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Yeast, a model organism for iron and copper metabolism studies

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Abstract

Virtually all organisms on earth depend on transition metals for survival. Iron and copper are particularly important because they participate in vital electron transfer reactions, and are thus cofactors of many metabolic enzymes. Their ability to transfer electrons also render them toxic when present in excess. Disturbances of iron and copper steady-state levels can have profound effects on cellular metabolism, growth and development. It is critical to maintain these metals in a narrow range between utility and toxicity. Organisms ranging from bacteria and plants to mammals have developed sophisticated mechanisms to control metal homeostasis. In this review, we will present an overview of the current understanding of iron and copper metabolism in yeast, and the utility of yeast as a model organism to investigate iron and copper metabolism in mammals and plants.

Introduction

Transition metals are indispensable for life because of their ability to donate and accept electrons. Iron, copper and manganese, for example, are found as prosthetic groups of proteins that catalyze redox reactions. However, these metal ions can be toxic to cells when present in excess, by inducing the formation of toxic reactive oxygen species. To maintain levels of metal ions in tight homeostasis, organisms have evolved complex regulatory mechanisms that have been conserved through evolution. Multiple transport systems exist for these metals allowing organisms to obtain these metals under a variety of conditions from different sources. This review focuses on iron and copper homeostasis in yeast. More information on manganese can be found at the following references (Cohen *et al.* 2000; Pinner *et al.* 1997). There is a remarkable concordance of cellular pathways of copper and iron metabolism in distantly related organisms. Individual proteins and entire pathways of copper and iron metabolism, including import, distribution and export, are conserved from *Saccharomyces cerevisiae* to humans (Valentine & Gralla 1997) and plants (Himelblau & Amasino 2000) (Fox & Guerinot 1998). Understanding copper and iron metabolism in yeast has lead to fundamental insights into both mammalian and plant metabolic pathways. The information gained has direct applications for improving the bioremediation of metal contaminated sites, improving dietary copper and iron availability in mammals, and treating medical disorders related to metals.

Human disorders of copper and iron metabolism

Several human diseases are directly associated with imbalances of iron and copper ions. For example, familial hemochromatosis and Friedreich's ataxia are associated with perturbation in iron pools. Menkes and Wilson diseases are associated with copper deficiency and overload, respectively (Waggoner *et al.* 1999; De Freitas & Meneghini 2001). Several neurodegenerative diseases show deposits of iron and copper at the affected region in the brain (Waggoner *et al.* 1999). In humans, alterations in the activity of copper containing proteins or copper transporters have also been associated with diseases. Cu,Zn-SOD is associated with the familial form of Amyotrophic Lateral Sclerosis (ALS) (Lyons *et al.* 1999). Inter-

estingly, two inherited human disorders, the Menkes and the Wilson's diseases, are caused by mutations in the yeast CCC2 homologues, ATP7A and ATP7B, respectively. In Menkes patients, a mutation in the ATP7A blocks copper efflux from the intestine. Copper becomes trapped in enterocytes, resulting in severe copper deficiency elsewhere (Vulpe & Packman 1995; Ambrosini & Mercer 1999). On the other hand, in Wilson's patients, a mutation in ATP7B blocks copper efflux from the liver, resulting in copper accumulation in hepatocytes (Bull & Cox 1994).

Iron, copper and oxidative stress

The ability of iron and copper to transfer and gain electrons from molecular oxygen and form reactive oxygen species (ROS), makes these metals potentially toxic (Valentine *et al.* 1998; Fridovich 1999). 'Free' copper and iron can rapidly catalyze superoxide radical (O_2^-) production, which is a precursor of hydrogen peroxide (H_2O_2) (Liochev & Fridovich 1999; Koppenol 2001). This hydrogen peroxide can then react with both 'free' iron(II) and copper (I) by the Fenton reaction to generate the powerful oxidant hydroxyl radical ($^{\bullet}OH$). Recent studies have shown that superoxide radicals can in turn promote oxidation of iron-sulfur [4Fe-4S] clusters of several enzymes and cause loss of 'free' iron, which in turn propagates the Fenton reaction further in a chemical feedback loop (Liochev & Fridovich 1994, 1999). Hydroxyl radicals can attack nearby bio-molecules, including protein, lipids and DNA, and cause DNA damage, mutations, stress and cellular apoptosis (Mello & Meneghini 1991; Bertoncini & Meneghini 1995; Meneghini 1997; DavisKaplan *et al.* 1998; Nunez *et al.* 2001). Cells defend themselves against oxidative damage by tightly controlling the activity of free copper and iron, as well as by detoxifying ROS. As a result, the levels of unbound (free) copper and iron (defined as the 'activity' of copper and iron) are kept exceedingly low (Rae *et al.* 1999).

Cu,Zn-superoxide dismutase (SOD) is a primary enzyme in the defense against oxidative stress by catalyzing the dismutation of superoxide radicals (O_2^-) into hydrogen peroxide (Fridovich 1995, 2001). Cu,Zn-SOD thus protects both inactivation of [4Fe-4S]-enzymes and prevents a chemically induced increase in 'free' iron. Peroxidases such as catalase, glutathione peroxidase, and cytochrome-c oxidase help eliminate the excess of hydrogen peroxide (H_2O_2). In addition to using enzymes that destroy ROS, multiple, complex systems keep transition metals such as iron and copper under tight homeostatic regulation by regulating iron and copper uptake, delivery and storage (Kaplan & Ohalloran 1996).

Iron and copper uptake in the yeast *Saccharomyces cerevisiae*

Iron and copper are both reduced by the plasma membrane reductases, Fre1p and Fre2p, prior to uptake. Reduced copper is transported by high affinity (Ctr1p and Ctr3p) or low affinity (Ctr2p) transporters (Eide 1998; Pena *et al.* 1999). The availability of copper in the medium modulates the expression of these proteins. Under low copper conditions, transcription of Ctr1p and Ctr3p is enhanced, facilitating copper uptake. Conversely, copper overloaded cells cause both Ctr1p and Ctr3p to be down-regulated to decrease copper uptake. Mac1p is essential for down- or up-regulation of these genes (Labbe *et al.* 1997). Ctr1p is also modulated by copper level through an unknown mechanism. The transporter is stable when cells are grown in low concentrations of copper, but high concentrations of copper trigger degradation of cell surface Ctr1p, possibly at the plasma membrane (Ooi *et al.* 1996). Unlike Ctr1p, Ctr3p does not undergo degradation in response to toxic copper levels (Pena *et al.* 2000). It is important to note that *S. cerevisiae* laboratory strains contain a Ty2 transposon insertion that abolishes CTR3 expression (Knight *et al.* 1996). Copper is also transported through Fet4p (Hassett *et al.* 2000). However, the role of this divalent metal transporter in copper homeostasis remains unclear.

S. cerevisiae utilizes two different uptake systems to obtain iron from the environment. Iron can be transported into the yeast cells bound to low molecular weight compounds or as ions. The high affinity system is specific for iron. Reduced iron is trapped by the Fet3p/Ftr1p complex and is re-oxidized by the Fet3p multicopper oxidase for subsequent transport through the Ftr1p permease at the surface of the plasma membrane (Stearman *et al.* 1996). Reduced iron is also transported through a less specific, lower affinity plasma membrane transporter, Fet4p. This protein is not specific for iron and is capable of transporting other divalent cations (Dix *et al.* 1997).

Copper is required for iron homeostasis in yeast, plants and mammals. The Fet3p/ Ftr1p complex is

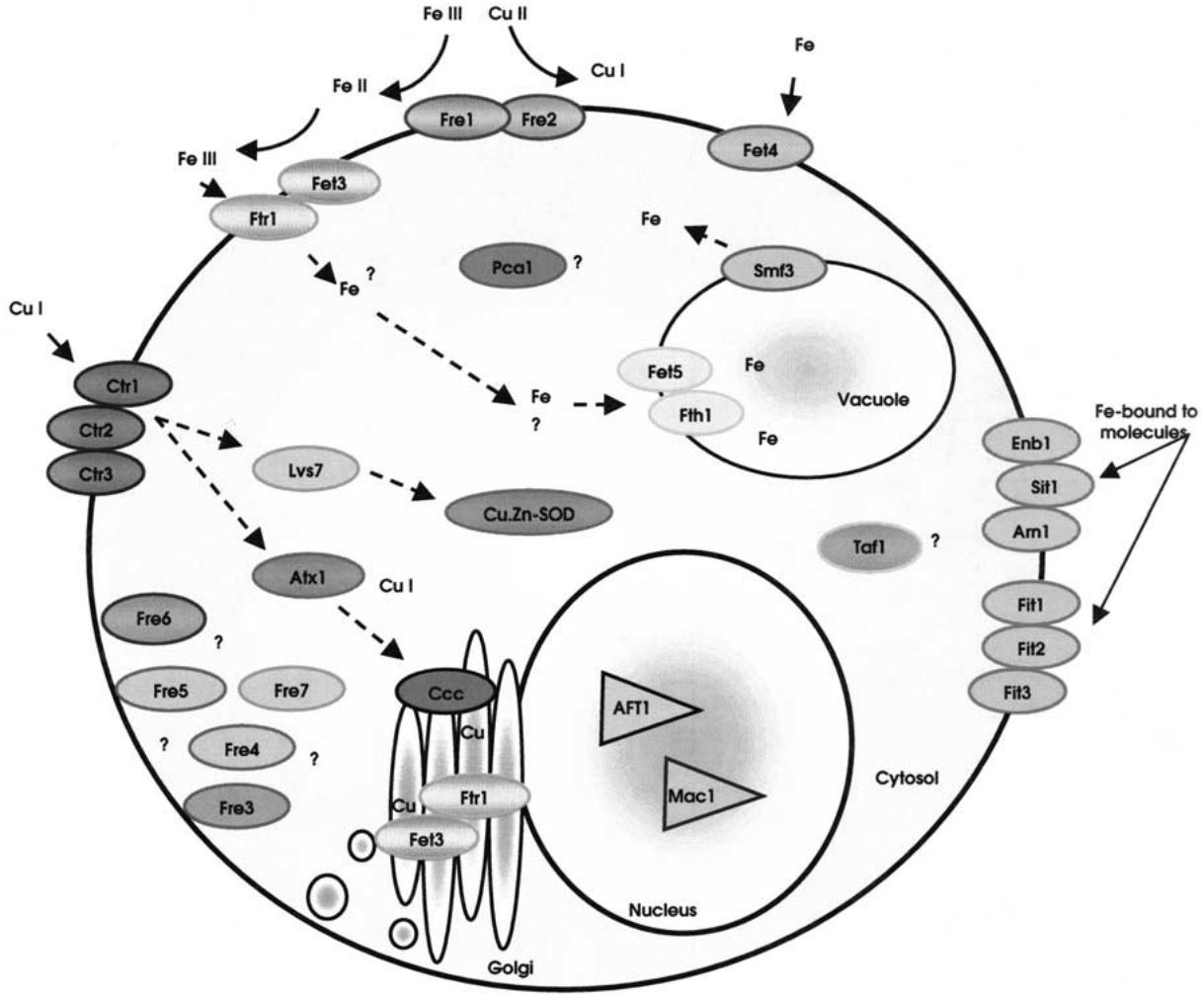


Fig. 1. Copper and iron metabolism in yeast. Copper and iron ions are reduced by cell surface reductases Fre1p/Fre2p prior to uptake. High affinity Copper uptake is mediated by the Ctr1 and Ctr3 proteins. Within the cell, copper is bound to cytosolic copper chaperones Atx1p, Cox17p and Lys7p for specific delivery to the secretory pathway, mitochondria and Cu,Zn-SOD, respectively. Within the secretory pathway, Ccc2 accepts and transports copper ions into Golgi related vesicles, where copper is incorporated in multicopper ferroxidase, Fet3p. Fet3 forms a complex with the iron permease Ftr1p and re-locates to the plasma membrane (high affinity iron uptake). In mitochondria copper delivered by Cox17p is incorporated into cytochrome oxidase, a process that requires the integral inner-membrane protein Sco1p and possibly its homologue, Sco2p. Lys7p delivers copper specifically to Cu,Zn-SOD1 in the cytosol. Ionic iron and copper transport occurs also through Fet4p. Several members of FRE family are located in the plasma membrana, for example Fre3, Fre4, Fre5 and Fre6, however their role in iron/copper metabolism is unknown. Fre7p is localized in unspecified membranes and its function is also unknown. Pca1p is a potential pATPase copper transporter of unknown function. The complex Fet5p/Fth1p may transport iron out of the vacuole, *whereas, Smf3p Cccl may transport* iron in. The uptake of iron bound to low molecular weight is made to several different siderophore transporters, Enb1p, Sit1p, Arn1p with help of cell wall manoproteins, Fit1, Fit2 and Fit3. Taf1p is a protein of unknown function that could play a role in siderophore transport.

assembled in Golgi vesicles, where apoFet3p obtains copper transported into vesicles by Ccc2p. This copper incorporation is required for the Fet3p/Ftr1p complex to traffic to the plasma membrane when cells are iron deficient (Lin *et al.* 1997; O'Halloran & Culotta 2000). The lack of Ccc2p disables import of copper into Golgi vesicles and blocks the incorporation of copper into Apo-Fet3p (Yuan *et al.* 1995). Thus, copper deficiency leads to iron deficiency, which in turn activates Aft1p, an iron-responsive transcriptional regulator factor. In fact, recent studies from our laboratory have shown that Aft1p targets are highly up-regulated under low copper conditions (de Freitas *et al.*, Manuscript in preparation).

S. cerevisiae has retained mechanisms to obtain iron bound to low molecular weight molecules such

as siderophores, despite lacking the ability to synthesize and secrete these strong iron chelators. Lesuisse and co-workers have described this mechanism as an opportunistic strategy of iron uptake, since yeast uses siderophores excreted by other microorganisms (Lesuisse *et al.* 2001). Several genes encoding siderophore transporters have been identified in *S. cerevisae*. ENB1, SIT1, ARN1 and TAF1 encode the transporters for ferric ions chelated to enterobactin, ferrioxamine, ferrichromes and triacetylfusarinine C, respectively (Heymann *et al.* 2000).

Siderophore transport is regulated by Aft1p and Tup1p/Ssn6p. The $AFT1^{up}$ mutant, which constitutively expresses iron deficiency responses due to the lack of repression by Aft1p, accumulates large amounts of ferric siderophores. Similarly, cells disrupted in the TUP1 or SSN6 genes are constitutively de-repressed for the uptake of iron bound to ferrichrome, or ferrioxamine B (Lesuisse *et al.* 2001). Although the regulation of ferric siderophore uptake in yeast is not completely elucidated, it is fascinating that both systems of iron uptake, elemental iron uptake and chelated iron uptake, share the common Aft1 regulatory pathway.

Iron and copper storage

Cellular iron and copper is sequestered or bound to prevent toxicity or for later use. Bacteria and mammals sequester iron into ferritin in the cytosol (Aisen *et al.* 2001) and plants sequester iron in plastidic phytoferritin (Briat *et al.* 1999). *S. cerevisiae*, however, lacks ferritin. Instead, iron is likely sequestered into vacuoles. The Fet5p/Fth1 complex may mobilize iron for use during iron limitation (Eide *et al.* 1993; Urbanowski & Piper 1999). Copper may actually play a role in iron detoxification (Szczypka *et al.* 1997). Copper itself is detoxified by CuS mineralization at the cell surface (Yu *et al.* 1996), is bound to cytosolic metallothionein or sequestered into vacuoles (Andrews 2000; Aisen *et al.* 2001; Eide *et al.* 1993). How iron is safely carried within the cytosol to subcellular compartments is not fully understood, but three cellular copper chaperones have been identified (discussed below).

Copper and its chaperones

Three copper chaperones have been identified in the *S. cerevisiae* (Pena *et al.* 1999). Chaperones deliver metal to target molecules or into organelles and serve to minimize the toxicity of copper inside cells. Atx1p delivers copper to Golgi related vesicles where Ccc2p, a copper-ATPase, pumps copper into the organelle. Lys7p delivers copper to the antioxidant Cu,Zn-superoxide dismutase. Finally, Cox17p delivers copper to mitochondria. Orthologs of these Atx1, Lys7 and Cox17 chaperones have been identified in humans and have been called HAH1, CCS and hCox17, respectively. In humans, CCS delivery of copper to Cu,Zn-SOD may be inhibited by CCS interaction with X11α, a protein that also interacts with the Alzheimer's peptide APP (McLoughlin *et al.* 2001), but an X11α has not been found in yeast.

Transcriptional regulators of iron and copper homeostasis

Copper and iron availability affect three known transcription factors; Mac1, Ace1 and Aft1. Mac1p and Ace1p are specifically controlled by copper status, whereas Aft1p senses iron. Mac1p is a copper deficiency inducible transcription factor that activates genes involved in copper uptake (Jungmann *et al.* 1993; Yamaguchi-Iwai *et al.* 1997; Zhu *et al.* 1998). Ace1p is activated by copper overload and induces the expression of genes involved in scavenging copper and ROS resulting from copper overload.

Activated Mac1p mediates the activation of various genes by binding two CuRE (Copper responsive element, 5′-TTTG(T/G)C(A/G)-3′) in the promoters of CTR1, CTR3, FRE1, and FRE7, (and possibly YFR055w and YJL217w of unknown function (Gross *et al.* 2000)). The presence of copper inactivates Mac1p but the exact mechanism is not well understood. It has been suggested that Mac1p is able to sense copper ions through direct coordination of copper in its metal clusters. Additionally, Mac1p undergoes phosphorylation, which is required for Mac1p to bind to CuRE (Zhu *et al.* 1998). Interestingly, under copper deficiency Mac1p is a stable protein, but it is rapidly degraded by a yet unclear mechanism when cells are overloaded with copper (Zhu *et al.* 1998).

High copper levels activate Ace1p which in turn increases expression of CUP1, CRS5 and SOD1, coding for a metallothionein, a metallothionein-like protein and Cu,Zn-SOD, respectively (Jensen *et al.* 1996; Strain & Culotta 1996; Pena *et al.* 1998). Metallothioneins chelate excess copper, whereas Cu,Zn-SOD control the levels of ROS preventing oxidative

stress. Under copper deficiency Ace1p is inactive and transcription of these genes is minimal.

High affinity iron transport is regulated by iron status via AFT1, an iron responsive transcription factor, by regulating FTR1 and FET3 transcript levels (Yamaguchiiwai *et al.* 1995). Aft1p regulates many other genes involved in iron homeostasis including ferric reductases (FRE1, FRE2), cytosolic copper chaperones (CCC2, ATX1), and siderophore-mediated iron transporters (Table 1). Interestingly, the low affinity iron transporter FET4 is not regulated by Aft1p (Casas *et al.* 1997; Dix *et al.* 1997; Hassett *et al.* 1998).

AFT1 encodes a 98 kD protein that is histidine rich toward the N-terminus and glutamine rich toward the C-terminus. The structural domains of Aft1p are summarized in Figure 2. Other interesting characteristics include a basic region between residues 140 and 280, and 4 cysteine residues, which may confer an iron binding site. A single mutation of cys291phe causes Aft1p to be constitutively active (Yamaguchi-Iwai *et al.* 1995). Aft1p does not show a strong homology to any previously characterized transcription factors, but a homologue, AFT2, has been identified. Although many studies have concentrated on uncovering genes regulated by Aft1p, little work has been done to understand the how Aft1p responds to iron concentrations and subsequently modulates transcription of its target genes. Two early studies by of Aft1p function provide some insight into the mechanism of Aft1p transactivation. Yamaguchi and coworkers (Yamaguchi *et al.* 1996; Yamaguchi-Iwai *et al.* 1996) discovered a consensus sequence in the promoter region of FET3, PyPuCACCCPu, to which Aft1p binds. This binding occurs only under iron deficiency (Yamaguchi-Iwai *et al.* 1996), but the transactivation activity of Aft1p is independent of cellular iron status (Casas *et al.* 1997). The putative domains for these separate binding and activation functions remain to be discovered.

Aft1p responds not only to iron status, but also to nutrient status. It is phosphorylated in yeast grown on a fermentable carbon source and during the diauxic shift. This phosphorylation, however, does not correlate with changes in the expression of Aft1p target genes (Casas *et al.* 1997). In anaerobic conditions transcripts of Aft1p targets are undetectable, but they quickly accumulate upon addition of oxygen to the media (Hassett *et al.* 1998). Hassett *et al.* found that this phenomenon was caused by alterations in cellular iron concentrations in anaerobic and aerobic conditions. They postulated that changes in intracellular iron concentration could be detected by Aft1p, which then regulates the expression of its target genes.

Additional evidence collected from frataxin null mutants, *yfh1*, supports the theory that Aft1p responds to cellular iron concentrations. In the frataxin mutant, iron accumulates in mitochondria causing mitochondrial damages (Babcock *et al.* 1999; Radisky *et al.* 1999). Despite a global iron overload, cytosolic pools of iron are low and Aft1p target genes are highly upregulated leading to increased iron transport (Foury & Talibi 2001). Upregulation of these genes also occur in a phenotypically similar mutant overexpressing CCC1 (Chen & Kaplan 2000).

Recently, another iron regulated transcription factor AFT2 was discovered that shares 39% sequence similarity with AFT1. This gene encodes a smaller protein of 47 kD. Like Aft1p, Aft2p contains four cysteine residues, which may create an iron-binding site. Aft1p and Aft2p both respond to iron status but the mechanisms may differ. Aft2p, unlike Aft1p, has a trans-activation activity which responds to iron status (Blaiseau *et al.* 2001). The discovery of Aft2p helps to explain some gaps in our understanding of iron dependent gene regulation. For example, FET3 is expressed at low levels in the absence of Aft1p. This low level of transcription is activated by Aft2p, which requires the same consensus sequence as Aft1p. Aft2p may be involved in the regulation of other proteins as well that do not respond to Aft1p. It has been postulated that the viability of the Δ*aft1* is due to Fet4p-mediated iron uptake. The Fet4p low affinity transporter is upregulated by iron deficiency independently of Aft1p (Dix *et al.* 1997). Thus, it will be interesting to determine whether Fet4p is under Aft2p regulation, since the deletion of both AFT1 and AFT2 arrests yeast growth in iron deficient media. Together, Aft1p and Aft2p help regulate iron levels in the cell by controlling transcription of genes involved in iron homeostasis (Blaiseau *et al.* 2001).

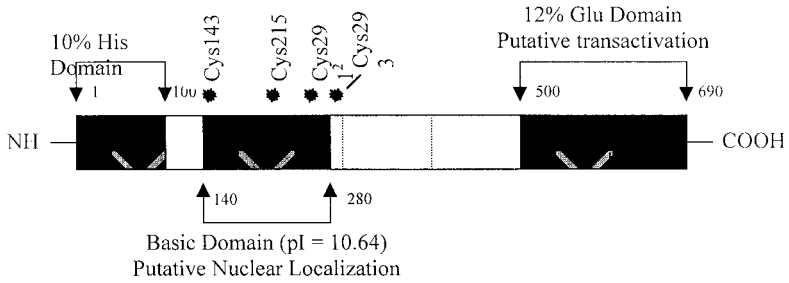
Iron and copper in mitochondria

Iron and copper are major components of enzymes involved in oxido-reduction because of their ability to lose or gain electrons. Thus, it is not a surprise that these two metals are found in mitochondria where they participate in oxidative phosphorylation and are essential components of the respiratory chain. While iron is abundant in mitochondria, mostly in the form of Fe/S clusters and cytochromes, the only known role for

Table 1. Aft1p Target Genes.

Gene	Function	Reference
FRE1	Ferric reductase involved in iron uptake	(Yamagushi-Iwai *et al.* 1995)
FRE2	Ferric reductase involved in iron uptake	(Yamagushi-Iwai *et al.* 1995)
FET3	Cuproprotein involved in high affinity iron uptake	(Yamagushi-Iwai *et al.* 1995)
CCC2	PATPase Cu transporter, delivers Cu to Fet3p	(Yamaguchi-Iwai *et al.* 1996)
FTR1	High affinity iron permease	(Yamaguchi-Iwai *et al.* 1996)
FTH1	Vacuolar iron transport	(Yamaguchi-Iwai *et al.* 1996)
FRE3	Siderophore-iron reductase	(Martins *et al.* 1998; Yun *et al.* 2001)
FRE4	Siderophore-iron reductase	(Martins *et al.* 1998; Yun *et al.* 2001)
FRE5	Homology to ferric reductase	(Martins *et al.* 1998)
FRE6	Homology to ferric reductase	(Martins *et al.* 1998)
ATX1	Cytocolic Cu chaperone responsible for delivering Cu to Fet3p	(Lin *et al.*, 1997)
ARN1	Siderophore-mediated iron transport	(Yun *et al.* 2000; Lesuisse *et al.* 2001)
ARN2/TAF1	Siderophore-mediated iron transport	(Yun *et al.* 2000; Lesuisse *et al.* 2001)
ARN3/SIT1	Siderophore-mediated iron transport	(Yun *et al.* 2000; Lesuisse *et al.* 2001)
ARN4/ENB1	Siderophore-mediated iron transport	(Yun *et al.* 2000; Lesuisse *et al.* 2001)
YOR382w	Gp1-anchor glycoprotein involved in siderophore-mediated iron uptake	(Foury & Talibi 2001)
YOR383c	Gp1-anchor glycoprotein involved in siderophore-mediated iron uptake	(Foury & Talibi 2001)
YOR534c	Gp1-anchor glycoprotein involved in siderophore-mediated iron	(Foury & Talibi 2001)
YLR136c	Post-transcriptional regulator of iron responsive genes	(Foury & Talibi 2001)
ISU1	Mitocondrial assembly of iron sulfur clusters	(Garland *et al.* 1999)
ISU2	Mitocondrial assembly of iron sulfur clusters	(Garland *et al.* 1999)
FET5*	Vacuolar iron transport	(Urbanowski & Piper 1999)
YLR205c**	Homology to heme oxygenase	(Foury & Talibi 2001)

*putative target gene based on its promoter dependent induction in an iron deficient state
**putative target gene based on the identification of a consensus sequence in its promoter region



[1](Yamaguchi-Iwai, Dancis et al. 1995) (Blaiseau, Lesuisse et al. 2001)
[2]Mutating cys291 to phe creates the Aft1-1[up] mutant, which is not responsive to iron concentrations

Fig. 2. Structural domains of the Aft1p Transcription Activator.

Table 2. Genes involved in mitochondrial iron metabolism.

Gene	Function	Reference
HEM15	Ferrochelatase- incorporation of iron in heme	(Lange *et al.* 1999)
SDH2	Fe/S subunit of succinate dehydrogenase	(Lombardo *et al.* 1990)
ARH1	Adrenodoxin Reductase	(Li *et al.* 2001a)
YAH1	Ferrodoxin synthesis	(Lange *et al.* 2000)
NFS1	Cysteine desulfurase	(Kispal *et al.* 1999)
NFU1	Homolog to C-terminus of NifU	(Schilke *et al.* 1999)
ISU1	IscU homolog, Fe/S assembly	(Garland *et al.* 1999)
ISU2	NifU homolog, Fe/S assembly	(Garland *et al.* 1999)
ISA1	IscA homolog, Fe/S assembly	(Jensen & Culotta 2000; Pelzer *et al.* 2000)
ISA2	IscA homolog, Fe/S assembly	(Jensen & Culotta 2000)
SSQ1	Mitochondrial HSP 70, Fe/S assembly	(Baumann *et al.* 2000; Voisine *et al.* 2000; Lutz *et al.* 2001)
JAC1	J-type chaperone , Fe/S assembly	(Kim *et al.* 2000; Kim *et al.* 2001; Lutz *et al.* 2001; Voisine *et al.* 2001)
ATM1	ATP binding cassette- mitochondrial export of cytosolic Fe/S clusters	(Leighton & Schatz 1994; Kispal *et al.* 1997)
ERV1	Sulfhydryl oxidase, mitochondrial export of cytosolic Fe/S clusters	(Lange *et al.* 2001)
YFH	Frataxin Homolog	(Babcock *et al.* 1997)

Table 3. Genes involved in mitochondrial copper transport.

Gene	Function	Reference
COX1	Cytochrome Oxidase Subunit 1 – CuA center	(Netter *et al.* 1992)
COX2	Cytochrome Oxidase Subunit 1 – CuB center	
COX17	Mitochondrial copper chaperone	(Glerum *et al.* 1996a)
SCO1	Inner membrane - Copper binding protein	(Buchwald *et al.* 1991; Glerum *et al.* 1996b)
SCO2	Inner membrane protein	(Glerum *et al.* 1996b)
COX 11	Assembly of Cu in CuB center	(Hiser *et al.* 2000)

copper is in cytochrome oxidase where it is involved in dioxygen reduction, the last step of respiratory electron transfer. Recent work in yeast, elaborated below, demonstrates that mitochondrial iron metabolism plays a central role in cellular iron homeostasis and copper is critical for oxygen consumption.

Iron homesostasis and mitochondria

In mitochondria, Fe/S clusters are associated with complex I, complex II and the Rieske Fe/S protein located in complex III. The Fe/S clusters are not limited to mitochondria, but are exclusively synthesized in that organelle (Lill & Kispal 2000; Muhlenhoff & Lill 2000). Thus, Fe/S clusters are either assembled into mitochondrial proteins or exported from mitochondria for incorporation into cytosolic proteins. Similarly, incorporation of iron into porphyrin by ferrochelatase, the last step step in heme biosynthesis, occurs in the mitochondrial matrix. Heme destined for cytosolic proteins must be exported out of mitochondria (Lange *et al.* 1999). These critical iron-incorporation roles cause mitochondria to act like magnets for iron in yeast and thus disruption of Fe/S cluster synthesis perturbs cellular iron homeostasis leading to the accumulation of iron in mitochondria. Excess mitochondrial iron leads to a loss of metabolic and respiratory functions through the depletion of cytosolic iron and generation of free radicals causing oxidative stress in mitochondria. Normally, proper mitochondrial iron levels are maintained by balancing intake of iron and egress of Fe/S clusters and heme. Despite the central importance of iron in mitochondria, the molecular basis of its transport into mitochondria is

not known. Two proteins, Mft1 and Mft2, have been suggested to play a role in iron transport into mitochondria, but their exact role is unknown (Li & Kaplan 1997; Lange *et al.* 1999). One major component of cellular and mitochondrial iron homeostasis in yeast is the yfh1 gene, a homologue of the human frataxin gene responsible for the Friedreich Ataxia syndrome. Mutations in yfh cause an overload of iron in mitochondria in both yeast as well as depletion of cytosolic iron pools (Babcock *et al.* 1997; Radisky *et al.* 1999; Lodi *et al.* 2001; Palau 2001). The role of frataxin is not yet elucidated but in mammalian cells frataxin activates oxidative phosphorylation (Ristow *et al.* 2000). Frataxin, however, is not indispensable for respiration in yeast (Foury & Talibi 2001). Recently the yeast gene CCC1, involved in vacuolar iron transport, was shown to restore normal respiration to yeast yfh1 null mutants by preventing excessive iron accumulation in mitochondria and lowering free iron levels in the cytosol (Chen & Kaplan 2000; Li *et al.* 2001). Several other genes playing a role in mitochondrial iron homeostasis have been identified in yeast by genetic screens (Muhlenhoff & Lill 2000). Mitochondria possess proteins that evolutionarily derive from both the Nif operon (involved in nitrogen fixation in such microorganisms as *Azotobacter vinelandii*) and the Isc operon (involved in the assembly of Fe/S clusters for enzymes other than nitrogenases) (Schilke *et al.* 1996). These proteins are all involved in synthesis or repair of Fe/S clusters, and include Nfs1p, a cysteine desulfurase (Strain *et al.* 1998; Kispal *et al.* 1999); Isu1p (IscU homolog), Isu2p (NifU homolog) (Garland *et al.* 1999), Jac1 and Ssq1 protein chaperones (Strain *et al.* 1998; Kim *et al.* 2001; Lutz *et al.* 2001; Voisine *et al.* 2001), Isa1 and Isa2 (Jensen & Culotta 2000; Kaut *et al.* 2000; Pelzer *et al.* 2000). Disruption of any of these genes involved in Fe/S assembly leads to perturbation in iron homeostasis and mitochondrial iron accumulation in yeast. Atm1, an ABC transporter, and a newly identified gene Erv1, a sulfhydryl oxidase, are both responsible for the export of iron sulfur clusters from mitochondria to the cytosol (Kispal *et al.* 1997, 1999; Lange *et al.* 2001; Lill & Kispal 2001). Their disruption leads, like other proteins involved in Fe/S assembly, to mitochondrial iron accumulation. Finally Yah1 (ferredoxin synthesis) and Arh1p (adrenodoxin reductase homolog) are required for cellular and mitochondrial iron homeostasis and their roles are being investigated (Lange *et al.* 2000; Li *et al.* 2001).

Our current knowledge of iron metabolism in yeast clearly indicates that mitochondria are critical for cellular iron homeostasis. While the cell can control iron levels by regulating iron intake and sequestration in response to nutritional iron conditions, it appears that the proper processing of iron in mitochondria is a key factor for maintaining cellular iron balance. The means by which iron is delivered to the mitochondria are not known.

Copper transport to mitochondria

Copper is not as abundant as iron in cells but its role in respiration is critical. Copper is a cofactor of terminal oxidases in various metabolic pathways. In mitochondria, copper is located exclusively in two cytochrome oxidase centers, CuA in subunit I and CuB in subunit II, containing a total of three atoms of copper. Copper is delivered to mitochondria by Cox17p, one of three characterized copper chaperones in yeast (Glerum *et al.* 1996). Cox17p is a small soluble cysteine-rich protein that binds two atoms of copper in a cluster and can self-associate into oligomeric polycopper complexes (Beers *et al.* 1997). Cox17p is found both in the cytosol and in the mitochondrial intermembrane space (Beers *et al.* 1997; Srinivasan *et al.* 1998; Punter *et al.* 2000; Heaton *et al.* 2000; Heaton *et al.* 2001). Cox17 is required for normal growth of yeast, since a mutation in Cox17 results in a respiratory deficiency, but it is not indispendable as excess copper allows the Cox17 null mutant to grow (Glerum *et al.* 1996). How copper is transferred from Cox17p in the inner mitochondrial membrane space to membrane-bound cytochrome oxidase is not known. Two mitochondrial membrane proteins, Sco1p and Cox11p are believed to be involved (Glerum *et al.* 1996). Sco1 can rescue a Cox17 mutant when overexpressed, but its role in the mitochondrial copper pathway is not known and there is no evidence yet that it interacts with Cox17p. Sequence similarities between the copper binding sites of Cox2p (subunit 2 of cytochrome oxidase) and Sco1p suggest an interaction involving copper, between the two proteins (Glerum *et al.* 1996; Lode *et al.* 2000). A Sco1p homolog, Sco2p, can also restore respiratory growth of the COX17 mutant but again its role in copper transport and cytochrome oxidase assembly is unknown. Cox11p is a homolog of Cox11p of *Rhodobacter sphaeroides* that is involved in a late step of cytochrome oxidase assembly, most likely the insertion of CuB. It remains to be shown whether the yeast homolog of Cox11p is involved in copper assembly in cytochrome oxidase.

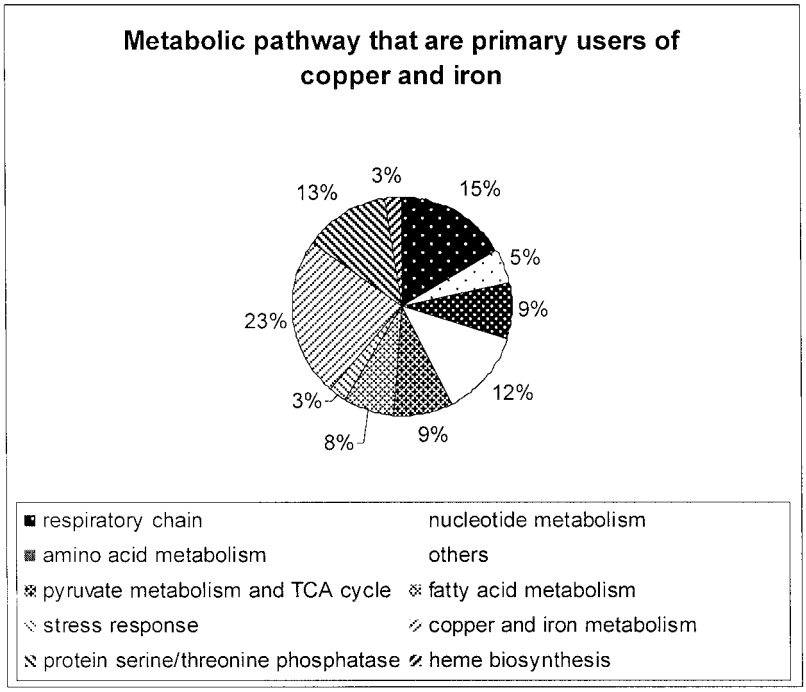


Fig. 3. Pie chart illustrating the repartition of iron and copper containing enzymes into functional groups.

Iron and copper in cellular metabolism

The major metabolic pathways requiring copper or iron prosthetic groups are summarized in Figure 3. Copper and iron play predominating roles in energy metabolism pathways. Pyruvate metabolism, the TCA cycle and the respiratory chain employ most iron and copper containing proteins, such that these pathways are likely to be most vulnerable to disturbances of iron and copper homeostasis. In the TCA cycle, aconitase and succinate dehydrogenase require iron-sulfur or heme prosthetic groups as cofactors. Even though these enzymes are not normally rate-limiting, copper deficiency may cause these enzymes to become rate-limiting upon diauxic-shift (DeRisi *et al.* 1997). Carbon shunted from these pathways to amino acids and other compounds, caused by a decrease in the activity of these copper enzymes, could have dire consequences for the cell. Biosynthetic pathways for branched chain amino acid, lysine, arginine, aromatic amino acids, cysteine and methionine can all be affected by copper deficiency (Hughes *et al.* 2000). In support of this, YFR055W one of the enzymes involved in cysteine and methionine synthesis is transcriptionally regulated by Mac1 (Gross *et al.* 2000; Hughes *et al.* 2000). Many serine/threonine phosphatases also have potential iron binding sites, and are involved in various cellular functions shown in Figure 3. Fifteen percent of the known proteins involved in respiratory functions contain iron, and in the case of cytochrome c oxidase, copper as well. For example, subunits of succinate dehydrogenase, complex III, cytochrome c and cytochrome c oxidase, require iron, iron-sulfur clusters or copper. Therefore, copper and iron deficiency result in defects in respiration, leading to *petit* phenotypes (Jungmann *et al.* 1993). Iron and copper are also involved in responses to oxidative and non-oxidative stress (discussed above). Ergosterol, known to be involved in stress responses, is required for growth under iron deficiency (Li & Kaplan 1996), and many enzymes in sterol metabolism have iron or iron-sulfur clusters as cofactors. Furthermore, DNA repair and the *de novo* synthesis of nucleotides (ribonucleotide reductase) require iron-sulfur and iron cofactors, respectively. Since iron and copper are critical for so many cellular processes, alterations in homeostasis of these two metals affect many different biochemical pathways.

Table 4. Genes containing copper and iron[a].

Gene ID	Gene name	Function
Copper containing proteins		
Q0045	COX1	Cytochrome c oxidase subunit I
Q0250	COX2	Cytochrome c oxidase subunit II
YBR295W	PCA1[b]	Copper-transporting ATPase
YDR270W	CCC2	Copper transporting P-type ATPase
YDR506C	YDR506C[b]	Protein with similarity to Fet5p
YFL041W	FET5[b]	Multicopper ferroxidase
YGL166W	ACE1	Transcription factor
YHR053C	CUP1A	Metallothionein
YHR055C	CUP1B	Metallothionein
YJR104C	SOD1	Copper-zinc superoxide dismutase
YLL009C	COX17	Copper chaperone
YMR021C	MAC1	Transcription factor
YMR038C	LYS7	Copper chaperone
YMR058W	FET3	Multicopper ferroxidase
YNL259C	ATX1	Antioxidant protein
YPR008W	HAA1	
Iron – sulfur cluster containing proteins		
YAL015C	NTG1	Repair of oxidative DNA damage
YDL171C	GLT1[b]	Glutamate synthase
YDR091C	RLI1	
YDR234W	LYS4[b]	Lysine biosynthesis
YEL024W	RIP1	Rieske iron-sulfur protein
YFR030W	MET10	Sulfate assimilation pathway
YGL009C	LEU1	Leucine biosynthesis
YGR286C	BIO2[b]	Biotin synthetase
YJL200C	YJL200C[b]	Similarity to aconitase
YJR016C	ILV3[b]	BCAA biosynthesis
YJR137C	ECM17	Sulfite reductase
YLL041C	SDH2	succinate dehydrogenase
YLR304C	ACO1	Aconitase
YOL043C	NTG2	DNA damage repair
YOR226C	ISU2	Iron- sulfur cluster formation
YPL135W	ISU1	Iron- sulfur cluster formation
YPL252C	YAH1	Mitochondrial ferrodoxin
Heme containing proteins		
Q0045	COX1	Cytochrome c oxidase subunit I
Q0105	COB	Cytochrome b
YAL039C	CYC3	Cytochrome c heme lyase
YDR178W	SDH4	Succinate dehydrogenase membrane anchor subunit
YDR256C	CTA1	Catalase A; peroxisomal
YDR402C	DIT2[b]	Cytochrome P450 56
YEL039C	CYC7	Cytochrome-c isoform 2 (anaerobic condition)
YFR030W	MET10	Sulfate assimilation pathway
YGL055W	OLE1	Unsaturated fatty acids synthesis
YGR088W	CTT1	Catalase T; cytosolic
YHR007C	ERG11[b]	Ergosterol biosynthesis
YJR048W	CYC1	Cytochrome-c isoform 1
YKL087C	CYT2	Cytochrome c1 heme lyase
YKL141W	SDH3	Succinate dehydrogenase
YKL220C	FRE2	Ferric and cupric reductase
YKR066C	CCP1	Cytochrome-c peroxidase
YLR256W	HAP1	Transcription factor with heme-dependent DNA-binding activity
YML054C	CYB2	Cytochrome b2
YMR015C	ERG5	Ergosterol biosynthesis
YMR073C	YMR073C[b]	
YMR272C	SCS7	Ceramide hydroxylase
YNL111C	CYB5	Cytochrome b5
YNR060W	FRE4	Protein involved in iron uptake
YOR065W	CYT1	Cytochrome c1
YOR381W	FRE3	Protein involved in iron uptake
YOR384W	FRE5	
Iron Containing Proteins; no specification of prosthetic group		
YDL047W	SIT4[b]	Protein serine/threonine phosphatase
YDL134C	PPH21[b]	Protein serine/threonine phosphatase
YDL188C	PPH22[b]	Protein serine/threonine phosphatase
YDR035W	ARO3	Aromatic amino acid biosynthesis
YDR044W	HEM13	Heme biosynthesis
YDR075W	PPH3[b]	Protein serine/threonine phosphatase
YDR436W	PPZ2[b]	Protein serine/threonine phosphatase
YER133W	GLC7[b]	Protein serine/threonine phosphatase
YGL256W	ADH4[b]	Alcohol dehydrogenase IV
YGR060W	ERG25	Ergosterol biosynthesis
YGR123C	PPT1[b]	Protein serine/threonine phosphatase
YGR180C	RNR4[b]	Ribonucleotide reductase
YGR234W	YHB1[b]	Flavohemoglobin
YJL026W	RNR2	Ribonucleotide reductase
YJR078W	YJR078W[b]	
YLR056W	ERG3	Ergosterol biosynthesis
YLR214W	FRE1	Ferric and cupric reductase
YML016C	PPZ1[b]	Protein serine/threonine phosphatase
YNR032W	PPG1[b]	Protein serine/threonine phosphatase
YOR176W	HEM15	Ferrochelatase
YPL179W	SAL6[b]	Protein serine/threonine phosphatase

[a]Identified at http://www.proteome.com, http://pedant.gsf.de, & http://mips.gsf.de/proj/yeast/
[b]Proteins with potential metal binding motifs based on similarity

Conclusion and perspectives

The conserved pathways for iron and copper homeostasis mechanisms between yeast, plants and animals

allows the use of yeast as a model system to elucidate many of the major metabolic pathways affected by iron and copper in mammals and plants. Genomic approaches, collections of gene knockouts in yeast and facile transformation of yeast can be used to accelerate our understanding of iron and copper homeostasis in more complex eukaryotic organisms. Hypotheses of the functions of genes identified in plants and animals can be made based on the functions of their yeast homologs. These hypotheses can be directly tested in yeast by complementation or overexpression followed by phenotypic and genomic analyses.

Understanding mechanisms of eukaryotic cellular iron and copper uptake and homeostasis is a prerequisite to understanding many medical, nutritional, agricultural and aquatic processes. Iron and copper metabolism are intertwined in eukaryotic organisms. Iron and copper metabolism disorders are being implicated in an increasing number of disease entities and pathologic processes. Iron deficiency is the most significant global dietary problem people face today and crop yields are also negatively impacted by iron deficiency. Increasing the selective uptake of desired metals therefore benefits both human nutrition and crop yields. Furthermore, developing plants and microbes capable of selectively extracting metals from soil and water is a feasible approach for detoxifying metal contamination, but implementation is difficult without a full understanding of the major metabolic pathways affected by altering metal homeostasis.

References

Aisen P, Enns C *et al.* 2001 Chemistry and biology of eukaryotic iron metabolism. *Intern J Biochem Cell Biol* **33**(10), 940–959.

Ambrosini L, Mercer JFB. 1999 Defective copper-induced trafficking and localization of the Menkes protein in patients with mild and copper-treated classical Menkes disease. *Hum Mol Gen* **8**(8), 1547–1555.

Andrews GK. 2000 Regulation of metallothionein gene expression by oxidative stress and metal ions. *Biochem Pharmacol* **59**(1), 95–104.

Andrews NC, Fleming MD *et al.* 1999 Iron transport across biologic membranes. *Nutri Rev* **57**(4), 114–123.

Babcock M, deSilva D *et al.* 1997 Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science* **276**(5319), 1709–1712.

Beers J, Glerum DM *et al.* 1997 Purification, characterization, and localization of yeast Cox17p, a mitochondrial copper shuttle. *J Biol Chem* **272**(52), 33191–33196.

Bertoncini CRA, Meneghini R. 1995 DNA strand breaks produced by oxidative stress in mammalian cells exhibit 3′-phosphoglycolate termini. *Nucl Acids Res* **23**(15), 2995–3002.

Blaiseau PL, Lesuisse E *et al.* 2001 Aft2p, a novel iron-regulated transcription activator that modulates, with Aft1p, intracellular iron use and resistance to oxidative stress in yeast. *J Biol Chem* **276**(36), 34221–34226.

Briat JF, Lobreaux S *et al.* 1999 Regulation of plant ferritin synthesis: how and why. *Cell Mol Life Sci* **56**(1–2), 155–166.

Bull PC, Cox DW. 1994 Wilson Disease and Menkes Disease – New Handles on Heavy-Metal Transport. *Trends Gen* **10**(7), 246–252.

Casas C, Aldea M *et al.* 1997 The AFT1 transcriptional factor is differentially required for expression of high-affinity iron uptake genes in *Saccharomyces cerevisiae*. *Yeast* **13**(7), 621–637.

Chen OS, Kaplan J. 2000 CCC1 suppresses mitochondrial damage in the yeast model of Friedreich's ataxia by limiting mitochondrial iron accumulation. *J Biol Chem* **275**(11), 7626–7632.

Cohen A, Nelson H *et al.* 2000 The family of SMF metal ion transporters in yeast cells. *J Biol Chem* **275**(43), 33388–33394.

DavisKaplan SR, Askwith CC *et al.* 1998 Chloride is an allosteric effector of copper assembly for the yeast multicopper oxidase Fet3p: an unexpected role for intracellular chloride channels. *Proc Natl Acad Sci USA* **95**(23), 13641–13645.

De Freitas JM, Meneghini R. 2001 Iron and its sensitive balance in the cell. *Mutat Res Fund Mol Mech Mutagen* **475**(1–2 SI), 153–159.

DeRisi JL, Iyer VR *et al.* 1997 Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**(5338), 680–686.

Dix D, Bridgham J *et al.* 1997 Characterization of the FET4 protein of yeast – Evidence for a direct role in the transport of iron. *J Biol Chem* **272**(18), 11770–11777.

Eide DJ. 1998 The molecular biology of metal ion transport in *Saccharomyces cerevisiae*. *Ann Rev Nutri* **18**, 441–469.

Eide DJ, Bridgham JT *et al.* 1993 The vacuolar H+-Atpase of *Saccharomyces-Cerevisiae* is required for efficient copper detoxification, Mitochondrial Function, and Iron Metabolism. *Mol General Gen* **241**(3–4), 447–456.

Foury F, Talibi D. 2001 Mitochondrial control of iron homeostasis – A genome wide analysis of gene expression in a yeast frataxin-deficient strain. *J Biol Chem* **276**(11), 7762–7768.

Fox TC, Guerinot ML. 1998 Molecular biology of cation transport in plants. *Ann Rev Plant Physiol Plant Mol Biol* **49**, 669–696.

Fridovich I. 1995 Superoxide Radical and Superoxide Dismutases. *Ann Rev Biochem* **64**, 97–112.

Fridovich I. 1999 Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? Annals of the New York Academy of Sciences; Oxidative/energy metabolism in neurodegenerative disorders. In Blass JP and McDowell FH (eds) New York, Academy of Sciences; New York: 13–18.

Fridovich I. 2001 Reflections of a fortunate biochemist. *J Biol Chem* **276**(31), 28629–28636.

Garland SA, Hoff K *et al.* 1999 *Saccharomyces cerevisiae* ISU1 and ISU2: Members of a well-conserved gene family for iron-sulfur cluster assembly. *J Mol Biol* **294**(4), 897–907.

Glerum DM, Shtanko A *et al.* 1996 Characterization of Cox17, a Yeast Gene Involved in Copper Metabolism and Assembly of Cytochrome Oxidase. *J Biol Chem* **271**(24), 14504–14509.

Glerum DM, Shtanko A *et al.* 1996 Sco1 and Sco2 Act as High Copy Suppressors of a Mitochondrial Copper Recruitment Defect *Saccharomyces Cerevisiae*. *J Biol Chem* **271**(34), 20531–20535.

Gross C, Kelleher M *et al.* 2000 Identification of the copper regulon in *Saccharomyces cerevisiae* by DNA microarrays. *J Biol Chem* **275**(41), 32310–32316.

Hassett R, Dix DR *et al.* 2000 The Fe(II) permease Fet4p functions as a low affinity copper transporter and supports normal copper trafficking in *Saccharomyces cerevisiae*. *Biochem J* **351**(PT2), 477–484.

Hassett RF, Romeo AM *et al.* (1998). Regulation of high affinity iron uptake in the yeast *Saccharomyces cerevisiae* – Role of dioxygen and Fe(II). *J Biol Chem* **273**(13), 7628–7636.

Heaton D, Nittis T *et al.* 2000 Mutational analysis of the mitochondrial copper metallochaperone Cox17. *J Biol Chem* **275**(48), 37582–37587.

Heaton DN, George GN *et al.* 2001 The mitochondrial copper metallochaperone Cox17 exists as an oligomeric, polycopper complex. *Biochemistry* **40**(3), 743–751.

Heymann P, Ernst JF *et al.* 2000 Identification and substrate specificity of a ferrichrome-type siderophore transporter (Arn1p) in *Saccharomyces cerevisiae*. *Fems Microbiol Lett* **186**(2), 221–227.

Himelblau E, Amasino RM. 2000 Delivering copper within plant cells. *Curr Opin Plant Biol* **3**(3), 205–210.

Hughes TR, Marton MJ *et al.* 2000 Functional discovery via a compendium of expression profiles. *Cell* **102**(1), 109–126.

Jensen LT, Culotta VC. 2000 Role of *Saccharomyces cerevisiae* ISA1 and ISA2 in iron homeostasis. *Mol Cell Biol* **20**(11), 3918–3927.

Jensen LT, Howard WR *et al.* 1996 Enhanced effectiveness of copper ion buffering by Cup1 metallothionein compared with Crs5 metallothionein in *Saccharomyces Cerevisiae*. *J Biol Chem* **271**(31), 18514–18519.

Jungmann J, Reins HA *et al.* 1993 Mac1, a nuclear regulatory protein related to Cu-dependent transcription factors is involved in Cu/Fe utilization and stress resistance in yeast. *Embo J* **12**(13), 5051–5056.

Kaplan J, Ohalloran TV 1996 Iron metabolism in eukaryotes – Mars and Venus at it again. *Science* **271**(5255), 1510–1512.

Kaut A, Lange H *et al.* 2000 Isa1p is a component of the mitochondrial machinery for maturation of cellular iron-sulfur proteins and requires conserved cysteine residues for function. *J Biol Chem* **275**(21), 15955–15961.

Kim R, Saxena S *et al.* 2001 J-domain protein, Jac1p, of yeast mitochondria required for iron homeostasis and activity of Fe-S cluster proteins. *J Biol Chem* **276**(20), 17524–17532.

Kispal G, Csere P *et al.* 1997 The ABC transporter Atm1p is required for mitochondrial iron homeostasis. *Febs Lett* **418**(3), 346–350.

Kispal G, Csere P *et al.* 1999 The mitochondrial proteins Atm1p and Nfs1p are essential for biogenesis of cytosolic Fe/S proteins. *Embo J* **18**(14), 3981–3989.

Knight SAB, Labbe S *et al.* 1996 A widespread transposable element masks expression of a yeast copper transport gene. *Genes Develop* **10**(15), 1917–1929.

Koppenol WH. 2001 The Haber-Weiss cycle–70 years later. *Redox Rep* **6**(4), 229–34.

Labbe S, Zhu ZW *et al.* 1997 Copper-specific transcriptional repression of yeast genes encoding critical components in the copper transport pathway. *J Biol Chem* **272**(25), 15951–15958.

Lange H, Kaut A *et al.* 2000 A mitochondrial ferredoxin is essential for biogenesis of cellular iron-sulfur proteins. *Proc Natl Acad Sci USA* **97**(3), 1050–1055.

Lange H, Kispal G *et al.* 1999 Mechanism of iron transport to the site of heme synthesis inside yeast mitochondria. *J Biol Chem* **274**(27), 18989–18996.

Lange H, Lisowsky T *et al.* 2001 An essential function of the mitochondrial sulfhydryl oxidase Erv1p/ALR in the maturation of cytosolic Fe/S proteins. *Embo Rep* **2**(8), 715–720.

Lesuisse E, Blaiseau PL *et al.* 2001 Siderophore uptake and use by the yeast *Saccharomyces cerevisiae*. *Microbiol-Uk* **147**(PT2), 289–298.

Li J, Saxena S *et al.* 2001 Adrenodoxin reductase homolog (Arh1p) of yeast mitochondria required for iron homeostasis. *J Biol Chem* **276**(2), 1503–1509.

Li LT, Chen OS *et al.* 2001 CCC1 is a transporter that mediates vacuolar iron storage in yeast. *J Biol Chem* **276**(31), 29515–29519.

Li LT, Kaplan J. 1996 Characterization of yeast methyl sterol oxidase (Erg25) and identification of a human homologue. *J Biol Chem* **271**(28), 16927–16933.

Li LT, Kaplan J 1997 Characterization of two homologous yeast genes that encode mitochondrial iron transporters. *J Biol Chem* **272**(45), 28485–28493.

Lill R, Kispal G. 2000 Maturation of cellular Fe-S proteins: an essential function of mitochondria. *Trends Biochem Sci* **25**(8), 352–356.

Lill R and Kispal G. 2001 Mitochondrial ABC transporters. *Res Microbiol* **152**(3–4), 331–340.

Lin SJ, Pufahl RA *et al.* 1997 A role for the *Saccharomyces cerevisiae* ATX1 gene in copper trafficking and iron transport. *J Biol Chem* **272**(14), 9215–9220.

Liochev SI, Fridovich I. 1994 The role of O-2-center-dot- in the production of Ho-center-dot - *in vitro* and *in vivo*. *Free Rad Biol Med* **16**(1), 29–33.

Liochev SI, Fridovich I. 1999 Superoxide and iron: partners in crime. *Iubmb Life* **48**(2), 157–161.

Lode A, Kuschel M *et al.* 2000 Mitochondrial copper metabolism in yeast: interaction between Sco1p and Cox2p. *FEBS Lett* **485**(1), 19–24.

Lodi R, Taylor DJ *et al.* 2001 Mitochondrial dysfunction in friedreich's ataxia. *Biol Signals Recept* **10**(3–4), 263–270.

Lutz T, Westermann B *et al.* 2001 The mitochondrial proteins Ssq1 and Jac1 are required for the assembly of iron sulfur clusters in mitochondria. *J Mol Biol* **307**(3), 815–825.

Lyons TJ, Gralla EB *et al.* 1999 Biological chemistry of copper-zinc superoxide dismutase and its link to amyotrophic lateral sclerosis. *Met Ions Biol Syst* **36**, 125–177.

McLoughlin DM, Standen CL *et al.* 2001 The neuronal adaptor protein X11 alpha interacts with the copper chaperone for SOD1 and regulates SOD1 activity. *J Biol Chem* **276**(12), 9303–9307.

Mello AC, Meneghini R. 1991 Iron is the intracellular metal involved in the production of DNA damage by oxygen radicals. *Mutat Res* **251**(1), 109–113.

Meneghini R. 1997 Iron homeostasis, oxidative stress, and DNA damage. *Free Rad Biol Med* **23**(5), 783–792.

Muhlenhoff U, Lill R. 2000 Biogenesis of iron-sulfur proteins in eukaryotes: a novel task of mitochondria that is inherited from bacteria. *Biochim Biophys ActaBioenerg* **1459**(2–3), 370–382.

Nunez MT, Tapia V *et al.* 2001 Iron-induced oxidative damage in colon carcinoma (Caco-2) cells. *Free Rad Res* **34**(1), 57–68,U17,U18.

O'Halloran TV, Culotta VC. 2000 Metallochaperones, an intracellular shuttle service for metal ions. *J Biol Chem* **275**(33), 25057–25060.

Ooi CE, Rabinovich E *et al.* 1996 Copper-dependent degradation of the *Saccharomyces cerevisiae* plasma membrane copper transporter Ctr1p in the apparent absence of endocytosis. *Embo J* **15**(14), 3515–3523.

Palau F. 2001 Friedreich's ataxia and frataxin: molecular genetics, evolution and pathogenesis (Review). *Interl J Mol Med* **7**(6), 581–589.

Pelzer W, Muhlenhoff U *et al.* 2000 Mitochondrial Isa2p plays a crucial role in the maturation of cellular iron-sulfur proteins. *FEBS Lett* **476**(3), 134–139.

Pena MMO, Koch KA *et al.* 1998 Dynamic regulation of copper uptake and detoxification genes in *Saccharomyces cerevisiae*. *Mol Cell Biol* **18**(5), 2514–2523.

Pena MMO, Lee J *et al.* 1999 A delicate balance: homeostatic control of copper uptake and distribution. *J Nutrit* **129**(7), 1251–1260.

Pena MMO, Puig S *et al.* 2000 Characterization of the *Saccharomyces cerevisiae* high affinity copper transporter Ctr3. *J Biol Chem* **275**(43), 33244–33251.

Pinner E, Gruenheid S *et al.* 1997 Functional complementation of the yeast divalent cation transporter family SMF by NRAMP2, a member of the mammalian natural resistance-associated macrophage protein family. *J Biol Chem* **272**(46), 28933–28938.

Punter FA, Adams DL *et al.* 2000 Characterization and localization of human COX17, a gene involved in mitochondrial copper transport. *Hum Gen* **107**(1), 69–74.

Radisky DC, Babcock MC *et al.* 1999 The yeast frataxin homologue mediates mitochondrial iron efflux – Evidence for a mitochondrial, iron cycle. *J Biol Chem* **274**(8), 4497–4499.

Rae TD, Schmidt PJ *et al.* 1999 Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. *Science* **284**(5415), 805–808.

Ristow M, Pfister MF *et al.* 2000 Frataxin activates mitochondrial energy conversion and oxidative phosphorylation. *Proc Natl Acad Sci USA* **97**(22), 12239–12243.

Schilke B, Forster J *et al.* 1996 The cold sensitivity of a mutant of *Saccharomyces cerevisiae* lacking a mitochondrial heat shock protein 70 Is Suppressed by Loss of Mitochondrial DNA. *J Cell Biol* **134**(3), 603–613.

Srinivasan C, Posewitz MC *et al.* 1998 Characterization of the copper chaperone Cox17 of *Saccharomyces cerevisiae*. *Biochemistry* **37**(20), 7572–7577.

Stearman R, Yuan DS *et al.* 1996 A permease-oxidase complex involved in high-affinity iron uptake in yeast. *Science* **271**(5255), 1552–1557.

Strain J, Culotta VC. 1996 Copper ions anti the regulation of *Saccharomyces Cerevisiae* metallothionein genes under aerobic and anaerobic conditions. *Mol Gen Gen* **251**(2), 139–145.

Strain J, Lorenz CR *et al.* 1998 Suppressors of superoxide dismutase (SOD1) deficiency in *Saccharomyces cerevisiae* – Identification of proteins predicted to mediate iron-sulfur cluster assembly. *J Biol Chem* **273**(47), 31138–31144.

Szczypka MS, Zhu ZW *et al.* 1997 *Saccharomyces cerevisiae* mutants altered in vacuole function are defective in copper detoxification and iron-responsive gene transcription. *Yeast* **13**(15), 1423–1435.

Urbanowski JL, Piper RC. 1999 The iron transporter fth1p forms a complex with the Fet5 iron oxidase and resides on the vacuolar membrane. *J Biol Chem* **274**(53), 38061–38070.

Valentine JS, Gralla EB. 1997 Delivering copper inside yeast and human cells. *Science* **278**(5339), 817–818.

Valentine JS, Wertz DL *et al.* 1998 The dark side of dioxygen biochemistry. *Curr Opin Chem Biol* **2**(2), 253–262.

Voisine C, Cheng YC *et al.* 2001 Jac1, a mitochondrial J-type chaperone, is involved in the biogenesis of Fe/S clusters in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **98**(4), 1483–1488.

Vulpe CD, Packman S. 1995 Cellular copper transport. *Ann Rev Nutrit* **15**, 293–322.

Waggoner DJ, Bartnikas TB *et al.* 1999 The role of copper in neurodegenerative disease. *Neurobiol Dis* **6**(4), 221–230.

Yamaguchi Y, Heiny Me *et al.* 1996 Biochemical characterization and intracellular localization of the Menkes disease protein. *Proc Natl Acad Sci USA* **93**(24), 14030–14035.

Yamaguchi-Iwai Y, Serpe M *et al.* 1997 Homeostatic regulation of copper uptake in yeast via direct binding of MAC1 protein to upstream regulatory sequences of FRE1 and CTR1. *J Biol Chem* **272**(28), 17711–17718.

Yamaguchi-Iwai Y, Stearman R *et al.* 1996 Iron-regulated DNA binding by the Aft1 protein controls the iron regulon in yeast. *Embo J* **15**(13), 3377–3384.

Yamaguchiiwai Y, Dancis A *et al.* 1995 Aft1 – a mediator of iron regulated transcriptional control in *Saccharomyces cerevisiae*. *Embo J* **14**(6), 1231–1239.

Yu W, Farrell RA *et al.* 1996 Identification of Slf1 as a new copper homeostasis gene involved in copper sulfide mineralization in *Saccharomyces Cerevisiae*. *Mol Cell Biol* **16**(5), 2464–2472.

Yuan DS, Stearman R *et al.* 1995 The Menkes Wilson disease gene homologue in yeast provides copper to a ceruloplasmin-Like oxidase required for iron uptake. *Proc Natl Acad Sci USA* **92**(7), 2632–2636.

Zhu ZW, Labbe S *et al.* 1998 Copper differentially regulates the activity and degradation of yeast Mac1 transcription factor. *J Biol Chem* **273**(3), 1277–1280.

Web references

http://www.proteome.com
http://pedant.gsf.de
http://mips.gsf.de/proj/yeast/
http://www.expasy.ch
http://pedant.gsf.de
http://genome-www4.stanford.edu/cgi-bin/SGD