-64 in plasma after oral administration indicates absorption in the stomach and upper intestine in humans[16] though the ileum has been suggested as the predominant copper absorption site from food.[17] In the absorptive canal, dietary copper is bound to various food sources, and when digested in the stomach with a low pH, the metal naturally exists in a cuprous state Cu(I)[18] that can be absorbed by specific cupro-sensitive transporters.[19] When copper travels down the gut canal, the pH will gradually rise and favour the cupric form Cu(II). Uptake here will need reduction of the metal or the possible use of a divalent ion transporter.

Each segment of the gut canal has variable absorptive properties. Lower segments are important for water balance and expelling of copper secreted *via* bile. In the upper entero-segments, fluid flow is in the opposite direction of nutrients, while in the lower distal segments the net-uptake of water occurs.

Thus, copper transport varies along the digestive canal as well as with the maturity of individual absorptive cells. When generated at the bottom of the crypt of villi, enterocytes are joined together by immature tight junctions (TJs) allowing easy flow of water and uptake of His–Cu–His.[9,20] The turn-over of the human gut epithelium is about three days[21] enabling a constant though minute His–Cu–His uptake. When gut mucosal cells are sloughed off, significant amounts of copper leave with the faeces.[22] High turn-over of enterocytes is exploited in Wilson patients when treated with zinc to induce metallothionein (MT) binding of copper followed by sloughing of the mucosa and excretion *via* faeces.[23]

The brush border of the apical surface of intestinal cells is equipped with metal transporters and related enzymes,[20] each specific for a single metal or a selected group of similar metals. This selectivity is based on the metal's ability to coordinate with certain amino acids, *e.g.* methionine and cysteine. The plasma membrane of absorptive cells contains an integral transporter, CTR1 (copper transporter 1) (SLC31A1) (OMIM 603085), facilitating copper uptake.[8,24]

In vitro studies have suggested that DMT1 (Divalent Metal Transporter 1) (SLC11A1) (OMIM 600266) may contribute to dietary copper uptake.[25,26] In contrast *in vivo* studies using mice and rats lacking functional SLC11A1 show no evidence of altered copper transport[27,28] indicating an insignificant role in copper homeostasis.

In the abluminal end of the enterocyte at the serosal side, also named the basolateral border, an energy dependent transporter, ATP7A, pumps copper ions out into the portal blood.[9] During transfer the fully copper loaded transporter undergoes a conformational flip-flop change that finally dumps the metal into plasma, followed by a remodelling back into its original form.[29] Release of copper from enterocytes into the blood is accompanied by an electrochemical shift from cuprous to cupric forms to allow binding to histidine-rich molecules that carry the metal to various organs and tissues.

Liver

The liver is the central body copper storage site and can respond to increased copper needs in the periphery.[30] Being the master organ of whole body copper homeostasis, the liver must control the storage of copper, mobilize copper to peripheral tissues, incorporate copper into ceruloplasmin[31] (CP) (OMIM 117700) and other copper-dependent proteins, and when in excess excrete copper *via* bile into faeces. Hepatocytes are polarized cells with basolateral (sinusoidal) and apical (canalicular) membranes. They import copper from the blood *via* basolaterally expressed SLC31A1/CTR1.[32,33] Mice with a targeted liver-specific loss of SLC31A1/CTR1 have reduced activity of copper dependent enzymes, decreased copper levels in the liver, and diminished copper excretion through bile.[34,35] Copper levels in other organs are not reduced, suggesting a functional whole body copper homeostasis, despite diminished copper hepatic stores,[34] stressing that the liver can be circumvented in delivery to extra-hepatic sites (Fig. 2).

Copper is excreted from the body *via* bile, and the system can be viewed as a secretory extension of the gut. Bile is secreted by hepatocytes into minute channels (bile canaliculi), and the excreted copper is complexed making the metal unavailable for reabsorption.[36] No enterohepatic copper circulation exists, and in practice, biliary excretion serves as the main route for copper elimination.

Secretory hepatocytes are polarized parenchymal cells and the surface towards the canaliculi possesses microvilli and is

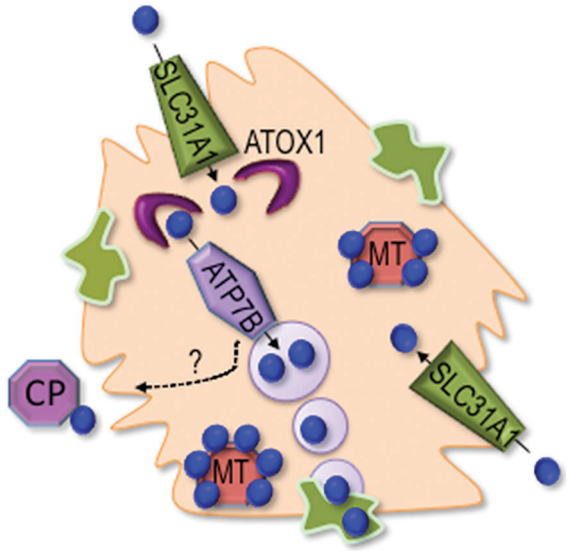


Fig. 2 Hepatic copper navigation: copper (blue) is taken up at the basolateral sinusoidal membrane by the copper importer SLC31A1/CTR1. ATP7B promotes copper biliary (light olive green) excretion through the apical canalicular membrane by pumping the metal into an excretory subset of lysosomes (light lilac). ATP7B biliary excretion is regulated by a complex interplay of several molecules (*e.g.* COMMD1, XIAP, and DCTN4). The arrow indicates the ATP7B sinusoidal route before either canicular excretion or extra-hepatic copper delivery.

hence an apical surface. At high copper concentrations ATP7B localizes in vesicles near the apical membrane,[37] and apical copper excretion[38] is supported by the presence of an apical targeting sequence in the N-terminus containing the copper binding aromatic amino acids tryptophan, phenylalanine, and tyrosine.[39,40] Copper is excreted *via* the endosomal–lysosomal pathway through a transient XIAP regulated interaction between ATP7B and COMMD1 (OMIM 607238).[41,42] Lysosomal copper exocytosis is promoted through an interaction between ATP7B and the dynactin 4 subunit (DCTN4) (OMIM 614758) of the dynactin motor allowing translocation towards the canalicular pole of hepatocytes by microtubule-dependent vesicular transport.[43] This interaction requires copper loading of metal-binding domains (MBDs) of ATP7B and the C-terminal RING zinc-finger motif in DCTN4,[44] and is crucial for excretion of excessive copper.[45] ATP7B recycles back, when sufficient copper has been released, and COMMD1 participates in the retrieval of ATP7B from lysosomal vesicles.[42]

Steady state investigations of human samples have, however, showed varying and different ATP7B pump localizations.[37] The presence of ATP7B at the sinusoidal pole of hepatocytes[37] indicates that the protein also participates in trafficking of copper to the bloodstream. Hepatic sinusoidal capillaries secrete the copper carrying proteins (CP), albumin (ALB) (OMIM 103600), and α2-macroglobulin (A2M) (OMIM 103950) into the bloodstream.

Kidneys

Urinary copper is the net result of glomerular filtration, tubular reabsorption and secretion. In the kidneys, the diffusible part of the blood copper pool is filtered out into crude urine by leaky vessels and the ependymal cell layer in glomeruli. Glomeruli are in contact with polarized podocytes that form a crucial part of the glomerular filtration barrier.[46] They possess multiple dynamin containing foot processes[47] that have a location as astrocytic endfeet in the brain (see later). They are surrounded by Bowman's capsule and together they constitute a functional unit. The capsules empty the filtrate into proximal convoluted tubules that are part of the duct system of the nephron. The structure of kidney tubular cells is analogous to absorptive enterocytes, and the cells of the proximal tubules contain numerous microvilli. SLC31A1/CTR1 is expressed in both proximal and distal kidney tubular cells and may import glomerular-filtered copper from primary urine back into the blood.[32] In support, kidney SLC31A1/CTR1 levels are elevated under systemic copper deficiency,[32] which may serve to increase copper reabsorption from urine. SLC31A1/CTR1 can also promote urinary excretion of excess metal in proximal tubular cells.[48]

Like the gut, absorptive cells are segmented along the tubules of the nephron. ATP7A and ATP7B are located in tubular cells both proximally and distally indicating that both pumps are necessary for normal renal copper balance.[49,50] However, inconsistent results have been obtained with regard to the localization of ATP7A and ATP7B in the kidneys, which probably is due to different experimental techniques, variable ages of animals tested, and different copper loads. ATP7A has been detected in glomeruli, podocytes, proximal and distal tubular cells, and the loop of Henle.[51–53] ATP7B has been found in glomeruli and, proximal and distal tubules.[49,52,54,55] ATP7A location in glomeruli could not be confirmed and might be an artefact due to the use of improper controls as pointed out by another study.[54]

ATP7A is shown to be located at the basolateral border of kidney tubular cells[54] indicating that the most likely pumping direction is into the tissue for reabsorption. In analogy with hepatic canaliculi, ATP7B has been found at the brush border membrane of tubular cells.[55]

When the hepatic route is blocked in the case of ATP7B deficiency, urinary excretion serves as an additional route for elimination of excess copper.[35] Elevated blood copper is bound to low molecular weight (MW) molecules and filtered through glomeruli.[56] Kidney transcellular tubular elimination is not used because SLC31A1/CTR1 cannot uptake copper from the low MW molecule.[35]

Brain

Within the brain (CNS), several structures and cells have their own specialized role in copper metabolism.[57,58] We primarily cover transfer across the blood brain barriers (BBBs) that play crucial roles in maintaining brain copper homeostasis by permitting selective entry into the brain. The two main barriers, blood–endothelial barrier (BEB) and blood–cerebrospinal-fluid barrier (BCB), each possess specific cells and roles in copper homeostasis, and both barriers are characterized by cells derived from nerve tissue working together with endothelial cells.

The quantitatively most important blood exchange site is the BEB, often referred to as the blood–brain barrier.[59] Brain capillaries with surrounding cells form a functional unit controlling uptake into the neuronal compartment. Vascular endothelial cells are glued together by TJs that restrict passage between them, and astrocytic endfeet surround capillaries like podocytic foot processes surround glomeruli. Astrocytes are also polarized and may similarly modulate the BEB function. SLC31A1/CTR1 promotes uptake into astrocytes and is expressed in high amounts.[60,61] ATP7A controls copper transport from astrocytes into the brain tissue,[62] and excess copper induces MT synthesis to restrict release from the cells.[61,63] Secretion of CP from perivascular astrocytes indicates the expression of ATP7B that likely promotes export from the brain tissue to the blood.[64–66]

The choroid plexi of the BCB mimics the villi of the gut and the ependymal lining has a structure similar to absorptive cells and creates a tight barrier. Specialized ependymal cells similarly possess a brush border towards brain ventricles, and once they are mature and fully differentiated their life span is life-long,[67] in contrast to the short life span of gut epithelial cells.

Like the gut and kidneys, brain ependymal cells are segmented and consist of four distinct and separated structures.[68] Each segment contains similar but morphologically discrete choroid plexi, which are formed at different stages of fetal development and derived from two different fetal stem cells.[68–71] Choroid epithelial cells and adjacent ependymal cells function to regulate the content of cerebrospinal fluid (CSF) and thereby provide micronutrients, proteins, hormones and metals for neuronal and glial development, maintenance and function.[72] Re-uptake of important nutrients including copper occurs *via* the polarized ependymal cells similar to kidney tubular cells and active secretion can also occur. It is still debated whether the major role of CSF transport is for nutrition of various brain areas or elimination of waste products, or both.[72] Tiny fenestrated blood vessels located beneath the ependymal epithelium penetrate into the brain tissue and likely regulate the flow of important nutrients to and from the brain parenchyma. The architecture of each tuft of choroid plexi is reminiscent of glomeruli and the ependymal vessels are like vessels intertwining the fluid-collecting tubes of nephrons.

Water flows easily across the epithelial cell layer in the choroid plexus proper into lateral ventricles and is said to produce CSF which, like in the gut and kidneys, is drained back distally into the venous system.[73,74] The liquid flow goes from lateral ventricles through third and fourth ventricles that are interconnected by narrow passages. From the fourth ventricle the CSF flows into the subarachnoid space surrounding the brain and spinal cord. The CSF is absorbed through blood vessels over the surface of the brain back into a newly discovered brain lymphatic network of *dura mater*.[73–76]

Interstitial fluid around glia and nerve cells is easily filtered into the ventricles by the ependymal lining and flushes nerve tissue free of waste.[73,77] The brain parenchyma contains numerous water channels (aquaporins) that function as the brain's glymphatic system for fluid exchange.[73] The brain does not contain a proper lymphatic system. The liquid flow is regulated by pressure and the pulsation is created by the heart beat and respiratory ventilation. When pressure is low during the night the flow will be at its highest implying that the brain is primarily cleared during sleep while nurtured during waking hours.[78]

In parallel to the gut, most copper will take the transcellular route. Copper transport by the paracellular route is unlikely due to the TJs, but may occur to a minor extent through the more leaky TJs in the developing brain.[73] The apical membrane of choroid plexi cells expresses high levels of SLC31A1/CTR1[32] and copper deficiency increases the SLC31A1/CTR1 expression facilitating uptake from the CSF to meet brain copper demands.[32,79]

In choroid plexi there is no consensus about the location of the copper pumps, ATP7A and ATP7B.[59,80–83] The important question is in which membranes and perhaps segments the pumps act. Like in the kidneys, the two pumps may be located both together and in different segments.

Localization of any membrane transporter in choroid plexi is not straight forward, because molecules creating cell polarity are differently located.[84] Many transporters are localized similarly to other transporting epithelia, while others have opposite localization.[85] Therefore, it is not possible to foresee the correct localization of ATP7A and ATP7B, without knowing the specific membrane molecules anchoring the pumps. The possibility of flexible membrane integrations should be considered.

Mutations in ATP7B cause high cerebral and CSF copper,[9] while Menkes boys have low brain copper including low CSF copper. Thus, ATP7A seems to be the rate-limiter in the uptake of copper across the BEB as well as the BCB into the brain and ATP7B in extrusion both at the BCB and BEB.

Using *in situ* hybridization on human *post mortem* samples from an unspecified region of the choroid plexi, ATP7A and ATP7B showed basolateral and apical localizations, respectively.[57] However, animal studies have localized the two pumps opposite other transporting epithelia. At a high copper concentration ATP7A travels to the luminal border, while ATP7B is directed towards the basolateral membrane.[86]

More evidence is needed to firmly establish correct membrane integration. It is necessary to know the exact plexus origin of the epithelia studied and *in situ* animal studies using *in vivo* fixation techniques may give more valid results. It is also important to use antibodies raised against distinctly different epitopes in the two pumps.[54] ATP7A and ATP7B are so alike that cross-reactions are very likely. In addition steady state and copper induced localizations may vary.

Extra- and intracellular copper buffers and complexes

Copper tightly complexed in a protein is used for enzymatic reactions (transfer of electrons), scavenging of the metal or regulation of specific pathways. For transient binding, copper needs buffering rather than complexing.[6]

Blood buffers and pools

Extracellularly copper exists mostly in the cupric form Cu(II), and in plasma copper trafficking and buffering is achieved mainly by histidine-rich sites[87] meaning a rather loose binding compared to the cytoplasm. The labile copper pool comprises His–Cu–His in equilibrium with ALB[88] and A2M, which rapidly exchange copper.[89] A major unavailable pool is tightly bound in CP[15,89] that may participate in copper redox regulation.[9,15,90]

ALB belongs to proteins with N-terminal imidazol groups of histidines that are highly selective for Cu(II).[91] They often form an ordered site of varying sizes where more imidazol groups cooperate, and the cooperation may result in enhanced copper redox cycling.[92] Imidazol cluster motifs are found in numerous extracellular proteins and enzymes *e.g.* ALB, A2M, Cu/Zn superoxide dismutases 1 (SOD1) (OMIM 147450)[93] and 3 (SOD3) (OMIM 185490), amyloid precursor protein (APP) (OMIM 104760), and prion protein (PRNP) (OMIM 176640). Besides binding copper, they have additional roles in whole body homeostasis.[9,89,90]

The role of ALB as a copper buffer and carrier has been debated. Circumstantial evidence comes from canine copper toxicosis susceptibility. Besides a specific COMMD1 defect in the best known model,[94,95] all dogs lack one of the histidines in the ALB terminal imidazole metal binding site[96] that renders them particularly prone to copper toxicosis. Also labrador retrievers with changes in one or the other copper ATPase show severe copper disturbances.[97–99] The canine copper toxicosis susceptibility trait has also helped in identifying a lipid ABC transporter (ABCA12) (OMIM 607800) of importance for hepatic copper homeostasis.[100]

Likely ALB participates in extracellular copper buffering. However, analbuminemia (OMIM 616000) in humans is not accompanied by obvious aberrant copper handling[101] emphasizing that ALB is not the sole imidazol containing copper carrier in plasma and pointing to a broader extracellular buffering flexibility. Extracellular SOD3 constitutes a large blood copper pool and has been extensively studied in relation to atherosclerosis.[102,103]

CSF buffers and pools

The CSF and brain interstitial fluid contain the same proteins and peptides as plasma though at much lower levels.[104,105] CP are synthesized by the choroid plexi[105] and the GPI-anchored form by astrocytes,[58,65,106] but only a small part of CSF copper is CP-bound,[107] implying that histidine-rich proteins are important.[80] A2M is synthesized by the choroid plexi[105] and astrocytes[108] and may constitute the principal copper buffer in the CSF and neuronal interstitial fluid. Other membrane-attached copper containing proteins important for extracellular brain copper homeostasis are APP and PRNP. The level of GSH in the CSF, as in plasma, is negligible. Recently extracellular MT has been implicated in brain redox homeostasis[109] and may substitute for GSH as a copper sensor.[3]

APP is an extracellular membrane bound protein[3,110] most abundantly found in the brain. It has been hypothesized to act as a copper redox buffer and reservoir.[111] APP contains an imidazol site within its extracellular domain[112] showing dismutase activity that can produce various oxidation species of copper and iron when not regulated properly.[113] APP may pre-exist as an apo-pool that is metallated when needed. This could account for APP's apparent copper buffering capacity. APP gene defects lead to Alzheimer disease (OMIM 104300) characterized by the accumulation of misfolded abeta aggregates.[114]

APP is regulated by CCS through β-site APP-cleaving enzyme 1 usually named β-secretase (BACE1) (OMIM 604252). A2M may be a copper donor since APP and A2M appear to be functional partners based on the predicted interaction network and confident hit of 0.971 from String analysis (String-db.org). A strong relationship is underlined by A2M being an Alzheimer susceptibility gene (OMIM 104300).[115]

PRNP is a ubiquitously expressed cell surface protein with high conservation among mammalian species pointing to an important function possibly in copper homeostasis. PRNP binds Cu(II) through four octapeptide sequences in the N-terminal end forming a four imidazol cluster motif that stabilizes the structure and is necessary for normal conformation.[109] *In vitro* PRNP possesses antioxidant activity and has been implicated in copper availability to cells, but further studies are required to elucidate its physiological functions.[116,117] PRNP regulates BACE1 activity[117] and hereby APP. Deficient misfolded PRNP gives progressive spongiform encephalopathies, such as Creutzfeldt–Jakob disease (CJD) (OMIM 123400).

Cytosolic copper complexes and pools

Within cells Cu(I) is favoured. Thiol groups in cysteine and methionine coordinate cuprous ions, and the small tripeptide GSH (γ-Glu–Cys–Gly) is very abundant in the cytoplasm of all cells. GSH is present in millimolar concentrations, but distributed differently among cellular compartments.[118] Significant pools are found within the mitochondrial matrix, endoplasmic reticulum (ER) and nuclear matrix.[119] It is a major redox buffer and sensor in cells usually present as the redox pair 2GSH/GSSG. Oxidized GSSG is rapidly reduced by NADPH-dependent glutathione reductase (GSR) (OMIM 138300)[119] and GSH is hence the predominant cytosolic form.[120] The redox potential between 2GSH/GSSG may vary among different locations *e.g.* the cytoplasm, mitochondrial matrix, peroxisomes, and lysosomes.[121,122] Copper chaperones and GSH may form an exchangeable cytoplasmic copper pool.[123,124]

Metallothioneins (MTs) comprise a large family of cysteine-rich (up to 30%), low MW proteins.[125] They are principally localized intracellularly, usually attached to Golgi membranes, but recently MTs have been found extracellularly, and under certain conditions they translocate to the nucleus.[126] MT transcription is regulated by zinc, but the protein can bind both essential (*e.g.* zinc and copper) and non-essential metals through the thiol groups.[125] The peptide chain folds into two distinct thiolate clusters with strong metal avidity, and MT is a biologically important zinc pool for rapid availability.[120,127,128] Several metals will displace zinc, but copper has the highest

avidity and is not easily mobilized again.[109,125,129,130] The role in copper homeostasis is a scavenging one, and MTs participate in regulation of the bioactive copper pool. To release copper, proteolytic degradation of Cu-thionein will possibly be required,[131,132] though some exchange with other SH containing molecules has been demonstrated.[3,109]

When displaced by copper or other potentially harmful metals, zinc becomes available for induction of more MT and regulation of other pathways *e.g.* the formation of the thiol buffer GSH by zinc dependent glutathione synthetase (GSS) (OMIM 601002). Thus MT provides protection against metal toxicity directly and indirectly by facilitating the production of GSH. MT can like GSH form metal-bridge dimers as well as S–S linked dimers that can be reduced to the monomeric form by GSR.[120] Thus MT works synergistically with GSH.

The importance of MT is underscored by the existence of more than 30 different MT genes and pseudogenes clustered in the human genome (OMIM Database). MTs are grouped into four different classes named MT1, 2, 3, and 4. MT1 and MT2 are ubiquitously expressed, while MT3 and 4 are tissue specific for the CNS and epithelial cells, respectively.[109,130] The binding dynamics between different MT classes vary towards various metals and also with stepwise binding.[3]

SOD1 was earlier viewed as a cytosolic copper buffer or store (cytocuprein/erythrocuprein)[133] because copper induces *de novo* synthesis[134] and the unmetallated apo-pool can take up copper.[135] SOD1 stores significant amounts of copper underlining the importance of this enzyme. Construction of the SOD1 active copper site involves intermolecular cluster formation and provides the basis for dimerization and tetramerization of the enzyme. Genetic disturbances of SOD1 lead to amyotrophic lateral sclerosis (ALS) (OMIM 105400).[136]

Alpha-synuclein (SNCA) (OMIM 163890) binds two coppers per monomer and contains seven imperfect repeats, which may mediate multimerization.[137] SNCA is primarily found in nerve tissue where it interacts with tubulin in the cytoskeleton[138] and may be involved in copper trafficking.[139] SNCA plays a role in the regulation of the dopamine pathway by stabilizing tyrosine hydroxylase (TH) (OMIM 191290).[140] Accumulation of abnormal SNCA is associated with Parkinson disease (PD) and Lewy body dementia,[141] and point mutations and gene multiplications lead[142] to autosomal dominant forms (OMIM 127750, 168601, 605543).

Mitochondrial copper sequestration and pools

GSH constitutes a very large mitochondrial pool of reducing equivalents and is the principal redox buffer of this organelle.[118,143] Maintenance of the mitochondrial GSH pool is critical for mitochondrial redox homeostasis. Despite high GSH concentration in the mitochondrial matrix, this organelle lacks the enzymes necessary for GSH production.[118,143] Mitochondrial GSH originates from cytosolic GSH, and an import mechanism must exist. A number of eukaryotic GSH transporters have been identified, and molecular mechanisms of GSH transport into various organelles are currently being defined.[144,145]

Solute carriers (SLCs) in the mitochondrial membranes may participate in GSH transport into the matrix.[143] Two human inner mitochondrial membrane GSH transporters have been identified, SLC25A10 (OMIM 606794)[146] and SLC25A11 (OMIM 604165).[147] Besides GSH transfer, these transporters possibly also transfer glutathione–metal complexes and glutathione S-conjugates.[148] Peptide-like polymers of GSH, chelatins [(Glu–Cys)$_{n=2-11}$–Gly], are likely also transported into the mitochondrial matrix.

Evidence (in yeast) suggests that copper may be stored in a labile matrix pool (CuL), but the nature of this storage pool is still obscure.[149,150] However, evidence from plants and nematodes[151–153] indicates that it may consist of GSH polymers, often referred to as phytochelatins. The formation of chelatins constitutes an important mechanism for heavy metal storage and detoxification in plants, and nematodes also contain a fully functional chelatin pathway.[152,154,155] Like phytochelatin peptides,[156] the matrix copper ligand (CuL) shows an absorbance indicating a non-protein metal-binding polypeptide.[149] Chelatins with 9–11 repetitive Glu–Cys moieties have a similar cysteine composition to MT thiolate clusters and may curl up similarly. Earlier, chelatins were confused with MTs and at a point named MTIII.[154,157]

Cellular and subcellular copper transporters and chaperones

For copper ions to traverse a membrane of lipid bilayers, specific copper binding amino acids need to form a backbone or a pore for the transfer. The binding needs to be transient, possibly regulated by the oxidation state of copper as well as donor and acceptor molecules at membrane interfaces, and perhaps also pH and the redox state.[158]

Cellular copper uptake

Copper enters cells through SLC31A1/CTR1 channels by a series of ligand exchange reactions.[159] The SLC31A1/CTR1 trimer contains a narrow pore at the plasma membrane that widens on the intracellular side,[159] and the pore shows a copper binding gradient with increasing affinity from the entrance to exit.[160,161] Transport occurs along a concentration gradient as intracellular copper is efficiently sequestered while extracellular copper is loosely bound. The SLC31A1/CTR1 ectodomain acquires copper using high affinity histidine and methionine rich sites,[162,163] and to control copper uptake methionine sites at the cytoplasmic gate regulate vesicular endocytosis and recycling of the transporter.[162,164,165] Copper Chaperone for Superoxide Dismutase (CCS) (OMIM 603864) and antioxidant 1 copper chaperone (ATOX1) (OMIM 602270) can both dock with the cytoplasmic end of SCL31A1/CTR1 and receive Cu(I) directly from the transporter.[161,166] CTR2 (copper transporter 2) (SLC31A2) (OMIM 603088) was identified by homology to SLC31A1/CTR1.[167]

In cell culture systems SLC31A2/CTR2 can act as a low affinity copper importer,[168] regulator of macro-pinocytosis,[169] and lysosomal copper exporter.[170] Systemic SLC31A2/CTR2 knockout mice demonstrated that the protein has a regulatory function facilitating the cleavage of the SLC31A1/CTR1 copper binding ectodomain.[171] Recently a subclass of lysosomal cysteine proteases, cathepsin B (CTSB) (OMIM 116810) and cathepsin L (CTSL) (OMIM 116880), were shown to be responsible for the cleavage of the ectodomain.[172] How the interaction occurs between SLC31A1/CTR1, SLC31A2/CTR2, and CTSB and/or CTSL remains elusive, as is regulation of the cleavage event. CTSB is also known as APP-secretase, and amyloid peptide cleavage occurs at a site that prevents the formation and deposition of APP. It has been suggested that genetic defects in CTSB might be related to Alzheimer disease.[173]

Once inside the cell, copper chaperones deliver the metal to distinct targets by direct protein–protein interactions[2,174] and the vectorial movement follows a thermodynamic binding gradient.[10,122] GSH likely bridges copper transfer from SLC31A1/CTR1 depending on chaperone concentrations.[161,175]

Copper and proteins both need specific mechanisms for crossing organelle membranes, and three different copper chaperoning principles exist that will be exemplified by ATOX1, CCS and cytochrome *C* oxidase copper chaperone 17 (COX17) (OMIM 604813). It is often assumed that copper and the chaperone are delivered together to the site of action, but this is the exception rather than the rule.

Copper in the secretory pathway

ATOX1 routes copper to the secretory pathway where the energy requiring transporters ATP7A and ATP7B reside. They pump copper into this compartment to load enzymes and also export the metal from the cell *via* excretory vesicles budding from the TGN. ATOX1 docks with and transfers copper to the MBDs in the N-terminal end[176] to regulate their conformation. In analogy with SERCA1, the MBDs are suggested to interact with the A domain during the catalytic cycle.[177,178] Being copper loaded, the six MBDs in ATP7A curl up to allow access to the channel entrance.[29,178] It is debatable whether the MBDs and ATOX1 curl up 'head to tail'[179,180] or 'side by side'. Certainly, they will curl up in the best possible way for sharing and exchanging copper electrons (cluster formation) and keep the thermodynamic energy as low as possible favouring a 'side by side' arrangement.

ATOX1 senses a need for copper extrusion and regulates the opening of the ion gate in consortium with the MBDs.[180] Besides direct regulation of ATP7A and ATP7B, ATOX1 has been shown to regulate other copper related molecular pathways, especially in the nucleus.

In the two copper ATPases, three channel binding sites have been identified, one at the cytoplasmic inner membrane face, one deeply embedded in the pore, and one at the outer membrane face.[29] During the catalytic cycle the molecule undergoes a remodelling to allow transient metal binding first at the cytoplasmic gate, next to the embedded middle site[181] and finally released from the outer gate.[182]

Transfer through the ion channel requires copper in an easily exchangeable form and not tightly complexed in ATOX1. Free copper ions need to be released or exchanged at the ion gate to avoid adverse reactions. GSH has a lower binding constant for Cu(I) than ATOX1[10] and is more suitable for intermediate shuttling.[122]

Previously the MBDs were supposed to deliver copper directly to the ion gate. New evidence suggests that the necessary signal for access to the ion gate is docking of ATOX1 with the kink platform.[29,178,183] Besides, the numbers of MBDs differ in different species and experimental removal of them does not abrogate copper transport. PINA, a possible alternative spliced form of ATP7B, consists of the A-domain, the P-domain, and the N-domain plus the ion channel, but lacks the MBDs and still shows copper transport capabilities.[184] Hence, the MBDs are not indispensable for copper transfer,[185] but are required for full activity. Metallated MBD5 and MBD6 are possibly necessary for the interaction with the A domain,[186] but it is still unresolved exactly how this interaction occurs.[178,181]

The MBDs in the copper ATPases play a crucial role in regulation of trafficking and initiation of the transport cycle together with ATOX1. A cascade of conformational changes is initiated by binding of copper to the MBDs. First, ATOX1 docks by the use of electrostatic forces with the kink platform,[29,178] secondly, copper binding changes the MBD conformation facilitating partner recognition between the other side of the chaperone and the side of the first MBD[178,183] sealing the domain in a loop. The MBDs are gradually in an ordered fashion filled with copper[10,179] leading to a tightening of the copper sphere-like tightening of an old fashioned purse with a string through holes in the lining. Glutathionylation of thiol groups or the formation of disulphide bonds[187] in one or more MBDs will loosen the sphere. GSH can effectively reduce ATOX1 presumably by a glutaredoxin-catalyzed reaction.[122] Furthermore, glutaredoxin 1 (GLXR) (OMIM 600443) can deglutathionylate the inactivated ATP7A/7B-SG mixed disulfides, releasing the reduced ATP7A/7B-SH protein that can then coordinate Cu(I) ions for subsequent transport.[188]

Shifts in copper's redox state

Redox potential within and outside cells differ, and when taken up or exported from a cell copper needs to undergo a valence shift. At the plasma membrane the STEAP family with ferri-cupri-reductase activity has been identified.[189,190] STEAP4 (OMIM 611098) expressed in the intestine is possibly copper loaded by ATP7A. Circumstantial evidence comes from studies on intestinal cells from a Menkes boy and a mottled mouse model. In the Menkes patient, silver heavy metal staining for copper showed marked accumulation at or in the brush border. Cyanid treatment that specifically removes copper almost completely removed the stain.[191] Similar experiments in the mouse model showed intracellular copper accumulation in a vesicular

compartment at the brush border.[192] The use of *in vivo* fixation in sedated mice makes their result more valid with respect to localization.

Novel data suggest that SCL31A1/CTR1 may facilitate cupric reduction, making a separate reductase less necessary.[193] The SCL31A1/CTR1 reducing ability may be sufficient in most cells while copper uptake at microvilli in the gut may be too abundant and require separate reducing capacity from STEAP4.

When copper is pumped into the lumen of exporting vesicles, a need for re-oxidation from cuprous to cupric ions exists, before metal release and transport in plasma.[9] This may happen spontaneously, but copper oxidation is likely required before depolarization of the plasma membrane and release of the metal. Hephaestin (OMIM 300167), an intracellular vesicular enzyme, and CP in plasma are both multicopper oxidases suited for oxidizing copper without the formation of ROS,[90] though their main role is in iron metabolism.[90,194] In a HEPH deficient mouse model copper investigations were not reported while severely disturbed iron metabolism was demonstrated.[195]

A possible role of HEPH in oxidizing Cu(I) to Cu(II) is suggested by Cu-64 accumulation in a fibroblast 120–130 kDa membrane fraction from a classic Menkes patient and silver staining showing marked membrane accumulation of copper.[191] Both HEPH and CP have a molecular density in this vicinity.[90] In theory, CP may have originated from the tissue culture medium, but due to a strong CP–copper complexing constant radioactive copper exchange is unlikely, leaving HEPH as the main candidate. In serum, CP may help keep copper in the right oxidation state,[90] and the predicted interaction network and confident hit of 0.893 based on String analysis indicate that ATP7A and CP may be functional partners (String-db.org). The role of HEPH and CP in Cu(I) oxidation needs experimental evidence.

Mitochondrial copper uptake

In mitochondria, copper is required mainly for the maturation and activity of cytochrome *C* oxidase (COX) of the respiratory chain, and an elaborate machinery exists to insert copper into its two catalytic centres.[12,196–198] COX is localized within the inner mitochondrial membrane and acquires copper from the intermembrane space (IMS) by a series of soluble and membrane bound molecules. The delivery and insertion of copper ions into COX appear to require at least nine copper chaperones[199] and has been extensively studied.[12,196–198] COX17 receives copper from the matrix pool[149] and donates it within the IMS to the two copper oxidoreductases SCO1/SCO2 and to COX11.[200–202]

COX17, COX19, and COX23 have been found both within the IMS and cytoplasm,[51,197] but cytosolic copper transport roles are debatable while retrograde transport of folded protein for regulation and quality control seems plausible.[203–205] Even though the function of COX23 is not well understood, it is known to work upstream of COX17.[206] COX19 works downstream of COX17 and has been implicated in the regulation of cellular copper export.[207] However, cytosolic COX17 is still almost invariably mentioned as the primary regulator of mitochondrial copper.[12,199]

We here aim to discuss the uptake and export of copper to and from the mitochondrial matrix whereas detailed mechanisms for the insertion of copper into COX are discussed in several papers.

Nuclear-encoded subunits of the electron transport chain and copper chaperones necessary for mitochondrial copper transport are imported as nascent peptides before folding.[196] TOM (Translocator of Outer Membrane) complexes form together with TIM (Translocator of Inner Membrane) complexes a dynamic ''pore'' from the cytoplasm to the matrix and secure delivery of newly synthesized proteins to the matrix where the leader sequence is removed and the unfolded protein is guided back by other TIM complexes into the IMS.[196] A number of IMS located proteins including COX17, COX19, COX23, and CCS lack a leader sequence and are trapped within the IMS by the mitochondrial MIA40 (CHCHD4) (OMIM 611077) machinery.[197,204,208–210] This mechanism delivers peptides directly from the cytoplasm to the IMS skipping the matrix step. The precursor peptide is translocated across the outer membrane and within the IMS intramolecular disulfide bond formation results in folding and retention. Reduced CHCHD4/MIA40 is then reoxidized *via* a disulfide relay system.[209] When CCS is in place it similarly folds and stabilizes the incoming SOD1 to secure retention in the IMS.[211]

Like other COX assembly factors, COX17 is transcribed in the nucleus and synthesized on free ribosomes for delivery to the mitochondria without the metal incorporated.[196,201,202] COX17 contains a copper-binding site similar to other copper chaperones and exchanges the metal by direct interaction with its acceptors within mitochondria.

If copper insertion into COX17 and other mitochondrial copper chaperones always happens within mitochondria, how does the metal get in and how does it get out again?

GSH and chelatins are likely transported into the matrix by specific TOM and TIM complexes and carries copper at the same time. While spanning the IMS longer chelatins may possibly form a backbone for copper transfer, and the metal could in theory travel from one thiol group to the next. Thus an attractive but unexplored hypothesis is that chelatins constitute a simple pore for the uptake and release of copper to and from the matrix.

PIC2, the yeast homolog of the human phosphate carrier SLC25A3 (OMIM 600370),[203] has recently been implicated in the import of copper to CuL in the mitochondrial matrix.[212] This may reflect an energy requirement for Cu–GSH transfer like amino acid transfer in the γ-glutamyl cycle.[119] SLC25A3 exists in two alternatively spliced forms expressed in different tissues.[213] Gene defects lead to hypertrophic cardiomyopathy and accumulation of lipids in muscles (OMIM 610773).[213] Similarly, defects in mitochondrial copper chaperones SCO1 (OMIM 603644) and SCO2 (OMIM 604272) are characterized by hypertrophic cardiomyopathy.[214,215]

Obviously a need exists for specific copper transporters to secure delivery of the metal from the matrix to the IMS and for export from the IMS. Copper accumulates in mitochondria in ATP7A and ATP7B deficient cells indicating a role for the two copper pumps in mitochondrial copper homeostasis.[216,217]

A concomitant mitochondrial GSH oxidation and general cellular GSH depletion points to matrix copper accumulation by ATP7A mutations[216,218] while copper in ATP7B deficient rats accumulates in the IMS.[217] Specific mechanisms ensuring delivery of copper to and from mitochondria as well as between mitochondrial compartments await identification.

Peroxisomal copper import

CCS regulates the activity of SOD1 in four different cellular compartments with different redox biology, peroxisomes, nuclei, and the IMS of mitochondria, but the cytoplasm is the principle site of location;[219] while at steady state the IMS contains 1–5% of the cellular SOD1 pool.[220]

Peroxisomes are organelles secluded from the cytoplasm by a single lipid bilayer making a specific copper transport mechanism needed. Similar to mitochondria, peroxisomes may divide as ''independent'' micro-bodies and new peroxisomes are often formed by division of the existing ones.[221] They have their own import machinery for peptides transcribed on free ribosomes, but close interaction between peroxisomes and ER for delivery of folded proteins has been demonstrated.[221] The peptide import mechanism is usually used for matrix proteins while ER import is mostly used for membrane proteins.

Recently CCS has been demonstrated to guide metallated and folded SOD1 to peroxisomes. CCS contains a peroxisomal targeting signal (PTS) and during sorting it directs the whole complex to the right position in a piggy-back way.[222,223] CCS possesses an ability to bind to lipid bilayers and this membrane affinity is important for the distribution of SOD1 to membrane secluded compartments.[166] Thus piggy-back transport by CCS explains the ER delivery of peroxisomal matrix located SOD1, and could theoretically also be used for SOD1 delivery to the nucleus. Probably cytoplasmic SOD1 is contained within membrane-enclosed vesicles released from the ER–Golgi complex. IMS activity is dependent on the presence of CCS in this compartment during uptake and folding of SOD1.[211] CCS is itself imported into the IMS by the CHCHD4/MIA40 relay system as mentioned earlier.[209]

CCS interacts with other molecules in the body *e.g.* X-linked inhibitor of apoptosis (XIAP) (OMIM 300079),[224] and BACE1 that contains a copper regulatory site in addition to the active protease site,[225] underlining that copper chaperones have a regulatory role apart from a transfer role. XIAP, a multifunctional RING zinc-finger protein, regulates CCS stability.[224] XIAP is normally located in the cytoplasm, but at hypoxia it redistributes into the nucleus to enhance CCS–SOD1 interaction as well as promoting cell repair and survival.[226] BACE1 regulates the activity of APP.[225]

Lysosomal copper uptake and export

Lysosomes are single membrane-enclosed organelles that may be a yet rather overlooked site in cellular metal handling.[132,227,228] The lysosomal system belongs to an extended vesicular network that functions as a dynamic and highly regulated cellular quality control and recycling station between the secretory pathway and the endocytic pathway.[229,230] Within the acidic lysosomal compartments molecules will be enzymatically degraded, and sorting decisions during trafficking determine whether a protein will be targeted for degradation or pass.[229] The process involves an elaborate interplay between numerous stabilizing, guiding and tethering molecules. Degradation and preservation of the acidic milieu require energy, and mitochondria are found close to the endosomal–lysosomal system often forming specific attachment points.[231]

Damaged or misfolded proteins, used organelles and foreign invaders, cell membrane debris with attached glycoproteins and cellular molecules like Cu-thionein are degraded in the lysosomal system. Reusable ions like copper are recirculated, while other molecules may be exported for further degradation in the liver.[229]

Lysosomes can acquire copper by different mechanisms. Copper separates from degraded proteins and induces compensatory mechanisms to rid lysosomes of excess.[228] SLC31A1/CTR1 is removed from the plasma membrane by endocytosis and the protein is guided *via* the endosomal–lysosomal pathway for either recycling or degradation.[171,232] Copper binding ectodomains are cleaved off in the lysosomal pathway and copper is released. A functional truncated form of SLC31A1/CTR1 plays an active role in mobilizing copper from endosomal–lysosomal stores and the cleavage of holo SLC31A1/CTR1 is dependent upon the structurally related SLC31A2/CTR2.[171] SLC31A2/CTR2 is suggested to release copper from the lysosomal compartment.[170]

Hepatocytes use ATP7B and a special secretory lysosomal pool for whole body biliary copper excretion. Other cell types also make use of the lysosomal excretory system,[229] but a role in copper homeostasis has not been defined. In hepatic secretory lysosomes active copper uptake by ATP7B occurs[45,227] while at present there is no evidence of a physiological role for ATP7A in the lysosomal system.[132]

Transporters passing the lysosomal quality control will be directed for use or reuse and if defective they will be withheld and degraded. A functional lysosomal system is hence needed to regulate correct trafficking and retrieval of copper transporters ATP7A, ATP7B, SLC31A/CTR1, and SLC31A2/CTR2 to and from vesicular membrane systems.

Molecules destined for lysosomal quality control are ubiquitylated, which refers to attaching ubiquitin to a protein. However, the two major pathways for protein quality control include both the lysosomal and the proteasomal systems.[233,234] Membrane proteins will often follow the endocytotic lysosomal-dependent pathway. But there appears to be a certain functional overlap between the two pathways and DCTN4 is part of both. Different levels of ubiquitylation exist; most labelling will be sent for degradation, but some are reversible and used for regulation of pathways. The process occurs in three steps using a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3).[235]

DCTN4 and XIAP are both RING zinc finger proteins indicating E3 ligase activity[236] and both play important roles in ATP7B

trafficking and homeostasis. DCTN4 interaction with the MBDs of ATB7B will likely change their conformation and down-regulate the pump activity to stop further copper uptake into the lysosomal compartment and promote the exocytosis of copper loaded vesicles.[45]

COMMD1 regulates the stability of ATP7B and is itself regulated by XIAP. XIAP binds COMMD1 *via* one of its zinc finger domains and XIAP-mediated COMMD1 ubiquitylation promotes its removal from ATP7B resulting in a compensatory decrease in copper export.[224] Substitution of zinc for copper will change the conformation of XIAP and make it unstable and susceptible to proteasomal degradation. Copper induced conformational changes are reversible suggesting that the XIAP–COMMD1 interaction is part of a copper regulated feedback loop.[237]

Relocalization of transporters

Cell-specific variations in sorting of membrane transporters occur, and SLC31A1/CTR1 can integrate into different membranes. Normally SLC31A1/CTR1 is found at the apical border of intestinal cells, while in hepatocytes SLC31A1/CTR1 is found at the basolateral sinusoidal membrane.[33,238] The orientation in the membrane is the same, and SLC31A1/CTR1 promotes uptake into the cell from different extracellular compartments. SLC31A1/CTR1 can under tissue culture conditions be found at the basolateral border in intestinal cells.[239] SLC31A1/CTR1 is usually located at the apical border in kidney tubular cells, but can integrate in the basolateral membrane when toxic metals need to be expelled.[48,239] How different membrane integrations of SLC31A1/CTR1 are achieved and regulated has not been addressed. Apical targeting can occur through *N*- and *O*-glycosylation[85] and SLC31A1/CTR1 contains glycosylation sites in the ectodomain.[240] Removal of the ectodomain by truncation of the protein will result in a functional copper pore, and the truncated form may possibly integrate in the basolateral or organelle membranes. *O*-Glycosylation prevents proteasomal removal of the ectodomain.[240]

Little need for variable membrane integration of ATP7A and ATP7B seems to exist. Possibly, the two mammalian pumps have evolved from one ancestral molecule to secure both efficient body uptake and extrusion of copper.[180] In mammals the speed and amount may be sufficient to determine the net-direction of copper transport.[180] The nematode *C. elegans* possesses one copper ATPase[241] and may be in need of relocalization mechanisms; some of these could be preserved in human Cu-ATPases though distinctly different regulatory mechanisms have evolved.[180]

When copper is mobilized from hepatic stores into the blood the metal needs to be released through the basolateral membrane. In adulthood, hepatic ATP7A is expressed in minute amounts and is an unlikely candidate. For ATP7B to promote hepatic copper release the protein needs basolateral integration. Histochemical studies on normal human liver biopsies show that ATP7B can be found at the sinusoidal pole[37] indicating that ATP7B travels to the basolateral border and releases copper to circulation *via* a normal TGN export mechanism. This implies that ATP7B can translocate to different membranes and would help explain deviating results in brain copper pump localizations.[57,86]

Hepatic ATP7B is constitutively sorted from the TGN to basolateral vesicles[42,242] but the copper sensitive apical targeting sequence in the N-terminus redirects the vesicles through the endosomal–lysosomal pathway towards the apical pole. If the apical targeting signal is experimentally removed, ATP7B will integrate in the basolateral membrane.[40] Release of copper from the apical signal would redirect ATP7B from the apical pole back to the TGN.

Recently focus has shifted from similarities between ATP7A and ATP7B to unique properties of each copper pump, and distinctly different protein sequences and regulation mechanisms are gradually being defined.[180]

ATP7B is the kinetically slower pump,[243] but it remains to be evaluated if this is true in all tissues and under different conditions. GSH is secreted into bile canaliculi in high amounts[244,245] and GSH concentration at the apical membrane may influence the rate of copper transfer. Lysosomes proteolytically degrade proteins and are thus in high need of thiols, but has a low reducing potential[121] favouring oxidized forms. Excretory copper is pumped into a lysosomal pool and the redirected copper complex filtered into urine when bile excretion is deficient possibly consists of small MW oxidized glutathione or cysteine complexes.[35,122] Kidney stones are prevalent in Wilson disease pointing to cystinuria precipitated by copper cystin complexes.

Transient glutathionylation will influence the speed and function of copper pumps. Both ATP7A and ATP7B can be glutathionylated in the MBD N-terminal region.[59,246] Glutathionylation or S–S cross linking of the MBDs in ATP7B will influence the interaction with ATOX1 and retains the pump in the Golgi complex,[187,247] reducing the excretion rate of copper into bile. At high copper levels, ATP7B redistributes in hepatoma cells to apical vacuolar vesicles reminiscent of bile canaliculi.[38]

In the liver, both pH[42,248,249] and GSH[218] will likely influence the trafficking of ATP7B, and under normal physiological conditions, both levels presumably change rapidly. However, most tissue culture experiments are performed under standard conditions (pH 7.4; CO_2 5%), and we may therefore only learn about a limited side of a protein's function and regulation. Applying non-standard culture conditions will possibly provide more information.

Roles of copper in the nucleus

Copper is essential for proliferation and has been implicated in reversible and flexible regulation of specific pathways. ATOX1 has been shown to act as a transcription factor[250] for an increasing number of pathways.[250–253] ATOX1 shows direct copper dependent interaction with metal regulatory elements (MREs) in the promoter region of cyclin D1 (CCND1) (OMIM 168461) which is a G1-S phase cell cycle regulator and required for copper-induced proliferation.[250] ATOX1 also interacts with the SOD3 promoter

that contains the same DNA sequence as CCND1.[254] SOD1 is induced by copper[134] through zinc release from MT and activation of MRE in the SOD1 promoter region.[255] Additional genes *e.g.* p47phox NADPH oxidase or neutrophil cytosol factor (NCF1) (OMIM 608512) required in superoxide production and inflammatory cell adhesion has been shown to possess the same potential ATOX1 transcription site as CCDN1 and SOD3.[251,256] Several of the genes are involved in cellular growth control,[257] and ATOX1 copper binding appears to depend on the 2GSH/GSSG redox state.[122] Recent yeast two-hybrid screening indicated that ATOX1 interacts with several genes involved in transcriptional and translational regulation, *e.g.* DNA methyltransferase (DNMT1) (OMIM 126375) that methylates CpG islands, translational regulator CPEB4 (OMIM 610607), and two zinc-finger proteins ZFHX3 (OMIM 104155) and ZNF521 (OMIM 610974).[258,259] Although, these findings require further biochemical follow-up, clearly they support additional roles for copper and ATOX1 in transcriptional regulation. The nuclear roles of ATOX1 are emphasized by the presence of a nuclear retention signal (NRS) (KKTGK).[250,251]

CCS is implicated in SOD1 delivery to various organelles including the nucleus.[260] XIAP will translocate to the nucleus under hypoxic conditions[226] and may be involved in nuclear regulation of CCS and in apoptosis.[237] Ubiquitylation of CCS leads to enhanced chaperone activity towards SOD1.[237]

Obviously a need exists for transporters to deliver copper to the perinuclear space and the lumen of the nucleus. ATP7A and/or ATP7B would be straightforward choices. ER is a direct continuation of the nuclear envelope and no further sophistication is needed to explain its use here, but it will need a NRS to stay in this compartment or it could be guided in a piggy-back fashion by ATOX1.[261] The interaction between ATP7B and DCTN4 provides circumstantial evidence for a nuclear role of ATP7B in copper homeostasis.[43,44] Nuclear roles of the RING zinc finger protein DCTN4 may involve gene regulation of vesicle transport, spindle assembly, and cell division.[43]

Copper, reactive oxygen species and antioxidant defence

ROS are naturally produced by our aerobic metabolism and participate in a vast amount of biological processes. Both ROS and RNS have important roles in signal transduction and gene expression. Copper ions with their labile electrons easily react with native oxygen and other oxygen species to form ROS. Copper ions also have catalytic roles in the enzymatic regulation of ROS levels, and copper chaperones exert antioxidant actions and regulate numerous defence pathways.

Three layers of antioxidant regulation exist in the human body: firstly, redox buffers and scavengers regulate the levels of ROS and RNS. Secondly, antioxidant enzymes detoxify toxic species to less reactive species. Thirdly, damaged cellular constituents are removed. Breakdown at any point of this fine-tuned system may result in deterioration of regulatory mechanisms and overload of proteasomal–lysosomal degradation with accumulation of damaged proteins and subsequent precipitation of disease states.

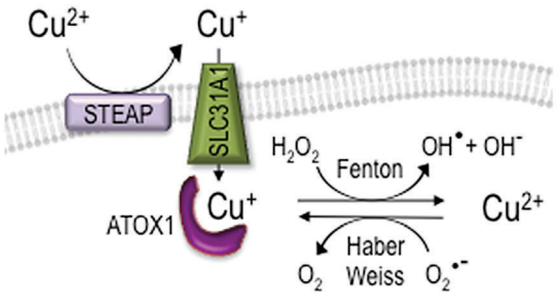


Fig. 3 Fenton/Haber–Weiss recycling: surplus Cu(I) catalyses the production of highly reactive hydroxyl radicals through the Fenton reaction. This is likely initiated extracellularly due to the abundance of free Cu(II) and the presence of numerous proteins (*e.g.* ALB, A2M, SOD3, APP, and PRNP) with imidazol cluster motifs that can disproportionate Cu(II) and generate Cu(I). Intracellularly, excessive superoxides will reduce Cu(II) to Cu(I) through the Haber–Weiss reaction and keep the vicious cycle.

Unbalanced ROS formation is often named 'oxidative stress' but may rather be called redox stress. Here we will discuss the roles of copper ions in formation, buffering, and enzymatic detoxification of ROS.

ROS production

Due to its chemical nature, copper participates in the production of radicals often called 'free' radicals. Radicals are chemical species (atoms, ions or molecules) containing unpaired electrons making them prone to 'fatal attractions'. Copper ions are radicals, many radicals are ROS, but not all ROS are radicals *e.g.* hydrogen peroxide (H_2O_2).[262] Metal catalysed production of highly reactive hydroxyl radicals takes place through the Fenton reaction,[263] that presents the critical step of the Haber–Weiss cycle.[264] Both copper and iron can participate, but due to higher electrochemical reactivity copper is more prone to form ROS and RNS (Fig. 3).

Physiologically ROS are used in cellular defence against infections and in intra- and intercellular signal transduction. If not directed correctly, ROS may act in an uncontrolled way, and will become harmful and precipitate pathological states.[265] ROS have been associated with DNA and RNA lesions, protein oxidation, and lipid peroxidation.[265] Copper induced oxidative stress may lead to organelle dysfunction and damage, resulting in enhanced lysosomal clearance.

H_2O_2 is substantially less toxic than superoxide and has a longer half-life enabling the molecule to act as a second messenger for increased ROS levels.[260] H_2O_2 uses a subset of aquaporins (peroxiporins) for movement across membranes[266,267] and is used for signalling within and between cells.[260] The presence of specific channels stresses the natural use of H_2O_2 in redox homeostasis.

Buffering of ROS

GSH is the master redox buffer within mammalian cells (see earlier) and is also secreted into bile and the lungs.[244,245,268]

Thiol groups have a particularly high affinity for Cu(I) and other thiophilic metals,[269] and GSH sequesters copper tightly[5] by scavenging its electrons from making radical attacks. GSH serves as a potent reductant eliminating hydroxyl radicals, peroxynitrites, and hydrogen peroxides, and also acts as a cofactor for glutathione peroxidase (GPX) (OMIM 138319, 138320). In mitochondria, GSH is particularly important because they lack catalase (CAT) (OMIM 115500), and endogenously produced H_2O_2 can instead be regulated by cytosolic GPX–GSH recycling systems.[270] GSH is likely imported into peroxisomes by a peptide import mechanism similar to the mitochondrial mechanism[221] and may partly substitute for the lack of CAT in Acatalasemia (OMIM 614097). In the nucleus GSH serves to create a proper redox environment[119] and as a zinc pool for transcription.

The MT–zinc pool becomes available when displaced by copper leading to induction of more MT and formation of GSH and SOD1 as well as stabilization of SOD3. Increased ROS leads to rapid translocation of MT to the nucleus,[128,271] and release of zinc by direct cross-linking of thiol groups. MT may provide protection against metal radicals directly and indirectly by protection against harmful actions of ROS.

Copper antioxidant enzymes

H_2O_2 signalling capability makes superoxide dismutases central players in antioxidant defence.[272] Superoxide dismutases convert superoxide to peroxide, and are classified based on their metal cofactor and their endogenous localization: SOD1, SOD3,[273] and Mn SOD2 (OMIM 147460). APP is a Cu/Zn containing protein with a dismutase-like fold,[113] but a natural substrate has not been identified.

CCS–SOD1 association with the cytoplasm, nucleus, and lumen of peroxisomes regulates partitioning of SOD1 between these different redox pools.[260] The location and activity of SOD1 is also regulated by oxygen tension.[274] High cytosolic oxygen maintains CCS in the cytoplasm where it folds and matures SOD1. At increased H_2O_2 levels CCS–SOD1 rapidly enters the nucleus where it initiates defence mechanisms[260] while at hypoxia SOD1 is directed to mitochondria.[220] ROS located in the IMS are generated by complex III and seem to play a role in signaling during cellular adaptation to hypoxia.[275] During hypoxia XIAP stabilizes CCS[250] and enhances SOD1 activity to produce H_2O_2 that can escape the IMS *via* peroxiporins. A major SOD1 pool is found in the cytoplasm while mitochondria normally contain a minor pool in the IMS (less than 5%),[220] and the matrix located SOD2 is the principal dismutase of this organelle. The active site of extracellular SOD3 shows strong homology with SOD1, but is copper loaded by ATP7A, and gene expression is regulated by ATOX1.[251,256]

Regulation of cellular antioxidant defence

Regulation of copper and redox homeostasis is a dynamic and inter-linked process. Intracellular cuprous ions are tightly chaperoned meaning an extremely low free copper ion concentration[5] making copper efficiently sequestered from forming ROS. The extracellular copper pool is much less tightly bound and free cupric ions are about 100 million times more prevalent. It is more likely that extracellular copper, naturally existing as Cu(II)–imidazol complexes, will disproportionate to Cu(I) and Cu(0) and cause disruption of cell membranes.[276] APP and PRNP have both been shown to disproportionate Cu(II).

Copper regulates the transcription of genes by increasing intracellular ROS and by inducing specific signalling molecules. ATOX1 has been shown to transcriptionally regulate an increasing number of pathways in oxidative defence and cellular growth.[158] The rediscovered role of ATOX1 as a central player in redox regulation has recently been reviewed,[122] and the ATOX1–GLXR connection secures the reduction of chaperone for its many activities.[122,188] ATOX1 regulates ATP7A and ATP7B and directs excess copper towards excretion, thereby lowering the pro-oxidant activity. XIAP interacts with COMMD1 and hereby regulates the stability of ATP7B to control whole body copper excretion.[277] It is likely that the redox state affects metal binding of COX17[278] to regulate respiration and oxygen consumption. Cytosolic COX17 may regulate translation on ribosomes attached to mitochondria.[197]

The master SOD1 contains both copper and zinc. Copper is used for the redox reactions while zinc confers stability to the molecule. Rapid availability of zinc is therefore vitally important.

In conclusion, ATOX1 primarily regulates the transcription of numerous genes while CCS regulates several expressed proteins in pathways related to redox stress implying that CCS stress regulation probably is the first line of defence. CCS appears to be crucial for redox regulation in the compartments and extracellular space making it central in antioxidant defence. CCS also affects whole body copper excretion *via* the XIAP–COMMD1 interaction, while ATOX1 primarily works at the cellular level. ATOX1 transcription and regulation of genes appear to guide towards renewal[158] and repair. CCS copper binding is about a million times stronger than ATOX1 binding,[10] and XIAP stabilizes the CCS pool[250] during hypoxia indicating that CCS regulation may be important as an initial post-translational response. Thus the CCS–SOD1 axis is extremely important.

Lysosomes and oxidative stress

Lysosomes play a significant role in quality control and removal of copper induced oxidatively damaged molecules. Defective organelles are broken down[279,280] and metals from enzymes, chaperones, and scavenging molecules are released. Organelles have different metal profiles,[281] but at normal metabolic control copper is reused in cellular pathways. Critical levels result in increased metal induced ROS and RNS and compromise the lysosomal function. However lysosomal dysfunction may also affect lysosomal handling of copper. Poor membrane integrity and the resulting disturbed acidification of lysosomes lead to altered redox states and eventually to disturbed metal homeostasis.[228]

Lysosomal degradation requires energy and the hallmark of a compromised system is an increase in mitochondria-associated membranes (MAMs) defined by interorganellar connections, the so-called ER–mitochondria attachment points.[231]

Metal dysregulation characterizing Zellweger syndrome (OMIM 214100)[282] caused by a compromised peroxisomal membrane system could be a result of H_2O_2 imbalance and increased activation of degradation pathways (pexophagy). Defects in an acetyl-CoA transporter, SLC33A1 in Huppke–Brendel syndrome (OMIM 614482), may result in copper disturbances by an analogous mechanism (ER-phagy).[283,284] Respiratory chain defects, *e.g.* SCO1 and SCO2, will result in increased ROS production and damaged mitochondria[214,215] that are removed by the lysosomal decay pathway (mitophagy). Lipid degradation (lipophagy)[285] perturbed in lipid storage disorders can result in disturbed copper homeostasis as shown in Niemann–Pick type C (NPC2) (OMIM 607625) and an animal model of Ceroid Lipofuscinosis (CLN6) (OMIM 601780).[286,287] A common crossroad of the aforementioned diseases resulting in metal disturbances appears to be the lysosomal capability for organelle phagocytosis,[288] and may group them among progressive lysosomal storage disorders.[289]

Antioxidant defence principles in the brain

High metabolic turn-over and oxygen consumption in the brain constantly produce ROS. The CNS is consequently in particular demand of efficient antioxidant regulatory mechanisms. Of interest is the extended influence of CCS. CCS location is guided by H_2O_2 signalling that is regulated by the redox state. This in turn helps in regulating and metallating a number of copper proteins *e.g.* SOD1 and BACE1 important for normal brain metabolic control. Besides the CCS–APP association, BACE1 regulates numerous other proteins and is itself regulated by numerous proteins.[290] CCS is abundantly expressed in astrocytes, neurons and foremost in Purkinje cells.[291]

The CNS may be more vulnerable to oxidative stress than other organs due to a high content of polyunsaturated fatty acids, which are susceptible to ROS and RNS oxidation. Thiol groups of the cytoskeleton, *e.g.* tubulin, are particularly prone to oxidation. In addition neurons are non-dividing and may have a propensity to accumulate damaged molecules and organelles making cerebral glia quality control particularly important.

Some structures are extremely rich in copper, particularly substantia nigra[57] making them prone to metal induced ROS generation in the case of imbalance of regulatory systems. This region contains metal requiring enzymes[57] *e.g.* two iron enzymes phenylalanine hydroxylase (PAH) (OMIM 612349) and the rate limiting TH, plus the copper containing dopamine β hydroxylase (DBH) (OMIM 609312). The amino acids, phenylalanine and tyrosine (as well as tryptophan), possess an aromatic ring structure of shared electrons with prevalent copper binding. Defects in TH lead to Segawa syndrome (OMIM 605407) and defective SNCA regulation of TH may lead to PD.[140]

The relevance of MT in neurophysiological and neuromodulatory functions has been stressed by very high levels found in astrocytes[61,63] and in choroid plexi during development[292] and by identification of a primarily brain-expressed isoform, MT3.[109,293] MT1 and MT2 are in particular expressed in astrocytes[294] and recently several isoforms have been found in brain interstitial fluid.[109] High MT levels indicate high metal turnover and a high need for zinc. The copper–zinc connection is becoming interesting.[3] Zinc ions interact with and stabilize several molecules of interest in redox stress.[295] MTs are the most direct connection and have become a centre of interest for future research into plaque forming diseases.[3]

Several proteins related to neurodegeneration, SOD1, SOD3, APP, and PRNP are redox-active imidazol cluster proteins. Changes in their primary, secondary or tertiary structure due to molecular destabilization are a hallmark of neurodegenerative diseases.[117] Defective lysosomal removal of damaged molecules appears be a core problem in the development of misfolding neurodegenerative diseases. If decay systems become overloaded and the concentration of damaged proteins increases above a certain threshold they may undertake a new conformation in order to be neutralized, packed and stored. Protein and lipid aggregation and packing are normal biological events[296] and are used here as defence mechanisms. If accumulation continues, the stored molecules may cause problems.

Therefore, metallo-ROS interaction rather than ROS alone may be the culprit that leads to neurodegeneration. Too much and too little oxygen will influence copper redox biology, but due to efficient control mechanisms neurological signs do not become evident until the system experiences an imbalance for a period of time. Genetic defects in any of the systems will shorten the time of onset. Likewise environmental factors including metal overload may precipitate disease symptoms, especially if a genetic susceptibility base is also present.

Conclusions and perspectives

In the present review we have discussed a broad though delimited view on copper handling and transport from a bioinorganic perspective. This angle points to a new role for HEPH in the oxidation of Cu(I), and STEAPS are likely copper loaded by ATP7A, but both suggestions need experimental proof.

Three main principles for cytoplasmic copper chaperoning exist. Upcoming conclusions are that chaperones orchestrate the organelle delivery of copper besides having a copper-loading role. In addition, regulatory roles are becoming evident. CCS regulates partitioning of SOD1 between the cellular compartments and functions as a piggy-back guide for the movement of the copper loaded enzyme. CCS also regulates folding and retention of nascent SOD1 within the IMS. ATOX1 regulates ATP7A and ATP7B by transferring copper to the MBDs, though delivery to the ion channel may be achieved by GSH. Piggy-backing may also apply to ATOX1 and help in moving the ATPases to another location.

Peptide bound copper emerges as the possible missing link for mitochondrial matrix uptake and storage. GSH and its polymers, chelatins, may play a role in the bidirectional movement of copper. COX17 is taken up directly into the IMS using the CHCHD4/MIA40-disulphide relay system for uptake and folding of nascent peptides. Cytosolic located chaperones may regulate translation of COX17 for uptake into mitochondria, but evidence is lacking. Copper transfer mechanisms between mitochondrial compartments are needed and additional roles for ATP7A and ATP7B may exist.

The membrane transporters SLC31A1/CTR1 and ATP7B appear to move to different membranes to fine-tune their functions. Evidence points to region- and function-specific distributions, but this needs further investigations.

Cellular organelles have emerged as important sites for copper induced redox regulations, and copper chaperones are right in the core of this regulation. ATOX1 plays an important role as a transcription factor for numerous defence and renewal pathways, while CCS functions as a posttranslational regulator and the first line of defence in oxidative stress.

The endosomal–lysosomal system has a significant role in quality control and removal of copper induced oxidatively damaged molecules. Dysfunction of the lysosomal system may in turn lead to disturbances in metal homeostasis.

Future directions and focal points

Cellular copper trafficking usually takes place in vesicles but interrelations between vesicles and target signals are in need of further investigations as well as their interactions with the cytoskeleton. The role of extracellular vesicles in copper homeostasis also needs to be explored.

List of abbreviations

ABCA12	ATP-binding cassette, subfamily A, member 12
ALB	Albumin
A2M	Alpha-2-macroglobulin
APP	Amyloid precursor protein
ATOX1	Antioxidant 1 copper chaperone
ATP	Adenosine triphosphate
ATP7A	Copper transporting ATPase A
ATP7B	Copper transporting ATPase B
BACE1	β-Site APP-cleaving enzyme 1
BBBs	Blood brain barriers
BCB	Blood–cerobrospinal fluid barrier
BEB	Blood–endothelial barrier
CAT	Catalase
CCS	Copper chaperone for superoxide dismutase
CCND1	Cyclin D1
CHCHD4	Coiled-coil–helix–coiled-coil–helix domain-containing protein 4
CNS	Central nervous system
CTR1	Copper transporter 1
CTR2	Copper transporter 2
COMMD1	COMM domain-containing protein 1
COX	Cytochrome *C* oxidase
COX17	Cytochrome *C* oxidase copper chaperone 17
CP	Ceruloplasmin
CPEB4	Cytoplasmic polyadenylation element-binding protein 4
CSF	Cerebrospinal fluid
Cu	Copper
DCTN4	Dynactin 4
DMT1	Divalent ion transporter no. 1
DNMT1	DNA methyltransferase 1
ER	Endoplasmic reticulum
GSH	Glutathione
GSSG	Oxidized dimer of GSH
GPI	Glycosylphosphatidylinositol
GPX	Glutathione peroxidase
GR	Glutathione reductase
GS	Glutathione synthetase
HEPH	Hephaestin
His	Histidine
HGNC	HUGO gene nomenclature committee
HUGO	Human genome organisation
H_2O_2	Hydrogen peroxide
IMS	Inter-membrane space
IUPAC	The International Union of Pure and Applied Chemistry; http://www.chem.qmul.ac.uk/iupac/
kDa	Kilo Dalton
MBDs	Metal binding domains
MIA40	Mitochondrial IMS assembly 40
Mn	Manganese
MRE	Metal regulatory element
MT	Metallothionein
MW	Molecular weight
NRS	Nuclear retention sequence
OMIM	Online Mendelian Inheritance in Man; http://www.omim.org/
PIC2	Phosphate inorganic carrier 2
PINA	Pineal night-specific ATPase
PRNP	Prion protein
PTS	Peroxisomal targeting sequence
RING	Really interesting new gene
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
SCO1	*S. cerevisiae* homolog of cytochrome *C* oxidase assembly protein 1
SCO2	*S. cerevisiae* homolog of cytochrome *C* oxidase assembly protein 2
SERCA1	Sarcoplasmic reticulum P2-type 1 Ca(II) ATPase
SLC	Solute carrier family
SLC11A1	Solute carriers number 10A1 = DMT1
SLC25A3	Solute carriers number 25A3
SLC25A10	Solute carriers number 25A10
SLC25A11	Solute carriers number 25A11
SLC31A1	Solute carriers number 31A1 = CTR1
SLC31A2	Solute carriers number 31A2 = CTR2
SLC33A1	Solute carriers number 33A1

SNCA	Alpha-synuclein
SOD1	Cu/Zn containing superoxide dismutase 1
SOD2	Mn containing superoxide dismutase
SOD3	Cu/Zn containing superoxide dismutase 3
STEAP	Six-transmembrane epithelial antigen of prostate
TF	Transcription factor
TIM	Translocator of inner membrane
TJ	Tight junction
TGN	Trans Golgi network
TOM	Translocator of outer membrane
XIAP	X-linked inhibitor of apoptosis

Nomenclature

The chemical terms and bioinorganic chemical concepts are based on IUPAC Gold Book, http://www.chem.qmul.ac.uk/iupac/. Details about the molecules involved and related diseases are compiled in the OMIM, http://www.omim.org/, and protein databases. HGNC and OMIM approved gene and disease names are used.

Conflicts of interest

None declared.

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