Published in final edited form as:

Biomed Pharmacother. 2003 November ; 57(9): 386–398.

Trace elements in human physiology and pathology. Copper

H. Tapiero^{a,*}, D.M. Townsend^b, and K.D. Tew^b

^aUniversité de Paris - Faculté de Pharmacie CNRS UMR 8612, 5, rue Jean-Baptiste-Clément, 94200 Chatenay-Malabry, France

^bDepartment of Pharmacology, Fox Chase Cancer Center, Philadelphia, PA, USA

Abstract

Copper is a trace element, important for the function of many cellular enzymes. Copper ions can adopt distinct redox states oxidized Cu(II) or reduced (I), allowing the metal to play a pivotal role in cell physiology as a catalytic cofactor in the redox chemistry of enzymes, mitochondrial respiration, iron absorption, free radical scavenging and elastin cross-linking. If present in excess, free copper ions can cause damage to cellular components and a delicate balance between the uptake and efflux of copper ions determines the amount of cellular copper. In biological systems, copper homeostasis has been characterized at the molecular level. It is coordinated by several proteins such as glutathione, metallothionein, Cu-transporting P-type ATPases, Menkes and Wilson proteins and by cytoplasmic transport proteins called copper chaperones to ensure that it is delivered to specific subcellular compartments and thereby to copper-requiring proteins.

Keywords

Copper; Bioavailability; Deficiency; Enzymes; Ceruloplasmin; Metallothionein

1. Introduction

The effects of trace element are heavily dependent on one another. Thus, high intakes of zinc, cadmium or copper interfere with the utilization and tissue storage of iron. Low concentrations of dietary iron enhance the absorption of not only dietary iron, but also of lead, zinc, cadmium cobalt and manganese. Zinc supplements have been shown to cause anemia secondary to hypocupremia and tetrathiomolybdate inhibits copper absorption. Moreover, little is known about the role of drugs especially diuretics, and intercurrent illness on the development of trace mineral deficiency and the interactions of trace elements with one another, particularly in the situation where the decision is made to replace a single trace element or in older individuals who may be on one trace element supplement.

Copper (Cu) is an essential trace metal found in all living organisms in the oxidized Cu(II) and reduced Cu(I) states. It is required for survival and serves as an important catalytic cofactor in redox chemistry for proteins that carry out fundamental biological functions that are required for growth and development [1]. The average intakes of copper by human

*Corresponding author: haimtapiero@aol.com (H. Tapiero).

adults, vary from 0.6 to 1.6 mg/d [1–5] and the main sources are seeds, grains, nuts, and beans (concentrated in the germ and bran), shellfish and liver. Drinking water does not normally contribute significantly to intake. The concentration of free copper ions has been estimated to be of the order of 10^{-18} – 10^{-13} M in yeast cells and in human blood plasma, respectively. In excess of cellular needs, Cu can be cytotoxic. Copper, similar to iron (Fe), can participate in reactions that result in the production of highly reactive oxygen species (ROS), responsible for lipid peroxidation in membranes, direct oxidation of proteins, and cleavage of DNA and RNA molecules (Table 1) [7]. The generation and action of ROS are major contributing factors to the development of different pathologies such as cancer, diseases of the nervous system and aging [8]. In addition to the generation of ROS, Cu may manifest its toxicity by displacing other metal cofactors from their natural ligands. The replacement of Zn(II) by Cu(II) in the zinc-finger DNA binding domain of the human estrogen receptor renders this protein defective, altering its role in hormone-dependent signal transduction in vivo [9]. Thus, precise regulatory mechanisms must be in place to prevent the accumulation of Cu ions to toxic levels [6]. The ingested Cu is absorbed and distributed to copper-requiring proteins. Excretion is the main factor controlling homeostasis. Although copper deficiency is rare, it may occur when there is a genetic defect in the functioning of a copper transporter (ATP7A), resulting in Menkes disease or the milder Occipital Horn Syndrome. Menkes and Wilson's diseases (WDs), human genetic diseases in Cu transport, have revealed the importance of maintaining appropriate Cu homeostasis [10–14]. Moreover, Cu is essential for efficient iron uptake and mobilization in mammals [15].

2. Dietary copper intake and absorption

2.1. Copper absorption

In humans and animals, dietary copper is absorbed across the mucosal membrane in cells, which line the stomach and the small intestine primarily by those of the small intestine. Cu diffuses through the mucous layer that covers the intestinal wall [1]. In humans with normal intakes (0.6–1.6 mg/d), 55–75% is absorbed and actively recycled between the digestive tract, body fluids and tissues (particularly the liver). Thus, dietary copper contributes only a small proportion of the total reabsorbed from saliva, gastric juices, the bile, pancreatic and duodenal fluids (4–7.5 mg). About 1 mg of Cu is excreted daily by adults, the bile being the main route for copper excretion, with very little excreted by other routes [1,5]. The transport of Cu(II) across the brush border involves both a non-energy-dependent saturable carrier, and diffusion (active at low or high Cu concentrations, respectively) [1]. The rates of copper transport increase with pregnancy and in cancer, and decrease (at least in female rats) upon repeated treatment with estrogen [16]. Within mucosal cells, most newly absorbed copper (about 80%) is retained in the cytosol, bound to glutathione, metallothioneins and/or proteins of similar size. Excess intracellular copper in the intestine, liver, and probably other tissues is immediately bound to glutathione and subsequently to metallothionein [17,18]. Glutathione has been shown to protect cells against copper toxicity [17–19] and inhibition of its synthesis in hepatocytes with buthionine sulfoximine (BSO) reduces the incorporation of ^{67}Cu into metallothionein by more than 50%. Metallothioneins are a group of low-molecular-weight cysteine-rich proteins found in vertebrates, invertebrates, and fungi [20].

Mammalian cells have multiple metallothionein proteins that can buffer the intracellular concentrations of several metal ions, such as copper, zinc, cadmium, and others, which induce and bind to metallothioneins to different extents [21–24]. In mammals, metallothionein induction is mediated by binding of metal-responsive transcription factor 1 (MRF-1) to metal-responsive elements (MRE), in metallothionein promoters. These promoters have multiple copies of MRE elements, which contain a core consensus sequence, 5'-TGCPuCXC-3' conserved in all higher eukaryotes [25]. In yeast, there are two metallothioneins: Cup1 (the most important) and Crs5. Only Cu and Ag induce the CUP1 and CRS5 genes and this induction is mediated through a copper binding transcription factor Ace1 [26–28]. The CUP1 gene promoter contains four Ace1 binding sites, whereas the CRS5 promoter contains only a single site [29,30]. Studies of both yeast and mammalian cells have shown that metallothioneins have no direct role in copper uptake, but are important for the storage of the metal ions [17,31,32] and for protection against copper toxicity. Since the affinity of these proteins for Cu(II) is higher than for most other abundant metal ions (notably Zn(II)), the incoming copper will displace these ions. If metallothionein concentrations in the mucosal cells are high (as when induced with high intakes of zinc), the binding of copper to metallothionein will interfere with its transfer across the serosal surface. Thus, in Wilson's disease, a high dose of zinc is used to inhibit intestinal copper absorption. Several genes and gene products have emerged with regard to the potential transporters and carrier systems for intestinal absorption [33–36]. CTR1, cloned in humans [37] and in mice [38] and a high-affinity Cu transport protein, hCtr1, have been identified in yeast cells defective in Cu transport due to inactivation of both the CTR1 and CTR3 genes. hCtr1 is a 190 amino acid protein with significant homology to yeast Ctr1 and Ctr3, suggesting that mammalian high-affinity Cu transporters may have evolved from both Ctr1 and Ctr3. The amino terminal domain of hCtr1 is rich in methionine and histidine residues. hCtr1 is expressed in many organs and tissues, with liver, heart and pancreas exhibiting the highest levels, with intermediate levels in intestine, while expression in the brain and muscle is low. Transfection experiments have confirmed that hCtr1 promotes copper uptake into mammalian cells [39–41]. A low affinity mammalian Cu transporter, hCtr2, was also identified by sequence homology with hCtr1 [33,37,42]. Similar to hCtr1, hCtr2 mRNA is detected in many organs and tissues; however, the highest levels were observed in the placenta with very low abundance observed in the liver, ovary, intestine and colon [43]. Unlike hCtr1, hCtr2 is unable to complement the respiratory defect in yeast strains defective in Cu transport. A second low affinity Cu transporter, is the Nramp2 protein, that transports divalent metal ions such as Fe(II), Zn(II) and Mn(II) [44]. Nramp2 protein is homologous to the Smf1/Smf2 yeast metal ion transport proteins [45,46]. Ubiquitously expressed in tissues and present in the intestinal brush border, its transport of metal ions is protein coupled and dependent on the membrane potential. A role in iron absorption, as well as homeostatic regulation of its expression by iron within the intestine, has been well established [47–50].

2.2. Uptake and transport of copper

In the portal blood plasma and interstitial fluid, there are proteins, which have specific and high-affinity copper binding. Although albumin is the most abundant plasma protein, only 10–12% of the total plasma copper is bound to albumin (100–150 ng/ml) [51–53]. High-affinity copper binding has been demonstrated for human [54,55] and bovine albumin [54].

Albumin, binds copper with the help of the three amino acids at its N-terminus (including a histidine in the third position). Albumin in some vertebrates, including dogs and pigs, lacks a histidine near the N-terminus. This does not eliminate binding, but lowers copper affinity by 10-fold [56]. In rats and humans, two other proteins: ceruloplasmin, and a macroglobulin, transcuprein were identified [53,57]. Transcuprein and albumin represent the bulk of the exchangeable copper pool in blood plasma [58,59]. Once in the blood plasma, the copper on ceruloplasmin is available for uptake by tissues throughout the rest of the organism [58]. Under normal circumstances, ceruloplasmin is probably the main source of copper for other tissues [60,61] and albumin is not required for copper uptake by the liver and kidney [62]. Ceruloplasmin-copper is not the only form of copper available to most non-hepatic tissues [63–66]. Using various cell lines, it was shown that copper can be taken up from ceruloplasmin, albumin, transcuprein, or Cu-dihistidine [63,67,68] (Table 2). Most non-hepatic tissues, and particularly the heart and placenta, show a preference for ceruloplasmin-copper that must involve interaction with specific receptors in the plasma membranes [69–71].

2.3. Intracellular distribution and metabolism of copper

In cells, due to its highly reactive nature, it would be extremely harmful for Cu(I) to exist as a free ion, where it can participate in reactions whose products ultimately damage cell membranes, proteins and nucleic acids. Thus, Cu is delivered to specific molecules by forming complexes with small cytosolic proteins known as copper chaperone proteins. Crossing the brush border of the enterocyte, most of the copper is shuttled to the trans-golgi network (TGN) and into its channels by ATOX1/HAH1 (corresponding to yeast Atx1) delivering copper to the P-type ATPases located in the TGN [72,73]. In the case of the enterocyte, it would be ATP7A or MNK (the protein defective in Menkes disease) and in the case of the liver, it would be ATP7B or WND (the protein defective in WD). The chaperone protein CCS, delivers copper to the Cu/Zn superoxide dismutase (SOD) in the cytoplasm [74,75], which protects cells against superoxide radicals, whereas, COX17 delivers copper to the mitochondria [76,77], where it is required for cytochrome-*c* oxidase, the terminal enzyme in respiration. Glutathione (GSH) may also play the role of a general chaperone for copper ions [78] by delivering Cu to CTR1 in the plasma membranes. In vitro studies have shown that GSH will reduce and bind Cu(I) and deliver it to metallothioneins and to some copper-dependent apoenzymes, like SOD and hemocyanin [78]. Observations that copper binds to chaperones after it has entered suggest that GSH mediation may be involved and direct binding of the chaperone to the membrane transporter might not be required [79]. GSH may not be needed directly for copper distribution within the cell, but might also be needed to restore the abilities of copper binding proteins (with thiol groups) to bind their copper. It might also provide electrons for reduction of copper during transport.

In mammalian serum, the predominant Cu containing protein is ceruloplasmin, a glycosylated multi-Cu ferroxidase synthesized primarily in the liver, which carries 95% of total serum Cu [80]. Ceruloplasmin coordinates seven Cu atoms that are incorporated during its biosynthesis and maturation in the secretory pathway [81]. In patients with aceruloplasminemia, the absence of ceruloplasmin does not alter Cu levels in the peripheral tissues [82,83]. The ability of copper to bind to transcuprein in the presence of abundant

albumin (with its high-affinity copper sites) emphasizes its high-affinity for this protein. Rodents express a different spectrum of macroglobulins in their blood plasma than do humans and most other mammals. Rat transcuprein appears to be α_1 -inhibitor3, a monomeric macroglobulin with a total molecular weight of about 200,000. The main human macro-globulin (α_2 -macroglobulin) and α_1 -Inhibitor3 both have a highly homologous, histidine-rich region, suggesting the conservation of specific metal binding domains.

2.4. Release of copper from cells and copper excretion

The regulation of copper excretion appears to be the main mechanism for homeostasis [4,6]. Except for tissues producing secretions for the gastrointestinal tract (salivary glands, the pancreas, and epithelia in the stomach and intestine), most copper must return to the liver for excretion. It is carried by the plasma carriers, transcuprein and albumin, which particularly target the liver and also by ceruloplasmin. Most ceruloplasmin enters hepatocytes after desialylation in endothelial cells [71]. The primary pathway for the excretion of copper from the body is from hepatocytes, via the bile. The importance of maintaining mechanisms for proper Cu homeostasis in the liver is underscored by the existence of the autosomal recessive disorder Wilson's disease (WND). The Wilson and Menkes proteins are highly homologous P-type ATPases. Both the proteins contribute to the cellular export of copper, by direct extrusion of copper from the cell. The biosynthetic loading takes place in the TGN, where the Wilson and Menkes proteins are normally located. The Wilson protein is expressed primarily in the liver, transporting copper to apoceruloplasmin, whereas the Menkes protein is predominant in all other tissues [84,85]. Direct excretion of copper appears to take place when cells are subjected to elevated copper levels. Under these conditions, the Menkes proteins move from TGN to the plasma membrane [86]. On the other hand, an increase in the concentration of copper in HepG2 cells results in the movement of the Wilson protein into a cytoplasmic vesicular compartment [87,88]. The Menkes protein is encoded by the ATP7A gene and mutations in this gene result in the Menkes disease. Menkes' disease is X-linked and characterized by severe neurodegeneration and connective tissue abnormalities, which can be ascribed to the reduced activity of several copper-requiring enzymes. Fibro-blasts from patients suffering from Menkes' disease accumulate copper, and this is used diagnostically [89]. WD, which is caused by mutations in the ATP7B gene, is characterized by copper toxicity resulting from the loss of ability to export copper from the liver to the bile and the inability to incorporate copper into ceruloplasmin [90–93]. Patients with WD accumulate Cu in the liver and brain, resulting in liver cirrhosis, neurodegeneration and the formation of apoceruloplasmin [94]. The ATP7B gene, which encodes the 160-kDa WND P-type ATPase, is required for biliary excretion of Cu and incorporation of Cu into ceruloplasmin in the liver [11,14,95]. WND ATPase (7B) has mainly been located in the TGN of liver and brain, where it likely functions to incorporate Cu into ceruloplasmin [96] and perhaps at the plasma membrane of hepatocytes for Cu excretion into the bile [97]. These vesicles are close to the canalicular membrane, where the bile is released [98]. Moreover, a cleaved form of the WND protein of 140 kDa was reported to be localized in the mitochondria of cultured hepatic cells and human tissues, rather than in TGN [99,100] where it is suggested to play a role in mitochondrial Cu ion homeostasis. Although most of the copper is recycled within given cells and tissues, some is released back into the blood. From non-hepatic cells, copper release occurs through MNK. There is

evidence that it can occur in two different ways, exocytosis and by trafficking to the plasma membrane particularly when large amounts of copper need to be exported [86,98]. The mechanism involves the cycling of MNK between the TGN and the plasma membrane. This was first demonstrated in CHO cells that developed resistance to copper toxicosis [101,102]. Copper set aside for permanent excretion would be directed to the bile. The route taken by copper to the bile involves HAH1/ATOX1, WND, and exocytosis or trafficking of WND to the brush border of the bile canaliculus [103]. However, copper in bile is less reabsorbable than that in other gastrointestinal fluids. Reabsorbability varies in relation to the amounts of copper in hepatocytes. In addition, it has been proposed that a large fragment of ceruloplasmin, high in copper and resistant to proteolysis, may furnish a means of excreting copper without intestinal reabsorption [104]. Thus, much of copper homeostasis is controlled by the level and form in which it is excreted through the bile. It is clear that no copper is present in cells (or in body fluids) as a free ion [105]. Evidence from human studies with stable copper isotopes indicates that relatively little copper enters and leaves the cells; most is recycled on a daily basis [4,6].

3. The role of copper in mammalian cells

Copper is essential as a cofactor in a number of critical enzymes in metabolism (Table 3).

3.1. Cytochrome-c oxidase

Cytochrome-*c* oxidase [106] sits within the inner mitochondrial membrane. It has four redox active metal sites, two heme sites (hemes α and α_3), and two copper sites (CuA and CuB) [107]. The heme is characterized by a hydroxyl farnesylethyl group at position 2 and a formyl group at position 8 of the porphyrin substituted groups. Cytochrome-*c* oxidase is the terminal oxidase in most aerobic organisms and reduces molecular oxygen (O_2) to water [107]. In addition to the O_2 reduction, cytochrome-*c* oxidase pumps protons from the inside to the outside of the membrane. Thus, in addition to the membrane potential produced by the net migration of the positive charges, it produces a proton gradient across the membrane [107,108]. The two heme sites are located within the single polypeptide of subunit I and the two heme iron sites are called hemes α and α_3 . In the oxidized state, a respiratory inhibitor, cyanide, binds specifically to heme α_3 and stabilizes the oxidized state. The cyanide-bound heme α_3 cannot be reduced even with an excess amount of dithionite. In contrast, heme α is unreactive to cyanide and is readily reduced by dithionite [109].

3.2. Copper/zinc superoxide dismutase

Copper/zinc superoxide dismutase (Cu/Zn-SOD) converts superoxide anions to peroxide for further disposal (by catalase and glutathione peroxidase). *Drosophila* and microorganisms lacking the enzyme have been shown to be more vulnerable to damage by ROS [110]. In mice where the gene has been knocked out, there is gradual damage to neuromuscular junctions in the hindlimbs [111]. Mutations of Cu/Zn-SOD have also been of interest in connection with amyotrophic lateral sclerosis (ALS), where a gain of function may be responsible for the underlying neurological symptomatology [112–114]. Normally, the expression of Cu/Zn-SOD appears to be fairly constitutive. However, when copper becomes

less available and hyperoxia induces the expression of SOD (along with metallothionein), it is (at least in certain cells) one of the first enzymes to lose its activity [1,17,115].

3.3. Metallothioneins

Metallothioneins (61 amino acid proteins, with 20 cysteines) come in at least two isoforms encoded by several genes and tightly binding divalent metal ions (except Fe). One characterized function for this group of proteins is to sequester metal ions in an innocuous form when they are present in excess amounts. Thus, cadmium accumulates in metallothionein complexes (particularly in the kidney) throughout life. Excess copper accumulates in MT in Wilson-disease-affected tissues. Zinc and cadmium are particularly good inducers of MT, although copper can also be effective in some tissues. Some other factors, notably the hormones glucagon and cortisol, as well as agents that induce inflammation and the acute-phase response, also enhance MT expression. Although MTs mainly bind Zn, Cu, and Cd ions they can also sequester Hg, Ag, or Ni. However, Cu is bound most tightly and can displace these other ions. Since Zn and Cd ions are not as reactive as Cu with regard to oxygen radical formation, binding of copper to MT is protective for the cell. In addition, Cu–MT, appears to have some SOD activity [116] and in the absence of SOD, oxidative stress induces the expression of MT [115,117].

3.4. Ceruloplasmin

Ceruloplasmin is a single polypeptide chain (about 120 kDa with 12 kDa carbohydrate). Besides its potential role in copper delivery to cells and excretion of copper from the body, ceruloplasmin is a ferroxidase, with the ability to oxidize Fe(II) to Fe(III). This change is helpful to provide iron in the form needed to bind transferrin, (iron plasma carrier) as it emerges from cells for further transport from the bone marrow to red blood cells, where most iron resides. In severe copper deficiency, there is little or no copper-containing ceruloplasmin in the plasma and in tissues and in the absence of active-ferroxidase, iron accumulates in the liver. IV infusion of ceruloplasmin (but not copper–albumin) results in the immediate release of liver iron into the blood [118–121]. However, only 1–2% of the normal plasma level of ferroxidase-active ceruloplasmin can play a role in iron efflux [122]. Since 22 mg (about 0.7%) of iron in the human body fluxes in and out of red blood cells every day, and a major portion of that enters and leaves liver cells, including hepatocytes, it seems that ceruloplasmin alone cannot be responsible for this process. Recently, a new glycosylphosphatidylinositol anchored form of ceruloplasmin, (GPI-linked ceruloplasmin) has been found in mammalian astrocytes [123] and glia [124,125] in the brain. This form of ceruloplasmin may also be involved in iron transport [126]. However, it appears unlikely that ceruloplasmin plays a role in releasing iron from enterocytes during iron absorption [127]. Ceruloplasmin concentration in plasma increases during inflammation or infection. It appears that the regulation of ceruloplasmin expression is controlled not only just by inflammatory cytokines, but also through hypoxia-inducible factor (HIF1), which is linked to iron metabolism [128].

3.5. Hephaestin

Hephaestin is a ceruloplasmin homolog and shares some of the characteristics of this protein. It is a transmembrane protein of about 134 kDa, primarily located in trans-golgi

vesicles. It has ferroxidase activity [129] and is involved in intestinal iron absorption [15]. It may oxidize Fe for binding to apotransferrin, allowing its release into the blood as holotransferrin (through exocytosis).

3.6. Cartilage matrix glycoprotein

Cartilage matrix glycoprotein (CMGP) is another intracellular ceruloplasmin homolog with ferroxidase and oxidase activities [130]. It is located in the vesicular portions of chondrocytes, as well as in the epithelial cells of the eye; composed of four disulfide-bonded subunits (each of 116 kDa), it may play a role in the formation of the extracellular matrix.

3.7. Lysyl oxidase (protein-6-lysine oxidase)

Lysyl oxidase plays a crucial role in the formation, maturation, and stabilization of connective tissue. It is part of the extracellular matrix of organs and tissues in the body (including cartilage and bone). Genes for lysyl oxidase were cloned from rat aorta [131] and human placenta [132]. Recently, several lysyl oxidase-like genes have been identified and cloned. Some of these are particularly expressed by placenta and other reproductive tissues [133,134]. Lysyl oxidase is a multimeric protein composed of 32 kDa subunits, that requires copper for its activity. In Menkes' disease, or in Occipital Horn Syndrome characterized by Cu deficiency, the development of normal connective tissue is altered. The cofactor, lysine tyrosyl quinone (LTQ), is part of the enzyme's active site. It catalyzes the cross-linking of elastin and collagen fibers. Recent evidence suggests that the role of copper may not be catalytic as much as supportive of cofactor formation and structure or enzyme integrity [135,136]. Although copper availability determines enzyme activity, nutritional copper status does not alter the expression of the protein or its mRNA [137].

4. Role of copper in embryogenesis

During embryogenesis, when cell proliferation is very active, respiration and cytochrome oxidase activity are essential. In the last part of gestation, considerable copper is transferred to the fetus from the maternal circulation via the placenta or by ingestion of the amniotic fluid [61]. It is bound to metallothionein and accumulates and is stored in the liver along with similar stores of iron and zinc to be used during the suckling period [138]. The placenta expresses both WND and MNK, but not ceruloplasmin. It was suggested that WND is involved in copper transport at the maternal side of the placenta [139,140], whereas, the MNK protein seems to be active on the fetal side [141]. During embryogenesis, the MNK protein is expressed in all tissues and particularly in the brain, whereas WND expression is initially confined to the central nervous system (CNS), liver, and heart [142]. After birth, much copper is delivered to the newborn via the milk. In human milk, copper is present in various components; ceruloplasmin contributes 20–25% of the copper [143,144]. Most of the ceruloplasmin in milk is produced by the lactating mammary gland [145–149].

5. Role of the pineal night-specific ATPase (PINA) in copper transport

Circadian rhythms are found in virtually all organisms. These rhythms dictate our daily sleep schedule and hormonal fluctuations [150] and even influence our susceptibility to disease

such as heart attack [151], strokes [152], and seizures [153]. The pineal gland, an organ situated deep within the brain exhibits dramatic diurnal fluctuations in the secretion of the hormone melatonin, which is known to link environmental light information to the body's physiological responses. Melatonin synthesis is ultimately controlled by the suprachiasmatic nucleus (SCN) of the brain, which uses a biological clock and lighting information to rhythmically control neural pathways. One role of the SCN is to influence neurons of the superior cervical ganglion (SCG), which sends axonal processes directly to the pineal gland. When stimulated, sympathetic SCG neurons release norepinephrine into the pineal, activating β -adrenergic receptors on the plasma membrane of pinealocytes. The receptors initiate a signaling cascade resulting in the production of cAMP, which stimulates the production of melatonin [154]. In animals, the only documented function of the pineal is the synthesis and regulation of melatonin. Melatonin is synthesized from dietary tryptophan by the actions of four enzymes. (1) Tryptophan hydroxylase (TPH), the rate-limiting enzyme in serotonin synthesis, is responsible for the 5'-hydroxylation of tryptophan, yielding 5-hydroxytryptophan (5-HTP). (2) A non-specific aromatic amino acid decarboxylase (AAADC) converts 5-HTP to 5-hydroxytryptamine (5-HT, serotonin). (3) A pineal/retina-specific enzyme serotonin *N*-acetyltransferase (NAT) acetylates serotonin to form *N*-acetylserotonin (NAS). (4) And another pineal/retina-specific enzyme, hydroxyindole-*O*-methyltransferase (HIOMT), catalyzes the conversion of NAS to melatonin (Fig. 1).

Sequence analysis of the pineal gland night-specific ATPase (PINA) revealed that it is an alternatively spliced form of the copper-transporting ATPase mutated in WD patients, ATP7B. Sequences encoding the N-terminal half of ATP7B are replaced by a unique untranslated 300-bp leader sequence. The PINA protein, therefore, represents only the C-terminal half of ATP7B. PINA completely lacks the metal binding repeats and the first four putative transmembrane segments of WND and despite these deletions, it is proposed to function as a Cu transporter in rat pinealocytes. It was found to be expressed in the pinealocytes and a subset of photoreceptors in adult rats, and transiently in the retinal pigment epithelium and ciliary body during retinal development [155]. PINA is expressed at 100-fold higher levels at night than in daytime.

6. Copper and human syndromes

6.1. Menkes and Wilson diseases

WD is an autosomal recessive disorder resulting from mutations in the ATP7B gene. Patients with WD suffer from brain disorders and liver disease. The cornea of the eye is also affected in many patients, resulting in the hallmark brown discoloration of the cornea, which is very specific for neurological WD, the "Kayser–Fleischer ring". The etiological significance of copper is supported by the efficacy of treatments, which are principally aimed at chelation of free copper. In the case of copper toxicity (Indian cirrhosis), there is clear liver failure, yet there have been no neurological symptoms described [148,149]. The genes responsible for WD [156,157] and Menkes' disease (and the less severe occipital horn syndrome) were cloned [12,13,158]. The corresponding normal proteins encoded by these genes are both P-type ATPases (ATP7B and 7A, for WND and MNK, respectively). They are usually expressed in different cell types (MNK widely, and WND primarily in hepatocytes and

certain areas of the brain). Located in the TGN and vesicular compartments, they can be translocated to the plasma membrane under conditions when copper secretion or efflux needs to be promoted. In contrast to Menkes' disease, WD occurs more gradually, and after birth. It results in the accumulation of excess copper in the liver with other tissues accumulating oxidative damages [110]. Due to the absence of the normal WND ATP7B protein, it is difficult for copper to reach the bile. Accumulation of excess copper in tissues (although mitigated by binding to metallothionein) promotes the formation of ROS, eventually resulting in liver cirrhosis [159–162]. The brain and some endocrine organs are also affected. Recently, a number of small cytosolic copper chaperones have been shown to transport copper to specific copper-dependent target proteins. HAH1, transports copper to the Wilson and the Menkes proteins [163]; CCS, transports copper to the cytoplasmic protein SOD [74] and COX17 transports copper to mitochondria for incorporation into cytochrome-*c* oxidase [164,165]. The presence of these copper chaperones is necessary to ensure that copper can reach its specific target protein. It is not known as to how the chaperones become loaded with copper and whether copper uptake proteins are directly involved in this process.

Menkes' disease is characterized by progressive neurological impairment and death in infancy [166]. Alteration in Cu transport, the entrapment of Cu in intestinal and kidney cells or vascular endothelial cells in the blood-brain barrier leads to Cu deficiency [167]. Menkes' disease gene (ATP7A) has been shown to be expressed in intestinal epithelial cells [168]. The Menkes protein (MNK) is involved in both providing Cu to secreted Cu-metalloproteins, and Cu efflux from intestinal epithelial cells. It contains six successive repeats of the Cu binding motif within the amino terminal region and is regulated by Cu ion concentrations [86]. In the presence of low Cu ion concentrations, the MNK protein is localized in the TGN and at elevated concentrations, Cu stimulates the transport of the MNK protein from the TGN to the plasma membrane to be involved in Cu efflux. It is suggested that in mammalian cells, the reduced form of Cu [Cu(I)], activates this process. The third transmembrane region of MNK functions as a TGN targeting signal [169] and a carboxyl terminal di-leucine is required for recycling from the plasma membrane back to the TGN [170].

6.2. Aceruloplasminemia

Ceruloplasmin has many functions, including antioxidant defense, copper transport, and iron transport. A genetic absence of the production of active ceruloplasmin has been detected in a few families and has been mimicked by knocking out the gene in mice. The absence of ceruloplasmin does not produce marked changes in copper metabolism. It does, however, produce a gradual accumulation of iron in the liver and other tissues [121,171].

Ceruloplasmin is the only way in which iron destined for transferrin could be oxidized and low levels of ceruloplasmin (1–2% of normal) are sufficient to prevent liver accumulation and promote iron release into the blood [172].

6.3. Alzheimer's disease

Although there is no direct cause–effect relationship for copper in Alzheimer's disease, both copper and zinc are associated with the b-amyloid protein, which forms the damaging

“tangles” in the brain pathology of the disease [173,174]. However, zinc precipitates aggregation of the amyloid protein, and copper works against the effect of zinc (except at high concentrations) [175].

6.4. Spongiform encephalopathies (prion diseases)

The prion protein, PrPC, which misfolds in bovine spongiform encephalopathy (mad cow disease) is the causative agent in Creutzfeld-Jacob disease (CJD), kuru, Gerstmann-Straussler-Scheinker (GSS) disease and fatal familial insomnia (FFI), collectively known as prion diseases [176]. PrPC is reported to have copper-dependent SOD-like activity [177–179]. It is a 33–35 kDa protein (varying in glycosylation) with four or five atoms of copper bound to four identical sequences of eight amino acids (“octarepeats”) in the N-terminal region of the protein, (probably via the imidazole and glycine or histidine residues [180]). There is evidence that copper lends structural stability not only to the N-terminal region of the protein but also to other parts of the molecule [181,182]. PrPC is expressed in neurons and other cells, including skeletal muscle. Protein concentration is particularly high in neuronal synapses although the two distinct neuronal forms are synaptic or non-synaptic [183].

The prion protein, PrPC is a GPI-anchored to the outside of the plasma membrane found in the brain, spinal cord and peripheral tissues. It has been shown to bind Cu(II) [184–186] and it constantly recycles between the plasma membrane and an early endosomal compartment [187]. Copper stimulates endocytosis of the prion protein [188] and facilitates the renaturation of guanidine-denatured PrPSc molecules to form the protease-resistant infectious prion particle [189]. Disturbance of normal prion protein metabolism, through infection with the protease-resistant form that accumulates in the spongiform encephalopathies (PrPSc), impairs the ability of neurons to respond to oxidative insults [190].

The abnormal form of the prion protein implicated in this disease (PrPSc), which accumulates and is resistant to proteolysis, probably functions normally in the transport of copper at synapses and perhaps also (directly and/or indirectly) in the scavenging of radicals. It has been reported that the enhanced expression of PrpC increases a cell’s stability to take up copper [191]. It also appears to increase its resistance to copper toxicity and oxidative stress [192]. Exposure to large non-physiological concentrations of Cu(II) or Zn(II) (but not Mn(II)) induced endocytosis of the protein [193], which would bring its copper (or zinc) into the cell. The addition of octarepeats to the prion protein (as occurs in some forms of spongiform encephalopathy) prevented copper-induced endocytosis, consistent with other evidence that the lack of removal/turnover of the prion protein results in (or contributes to) the brain damage seen in these diseases [194].

6.5. Inflammation, infection, and cancer

Copper metabolism is altered in inflammation, infection, and cancer. In contrast to iron levels that decline in serum in infection and inflammation, copper concentrations and ceruloplasmin rise.

Plasma ceruloplasmin synthesis and secretion by hepatocytes is stimulated by interleukin-1 (IL-1) and IL-6 [5]. Copper itself is important for immune response, including the production of IL-2 by activated lymphocytic cells [195], and supports the activity and effectiveness of cellular and humoral immunity [196,197]. In cancer, plasma ceruloplasmin antigen or oxidase activity are positively correlated with disease stage [198]. Malignant tumors have concentrations of copper that are often higher than those of their tissue of origin. Copper is absorbed by the tumors from ceruloplasmin and from non-ceruloplasmin sources in the blood [60]. Copper may also have a role in angiogenesis [199].

In addition, recent studies provide evidence that limiting the biological availability of copper, by penicillamine [200] or tetrathiomolybdate administration [201], slows tumor growth which is probably due to the inhibition of angiogenesis [199].

The amounts of copper that are normally ingested, and even intakes that are considerably higher, are usually not problematic for humans or rodents. Daily intakes of copper as high as 3 mg/d for children 4–8 years of age and 8–10 mg/d for adults are considered tolerable. (These intakes are 7–11 times higher than recommended daily intakes [202]). For most mammals, copper is a relatively benign trace element. Except in some genetically based special circumstances, copper is not implicated in pathologies. Moreover, copper-containing enzymes and even many copper complexes are capable of protecting the organism against ROS implicated in many chronic diseases. Nevertheless, individuals with inherited propensities to accumulate copper, have been vulnerable to toxicity from ingestion of high copper doses [203]. Moreover, dogs and sheep appear to have a limited ability to excrete excess copper in the bile [204]. Dogs tend to accumulate copper in their livers throughout their lifespan, and many die of copper toxicosis.

References

- [1]. Linder MC. Biochemistry of copper. New York: Plenum Press; 1991.
- [2]. Walker WR. The results of a copper bracelet clinical trial and subsequent studies In: Sorenson J, Jr, editor. Inflammatory diseases and copper. Totowa (NJ): Humana; 1982 p. 469–78.
- [3]. Turnlund JR, Keyes WR, Erson HL, Acord LL. Copper absorption and retention in young men at three levels of dietary copper using the stable isotope ⁶⁵Cu. Am J Clin Nutr 1989;49:870–8. [PubMed: 2718922]
- [4]. Scott KC, Turnlund JR. Compartment model of copper metabolism in adult men. J Nutr Biochem 1994;5:342–50.
- [5]. Linder MC, Hazegh-Azam M. Copper biochemistry and molecular biology. Am J Clin Nutr 1996;63:797S–811S. [PubMed: 8615367]
- [6]. Turnlund JR, Keyes WR, Peiffer GL, Scott KC. Copper absorption, excretion, and retention by young men consuming low dietary copper determined by using the stable isotope ⁶⁵Cu. Am J Clin Nutr 1998;67: 1219S–25S.
- [7]. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and diseases. Biochem J 1984;219:1–4. [PubMed: 6326753]
- [8]. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. Meth Enzymol 1990;186:1–85.
- [9]. Predki PF, Sarkar B. Effect of replacement of “zinc finger” zinc on estrogen receptor DNA interactions. J Biol Chem 1992;267:5842–6. [PubMed: 1556100]
- [10]. Bull PC, Cox DW. Wilson disease and Menkes disease: new handles on heavy-metal transport. Trends Genet 1994;10:246–52. [PubMed: 8091505]

- [11]. Bull PC, Thomas GR, Rommens JM, Forbe JR, Cox DW. The Wilson disease gene is a putative copper transporting P-typeATPase similar to the Menkes gene. *Nat Genet* 1993;5:327–37. [PubMed: 8298639]
- [12]. Chelly J, Tumer Z, Tonnesen T, Petterson A, Ishikawa-Brush Y, Tommerup N, et al. Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein. *Nat Genet* 1993;3:14–9. [PubMed: 8490646]
- [13]. Mercer JFB, Livingston J, Hall B, Paynter JA, Begy C, Chandrasekharappa S, et al. Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein. *Nat Genet* 1993;3:20–5. [PubMed: 8490647]
- [14]. Yamaguchi Y, Heiny ME, Gitlin JD. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. *Biochem Biophys Res Commun* 1993;197:271–7. [PubMed: 8250934]
- [15]. Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, Libina N, et al. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat Genet* 1999;21:195–9. [PubMed: 9988272]
- [16]. Cohen NL, Illowsky B, Linder MC. Altered Copper absorption in tumor bearing and estrogen treated rats. *Am J Physiol* 1979;236: E309–315. [PubMed: 426062]
- [17]. Freedman JH, Ciriolo MR, Peisach J. The role of glutathione in copper metabolism and toxicity. *J Biol Chem* 1989;264:5598–605. [PubMed: 2564391]
- [18]. Steinebach OM, Wolterbeek HT. Role of cytosolic copper, metal-lothionein and glutathione in copper toxicity in rat hepatoma tissue culture cells. *Toxicology* 1994;92:75–90. [PubMed: 7940570]
- [19]. Keogh JP, Steffen B, Siegers CP. Cytotoxicity of heavy metals in the human small intestinal epithelial, cell line I-407: the role of glutathione. *J Toxicol Environ Health* 1994;43:351–9. [PubMed: 7966443]
- [20]. Stillman MJ, Shaw III CF, Suzuki KT, editors. *Metallothioneins Synthesis, structure and properties of metallothioneins, phytochela-tins and metal-thiolate complexes*. NewYork: VCH; 1992 p. 55–127.
- [21]. Culotta VC, Hamer DH. Fine mapping of a mouse metallothionein gene metal response element. *Mol Cell Biol* 1989;9:1376–80. [PubMed: 2725504]
- [22]. Masters BA, Ouafie CJ, Ericksen JC, Kelly EJ, Froelick GJ, Zambrowicz BP, et al. Metallothionein III is expressed in neurons that sequester zinc in synaptic vesicles. *J Neurosci* 1994;14:5844–57. [PubMed: 7931547]
- [23]. Masters BA, Kelly EJ, Ouafie CJ, Brinster RL, Palmiter RD. Targeted disruption of metallothionein I and II genes increases sensitivity to cadmium. *Proc Natl Acad Sci USA* 1994;91:584–8. [PubMed: 8290567]
- [24]. McArdle HJ, Mercer JF, Sargeson AM, Danks DM. Effects of cellular copper content on copper uptake and metallothionein and ceruloplasmin mRNA levels in mouse hepatocytes. *J Nutr* 1990;120:1370–5. [PubMed: 2231026]
- [25]. Westin G, Schaffner W. A zinc-responsive factor interacts with a metal-regulated enhancer element (MRE) of the mouse metallothionein-1 gene. *EMBO J* 1988;7:3763–70. [PubMed: 3208749]
- [26]. Furst P, Hu S, Hackett R, Hamer D. Copper activates metallothionein gene transcription by altering the conformation of a specific DNA binding protein. *Cell* 1988;55:705–17. [PubMed: 3052856]
- [27]. Furst P, Hamer D. Cooperative activation of a eukaryotic transcription factor: interaction between Cu(I) and yeast ACEI protein. *Proc Natl Acad Sci USA* 1989;86:5267–71. [PubMed: 2664778]
- [28]. Butler G, Thiele DJ. ACE2, an activator of yeast metallothionein expression which is homologous to SWI5. *Mol Cell Biol* 1991;11: 476–85.
- [29]. Thiele DJ, Hamer DH. Tandemly duplicated upstream control sequences mediate copper-induced transcription of the *Saccharomyces cerevisiae* copper-metalllothionein gene. *Mol Cell Biol* 1986;6: 1158–63. [PubMed: 3537699]
- [30]. Culotta VC, Howard WR, Liu XF. CRS5 encodes a metallothionein-like protein in *Saccharomyces cerevisiae*. *J Biol Chem* 1994;269: 25295–302. [PubMed: 7929222]

- [31]. Suzuki KT, Karasawa A, Sunaga H, Kodama H, Yamanaka K. Uptake of copper from the blood-stream and its relation to induction of metallothionein synthesis in the rat. *Compar Biochem Physiol* 1989; 94:93–7.
- [32]. Lin CM, Kosman DJ. Copper uptake in wild type and copper metallothionein-deficient *Saccharomyces cerevisiae*. Kinetics and mechanism. *J Biol Chem* 1990;265:9194–200. [PubMed: 2188974]
- [33]. Dancis A, Yuan DS, Haile D, Askwith D, Eide D, Moehle C, et al. Molecular characterization of a copper transport protein in *S. cerevisiae*: an unexpected role for copper in iron transport. *Cell* 1994;76:393–402. [PubMed: 8293472]
- [34]. Dancis A, Haile D, Yuan DS, Klausner RD. The *Saccharomyces cerevisiae* transport protein (ctr1p). Biochemical characterization, regulation by copper, and physiological role in copper uptake. *J Biol Chem* 1994;269:25660–7. [PubMed: 7929270]
- [35]. Labbe S, Thiele DJ. Pipes and wiring: the regulation of copper uptake and distribution in yeast. *Trends Microbiol* 1999;7:500–5. [PubMed: 10603486]
- [36]. Eide DJ. Molecular biology of metal ion transport in *Saccharomyces cerevisiae*. *Annu Rev Nutr* 1998;18:441–69. [PubMed: 9706232]
- [37]. Zhou B, Gitschier J. hCTR1: a human gene for copper uptake identified by complementation in yeast. *Proc Natl Acad Sci USA* 1997;94: 7481–6. [PubMed: 9207117]
- [38]. Lee J, Prohaska JR, Dagenais SL, Glover TW, Thiele DJ. Isolation of a murine copper transporter gene, tissue specific expression and functional complementation of a yeast copper transport mutant. *Gene* 2000;254:87–96. [PubMed: 10974539]
- [39]. Kuo Y-M, Zhou B, Cosco D, Gitschier J. The copper transporter CTR1 provides an essential function in mammalian embryonic development. *Proc Natl Acad Sci USA* 2001;98:6836–41. [PubMed: 11391004]
- [40]. Moller LB, Petersen C, Lund C, Horn N. Characterization of the hCTR1 gene: genomic organization, function, expression and identification of a highly homologous processed gene. *Gene* 2000;257:13–22. [PubMed: 11054564]
- [41]. Lee J, Prohaska JR, Thiele DJ. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. *Proc Natl Acad Sci USA* 2001;98:6842–7. [PubMed: 11391005]
- [42]. Kampfenkel K, Kushnir S, Babychuk E, Inze D, Van Montagu M. Molecular characterization of a putative *Arabidopsis thaliana* copper transporter and its yeast homologue. *J Biol Chem* 1995;270:28479–86. [PubMed: 7499355]
- [43]. Pena MMO, Lee J, Thiele DJ. A delicate balance: homeostatic control of copper uptake and distribution. *J Nutr* 1999;129:1251–60. [PubMed: 10395584]
- [44]. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 1997;388:482–8. [PubMed: 9242408]
- [45]. Liu XF, Supek F, Nelson N, Culotta VC. Negative control of heavy metal uptake by the *Saccharomyces cerevisiae* BSD2 gene. *J Biol Chem* 1997;272:11763–9. [PubMed: 9115231]
- [46]. Liu XF, Culotta VC. Post-translation control of Nramp metal transport in yeast. Role of metal ions and the BSD2 gene. *J Biol Chem* 1999; 274:4863–8. [PubMed: 9988727]
- [47]. Fleming MD, Trenor III CC, Su MA, Foernsler D, Beier DR, Dietrick WF, et al. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet* 1997;16:383–6. [PubMed: 9241278]
- [48]. Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, Andrews NC. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. *Proc Natl Acad Sci USA* 1998;95:1148–53. [PubMed: 9448300]
- [49]. Roy CN, Enns CA. Iron homeostasis: new tales from the crypt. *Blood* 2000;96:4020–7. [PubMed: 11110669]
- [50]. Andrews NC. 2000 Intestinal iron absorption: current concepts circa. *Dig Liver Dis* 2000;32:56–61. [PubMed: 10975756]
- [51]. Wirth PL, Linder MC. Distribution of copper among multiple components of human serum. *J Natl Cancer Inst* 1985;75:277–84. [PubMed: 3860683]

- [52]. Barrow L, Tanner MS. Copper distribution among serum proteins in paediatric liver disorder and malignancies. *Eur J Clin Invest* 1988;18: 555–60. [PubMed: 3147183]
- [53]. Linder MC, Lomeli NA, Donley S, Mehrbod F, Cerveza P, Cotton S, et al. Copper transport in mammals In: Leone A, Mercer JFB, editors. *Copper transport and its disorders*. New York: Kluwer Academic/Plenum; 1999 p. 1–16.
- [54]. Masuoka J, Hegenauer J, Van Dyke BR, Saltman P. Intrinsic stoichio-metric equilibrium constants for the binding of zinc(II) and copper(II) to the high affinity site of serum albumin. *J Biol Chem* 1993;268: 21533–7. [PubMed: 8408004]
- [55]. Lau S, Sarkar B. Ternary coordination complex between human serum albumin, copper(II) and L-histidine. *J Biol Chem* 1971;246:5938–43. [PubMed: 5165635]
- [56]. Masuoka J, Saltman P. Zinc(II) and copper (II) binding to serum albumin. A comparative study of dog, bovine, and human albumin. *J Biol Chem* 1994;269:25557–61. [PubMed: 7929257]
- [57]. Weiss KC, Linder MC. Copper transport in rats involving a new plasma protein. *Am J Physiol* 1985;249:E77–88. [PubMed: 3925789]
- [58]. Linder MC, Wooten L, Cerveza P, Cotton S, Shulze R, Lomeli N. Copper transport. *Am J Clin Nutr* 1998;67:965S–71S. [PubMed: 9587137]
- [59]. Goforth J, Vivas E, Liu N, Askary HS, Lo LSL, Linder MC. Correspondence between rat transcuprein and human alpha-2-macroglobulin in copper binding. *FASEB J* 2001;15
- [60]. Campbell CH, Brown R, Linder MC. Circulating ceruloplasmin is an important source of copper for normal and malignant cells. *Biochim Biophys Acta* 1981;678:27–38. [PubMed: 7306577]
- [61]. Lee SH, Lancey R, Montaser A, Madani N, Linder MC. Ceruloplasmin and copper transport during the latter part of gestation in the rat. *Proc Soc Exp Biol Med* 1993;203:428–39. [PubMed: 8394586]
- [62]. Vargas EJ, Shoho AR, Linder MC. Copper transport in the Nagase analbuminemic rat. *Am J Physiol* 1994;267:G259–269. [PubMed: 8074226]
- [63]. Orena SJ, Goode CA, Linder MC. Binding and uptake of copper from ceruloplasmin. *Biochem Biophys Res Commun* 1986;139:822–5. [PubMed: 3767988]
- [64]. Harris ZL, Takahashi Y, Miyajima H, Serizawa M, MacGillivray RTA, Gitlin JD. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. *Proc Natl Acad Sci USA* 1995;92:2539–43. [PubMed: 7708681]
- [65]. Meyer LA, Durley AP, Prohaska JR, Harris ZL. Copper transport and metabolism are normal in Aceruloplasminemic mice. *J Biol Chem* 2001;276:36857–61. [PubMed: 11461924]
- [66]. Hilton M, Spenser DC, Ross P, Ramsey A, McArdle HJ. Characterization of the copper uptake mechanism and isolation of the ceruloplasmin receptor/copper transporter in human placental vesicles. *Biochim Biophys Acta* 1995;1245:153–60. [PubMed: 7492571]
- [67]. Mas A, Sarkar B. Uptake by ⁶⁷Cu by isolated human trophoblast cells. *Biochim Biophys Acta* 1992;1135:123–8. [PubMed: 1616932]
- [68]. Percival SS, Harris ED. Copper transport from ceruloplasmin: characterization of the cellular uptake mechanism. *Am J Physiol* 1990; 258:C140–146. [PubMed: 2301561]
- [69]. Stevens MD, DiSilvestro RA, Harris ED. Specific receptors for ceruloplasmin in membrane fragments from aortic and heart tissues. *Biochemistry* 1984;23:261–6. [PubMed: 6320863]
- [70]. Kataoka M, Tavassoli M. Identification of ceruloplasmin receptors on the surface of human blood monocytes, granulocytes, and lymphocytes. *Exp Hematol* 1985;13:806–10. [PubMed: 2995102]
- [71]. Tavassoli M, Kishimoto T, Kataoka M. Liver endothelium mediates the hepatocyte's uptake of ceruloplasmin. *J Cell Biol* 1986;102:1298–303. [PubMed: 3958047]
- [72]. Klomp LW, Lin SJ, Yuan DS, Klausner RD, Culotta VC, Gitlin JD. Identification and functional expression of HAH1: a novel human gene involved in copper homeostasis. *J Biol Chem* 1997;272:9221–6. [PubMed: 9083055]
- [73]. Larin D, Mekios C, Das K, Ross B, Yang AS, Gilliam TC. Characterization of the interaction between the Wilson and Menkes disease proteins and the cytoplasmic chaperone, HAH1. *J Biol Chem* 1999; 274:28497–504. [PubMed: 10497213]
- [74]. Culotta VC, Klomp LW, Strain J, Casareno RL, Krems B, Gitlin JD. The copper chaperone for superoxide dismutase. *J Biol Chem* 1997; 272:23469–72. [PubMed: 9295278]

- [75]. Casareno RL, Waggoner D, Gitlin JD. The copper chaperone CCS directly interacts with copper/zinc superoxide dismutase. *J Biol Chem* 1998;273:23625–8. [PubMed: 9726962]
- [76]. Glerum DM, Shtanko A, Tzagoloff A. Characterization of COX17: a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. *J Biol Chem* 1996;271:14504–9. [PubMed: 8662933]
- [77]. Amaravadi R, Glerum DM, Tzagoloff A. Isolation of a cDNA encoding the human homolog of COX17 a yeast gene essential for mitochondrial copper recruitment. *Hum Genet* 1997;99:329–33. [PubMed: 9050918]
- [78]. Harris ED. Cellular copper transport and metabolism. *Annu Rev Nutr* 2000;20:291–310. [PubMed: 10940336]
- [79]. Portnoy ME, Schmidt PJ, Rogers RS, Culotta VC. Metal transporters that contribute copper to metallochaperones in *Saccharomyces cerevisiae*. *Mol Genet Genom* 2001;265:873–82.
- [80]. Holmberg CG, Laurell CB. Investigations in serum copper. II. Isolation of the copper containing protein and a description of some of its properties. *Acta Chem Scand* 1948;2:550–6.
- [81]. Sato M, Gitlin JD. Mechanisms of copper incorporation during the biosynthesis of human ceruloplasmin. *J Biol Chem* 1991;266:5128–34. [PubMed: 2002050]
- [82]. Miyajima H, Nishimura Y, Mizoguchi K, Sakamoto M, Shimizu T, Honda N. Familial apoceruloplasmin deficiency associated with blepharospasm and retinal degeneration. *Neurology* 1987;37:761–7. [PubMed: 3574673]
- [83]. Harris ZL, Klomp LW, Gitlin JD. Aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis. *Am J Clin Nutr* 1998;67:972S–7S. [PubMed: 9587138]
- [84]. Paynter JA, Gimes A, Lockhardt P, Mercer JF. Expression of the Menkes gene homologue in mouse tissues lack of effect of copper on the mRNA levels. *FEBS Lett* 1994;351:186–90. [PubMed: 8082762]
- [85]. Yamaguchi Y, Heiny ME, Oitlin JD. Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. *Biochem Biophys Res Commun* 1993;197:271–7. [PubMed: 8250934]
- [86]. Petris MJ, Mercer JF, Culvenor JO, Lockhart P, Oleeson PA, Camakaris J. 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* 1996;15:6084–95. [PubMed: 8947031]
- [87]. Hung IH, Suzuki M, Yamaguchi Y, Yuan DS, Klausner RD, Oitlin JD. Biochemical characterization of the Wilson disease protein and functional expression in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* 1997;272:21461–6. [PubMed: 9261163]
- [88]. Schaefer M, Hopkins RO, Failla ML, Oitlin JD. Hepatocyte-specific localization and copper-dependent trafficking of the Wilson's disease protein in the liver. *Am J Physiol* 1999;276:G639–646. [PubMed: 10070040]
- [89]. Horn N. Copper incorporation studies on cultured cells for prenatal diagnosis of Menkes' disease. *Lancet* 1976;1(1):156–60.
- [90]. Schilsky ML. Identification of the Wilson's disease gene: clues for disease pathogenesis and the potential for molecular diagnosis. *Hepatology* 1994;20:529–33. [PubMed: 8045514]
- [91]. Schilsky ML, Stockert RJ, Sternlieb I. Pleiotropic effect of LEC mutation: a rodent model of Wilson's disease. *Am J Physiol* 1994;266: G907–913. [PubMed: 8203535]
- [92]. Dijkstra M, van den Berg OJ, Wolters H, In't Veld O, Slooff MJ, Heymans HS, et al. Adenosine triphosphate-dependent copper transport in human liver. *J Hepatol* 1996;25:37–42. [PubMed: 8836899]
- [93]. Terada K, Aiba N, Yang X-L, Iida M, Nakai M, Miura N, et al. Biliary excretion of copper in LEC rat after introduction of copper transporting P-type ATPase, ATP7B. *FEBS Lett* 1999;448:53–6. [PubMed: 10217409]
- [94]. Terada K, Nakako T, Yang X-L, Iida M, Aiba N, Minamiya Y, et al. Restoration of holoceruloplasmin synthesis in LEC rat after infusion of recombinant adenovirus bearing WND cDNA. *J Biol Chem* 1998; 273:1815–20. [PubMed: 9430732]

- [95]. Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, et al. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993;5:344–50. [PubMed: 8298641]
- [96]. Nakamura K, Urakami K-I, Umeyama K, et al. Intracellular distribution of the Wilson's disease gene product (ATPase7B) after in vitro and in vivo exogenous expression in hepatocytes from the LEC rat: an animal model of Wilson's disease. *Hepatology* 1998;27:799–807. [PubMed: 9500710]
- [97]. Dijkstra M, Vonk RJ, Kuipers F. How does copper get into bile? New insights into the mechanism(s) of hepatobiliary copper transport. *J Hepatol* 1996;24:109–20.
- [98]. Camakaris J, Voskoboinik I, Mercer JFB. Molecular mechanisms of copper homeostasis. *Biochem Biophys Res Commun* 1999;261:225–32. [PubMed: 10425169]
- [99]. Schaefer M, Roelofsen J, Wolters H, Hofmann WJ, Muller M, Kuipers F, et al. Localization of the Wilson's disease protein in human liver. *Gastroenterology* 1999;117:1380–5. [PubMed: 10579979]
- [100]. Lutsenko S, Cooper MJ. Localization of the Wilson's disease protein product to mitochondria. *Proc Natl Acad Sci USA* 1998;95:6004–9. [PubMed: 9600907]
- [101]. Camakaris J, Petris MJ, Bailey L, Shen P, Lockhart P, Glover TW, et al. Gene amplification of the Menkes (MNK ATP7 A) P-type ATPase gene of CHO cells is associated with copper resistance and enhanced copper efflux. *Hum Mol Genet* 1995;4:2117–23. [PubMed: 8589689]
- [102]. Petris MJ, Mercer JFB. The Menkes protein (ATP7A; MNK) cycles via the plasma membrane both in basal and elevated extracellular copper using a C-terminal di-leucine endocytic signal. *Hum Mol Genet* 1999;8:2107–15. [PubMed: 10484781]
- [103]. Roelofsen J, Wolters H, Van Luyn JA, Miura N, Kuipers F, Vonk RJ. Copper-induced apical trafficking of ATP7B in polarized hepatoma cells provides a mechanism for biliary copper excretion. *Gastroenterology* 2000;119:782–93. [PubMed: 10982773]
- [104]. Chowrimootoo GFE, Seymour CA. The role of ceruloplasmin in copper excretion. *Biochem Soc Trans* 1994;22:1905
- [105]. Rae TD, Schmidt PJ, Pufahl RA, Culotta VC, O'Halloran TV. Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. *Science* 1999;284:805–8. [PubMed: 10221913]
- [106]. Warburg O Über eisen, den sauerstoffübertragenden Bestandteil des Atrnungsfermentes. *Biochem Z* 1924;152:479–94.
- [107]. Ferguson-Miller S, Babcock GT. Heme/copper terminal oxidases. *Chem Rev* 1996;96:2889–907. [PubMed: 11848844]
- [108]. Wikstrom MKF. Proton pump coupled to cytochrome-c oxidase in mitochondria. *Nature* 1977;266:271–3. [PubMed: 15223]
- [109]. Keilin D, Hartree EF. Cytochrome a and cytochrome oxidase. *Nature* 1938;141:870–1.
- [110]. Linder MC. Copper and genomic stability in mammals. *Mutat Res* 2001;475:151–2.
- [111]. Flood DG, Reaume AG, Gruner JA, Hoffmann EK, Hirsch JD, Lin Y-G, et al. Hindlimb motor neurons require Cu/Zn superoxide dismutase for maintenance of neuromuscular junctions. *Am J Pathol* 1999; 155:663–72. [PubMed: 10433959]
- [112]. Valentine JS, Hart PJ, Gralla EB. Copper-zinc superoxide dismutase and ALS In: Leone A, Mercer JFB, editors. Copper transport and its disorders. New York: Kluwer Academic/Plenum; 1999 p. 193–203.
- [113]. Carri MT, Battistoni A, Ferri A, Gabbianelli R, Rotilio G. A study of the dual role of copper in superoxide dismutase as antioxidant and pro-oxidant in cellular models of amyotrophic lateral sclerosis In: Leone A, Mercer JFB, editors. Copper transport and its disorders. New York: Kluwer Academic/Plenum; 1999 p. 205–13.
- [114]. Johnson MA, Macdonald TL, Mannick JB, Conaway MR, Gaston B. Accelerated S-nitrosothiol breakdown by amyotrophic lateral sclerosis mutant copper, zinc-superoxide dismutase. *J Biol Chem* 2001;276: 39872–8. [PubMed: 11518706]
- [115]. Levy MA, Tsai YH, Reaume A, Bray TM. Cellular response of antioxidant metalloproteins in Cu/Zn SOD transgenic mice exposed to hyperoxia. *Am J Physiol* 2001;281:L172–182.

- [116]. Kang YJ. The antioxidant function of metallothionein in the heart. *Proc Soc Exp Biol Med* 1999;222:263–73. [PubMed: 10601885]
- [117]. Ghoshal K, Majumder S, Li Z, Bray TM, Jacob ST. Transcriptional induction of MT-I and II genes in the livers of Cu/Zn-SOD knockout mice. *Biochem Biophys Res Commun* 1999;264:735–42. [PubMed: 10544001]
- [118]. Ragan HA, Nacht S, Lee GR, Bishop CR, Cartwright GE. Effect of ceruloplasmin on plasma iron in copper deficient swine. *Am J Physiol* 1969;217:1320–3. [PubMed: 5346295]
- [119]. Osaki S, Johnson DA. Mobilization of liver iron by ferroxidase (ceruloplasmin). *J Biol Chem* 1969;244:5757–61. [PubMed: 5360468]
- [120]. Harris ZL, Durley AP, Man TM, Gitlin JD. Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. *Proc Natl Acad Sci USA* 1999;96:10812–7. [PubMed: 10485908]
- [121]. Yoshida K, Furihata K, Takeda S, Nakamura A, Yamamoto K, Hiyamuta S, et al. A mutation in the ceruloplasmin gene is associated with systemic hemosiderosis in humans. *Nat Genet* 1995;9:267–72. [PubMed: 7539672]
- [122]. Roeser HP, Lee GR, Nacht S, Cartwright GE. A role of ceruloplasmin in iron metabolism. *J Clin Invest* 1980;49:2408–17.
- [123]. Patel BN, David S. A novel glycosylphosphatidylinositol-anchored form of ceruloplasmin is expressed by mammalian astrocytes. *J Biol Chem* 1997;272:20185–90. [PubMed: 9242695]
- [124]. Salzer JL, Lovejoy L, Linder MC, Rosen C. Ran-2, a glial lineage marker, is a GPI-anchored form of ceruloplasmin. *J Neurosci* 1998; 54:147–57.
- [125]. Patel BN, Dunn RJ, David S. Alternative RNA splicing generates a glycosylphosphatidylinositol-anchored form of ceruloplasmin in mammalian brain. *J Biol Chem* 2000;275:4305–10. [PubMed: 10660599]
- [126]. Harris ZL, Klomp LW, Gitlin JD. Aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis. *Am J Clin Nutr* 1998;67:972S–7S. [PubMed: 9587138]
- [127]. Zerounian NR, Linder MC. Effects of copper and ceruloplasmin on iron transport in the CaCo₂ cell intestinal model. *J Nutr Biochem* 2001;13:138–48.
- [128]. Mukhopadhyay CK, Mazumder B, Fox PL. Role of hypoxia inducible factor-1 in transcription activation of ceruloplasmin by iron deficiency. *J Biol Chem* 1999;275:21048–54.
- [129]. Vulpe CD, Attieh ZK, Allaeddine RM, Su T. Identification of a ferroxidase activity for hephaestin. *FASEB J* 2001;15:A800
- [130]. Fife RS, Moody S, Houser D, Proctor C. Studies of copper transport in cultured bovine chondrocytes. *Biochim Biophys Acta* 1994;1201:19–22. [PubMed: 7918577]
- [131]. Trackman PC, Pratt AM, Wolanski A, Tang SS, Offner GD, Troxler RF, et al. Cloning of rat aorta lysyl oxidase cDNA: complete codons and predicted amino acid sequence. *Biochemistry* 1990;29: 4863–70. [PubMed: 1973052]
- [132]. Hamalainen ER, Jones TA, Sheer D, Taskinen K, Pihlajaniemi T, Kivirikko K. Molecular cloning of human lysyl oxidase and assignment of the gene to chromosome 5q23.2–31. *Genomics* 1991;2(11): 508–16.
- [133]. Jourdan-Le Saux C, Tronecker H, Bogic L, Bryant-Greenwood GD, Boyd CD, Csiszar K. The LOXL2 gene encodes a new lysyl oxidase-like protein and is expressed at high levels in reproductive tissues. *J Biol Chem* 1999;274:12939–44. [PubMed: 10212285]
- [134]. Maki JM, Kivirikko K. Cloning and characterization of a fourth human lysyl oxidase isoenzyme. *Biochem J* 2001;355:381–7. [PubMed: 11284725]
- [135]. Klinman JP. The catalytic function of bovine lysyl oxidase in the absence of copper. *J Biol Chem* 2001;276:30575–8. [PubMed: 11395477]
- [136]. Wang SX, Mure M, Medzihradszky KF, Burlingame AL, Brown DE, Dooley DM, et al. A crosslinked cofactor in lysyl oxidase: redox function for amino acid side chains. *Science* 1996;273:1078–84. [PubMed: 8688089]
- [137]. Rucker RB, Kosonen T, Clegg MS, Mitchell AE, Rucker BR, Uriu-Hare JY, et al. Copper, lysyl oxidase, and extracellular matrix protein cross-linking. *Am J Clin Nutr* 1998;67:996S–1002S. [PubMed: 9587142]

- [138]. Linder MC, Donley S, Dominguez D, Wooten L, Mehrbod F, Cerveza P, et al. Copper transport and ceruloplasmin during lactation and pregnancy In: Sarkar B, editor. Metals and genetics. New York: Kluwer Academic/Plenum; 1999 p. 117–29.
- [139]. Muramatsu Y, Tamada T, Moralejo DH, Suzuki Y, Matsumoto K. Fetal copper uptake and a homolog (Atp7b) of the Wilson's disease gene in rats. *Res Commun Mol Pathol Pharmacol* 1998;101:225–31. [PubMed: 9874280]
- [140]. Oga M, Matsui N, Anai T, Yoshimatsu J, Inoue I, Miyakawa I. Copper disposition of the fetus and placenta in a patient with untreated Wilson's disease. *Am J Obstet Gynecol* 1993;169:196–8. [PubMed: 8333453]
- [141]. Mann J, Camakaris J, Danks DM. Copper metabolism in mottled mouse mutants. Defective placental transfer of ⁶⁴Cu to foetal brindled (Mobr) mice. *Biochem J* 1980;186:629–31. [PubMed: 7378070]
- [142]. Kuo Y-M, Gitschier J, Packman S. Developmental expression of the mouse mottled and toxic milk genes suggests distinct functions for the Menkes and Wilson disease copper transporters. *Hum Mol Genet* 1997;6:1043–9. [PubMed: 9215673]
- [143]. Coni E, Bocca B, Galoppi B, Alimonti A, Caroli S. Identification of chemical species of some trace and minor elements in mature breast milk. *Microchem J* 2000;67:187–94.
- [144]. Wooten L, Shulze RA, Lancey RW, Lietzow M, Linder MC. Ceruloplasmin is found in milk and amniotic fluid and may have a nutritional role. *J Nutr Biochem* 1996;7:632–9.
- [145]. Olivares M, Pizarro F, Speisky H, Lonnerdal B, Uauy R. Copper in infant nutrition: safety of World Health Organization provisional guideline value for copper content of drinking water. *J Pediatr Gastroenterol Nutr* 1998;26:251–7.
- [146]. Olivares M, Araya M, Uauy R. Copper homeostasis in infant nutrition: deficit and excess. *J Pediatr Gastroenterol* 2000;31:102–11.
- [147]. Chierici R, Saccomandi D, Vigi V. Dietary supplements for the lactating mother: influence on the trace element content of milk. *Acta Paediatr* 1999;88:7–13. [PubMed: 10090539]
- [148]. Sethi S, Grover S, Khodaskar MB. Role of copper in Indian childhood cirrhosis. *Ann Trop Paediatr* 1993;13:3–5. [PubMed: 7681642]
- [149]. Muller T, Feichtinger H, Berger H, Muller W. Endemic Tyrolean infantile cirrhosis: an ecogenetic disorder. *Lancet* 1996;347:877–80. [PubMed: 8622397]
- [150]. Purcell H, Mulcahy D. Circadian rhythms and the onset of myocardial infarction: clinical implications. *J Cardiovasc Risk* 1995;2:510–4. [PubMed: 8665369]
- [151]. Vermeer SE, Rinkel GJ, Algra A. Circadian fluctuations in onset of subarachnoid hemorrhage. New data on aneurysmal and perimesencephalic hemorrhage and a systematic review. *Stroke* 1997;28:805–8. [PubMed: 9099200]
- [152]. Quigg M. Circadian rhythms: interactions with seizures and epilepsy. *Epilepsy Res* 2000;42:43–55. [PubMed: 10996505]
- [153]. Arendt J. Melatonin and the mammalian pineal gland. London: Chapman & Hall; 1995.
- [154]. Borjigin J, Li X, Snyder SH. The pineal gland and melatonin: molecular and pharmacologic regulation. *Annu Rev Pharmacol Toxicol* 1999; 39:53–65. [PubMed: 10331076]
- [155]. Borjigin J, Payne AS, Deng J, Li X, Wang MM, Ovodenko B, et al. A novel pineal night-specific ATPase encoded by the Wilson disease gene. *J Neurosci* 1999;19:1018–26. [PubMed: 9920665]
- [156]. Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type A TPase similar to the Menkes gene. *Nat Genet* 1993;5:327–37. [PubMed: 8298639]
- [157]. Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Toss B, et al. The Wilson disease gene is a copper-transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993;5:344–50. [PubMed: 8298641]
- [158]. Vulpe C, Levinson B, Whitney S, Packman S, Gitschier J. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nat Genet* 1993;3:7–13. [PubMed: 8490659]
- [159]. Wu J, Forbes JR, Chen HS, Cox DW. The LEC rat has a deletion in the copper transporting ATPase gene homologous to the Wilson disease gene. *Nat Genet* 1994;7:6541–5.

- [160]. Theophilos MB, Cox D, Mercer JFB. The toxic milk mouse is a murine model of Wilson disease. *Hum Mol Genet* 1996;5:1619–24. [PubMed: 8894697]
- [161]. Okayasu T, Tochimaru H, Takahashi T, Takekoshi Y, Li Y, Togashi Y, et al. Inherited copper toxicity in Long-Evans Cinnamon rats exhibiting spontaneous hepatitis: a model of Wilson's disease. *Pediatr Res* 1992;31:253–7. [PubMed: 1561010]
- [162]. Buiakova OI, Xu J, Lutsenko S, Zeitlin S, Das K, Das S, et al. Null mutation of the murine ATP7B (Wilson disease) gene results in intra-cellular copper accumulation and late-onset hepatic nodular transformation. *Hum Mol Genet* 1999;9:1665–71.
- [163]. Pufahl RA, Singer CP, Peariso KL, Lin SJ, Schmidt PJ, Fahrni CJ, et al. Metal ion chaperone function of the soluble Cu(I) receptor Atx1. *Science* 1997;278:853–6. [PubMed: 9346482]
- [164]. Olerum DM, Shtanko A, Tzagoloff A. SCO1 and SCO2 act as high copy suppressors of a mitochondrial copper recruitment defect in *Saccharomyces cerevisiae*. *J Biol Chem* 1996;271:20531–5. [PubMed: 8702795]
- [165]. Srinivasan C, Posewitz MC, George ON, Winge DR. Characterization of the copper chaperone Cox17 of *Saccharomyces cerevisiae*. *Biochemistry* 1998;37:7572–7. [PubMed: 9585572]
- [166]. Danks DM, Cartwright E, Stevens BJ, Townley RR. Menkes' kinky hair disease: further definition of the defect in copper transport. *Science* 1973;179:1140–2. [PubMed: 4120259]
- [167]. Danks DM. Disorders of copper transport In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The metabolic basis of inherited disease*. New York: McGraw-Hill; 1989 p. 1411–31.
- [168]. Murata Y, Kodama H, Abe T, Ishida N, Nishimura M, Levinson B, et al. Mutation analysis and expression of the mottled gene in the macular mouse model of Menkes disease. *Pediatr Res* 1997;42:436–42. [PubMed: 9380433]
- [169]. Francis MJ, Jones EE, Levy ER, Ponnambalam S, Chelly J, Monaco AP. A golgi localization signal identified in the Menkes recombinant protein. *Hum Mol Genet* 1998;7:1245–52. [PubMed: 9668166]
- [170]. Petris MJ, Camakaris J, Greenough M, LaFontaine S, Mercer JFB. A C-terminal di-leucine is required for localization of the Menkes protein in the trans-golgi network. *Hum Mol Genet* 1998;7:2063–71. [PubMed: 9817923]
- [171]. Gitlin JD. Aceruloplasminemia. *Pediatr Res* 1998;44:271–6. [PubMed: 9727700]
- [172]. Roeser HP, Lee GR, Nacht S, Cartwright GE. A role of ceruloplasmin in iron metabolism. *J Clin Invest* 1980;49:2408–17.
- [173]. Strausak D, Mercer JF, Dieter HH, Stremmel W, Multhaup G. Copper in disorders with neurological symptoms: Alzheimer's, Menkes, and Wilson diseases. *Brain Res Bull* 2001;55:175–85. [PubMed: 11470313]
- [174]. Cherny RA, Atwood CSA, Xilinas ME, Gray DN, Jones WD, McLean CA, et al. Treatment with a copper-zinc chelator inhibits β -amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 2001;30:665–76. [PubMed: 11430801]
- [175]. Suzuki K, Miura T, Takeuchi H. Inhibitory effect of copper(II) on zinc(II)-induced aggregation of amyloid β -peptide. *Biochem Biophys Res Commun* 2001;285:991–6. [PubMed: 11467850]
- [176]. Prusiner SB. Molecular biology of prion diseases. *Science* 1991;252: 1515–22. [PubMed: 1675487]
- [177]. Brown DR, Clive C, Haswell SJ. Antioxidant activity related to copper binding of native prion protein. *J Neurochem* 2001;76:69–76. [PubMed: 11145979]
- [178]. Brown DR, Wong B-S, Hafiz F, Clive C, Haswell SJ, Jones IM. Normal prion protein has an activity like that of superoxide dismutase. *Biochem J* 1999;344:1–5. [PubMed: 10548526]
- [179]. Brown DR, Hafiz F, Glasssmith LL, Wong B-S, Jones IM, Clive C, et al. Consequences of manganese replacement of copper for prion protein function and proteinase resistance. *EMBO J* 2000;19: 1180–6. [PubMed: 10716918]
- [180]. Aronoff-Spencer E, Burns CS, Avdievich NI, Gerfen GJ, Peisach J, Antholine WE, et al. Identification of the Cu^{2+} -binding sites in the N-terminal domain of the prion protein by EPR and CD spectroscopy. *Biochemistry* 2000;39:12760–3771.
- [181]. Miura T, Hori-I A, Takeuchi H. Raman spectroscopy study on the copper(II) binding mode of prion, octapeptide and its pH dependence. *FEBS Lett* 1996;396:248–52. [PubMed: 8914996]

- [182]. McMahon HEM, Mange A, Nishida N, Cerminon C, Casanova D, Lehmann S. Cleavage of the amino terminus of the prion protein by reactive oxygen species. *J Biol Chem* 2001;276:2286–90. [PubMed: 11060296]
- [183]. Brown DR. Prion or prejudice: normal protein and the synapse. *Trends Neurosci* 2001;24:85–90. [PubMed: 11164938]
- [184]. Hornshaw MP, McDermott JR, Candy JM. Copper binding to the N-terminal tandem repeat regions of mammalian and avian prion protein. *Biochem Biophys Res Commun* 1995;207:621–9. [PubMed: 7864852]
- [185]. Prince RC, Gunson DE. Prions are copper-binding proteins. *Trends Biochem Sci* 1998;23:197–8. [PubMed: 9644969]
- [186]. Viles JH, Cohen FE, Prusiner SB, Goodin DB, Wright PE, Dyson HJ. Copper binding to the prion protein: structural implications of four identical cooperative binding sites. *Proc Natl Acad Sci USA* 1999;96: 2042–7. [PubMed: 10051591]
- [187]. Shyng SL, Huber MT, Harris DA. A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. *J Biol Chem* 1993;268:15922–8. [PubMed: 8101844]
- [188]. Pauly PC, Harris DA. Copper stimulates endocytosis of the prion protein. *J Biol Chem* 1998;273:33107–10. [PubMed: 9837873]
- [189]. McKenzie D, Bartz J, Mirwald J, Olander D, Marsh R, Aiken J. Reversibility of scrapie inactivation is enhanced by copper. *J Biol Chem* 1998;273:25545–7. [PubMed: 9748215]
- [190]. Milhavet O, McMahon HEM, Rachidi W, Nishida N, Katamine S, Mange A, et al. Prion infection impairs the cellular response to oxidative stress. *Proc Natl Acad Sci USA* 2000;97:13937–42. [PubMed: 11095725]
- [191]. Brown DR. Prion protein expression aids cellular uptake and veratridine-induced release of copper. *J Neurosci Res* 1999;58:717–25. [PubMed: 10561700]
- [192]. Brown DR, Schmidt B, Kretzschmar HA. Expression of prion protein in PC12 is enhanced by exposure to oxidative stress. *Int J Dev Neurosci* 1997;15:961–72. [PubMed: 9641527]
- [193]. Sumudhu W, Perera S, Hooper NM. Ablation of the metal ion-induced endocytosis of the prion protein by disease-associated mutation of the octarepeat. *Curr Biol* 2000;11:519–23.
- [194]. Brockes JP. Topics in prion cell biology. *Curr Opin Neurobiol* 1999; 9:571–7. [PubMed: 10508744]
- [195]. Hopkins RG, Failla ML. Transcriptional regulation of interleukin-2 gene expression is impaired by copper deficiency in Jurkat human T lymphocytes. *J Nutr* 1999;129:596–601. [PubMed: 10082761]
- [196]. Percival SS. Copper and immunity. *Am J Clin Nutr* 1998;67:1064S–8S. [PubMed: 9587153]
- [197]. Huang ZL, Failla ML. Copper deficiency suppresses effector activities of differentiated U937 cells. *J Nutr* 2000;130:1536–42. [PubMed: 10827206]
- [198]. Linder MC, Moor JR, Wright K. Ceruloplasmin assays in diagnosis and treatment of human lung, breast and gastrointestinal cancer. *J Natl Cancer Inst* 1981;67:263–75. [PubMed: 6943365]
- [199]. Gullino PM, Ziche M, Alessandri G. Ganglioside, copper ions and angiogenic capacity of adult tissues. *Cancer Metastasis Rev* 1990;8: 239–51.
- [200]. Yoshida D, Ikeda Y, Nakazawa S. Copper chelation inhibits tumor angiogenesis in the experimental gliosarcoma model. *Neurosurgery* 1995;37:287–92. [PubMed: 7477781]
- [201]. Brewer GJ. Copper control as an antiangiogenic anticancer therapy: lessons from treating Wilson's disease. *Exp Biol Med* 2001;226: 665–7.
- [202]. Tanner MS. Indian childhood cirrhosis and Tyrolean childhood cirrhosis In: Leone A, Mercer JFB, editors. *Copper transport and its disorders*. New York: Kluwer Academic, Plenum; 1999 p. 127–37.
- [203]. Montaser A, Tetreault C, Linder MC. Comparison of copper binding proteins in dog serum with those in other species. *Proc Soc Exp Biol Med* 1992;200:321–9. [PubMed: 1615008]
- [204]. Moore-Ede MC, Sultzman FM, Fuller CA. *The clocks that time us-physiology of the circadian timing system* Cambridge (MA: Harvard University Press; 1982.

Fig. 1.
Melatonin synthetic pathways (see text for details).

Interactions of iron and copper ions with hydrogen peroxide and superoxide

Comparable reactions can be written in which H_2O_2 reacts with cuprous $[\text{Cu}^+]$ ion to yield $\text{OH}^\cdot$, $\text{O}_2^{\cdot-}$ reduces cupric (Cu^{2+}) ion to $\text{Cu}^\cdot$. Intermediate copper ion-oxygen complexes are formed

Table 2

Copper binding components in human blood plasma

Component	Contribution to total copper content		
	µg/l	µM	%
Ceruloplasmin	650–700	10–11	65–70
Albumin	120–180	2–3	12–18
Transcuprein (macroglobulin)	90	1.4	9
Ferroxidase II	10	0.16	1
Extracellular SOD and histidine-rich glycoprotein	< 10	< 0.16	< 1
Blood clotting factors V and VIII	< 5?	< 0.08	< 0.5?
Extracellular metallothionein and amine-oxidase	< 1?	< 0.02	< 0.1?
15–60 kDa components	40	0.63	4
Small peptides and amino acids	35	0.55	4
“Free” copper ions (source from Ref. [110])	0.0001	0.0000002	0

Table 3

Copper-dependent enzymes in mammals

Enzyme	Function
Cytochrome-c oxidase	Electron transport in mitochondria
Cu/Zn-SOD	Free radical detoxification
Metallothionein	Storage of excess Cu and other divalent metal ions [not Fe(II)]. Possible donor of Cu to certain apoproteins
Ceruloplasmin (extracellular)	Ferroxidase, promotes flow of Fe from liver to blood Scavenger of ROS, acute-phase reactant. Cu transport
Protein-lysine-6-oxidase	Cross-linking of collagen and elastin
Tyrosinase (catechol oxidase)	Formation of melanin
Dopamine-β-monooxygenase	Catecholamines production
α-Amidating enzyme	Modifies C-terminal ends of hypothalamic peptide hormones ending in glycine, leaving the COOH of the next to last AA amidated (necessary for hormone maturation)
Diamine oxidase	Inactivation of histamine and polyamines? (cellular and extracellular)
Amine oxidase (extracellular)	Inactivation of histamine, tyramine, dopamine, serotonin?
Peptidylglycine monooxygenase	Bioactivation of peptide hormones
Hephaestin	Ferroxidase, in trans-golgi of enterocytes; aids iron absorption homology to ceruloplasmin
CMGP	Ferroxidase/amine oxidase, homologous to ceruloplasmin (chondrocytes and eye ciliary epithelia)
β-Amyloid precursor protein	Normal function currently unknown
Prion protein (PrPC)	Copper binding properties suggests that it may protect against ROS; has SOD-like activity; may return copper to neurons at synapses (many cells)
S-Adenosylhomocysteine	Sulfur amino acid metabolism hydrolase
Angiogenin	Induction of blood vessel formation
Blood clotting factors V and VIII	Blood clotting