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REVIEW

Copper transport and Alzheimer's disease

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Abstract This brief review discusses copper transport in humans, with an emphasis on knowledge learned from one of the simplest model organisms, yeast. There is a further focus on copper transport in Alzheimer's Disease (AD). Copper homeostasis is essential for the well-being of all organisms, from bacteria to yeast to humans: survival depends on maintaining the required supply of copper for the many enzymes, dependent on copper for activity, while ensuring that there is no excess free copper, which would cause toxicity. A virtual orchestra of proteins are required to achieve copper homeostasis. For copper uptake, Cu(II) is first reduced to Cu(I) via a membrane-bound reductase. The reduced copper can then be internalised by a copper transporter where it is transferred to copper chaperones for transport and specific delivery to various organelles. Of significance are internal copper transporters, ATP7A and ATP7B, notable for their role in disorders of copper deficiency and toxicity, Menkes and Wilson's disease, respectively. Metallothioneins and Cu/Zn superoxide dismutase can protect against excess copper in cells. It is clear too, increasing age, environmental and lifestyle factors impact on brain copper. Studies on AD suggest an important role for copper in the brain, with some AD therapies focusing on mobilising copper in AD brains. The transport of copper into the brain is complex and involves numerous players, including amyloid precursor protein, Aβ peptide and cholesterol.

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Introduction

Survival of an organism depends on maintaining the proper balance of copper within all cells. Excess free copper is toxic but a deficiency of copper will also incapacitate proper functions, since copper is essential for numerous copper enzymes including cytochrome oxidase (EC 1.9.3.1), superoxide dismutase (EC 1.13.11.11), tryptophan-2,3-dioxygenase (EC 1.13.11.11), lysine oxidase (EC 1.4.3.12), monoamine oxidase (EC 1.4.3.4), tyrosinase (EC 1.14.18.1) and dopamine-β-hydroxylase (EC 1.14.17.1). In humans, these metalloenzymes are required in various organs or sites, and indeed some are essential in all cells. Thus, it can be seen that there is a need for correct transport of copper to organs distant from the site of copper absorption, as well as to cells and cellular components within those organs.

The purpose of this review is to discuss the mechanisms of copper transport and homeostasis throughout the body, and in addition, to discuss strategies to alter copper transport and homeostasis in the brain, which may affect outcomes of neurological disease. There is an orchestra of proteins involved in copper metabolism in humans and these proteins almost always have a counterpart in yeast, as shown in Table 1. Therefore, the study of copper metabolism in yeast, specifically *Saccharomyces cerevisiae*, has had a major impact on the knowledge we now possess. Throughout this review frequent reference will be made to these yeast studies (Fig. 1).

Distribution of copper from the diet to serum

It is estimated that a 70 kg adult contains 110 mg of copper, most of which is in the skeleton (46 mg) and skeletal muscle (26 mg). The liver (10 mg), brain (7 mg) and blood (6 mg) contain most of the remaining copper. There is a

Table 1 Major human proteins involved in copper uptake, efflux, transport and sequestration and their yeast equivalents

Function	Human protein	Yeast protein
Cu reduction	Steap1, Steap2, Steap3, Steap4	Fre1, Fre2
Multicopper oxidase/Fe transport	Ceruloplasmin, Hephaestin	Fet3
Cu uptake	hCtr1	Ctr1 Ctr3
	hCtr2 (late endosomes, lysosomes)	Ctr2 (vacuoles)
Cu chaperone	Atox1 (formerly Hah1)	Atx1 (→ Ccc2 → Fet3)
	Ccs1	Ccs1 (→ Sod1)
	Cox17	Cox17 (→ Sco1 & Cox11)
	Sco1	Sco1 (→ Cox2)
	Cox11	Cox11 (→ Cox1)
Internal Cu transport/efflux	Atp7A	Ccc2
	Atp7B	Ccc2
Cu sequestration	Metallothioneins	Metallothioneins
MT transcription factor	Mtf-1	Ace1

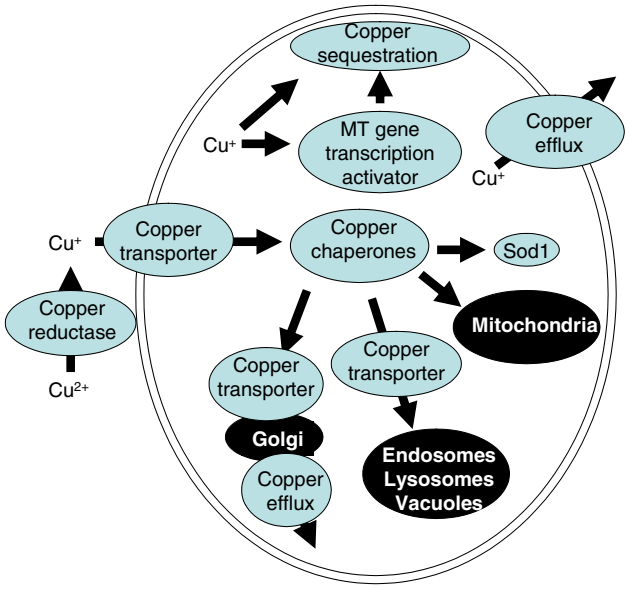

Fig. 1 Simplified schematic diagram of copper homeostasis in eukaryotic organisms. *Light shaded boxes* represent the classes of proteins described in Table 1. Cu(II) is first reduced by a copper reductase. Cu(I) can then be transported into the cell by a membrane-spanning copper transporter. Cu(I) is then carried by various chaperones to specific locations, including to Sod1, the mitochondrion and the *trans*-Golgi network. Another copper transporter is employed to deliver intracellular copper to late endosomes (*E*) and lysosomes (*L*) in mammals, or vacuoles (*V*) in yeast. Excess copper can be removed by efflux pumps, or can be sequestered into metallothionein. The synthesis of copper apothionein is controlled by a copper-sensing transcription factor that is itself a copper apothionein. *Arrows* indicate the passage of copper from one protein to the next

continual turnover of copper, with most of it being recycled. Each day more than 4 mg is excreted into the bile but most is reabsorbed in the intestines. Daily diets have around 0.6–1.6 mg of copper, about half of which is absorbed. The end result is for the organism to maintain copper homeostasis, with no net change in copper levels.

The uptake of copper from the diet has been reviewed by (Linder and Hazegh-Azam 1996). Absorption of copper occurs primarily (or solely) via the brush border cells of the small intestine into interstitial fluid and blood where copper then binds albumin (Linder and Hazegh-Azam 1996). Monitoring the distribution of injected radiolabelled copper sulfate from serum (Weiss and Linder 1985), showed that labelled copper disappeared from plasma within 2 h, with 40% of it being localised in the liver after 6 h. After that time there was significant redistribution to the plasma and to other organs, with levels in plasma, heart brain, muscle and kidney peaking after one to 3 days. Although albumin can accommodate ~40 mg copper per litre of blood, it normally carries <1% of this amount. The major copper binding in blood is ceruloplasmin, which is synthesised in the liver where it binds copper. Ceruloplasmin binds most of the serum copper but is not necessary for copper transport; it is used in the iron transport system. Ceruloplasmin functions as a multicopper oxidase, oxidizing Fe^{2+} to Fe^{3+}. The Fe^{3+} is then bound by transferrin for transport. The equivalent protein in yeast is Fet3, a protein that also functions as a multicopper oxidase (Table 1) (Askwith et al. 1994; de Silva et al. 1997; De Silva et al. 1995; Yuan et al. 1995). People with a total deficiency of ceruloplasmin have an iron metabolism disorder and mid-life dementia (Harris et al. 1995).

Cellular uptake of copper

Copper transport is dependent on the oxidation state of the copper. Only reduced copper can be transported, yet oxidative environments result in copper frequently occurring as the oxidised copper, Cu(II). Reduction occurs through copper reductases. In yeast the copper transporters, Ctr1 and Ctr2, utilise the same metalloreductase, Fre1 (Rees and Thiele 2007) which is also a ferric reductase. Another ferric

reductase, Fre2, appears to have an equivalent role to Fre1. The human counterparts of Fre1, the Steap proteins, function as both cupric and ferric reductases (Ohgami et al. 2006).

In yeast the transport of reduced copper at the plasma membrane takes place through a high affinity copper transporter Ctr1 (Dancis et al. 1994a, b). Ctr3 also shares this function but is inactivated by a Ty2 element insertion in some lab strains (Knight et al. 1996). The human equivalent of Ctr1, hCtr1, was identified by complementation in yeast (Zhou and Gitschier 1997). The yeast *ctr1* mutant exhibited defects in respiratory growth, iron transport and Sod1p, all of which were rescued by the hCtr1, while overproduction of hCtr1 led to copper overload (Zhou and Gitschier 1997). An additional gene, hCtr2, found by a similarity search (Zhou and Gitschier 1997), was recently shown to be expressed in late endosomes and lysosomes where it facilitated cellular copper uptake (van den Berghe et al. 2007). In yeast an equivalent copper transporter, Ctr2, is found exclusively on the vacuolar membrane where it controls vacuolar copper levels (Kampfenkel et al. 1995; Rees et al. 2004). Ctr2 appears to be a non-essential vacuolar copper transporter (Portnoy et al. 2001).

Copper chaperones

Yeast studies provided the first functional identification of the copper chaperones, or metallochaperones. These are intracellular proteins that receive copper and deliver it to specific locations. Because of the various pathways to the ultimate destinations, there are a series of pathway-specific chaperones. The chaperone Ccs1 delivers copper to Cu/Zn superoxide dismutase (Sod1) in the cytosol (Culotta et al. 1997). Cox17 delivers copper to the mitochondrion for incorporation into subunits of cytochrome oxidase (Amaravadi et al. 1997; Glerum et al. 1996), via both Sco1, and Cox11 (Horng et al. 2004). Atx1 is involved in delivery of copper to the secretory pathway for copper enzymes at the cell surface or for release (Klomp et al. 1997; Lin et al. 1997; Pufahl et al. 1997). In the Golgi, this copper is utilised by the copper–transporting ATPases, ATP7A and ATP7B, that are described below.

Internal copper transporters

The two major inherited disorders of copper metabolism, Menkes disease and Wilson's disease, provide insights on cellular copper efflux. Menkes disease has been shown to result from mutations in the MNK transporter, ATP7A, a P-type ATPase. ATP7A is produced in mucosal cells of the intestine and used for systemic absorption of copper. At normal or low copper ATP7A delivers intracellular copper to enzymes in the *trans*-Golgi network (Yamaguchi-Iwai et al. 1996). At high copper levels ATP7A moves to the plasma membrane and performs copper efflux (Petris et al. 1996). Menkes disease is effectively a copper deficiency disease that causes growth failure, skeletal defects, degeneration of the central nervous system, and early death (Danks 1995).

Wilson's disease is a result of defects in the WND transporter, ATP7B, which has 54% sequence identity with ATP7A. At normal or low copper ATP7B is found only in the liver where it delivers intracellular copper to apoceruloplasmin in the *trans*-Golgi network (Yamaguchi et al. 1996). At high copper levels ATP7B moves copper to vesicles for delivery to the biliary caniliculus, performing copper efflux into the bile for excretion (Petris et al. 1996). ATP7B mutations result in copper accumulation in the liver and brain leading to liver disease and neurological problems including behavioural disturbances and movement disorders. Menkes and Wilson's disease are similar in that they utilise a similar pump, but they use it in different tissues. Yeast cells have an equivalent pump, Ccc2, which facilitates cation transport, including copper, in trans Golgi vesicles (Yuan et al. 1995).

Copper sequestration

Excess and free copper are toxic to cells so it is essential that mechanisms exist to sequester free intracellular copper. In healthy yeast cells free copper levels are incredibly low (less than one free ion per cell) (Rae et al. 1999). Metallothionein is a cellular copper binding protein that provides significant protection against elevated copper levels. Yeast metallothionein (Karin et al. 1984; Winge et al. 1985) is highly regulated by Ace1, a constitutively produced transcriptional activator of the yeast metallothionein gene (Buchman et al. 1989; Szczypka and Thiele 1989). In the presence of copper Ace1 binds copper, and activates transcription. In the absence of metallothionein, superoxide dismutase can play a major protective role against copper toxicity. It is significant that some cases of amyotrophic lateral sclerosis (ALS) have defects in Cu/Zn superoxide dismutase, suggesting some linkage between this form of motor neurone disease and copper metabolism.

Alzheimer's disease and copper transport

Links have been established between copper metabolism and late onset neurological diseases, however, much work still needs to be done to fully understand the relationships. In Parkinson's Disease, α-synuclein oligomerisation is affected by copper (Paik et al. 1999) and dopamine may be affected by copper to become toxic (Paris et al. 2001; Snyder and Friedman 1998; Spencer et al. 1994). Human prions have also been found to have copper binding sites (Brown

et al. 1997; Hornshaw et al. 1995). The remainder of this review will focus on Alzheimer's Disease (AD) where the amyloid precursor protein (APP) and the Aβ peptide derived from APP are strongly implicated in copper metabolism.

Amyloid precursor protein is the most important protein in AD and may be a copper transporter that is unique to mammals. It has two copper binding sites, including one in the Aβ peptide sequence (Atwood et al. 1998; Hesse et al. 1994). Overexpression of APP in transgenic mice appears to reduce levels of copper in brains (Bayer et al. 2003; Maynard et al. 2002; Phinney et al. 2003) leading to suggestions that APP may be a copper transporter (White et al. 1998). Even though APP has no yeast equivalent, it can cause copper efflux in yeast (Treiber et al. 2004) as it does in brains. Proteins of similar structure, Aplp1 and Aplp2, have copper binding domains and may similarly function in copper efflux (Hesse et al. 1994; White et al. 1998, 1999a, b). APP is unique among this family because it is processed by the proteases BACE (Vassar et al. 1999) and γ secretase, to produce the Aβ peptide, or β amyloid, a causative agent in AD. Aβ exhibits neurotoxicity, and is a major component of the extracellular Aβ plaques associated with AD. These plaques are a reservoir for a number of metals, including copper, and it is has been considered that the copper in these plaques may be available for the production of reactive oxygen species which could cause neuronal loss and brain damage (Bush 2000). However, in view of the overall decline in copper levels as brains age, the alternate possibility, that build up of Aβ causes a copper shortfall, should also be considered.

Can neuronal copper levels be manipulated? If so this could be an avenue to the treatment of neurological diseases (although the latter will not be discussed here at any length). One of the most promising drugs that have been trialled is clioquinol, a metal protein attenuating compound. Clioquinol reduces plaque in mouse AD models and it would appear to alter copper levels (Cherny et al. 2001). In a treatment of APP 750^{SL} transgenic mice, clioquinol was shown to improve survival (Schafer et al. 2007). While clioquinol reduced serum copper levels from around 800 µg/l to around 500 µg/l, even with added copper in the diet, brain copper significantly increased, from 3.9 to 4.2 µg/g with added dietary copper. The treated mice also exhibited normal survival and improved memory, suggesting that clioquinol enabled brain copper levels to increase, and this in turn gave the positive benefit. Interestingly clioquinol has also been shown to cause increased copper levels in yeast producing APP (Treiber et al. 2004).

A combination of copper with high dietary cholesterol has been linked to increased deposits of Alzheimer's Aβ brain plaque in rabbits (Sparks and Schreurs 2003). Plaques were induced at levels of 0.12 ppm copper, much lower than the levels of 1.3 ppm permitted by water supply agencies. Conversely could lower cholesterol levels lead to lower copper levels, reducing Aβ plaques? Studies of cholesterol-lowering drugs, known as statins, may provide answers to this question.

Statins inhibit HMG-CoA reductase, leading to reduced cholesterol levels as well as lower levels of several other products dependent on HMG-CoA reductase (Fig. 2). Many consider these blockbuster drugs to be the best drugs to lower the incidence of AD. However, there has been a lack of concordance in some studies leading to significant confusion. A recent study by (Wolozin et al. 2007) has now shown that simvastatin is unique with respect to reducing AD. Unlike many other statins, simvastatin is lipophilic and crosses the blood brain barrier (BBB), so it may be expected that simvastatin would reduce cholesterol synthesis in the brain.

A major question now is how does simvastatin reduce the incidence of AD? As predicted from the cholesterol-fed rabbit model for AD, simvastatin may decrease Aβ deposition in the brain by reducing brain cholesterol levels. A second possibility, is that statins reduce protein prenylation decreasing BACE activity, thereby leading to lower levels of Aβ (see Fig. 2); cell culture studies support this possibility (Cole et al. 2005). A third possibility is that statins can reduce mitochondrial respiratory function through decreased haem synthesis, coenzyme Q10 production (see Fig. 2) (Macreadie et al. 2006; Satoh et al. 1994; Westermeyer and Macreadie 2007) or reduced mtDNA levels (Westermeyer and Macreadie 2007). This could in turn lead to lower levels of reactive oxygen species, possibly reducing the threshold for Aβ-induced damage (Tabner et al. 2001). The idea that statins reduce AD through anti-inflammatory properties could be dismissed with the knowledge that simvastatin but not atorvastatin reduces the incidence of AD. Understanding exactly how simvastatin affects the brain will now be an even higher priority area of research.

Finally, vaccine approaches may have some merit in animal models of AD by clearing Aβ plaque. Whether this

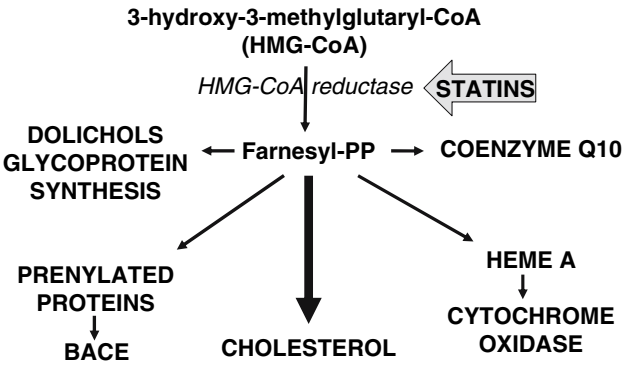

Fig. 2 Biochemical pathway affected by statins

affects brain copper has not been considered but presumably metals associated with the plaque would be removed along with plaque.

It is clear that much is now known about copper homeostasis, but there is still some way to go before we conquer neurological diseases that involve copper. It is likely that an increased understanding of copper homeostasis may help in unravelling the many important neurological diseases involving copper metabolism.

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