Biometals (2011) 24:785–809
DOI 10.1007/s10534-011-9451-4

Transition metal homeostasis: from yeast to human disease

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Received: 23 March 2011 / Accepted: 28 March 2011 / Published online: 10 April 2011

Abstract Transition metal ions are essential nutrients to all forms of life. Iron, copper, zinc, manganese, cobalt and nickel all have unique chemical and physical properties that make them attractive molecules for use in biological systems. Many of these same properties that allow these metals to provide essential biochemical activities and structural motifs to a multitude of proteins including enzymes and other cellular constituents also lead to a potential for cytotoxicity. Organisms have been required to evolve a number of systems for the efficient uptake, intracellular transport, protein loading and storage of metal ions to ensure that the needs of the cells can be met while minimizing the associated toxic effects. Disruptions in the cellular systems for handling transition metals are observed as a number of diseases ranging from hemochromatosis and anemias to neurodegenerative disorders including Alzheimer's and Parkinson's disease. The yeast *Saccharomyces cerevisiae* has proved useful as a model organism for the investigation of these processes and many of the genes and biological systems that function in yeast metal homeostasis are conserved throughout eukaryotes to humans. This review focuses on the biological roles of iron, copper, zinc, manganese, nickel and cobalt, the homeostatic mechanisms that function in *S. cerevisiae* and the human diseases in which these metals have been implicated.

Keywords Iron · Zinc · Copper · Manganese · Cobalt · Nickel · Transition metal homeostasis · Saccharomyces cerevisiae · Human disease

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Introduction

Metals are essential components of a variety of biological systems. Sodium, potassium, magnesium and calcium provide the basis for nerve conduction, muscle contraction, stabilizing nucleic acids and a plethora of other biological systems and thus are the dominant metal ions in a cell. The importance of metal ions in biology is further shown by one in four proteins in the Protein Data Bank (PDB) containing a metal ion coordinated to the protein (Shi et al. 2005). Often, the metal ions incorporated into proteins are not the abundant metal ions mentioned above but are the less abundant trace elements that include the transition metals iron, copper, manganese, zinc, cobalt and nickel. Proteins that bind transition metals are often catalytic enzymes with the transition metal ion being essential for activity. Under physiological conditions, most transition metals can exist in multiple valence states and thus can readily participate in single electron

transfer reactions, making them attractive for inclusion in biological systems. Metal ions are also found as components of prosthetic groups, cofactors and complexes prior to insertion into proteins. Although these six metals (iron, copper, manganese, zinc, cobalt and nickel) are all found grouped together in the periodic table, the biological systems in which each them are involved vary significantly. With the exception of iron and zinc, which are essential to virtually all organisms, the utilization of the metals is dependent on the organism and the corresponding environment. The number of protein families utilizing each metal ranges from over 300 for zinc to less than 10 for nickel (Zhang and Gladyshev 2009). These six transition metals have been chosen as the focus of this review as they are all biologically relevant particularly with respect to human health. Although the metals all have a specific biological functions essential for life, lack of regulation of intracellular metal pools can lead to toxicity often through the formation of oxygen radicals (including the hydroxyl radical) via the Fenton series of reactions (Valko et al. 2005) The resulting reactive oxygen species can go on to damage cellular components including proteins, lipids and nucleic acids eventually leading to cell death. Complex systems have evolved to maintain the delicate balance between transition metals as essential nutrients and their potential damaging role as cytotoxins. Perturbations in this balance are observed in the genetic diseases of transition metal metabolism that stem from both overload and deficiency that manifest in a variety of symptoms. Individuals who are subject to environmental overexposure to transition metals also exhibit a number of diseases related to the toxicity of the metals. Although much of the biology of transition metals has been described, there is still much to be learned about how cells establish and maintain metal levels and how the homeostatic mechanisms for each metal intersect with one another and pathways for other cellular processes.

Saccharomyces cerevisiae

The baker's yeast *S. cerevisiae* is a unicellular eukaryote that has been used as a model organism for countless studies. A major advantage of *S. cerevisiae* as an experimental system is that is has a life cycle which that provides for an ease of analysis using classical genetics and a fully mapped and sequenced genome that is relatively easy to manipulate. With approximately 12 million bases containing on the order of 6000 open reading frames (ORFs) arranged across 16 chromosomes, the yeast genome was the first eukaryotic genome to be completely sequenced (Goffeau et al. 1996). Compared to the human genome, the yeast genome is very compact with 70% of the yeast genome consisting of ORFs (Dujon 1996) whereas it is estimated that coding sequences make up less than 5% of the human genome (Lander et al. 2001). The compactness results from the yeast genome lacking the long stretches of non-coding DNA and only 4% of genes contain introns (Goffeau et al. 1996). As a result of the compactness of the genome, it is easier to identify genes and their functions experimentally in yeast and then probe the human genome for homologous sequences (with possibly homologous functions). In addition to the wealth of genetic and genomic advantages presented by yeast systems, it is also beneficial that yeast can grow on defined medium. For example, the transition metal content of Difco yeast extract is known to be: 57 μg/g Fe, 126 μg/g Zn, 11 μg/g Cu and 3 μg/g Mn (Bovallius and Zacharias 1971). This allows for manipulation of both the chemical and physical environment for the purpose of studying biochemical pathways and examining the interaction between the yeast genome and the environment.

Saccharomyces cerevisiae is generally regarded as the most well-understood eukaryotic organism (Guldener et al. 2005). This wealth of knowledge combined with the ease of working with yeast has led to the development of a vast arsenal of molecular biological tools for studying genetics, biochemistry and cell biology in the model organism. Among the most useful tools available to the yeast community is the systematic deletion library (Winzeler et al. 1999; Giaever et al. 2002). This deletion library is a collection of strains containing genetic knock-outs of over 95% of the predicted ORFs in the yeast genome. These deletions were made using a PCR based strategy (Gola et al. 2003) where each gene is replaced by a KanMX cassette and an ORF specific barcode of 20 nucleotides. Use of the deletion library has linked numerous genes to phenotypes and has aided in assigning function to previously undescribed ORFs and identifying new functions for known genes.

S. cerevisiae as a model organism for studies in transition metal metabolism

A high degree of conservation has been observed for genes of transition metal metabolism between yeast and humans. Obviously this is somewhat limited to the intracellular aspects of metal homeostasis as yeast would have no reason to develop intercellular systems for transporting metals such as the transferrin-transferrin receptor pair as seen in mammals for the transport of iron via the blood. In many cases, analysis of yeast genes has led to the identification of corresponding human homologs and in most cases, the genetics and biochemistry of the gene products are very similar between the homologs. A sample selection of known yeast-human homologous gene pairs with known activities related to transition metal homeostasis can be found in Table 1. One major difference in transition metal homeostasis between yeast and humans is the mechanism of storage of excess metal ions. Most excess transition metal ions are transported by yeast into the vacuole for storage from where they can be released when the cell encounters an environment with limited metal ions (Joho et al. 1995; Li et al. 2001; Culotta et al. 2005; Lin et al. 2008). On the other hand, humans have different mechanisms for coping with different metals. Copper and zinc are bound by cysteine rich metallothioneins (Blindauer and Leszczyszyn 2010), of which a yeast homolog does exist but the function in vivo appears to be limited to copper tolerance (Winge et al. 1985). Iron is stored in the lumen of the ubiquitous multimeric protein ferritin (Torti and Torti 2002) but *S. cerevisiae* is one of the few organisms that does not contain a ferritin-like protein (Li et al. 2001).

Despite these differences, the value of *S. cerevisiae* to the understanding of human transition metal metabolism has been substantial. Among the many experiments where *S. cerevisiae* has proved to be a useful model organism are then many high throughput studies that have aided in the identification of genes and gene products with novel implications in transition metal metabolism (for examples see Pagani et al. 2007; Jo et al. 2008; Arita et al. 2009; Shakoury-Elizeh et al. 2010; Takumi et al. 2010; Bleackley et al. 2011). The contribution of yeast based studies and the identification of homologous systems in humans will continue to facilitate advances in the diagnosis and treatment of diseases involving transition metals while also contributing to the general understanding of how cells maintain the balance of metals as essential nutrients versus toxic entities.

Iron

With an atomic number of 26, iron is a first row transition metal with oxidation states ranging from −II (as found in the $Fe(CO)_4^{2-}$ anion) to +VI (as found in the ferrate ion FeO_4^{2-}) (Harris 2002) with

Table 1 A selection of yeast metal homeostasis genes with the corresponding human homologs

Yeast gene	Human homolog	Function
ZRT1 (Eide 2004)	SLC39 family of transporters	Transport of Zn and other metals from the extracellular space or lumen of an organelle to the cytoplasm
SMF1 (Portnoy et al. 2000)	DMT1	Broad specificity divalent metal transport
YFH1 (Wilson and Roof 1997)	Frataxin	Regulation of mitochondrial iron availability. Thought to promote the maturation of heme and Fe/S clusters
ATX1 (Klomp et al. 1997)	HAH1	Cytosolic copper chaperone for delivering Cu ions to the Golgi
FET3	Hephaestin/Ceruloplasmin	Muliticopper ferroxidases implicated in iron homeostasis
MRS3/4 (Shaw et al. 2006)	Mitoferrin	Mitochondrial membrane iron transporter
CCC2 (Yuan et al. 1995)	Wilson's/Menke's protein	Golgi copper transporter
BSD2 (Howitt et al. 2009)	Ndfip1	Regulator of SMF1/DMT1
CTR1 (Puig et al. 2002)	CTR1	Plasma membrane copper transporter
PMR1 (Ton et al. 2002)	SPCA1/SPCA2	Golgi Mn^{2+}/Ca^{2+} transport ATPase

This table contains a small selection of genes that function in transition metal homeostasis in yeast that have a human homolog that performs a similar function. Studies of the biology of the yeast gene products have provided significant insight into the role of the protein in human biology and metal related disease

the two most biologically relevant oxidation states being Fe(II) and Fe(III). Electron transfer between Fe(II) and Fe(III) is readily accomplished by physiological reducing agents including ascorbate and molecular oxygen. Fe(IV) and Fe(V) are encountered in biology but only as reactive intermediates in the catalytic mechanisms of some iron-containing enzymatic processes (Pierre and Fontecave 1999). The potential of the Fe^{3+}/Fe^{2+} redox couple (0.77 V) (Harris 2002) is variable by up to 1.00 V in response to different environments (Lippard 1994; Radisky and Kaplan 1999), particularly when influenced by protein-bound coordination ligands. Variability of the redox potential contributes significantly to the role of iron as an essential biological metal. Within the range of biological oxidants and reductants (+0.820 to −0.320 V representing molecular oxygen and pyridine nucleotides, respectively), electron transfers facilitated by iron are reversible (Aisen et al. 2001). In the present day atmosphere, soluble Fe^{2+} spontaneously auto-oxidizes to Fe^{3+} which is essentially insoluble in water at neutral pH (Kosman 2003) and is therefore significantly less bio-available. This brings the question of why iron is so prevalent in biological systems if it is so hard for organisms to acquire from the environment. The answer can be found when considering the beginning of life and the advent of many cellular processes (or at least their evolutionary predecessors). It is believed that life first arose on earth in the absence of oxygen, a condition in which Fe(II) was stable and soluble. As a result, oceans contained a concentration of ferrous oxygen of about 50 μM. This abundance combined with the chemical properties that allow iron to readily participate in single electron transfer reactions made it ideal for incorporation of Fe(II) into biological systems. Evolution of photosynthetic cyanobacteria 1–2.5 $\times$ 10^9 years ago began the shift to the oxygen-rich atmosphere found today. However, it is estimated that it took 200–300 million years from the appearance of oxygen-producing bacteria for the atmosphere to reach a significant oxygen concentration because any O_2 produced was consumed in the oxidation of Fe(II) in the oceans (Crichton and Pierre 2001). Oxygenation of the atmosphere with the corresponding oxidation and precipitation of Fe in the oceans occurred in stages and can be observed in the geological banded iron formations seen in sedimentary rocks (Frei et al. 2009). In combination with the presence of O_2, which itself is a very toxic molecule, the limiting bio-availability of iron caused a massive extinction event. Organisms that survived in the newly oxygenated atmosphere developed mechanisms for scavenging and solubilizing iron while also combating the new issue of reactive oxygen species, a problem that is exacerbated by the presence of transition metal ions such as iron.

Iron in biology

With the exception of some members of *Lactobacillus* and *Bacillus*, iron is essential for all forms of life (Crichton and Pierre 2001). Iron proteins are essential for the catalysis of a variety of reactions in all cell types. Incorporation of iron into proteins is observed in three different classes. Insertion of iron into protoporphyrin IX yields the heme prosthetic group which is essential for the function of all aerobic cells and can be found in proteins including hemoglobin, myoglobin and cytochromes (Ponka 1999). Combination of iron and sulfur (from cysteine residues in scaffold proteins) leads to the assembly of small inorganic cofactors known as Fe/S (iron–sulfur) clusters which are then inserted into apoproteins (Lill and Muhlenhoff 2006). Proteins that contain Fe/S clusters include ferredoxins, nitrogenases and hydrogenases (Beinert 2000). The final class of Fe proteins is known as non-heme iron proteins where iron is bound to the polypeptide via a variety of mechanisms; non-heme iron proteins include ribonucleotide reductases which are a limiting factor in DNA synthesis (Eklund et al. 2001) and mononuclear non-heme iron oxidases and oxygenases which catalyze essential steps in a number of reactions including the biosynthesis of antibiotics such as isopenicillin (Bruijnincx et al. 2008). The same chemical properties that make iron so useful in biology also lead to the propensity to catalyze the formation of free radicals in the presence of oxygen. Thus, cells have had to develop a system of tightly regulating and coordinating iron uptake with utilization, storage and trafficking with many of these components being observed from yeast to humans.

Iron homeostasis in *S. cerevisiae*

Iron uptake in *S. cerevisiae* occurs through four distinct pathways (Fig. 1). Three of these pathways

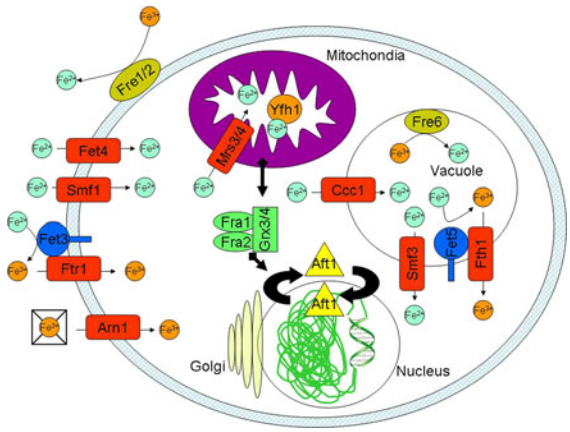

Fig. 1 Iron homeostasis in *S. cerevisiae*. See text for details of the proteins in this figure. Transporters are in *red*, oxidases in *blue*, transcription factors in *yellow*, reductases in *olive*. Yfh1 and Fre1/2, Grx3/4 do not fit into any of these categories and have been colored differently

depend on reduction of iron found in the environment as Fe^{3+} to soluble Fe^{2+} by membrane bound reductases Fre1p and Fre2p (Georgatsou and Alexandraki 1994). Fet4p was originally identified as a low affinity iron transporter in the yeast plasma membrane (Dix et al. 1994) but has also been shown to transport other metals such as copper (Hassett et al. 2000) and zinc (Waters and Eide 2002) and is responsible for the bulk uptake in iron replete cells (Dix et al. 1997). Smf1p is a broad specificity divalent metal transporter found in the plasma membrane that can transport iron with low affinity (Chen et al. 1999) and depends on a proton gradient to drive transport. Two homologs of the *SMF1* gene (*SMF2* and 3) are also found in the *S. cerevisiae* genome and all three are members of the natural resistance associated macrophage protein (NRAMP) family of metal transporters but the role of Smf2p and 3p in iron uptake from the environment is limited if any at all (Cohen et al. 2000). Under limiting iron conditions, the iron responsive transcription factor Aft1p (Yamaguchi-Iwai et al. 1995) induces expression of the iron regulon which includes the high affinity iron transport system consisting of the multicopper oxidase Fet3p (Askwith et al. 1994) and the high affinity iron transporter Ftr1p (Stearman et al. 1996) which interact in the membrane (Bonaccorsi di Patti et al. 2005). Fet3p oxidizes Fe^{2+} that had been previously reduced by Fre1/2p and directly transports the resulting Fe^{3+} to Ftr1p for transport into the cell. Ftr1p will only accept Fe^{3+} directly from the ferroxidase activity of Fet3p in a process known as iron channeling (Kwok et al. 2006). Membrane localization of each protein is dependant on the presence of the other—without Ftr1p, Fet3p will not localize to the membrane and vice versa (Stearman et al. 1996). In contrast, copper loading is not required for membrane localization of Fet3p and Ftr1p (Singh et al. 2006).

Iron uptake that is not dependent on the activity of membrane bound ferric reductases involves uptake of iron bound by siderophores. Siderophores are small organic molecules secreted by bacteria, fungi and grasses under iron-limiting conditions and are among the strongest Fe^{3+} chelating molecules known (Neilands 1995). *S. cerevisiae* does not produce any endogenous siderophores but it does express the necessary transporters (Arn1p, Taf1p (Arn2p), Sit1p (Arn3p) and Arn4p (Enb1p)) to scavenge siderophores produced by other organisms (Kosman 2003). In addition to the siderophore transporters, a family of cell wall mannoproteins (Fit1/2/3p) function to retain siderophore-bound iron in the cell wall (Philpott et al. 2002).

Approximately 20 target genes for the iron-responsive transcription factor Aft1p have been identified. These included genes encoding siderophore uptake proteins, the high affinity iron transport complex, and the metallo-reductases mentioned above along with other genes that code for other proteins that function in intracellular metal metabolism (Shakoury-Elizeh et al. 2004). Evidence has also been provided to support a role for Aft1p in the response to other metals including zinc (Pagani et al. 2007) and cobalt (Stadler and Schweyen 2002). More recently, the role of Aft1 has been shown to extend beyond the realm of metal homeostasis with implications ranging from cell cycle progression to cell wall stability being suggested (Berthelet et al. 2010). The Aft1p DNA binding site has been determined to be YRCACCR (where Y is a pyrimidine and R is a purine) and this sequence is found upstream of genes in the iron regulon (Yamaguchi-Iwai et al. 1996). However, the mechanism for iron regulation is not yet fully understood. When cells are iron replete, Aft1p is cytosolic, but upon iron deprivation Aft1p is translocated to the nucleus where it induces expression of the iron regulon. Translocation of Aft1p into the nucleus is thought to be dependent on an interaction with a complex consisting of Fra1p and Fra2p, which senses the Fe/S cluster levels in the cell (which are in turn indicative of

the iron status of the cell) and Grx3/4p (which are monothiol glutaredoxins). The interaction with the Fra1/2p-Grx3/4p complex retains Aft1p in the cytosol, preventing activation of the iron regulon (Kumanovics et al. 2008). In another different but not contradictory study, iron was shown to induce nuclear export of Aft1p by inducing multimerization. The Aft1p multimers are then recognized by the nuclear exportin Msn5p which exports Aft1p from the nucleus to the cytosol (Ueta et al. 2007). This model does suggest that Aft1p activity is regulated by iron levels and not Fe/S cluster levels as previously described (Chen et al. 2004). This situation is recapitulated in the IRE-IRP system of mRNA level regulation of some iron proteins in mammals where assembly of an Fe/S cluster in IRP1 prevents binding to IREs in target transcripts in iron replete cells (Pantopoulos 2004). A second iron responsive transcription factor Aft2p exhibits a partially redundant activity with Aft1p in activating genes in response to low iron levels (Rutherford et al. 2001) but there are distinct roles for each in iron homeostasis with Aft2 playing a larger role in the regulation of genes that function in vacuolar and mitochondrial iron homeostasis (Courel et al. 2005).

Synthesis of both heme and Fe/S clusters occurs within the mitochondria making the organelle a major site of intracellular iron metabolism (Ferreira et al. 1995; Muhlenhoff and Lill 2000). Two homologous membrane transporters Mrs3/4p are responsible for mitochondrial iron uptake in *S. cerevisiae* (Muhlenhoff et al. 2003). Studies of these yeast genes have provided significant insight into the human homolog mitoferrin. Once inside the mitochondria, the yeast frataxin homolog Yfh1p (Babcock et al. 1997) binds iron and acts as a mitochondrial iron chaperone, delivering iron to sites where it is required (Bulteau et al. 2004). In the absence of Yfh1p, iron accumulates in mitochondria as an inorganic precipitate and generates toxic oxygen species demonstrating the importance of Yfh1p as a chaperone in maintaining iron in a soluble form and preventing toxic effects (Lesuisse et al. 2003). Yfh1p interacts with the Fe/S cluster assembly complex made up of Isu1p and Nfs1p; this interaction is increased in the presence of ferrous iron suggesting a role for Yfh1p in iron loading of Fe/S clusters (Gerber et al. 2003). An interaction between ferrochelatase (the protein that inserts iron into protoporphyrin IX to form heme) and Yfh1p also occurs (Lesuisse et al. 2003) suggesting that Yfh1p also controls iron loading into heme. Genetic analysis of *MRS3/4* and *YFH1* have also confirmed to role for these genes in heme and Fe/S cluster assembly in the mitochondria (Zhang et al. 2005b, 2006).

Vacuoles are the largest organelles in yeast and function as storage compartments for divalent cations including iron. Yeast vacuoles are thought to correspond to the human lysosome (Matile and Wiemken 1967). Ccc1p is a membrane transport protein that functions in the vacuolar iron (and manganese) uptake from the cytosol (Li et al. 2001). Strains lacking *ccc1* are sensitive to increased iron concentrations in the medium, demonstrating the protective function of the vacuole and Ccc1p to excess iron concentrations. Iron sequestration in the vacuole is independent of vacuolar acidification; this makes iron unique among divalent cations as vacuolar uptake in generally dependent on a proton gradient (Bode et al. 1995). *S. cerevisiae* can survive in iron-deficient medium for a number of generations by mobilizing vacuolar iron stores. Vacuolar iron efflux is mediated by the reductase Fre6p (Singh et al. 2007), which belongs to the same family of proteins as the iron uptake membrane reductases Fre1/2p and, Smf3p (Portnoy et al. 2002), (a member of the Nramp family of divalent metal transporters) and a complex of Fth1p and Fet5p (Urbanowski and Piper 1999) (which are homologous to the high affinity iron transport complex of Ftr1p and Fet3p). Transport of iron from the vacuole to the cytoplasm is essentially a replication of iron uptake. Both processes are facilitated by a membrane bound reductase, broad specificity divalent metal transporter and a complex of an oxidase and a transporter.

Intracellular transport of iron from sites of uptake to cellular locales where iron is either utilized (mitochondria) or stored (vacuole) is a process that remains to be defined in yeast. PCBP1 is a member of an mRNA binding protein family that is also capable of delivering iron to ferritin in human cells (Shi et al. 2008). PCBP1 emerged after the mammalian radiation and is thus not found in other eukaryotes such as yeast (Makeyev and Liebhaber 2002). It is thought that an analogous iron chaperone system must exist for the safe handling and delivery of iron both in other organisms and for other cellular pathways—specifically delivery of iron to the mitochondrial transporters. An alternative system involving production of a

siderophore-like molecule in mammalian cells and binding by a lipocalin (which was previously described to bind bacterial siderophores as an immune function) has also been described as a mechanism for intracellular iron trafficking (Devireddy et al. 2010). Potential homologs for some of the genes that constitute this system exist in *S. cerevisiae* but no published data exists regarding a potential role in iron homeostasis and deletion of these genes has not been found to lead to an iron induced growth phenotype (Jo et al. 2008; Bleackley et al. 2011).

Iron in human disease

Both the essential nature and toxic effects of iron can be seen in human disease. The human body contains 3–4 g of iron, most of which is complexed in hemoglobin of red blood cells. Lack of sufficient iron-loaded hemoglobin or a decrease in the number or volume of red blood cells is known as anemia. Anemias can result from insufficient dietary iron uptake but there are also a multitude of other environmental causes (Umbreit 2005). Mutations in genes coding globin polypeptide chains, iron uptake and processing proteins and other proteins required for the synthesis and assembly of hemoglobin and red blood cells are among the many genetic bases for anemias (Rund and Fucharoen 2008; Camaschella 2009; Iolascon et al. 2009). Symptoms of anemias usually consist of a general feeling of weakness and fatigue due to a lack of oxygen delivery to skeletal muscles combined with other symptoms that are indicative of the underlying cause of the disease.

Hemochromatosis is an iron overload disease that results in tissue and organ failure as a result of the oxidative damage caused by excess iron deposition. The most commonly affected organs are the liver, heart and endocrine systems. Classic hereditary hemochromatosis (or type 1) is the most common genetic disease in Caucasians and is characterized by a mis-sense C282Y mutation in the HFE gene (Andrews 1999). HFE encodes the hemochromatosis protein which is thought to act as a sensor for body iron levels by sequestering the transferrin receptor when levels fall within the normal range (Andrews and Schmidt 2007). The HFE protein also competes with iron bound transferrin for binding sites in the receptor (West et al. 2001), lack of functional protein leads to unregulated iron uptake and results in iron overload in specific tissue types. It has also been proposed that the HFE protein is directly involved in regulating dietary iron uptake by DMT1 (West et al. 2006). Damage from type 1 hemochromatosis is usually not observed until later stages of life, which is probably a contributing factor to its prevalence, and can be avoided through dietary restrictions if the mutation is detected early. Mutations in genes for hemojuvelin and hepcidin that result in iron overload are classified as type 2 or juvenile hemochromatosis. Type 2 hemochromatosis is a severe form of the disease and is characterized by a comparatively early onset (Robson et al. 2004). Hemochromatosis type 3 is a rare disease caused by mutations in the transferrin receptor 2 gene which interacts with the HFE gene product in regulating iron metabolism (Roetto et al. 2001). Ferroportin disease, also known as type 4 hemochromatosis, is a result of loss of function mutations in the iron transporter ferroportin causing iron accumulation in reticuloendothelial cells and macrophages but normal to low transferrin saturation levels (Pietrangelo 2004; McGregor et al. 2005). The damaging effect of excess iron is seen in the common disease phenotype that results from this range of mutations.

Misregulation of intracellular iron also has a potential for cellular toxicity. Iron has been implicated in the pathology of a number of neurodegenerative disorders. Fredrich's ataxia is an autosomal recessive disease characterized by degeneration of Purkinje neurons in the cerebellum. The majority of patients are homozygous for an expansion of GAA triplet repeats in the frataxin gene with a smaller subset being heterozygous with a microdeletion in one allele and a point mutation in the other (Campuzano et al. 1996). Frataxin deficiency results in mitochondrial iron accumulation, decreased Fe/S cluster synthesis and an altered antioxidant response due to manganese deficiency and the resultant decrease in activity of the mitochondrial superoxide dismutase (Sod2) (Irazusta et al. 2006). Lack of oxidative protection results in lipid peroxidation and DNA damage both of which are characteristic of the disease (Bleackley et al. 2009).

Neuroferritinopathy is an autosomal dominant neurodegenerative disorder that affects both cognitive and motor function. A single nucleotide insertion in the region coding for the carboxy terminus of the ferritin light chain results in a frame shift that effects pore formation in the ferritin multimer and results in

free radical toxicity from iron and ferritin deposition in the brain (Curtis et al. 2001; Vidal et al. 2004).

Implication of iron in the etiology of other neurodegenerative diseases is becoming increasingly common. A dramatic increase in brain iron levels has been observed in both Parkinson's and Alzheimer's disease patients, and is thought to mediate neurodegeneration through the production of reactive oxygen species (Altamura and Muckenthaler 2009). A potential iron responsive element (IRE) has been identified in the 5′ UTR of the gene coding for α-synuclein, a major component of the abnormal protein aggregates known as Lewy bodies that develop in nerve cells in Parkinson's disease (Friedlich et al. 2007). Oligimerization of α-synuclein may be mediated by an interaction with iron and the resultant free radical damage (Ostrerova-Golts et al. 2000) which implicates iron in pathology of Parkinson's disease both at the translational and degradational levels. A putative IRE has also been identified in the 5′ UTR of the amyloid β precursor protein which is cleaved to yield the α-amyloid protein that accumulates in the neuritic plaques found in Alzheimer's disease (Rogers et al. 2002). Much like in Parkinson's disease, iron (and other metals) catalyze the aggregation of α-amyloid protein (Atwood et al. 2003) once again regulating the disease on multiple levels.

Regulation of intracellular iron homeostasis is essential for the survival of an organism. Exceptional physiological reactivity of iron has made it a key stone in a plethora of biochemical systems. The same properties that make iron such a useful biological molecule also make it extremely cytotoxic as can be observed in the array of diseases in which it is involved. Organisms have developed complex systems for establishing and maintaining iron homeostasis. Although there is a vast wealth of knowledge related to iron homeostasis and large portions of the systems for biological regulation of iron have been previously described, there are still gaps in the knowledge base leaving a number of genes and proteins yet to be identified as key players in iron homeostasis.

Copper

Copper is a first row transition metal with the atomic number 29 that is found predominantly in two oxidation states, Cu(I) and Cu(II). As a result of electron affinity, Cu(II) is the most effective bioavailable divalent cation for binding organic molecules as is Cu(I) for monovalent cations. Cu(I) and Cu(II) both readily form complexes with organic ligands which makes the redox pair extremely useful for biological oxidizing reactions with a potential ranging from +0.2 to +0.8 V (Frausto da Silva and Williams 2001a). As with other transition metals, the electron transfer properties of copper also allow for catalysis of hydroxyl radical formation from hydrogen peroxide through the Fenton reactions (Valko et al. 2005). Toxicity is also caused by inappropriate binding of copper to sulfur, oxygen and imidazole ligands in cells (Culotta 2010). In contrast to iron, copper is insoluble in the reduced (Cu(I)) form and was therefore not bioavailable during the early stages in the evolution of life. With the oxygenation of the atmosphere copper was oxidized to the bioavailable Cu(II) and a new biochemical redox couple was now available for incorporation into biological systems (Crichton and Pierre 2001).

Copper in biology

As with other trace elements, copper is essential to many forms of life. While not as prevalent as iron or zinc, specific properties of copper have made Cu extremely important for cellular redox reactions. Most enzymes employ copper in electron transfer reactions, with these enzymes predominantly being oxidative or involved in energy capture (mitochondria and chloroplasts). When examining the cellular localization of the approximately 30 copper-containing proteins, it is striking to notice the vast predominance of extracellular proteins. The few copper-containing proteins found in the cytoplasm are involved in protection and detoxification of copper and the cellular response to copper levels. Biologically-active copper centers can be grouped into distinct classes. Type 1 copper centers (also known as blue copper centers) have the copper bound by two nitrogen atoms and two sulfur atoms and are found in small electron transfer proteins such as cupredoxins. Type 2 copper centers have the copper coordinated by two or three nitrogen atoms and/or oxygen atoms and are most often involved in catalysis of oxygen species. Type 3 centers have copper dimers bridged by O_2 or a hydroxyl and are found along with a type-2 site in multicopper oxidases (MacPherson and Murphy 2007). A unique Cu_A site is found in the final

protein in the electron transport chain—cytochrome *c* oxidase. Cu_A sites consist of a dimer of type 1 sites linked by a Cu–Cu bond (Larsson et al. 1995).

Copper homeostasis in *S. cerevisiae*

Ctr1p is the high affinity copper transporter responsible for the bulk of copper uptake in yeast (Fig. 2) (Dancis et al. 1994a). Oligimerization of Ctr1p occurs in the membrane to form the functional copper transporter; expression is regulated by copper levels (Dancis et al. 1994b). *CTR3* encodes a second high affinity copper transporter but the insertion of a Ty2 transposable element that displaces the promoter from the transcription start site in the common laboratory strain S288C (Knight et al. 1996). Homologous to *CTR3*, *CTR2* encodes an expressed vacuolar copper transporter that functions to mobilize vacuolar copper stores (Rees et al. 2004). All three transport Cu(I) and thus are dependent on the activity of a corresponding reductase. In the case of Ctr1p the reductase is Fre1/2p, the same reductase involved in iron uptake (Georgatsou and Alexandraki 1994).

Expression of *CTR1*, *CTR3*, *FRE1*, *FRE2* and *FRE7* is controlled by the copper sensing transcription factor Mac1p (Labbe et al. 1997). In the absence of copper, Mac1p binds copper responsive elements (CuREs) in the promoters of target genes. Binding to two CuREs with specific spacing is required for activation by Mac1p (Martins et al. 1998). Under high copper levels Ctr1p is degraded at the plasma membrane, not internalized to the vacuole prior to degradation as with other proteins (Ooi et al. 1996). Mac1p also seems to be involved in regulating the degradation of Ctr1p which presents a unique situation where a transcription factor regulates both expression of a target gene and the stability of the gene product (Yonkovich et al. 2002).

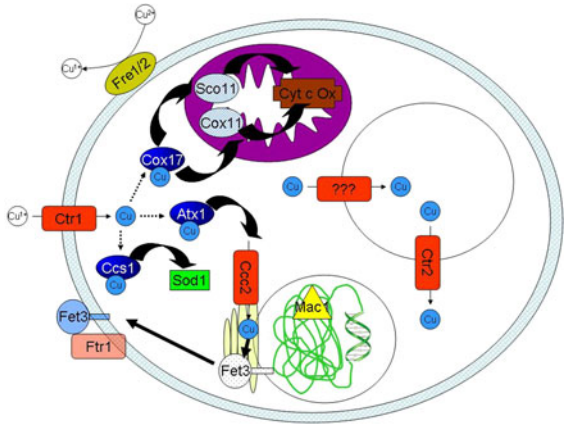

Fig. 2 Copper homeostasis in *S. cerevisiae*. See text for details of the proteins in this figure. Transporters are in *red*, copper chaperones in *dark blue*, transcription factors in *yellow*. Sco11 and Cox11 are not chaperones but do function in copper loading of Cyt *c* Ox and have been hashed in *blue*

Once inside the cell, there are three primary destinations for copper with transport to each destination from the transporter facilitated by a distinct copper metallochaperone (Robinson and Winge 2010). Each chaperone functions specifically for its pathway. Complementation is observed using the same chaperone from a different species but not between the different pathways (Harrison et al. 1999). Delivery of copper from the chaperone to the cognate receptor is dependent on specific protein–protein interactions.

Cytochrome *c* oxidase is the terminal protein in the electron transport chain in the inner mitochondrial membrane that reduces molecular oxygen and translocates four protons across the mitochondrial membrane. Subunits for the enzyme are encoded by three mitochondrial genes (*COX1/2/3*) and eight nuclear genes (*COX4/5A* or *5B/6/7/8/9/12/13)* (Geier et al. 1995). Cox1p contains a heme prosthetic group and the Cu_B copper, Cox2p contains the Cu_A copper dimer and cytochrome c binding site (Cooper et al. 1991). Copper is incorporated into the subunits in the mitochondria with the inner mitochondrial proteins Sco1p and Cox11p acting as copper donors to the Cu_A and Cu_B sites respectively. Cox17p acts as a copper chaperone, delivering copper from sites of import through the cytoplasm to both Sco11p and Cox11p in the mitochondria (Horng et al. 2004).

Incorporation of copper into the high affinity iron transport protein Fet3p occurs in the Golgi where Ccc2p functions as a copper transporting P-type ATPase (Fu et al. 1995; Yuan et al. 1995). Copper loading of Fet3p also requires the presence of chloride anions which bind to Fet3p and exhibit an allosteric effect on copper loading. Strains with mutations in *gef1*, (a voltage gated chloride channel localized to the Golgi) do not grow on medium deficient in iron as a result of a lack of copper loaded Fet3p (Davis-Kaplan et al. 1998; Braun et al. 2010). Atx1p is the cytosolic copper chaperone that delivers copper to Ccc2p for incorporation into Fet3p (Lin et al. 1997). Identification of Atx1p as a copper

binding protein was accomplished through a high copy suppressor screen for the oxygen toxicity in yeast lacking superoxide dismutase (Lin and Culotta 1995) which shows that although the functions of the chaperones are not redundant between copper trafficking pathways, they can compensate for toxic effects of excess copper in the cell. These proteins represent a link between copper and iron metabolism. Expression of the *CCC2*, *FET3* and *ATX1* genes is regulated by the iron responsive transcription factor Aft1p; strains deficient in these genes are sensitive to limiting iron conditions.

The third major function of copper in the yeast cell is in protecting against oxidative stress, a process that Cu can also contribute to. Sod1p is the cytosolic Cu–Zn superoxide dismutase in yeast which is a homodimer containing one atom each of Cu and Zn per subunit, which functions to break down superoxide to oxygen and hydrogen peroxide (Bermingham-McDonogh et al. 1988). The metallochaperone for insertion of copper into Sod1p is Ccs1p which contains a region of high homology (approximately 50% identity) to Sod1p itself (Schmidt et al. 1999) suggesting a gene duplication event in the evolution of this system. Copper is the enzymatically active metal ion in Sod1p; binding of the zinc ion stabilizes the fold of the protein around the copper atom.

These copper chaperones provide the clearest picture for how cells maintain the balance between the requirement for metals in biology and the detrimental effects that can be caused by metal ions that are not properly regulated. Experiments to characterize the three distinct systems and the requirement for protein–protein interactions for the donation of copper ions from chaperone to receptor provide an elegant framework on which to base hypotheses of how the distributions of other metals are regulated by cells.

Copper in human disease

Copper is a relatively scarce metal in humans with an average concentration of 1.4–2.1 mg/kg in healthy individuals. Aside from the low body concentration, disruptions in copper homeostasis have implications in a number of diseases, and deficiencies in copper absorption have a number of detrimental effects. Insufficient copper uptake during development interferes with the activity of cuproenzymes that leads to increased oxidative stress, decreased availability of nitric oxide, abnormal iron metabolism, problems with cross linking in the extracellular matrix and altered cell signaling; in turn, these reactions affect a variety of tissues and organs including the brain, heart, lungs and skin (Uriu-Adams et al. 2010). Acquired copper deficiency can be caused by gastric surgery, malabsorption and excess zinc, and results in both hematologic and neurologic abnormalities (Kumar 2006).

Mutations in *ATP7A* and *ATP7B*, which encode proteins homologous to the yeast Golgi copper transporter Ccc2p, result in Menkes and Wilson disease respectively (La Fontaine and Mercer 2007). Menkes disease is an X-linked recessive disorder associated with copper deficiency (de Bie et al. 2007) with over 200 mutations in *ATP7A* identified as causing the disease (Tumer et al. 1999). Symptoms of Menkes disease include neurological defects, growth retardation, hypopigmentation and "kinky" or "steely" hair and result from deficiencies in a number of cuproproteins (de Bie et al. 2007). Wilson disease is characterized by a toxic accumulation of copper in the liver and brain that result in cirrhosis and chronic hepatitis leading to eventual liver failure, neurological defects similar to those seen in Parkinson's disease and psychiatric symptoms (de Bie et al. 2007). Wilson disease is inherited as an autosomal recessive disorder (Ala et al. 2007) with two mutations accounting for most cases—H1069Q is prevalent in European and North American patients while R788L is prevalent in South East Asia (Ferenci 2006). Most other identified mutations are unique and limited to single families. *ATP7A* and *ATP7B* are highly homologous (Bull et al. 1993); the differences in the diseases caused by mutations in these genes is the result of differential tissue expression. *ATP7A* is expressed in almost all tissue types with a noticeable drop in expression seen in the liver while *ATP7B* is predominantly expressed in the liver with lower levels detected in kidney, brain, heart, lung, mammary gland and placenta (de Bie et al. 2007).

Ceruloplasmin is a multi-copper oxidase that binds approximately 95% of plasma copper. Mutations in the ceruloplasmin gene result in the autosomal recessive disease aceruloplasminemia which is characterized by the degeneration of the retina and basal ganglia together with diabetes mellitus (Harris et al. 1998). Although the disease is caused by mutations in genes for a copper protein, the pathological effects observed are the result of iron accumulation that occurs due to a

lack of functional protein. No accumulation of copper is observed in the liver, the major site of ceruloplasmin synthesis copper does not directly affect expression of the ceruloplasmin gene; however, improper copper loading leads to protein instability and rapid degradation of the protein (Desai and Kaler 2008).

Amyotrophic lateral sclerosis (ALS) is a fatal disease that is caused by degradation of motor neurons that leads to muscle atrophy and the potential loss of all voluntary muscle control. Familial ALS (fALS) have been linked to mutations in the Cu/Zn superoxide dismutase (Sod1) gene that are inherited as autosomal dominant alleles (Deng et al. 1993; Rosen et al. 1993). Corresponding mutations were studied in the yeast homolog Sod1p and variable metal-incorporation and activity were observed within the mutants suggesting that free radical damage is likely not the only cause of fALS related to Sod1p mutations (Nishida et al. 1994). Further studies in transgenic mice expressing both endogenous Sod1 and human fALS Sod1 provided evidence that the mutations in fALS were actually gain of function mutations as the endogenous Sod1 did not protect against the fALS alleles (Gurney et al. 1994; Wang et al. 2005). Mouse models of Sod1 in ALS have also revealed that an accumulation of mutant protein is common among models and that mutant proteins are more prone to oligomerization than wild type protein (Wang et al. 2003). It has been suggested that fALS mutations in Sod1 hinder the interaction between Sod1 and the copper chaperone CCS which in turn prevents CCS dependent maturation of Sod1 which includes insertion of the copper ion and oxidation of a disulphide bond and the immature protein aggregates and leads to disease (Seetharaman et al. 2009).

The prion protein that probably plays an important role in transmissible spongiform encephalopathies such as Mad Cow disease, scrapie in sheep and variant Creutzfeld-Jakob disease in humans has been proposed to function in brain copper homeostasis. In its normal cellular form (PrPc) the protein is thought to transport copper into the cell, protect from oxidative stress and buffer copper in the cell (Westergard et al. 2007) and has been shown to bind copper in vitro (Pan et al. 1992). Conversion of the protein to the scrapie form (PrPsc) is induced by interaction with copper and other metal ions such as zinc, iron and manganese (Rana et al. 2009) and altered metal binding by PrPsc contributes to the pathology of the consequent diseases (Wong et al. 2001).

Copper (and iron) are found in increased concentrations in the striata of Huntington's disease (HD) patients (Dexter et al. 1991). HD is characterized by progressive motor, cognitive and psychiatric deterioration caused by a dominant glutamine expansion in the N-terminus of the protein huntingtin. Mutant huntingtin is cleaved by caspase 6 and the cleavage fragments are thought to mediate the disease (Graham et al. 2006). Both normal and mutant huntingtin interact with copper but not other metal ions. However, copper has been found to promote, while metal chelation prevents, the aggregation of mutant huntingtin fragments possibly through the induction of structural changes in the transition from monomer to aggregate (Fox et al. 2007). Copper is also associated with accelerated aggregation of α-synuclein in the formation of Lewy bodies in Parkinson's disease (Rasia et al. 2005) and is known to interact with the amyloid precursor protein and the β-amyloid peptide in the plaques of Alzheimer's disease (Dingwall 2007).

Zinc

Zinc is essential to all forms of life and is the most the second most abundant transition metal in both seawater and humans (Outten and O'Halloran 2001) and is the most prevalent trace metal found in the cytoplasm with the cellular concentration of zinc falling between 0.1 and 0.5 mM in organisms ranging from *Escherichia coli* to man (Eide 2006). The Zn^{2+} ion has a full d orbital making it unique amongst biologically-relevant transition metals in that zinc does not exist in multiple valences under physiological conditions rendering it not redox active (Williams 1987) and making it generally considered less toxic than redox active metals such as iron and copper. However, Zn^{2+} is a strong Lewis acid due to the high electron affinity generated by the charge of 2^+ and its relatively small ionic radius. Utilization of zinc as a cofactor in catalysis is made more attractive by the rapid ligand exchange rate that allows for efficient turnover of reaction products (McCall et al. 2000). Although these properties are not individually unique for zinc, the combination of structural and thermodynamic properties with the abundant bioavailability have led to the incorporation of zinc in a variety of biological systems.

Zinc in biology

A vast array of macromolecules require zinc to maintain proper structure and function and zinc is know to participate in at least 300 enzymatic reactions (Tapiero and Tew 2003). Within the zinc proteins a number of distinct functional classes can be identified. A set of metalloproteases is know to bind zinc and includes (1) matrix metalloproteases with roles in embryonic development, cell motility, wound healing and reproduction (Amalinei et al. 2007) and (2) carboxypeptidases that function as digestive enzymes among other families (Hooper 1994). Zinc ions in metalloproteases are coordinated by specific motifs containing a number of histidine residues that differ between the families. In all cases, the zinc ion is required for enzymatic function.

Another diverse set of zinc binding proteins are zinc finger proteins which are only found in eukaryotes but have no enzymatic activity. Many zinc finger proteins are DNA (or RNA) binding proteins in which zinc finger domains are arranged in tandem where each domain is structurally similar but the nucleotide recognition sequence for each domain can be made unique by varying the amino acid side chains at a number of key positions (Klug 2010). Unlike other DNA binding proteins where amino acids interact with multiple bases and vice versa, DNA recognition and binding in zinc fingers occurs in a one to one ratio of amino acid to nucleotide (Pavletich and Pabo 1991). Three common folds are found among the majority zinc finger proteins: C_2H_2-like fold which is the classic fold found in most mammalian zinc finger proteins (Laity et al. 2001), treble clef and zinc ribbon (Krishna et al. 2003). Some zinc finger proteins do have enzymatic activity and include (1) protein kinase C (Ono et al. 1989) where the zinc finger binds to small molecules and is important in signal transduction, (2) alcohol dehydrogenase (Crow et al. 1995) and (3) nuclear hormone receptors (Matthews and Sunde 2002). A distinct family of zinc finger domains known as Really Interesting New Gene (RING) finger domains exists. RING finger domains contain two zinc atoms (Borden and Freemont 1996) and function in ubiquitination of proteins (Matthews and Sunde 2002) and in mediating protein–protein interactions (Saurin et al. 1996). Zinc finger proteins have been modified to act as artificial transcription factors for regulated gene expression (Sera 2009)and linked to a DNA cleavage domain from a restriction enzyme to form zinc finger nucleases which are used in genome engineering for site specific gene targeting (Durai et al. 2005).

A plethora of other zinc-containing proteins do not fall into these categories but are in a number of organisms and function in a multitude of physiological processes. Other zinc-containing proteins include: alkaline phosphatase (Plocke et al. 1962), phospholiase C (Ottolenghi 1965), carbonic anhydrase (Lindskog 1997) and insulin (Emdin et al. 1980). Recently zinc has also been shown to act a signaling molecule in a manner thought to be similar to calcium (Yamasaki et al. 2007).

Zinc homeostasis in *S. cerevisiae*

Uptake of zinc in *S. cerevisiae* is mediated by two specific zinc transporters Zrt1p, the high affinity zinc transporter which is active in zinc deficient cells (Zhao and Eide 1996a), and Zrt2p, a low affinity zinc transporter expression of which is time, temperature and concentration dependent and is active in zinc replete cells (Fig. 3) (Zhao and Eide 1996b). Both Zrt1p and Zrt2p are members of the ZIP family of metal transporters (Eng et al. 1998) and the two proteins share 44% amino acid sequence identity and 67% similarity (Eide 2003). A double mutant *zrt1zrt2* strain is viable (Zhao and Eide 1996a, b), this observation helped lead to the identification of the zinc transport function of Fet4p (Waters and Eide 2002). Transport by the phosphate transporter Pho84p has also been suggested, possibly in uptake as $ZnPO_4$ (Jensen et al. 2003).

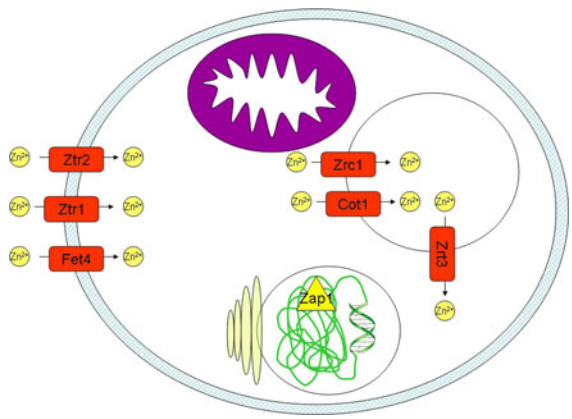

Fig. 3 Zinc homeostasis in *S. cerevisiae*. See text for details of the proteins in this figure. Transporters are shown in *red*, transcription factors are shown in *yellow*

The zinc deficiency response is mediated by the transcription factor Zap1p (Zhao and Eide 1997). Zap1p is localized to the nucleus irrespective of the zinc status of the cell and is regulated directly by zinc binding (Bird et al. 2003). Two independently-regulated activating domains (AD) and a DNA binding domain (DBD) containing seven zinc fingers (Bird et al. 2000) are found in the protein. AD1 lacks a known zinc binding motif but does bind zinc ions in vitro; binding of zinc to AD1 is thought to promote an interaction with the DBD of the protein which in turn inactivates the activation function of AD1 (Herbig et al. 2005). Zinc binding to two zinc fingers in AD2 leads to an interaction between the two fingers that prevents AD2 from recruiting co-activators to promoters (Wang et al. 2006). Zap1p has been implicated in the regulation of the expression of more than 80 genes (Eide 2009); this is accomplished through binding to zinc responsive elements (ZREs) in the promoters of target genes (Zhao et al. 1998) which include the genes for the zinc transporters *ZRT1/2* and *FET4* along with genes involved in vacuolar release of zinc stores (Lyons et al. 2000). The role of Zap1p in the response to limiting zinc conditions goes beyond just a transcriptional activator. Isoenzyme switching between zinc dependent alcohol dehydrogenases Adh1/3p, which are among the most abundant zinc proteins in the cell, and the iron dependent Adh4p is mediated by Zap1p (Eide 2009). Activation of genes by Zap1p is also concentration and time dependent, Zap1 mediates a coordinated response to zinc deficiency where particular genes are induced depending on the severity and duration (Wu et al. 2008). Part of this mechanism is controlled by the auto-activation of *ZAP1* and can be seen in the response of *ZRT2* to varying zinc levels. *ZRT2* is both activated and repressed by Zap1p (Bird et al. 2004). Two ZREs found upstream of the TATA box in the *ZRT2* promoter mediate activation whereas a third ZRE with a lower affinity for Zap1p is found down stream of the TATA box and functions in Zap1p mediated repression of *ZRT2* thus when exposed to moderate zinc deficiency Zap1p binds to the activating ZREs and turns on *ZRT2* but when zinc deficiency becomes severe, *ZAP1* auto-activates and the increased level of Zap1p leads to binding of the repressor ZRE which prevents *ZRT2* expression when it will not aid in zinc uptake when it will be accomplished solely through the high affinity zinc transporter Zrt1p (Eide 2009).

Upon the shift from zinc limiting to zinc replete conditions, Zrt1p levels drop rapidly in a process involving zinc-induced endocytosis and degradation in the vacuole (Gitan et al. 1998). This process protects cells from over-accumulation of zinc when shifted from low zinc to high zinc medium and is thought to function more in extreme shifts in zinc availability where as transcriptional control regulates the response to moderate shifts in zinc availability (Eide 2003).

Under zinc replete conditions, excess zinc is stored in the yeast vacuole. Zrc1p and Cot1p are the transporters that facilitate vacuolar zinc uptake (MacDiarmid et al. 2000). A proton gradient established by the V-ATPase is required for zinc transport by Zrc1p (MacDiarmid et al. 2002). The role of the vacuole in zinc metabolism is three fold; the vacuole and the transporters Zrc1p and Cot1p are essential for zinc detoxification, when zinc becomes limited Zrt3p, a protein similar to the membrane zinc transporters Zrt1/2p, is induced by Zap1p to mobilize zinc stores (MacDiarmid et al. 2000) and with the activation of the high affinity zinc uptake system through the activity of Zap1p the vacuole also functions in resistance to "zinc shock" where by the vacuolar uptake gene *ZRC1* is also induced by Zap1p in a preemptive mechanism to protect against the massive influx of zinc ions caused by activation of Zrt1p (MacDiarmid et al. 2003). Vacuolar zinc stores are sufficient to provide adequate zinc for subsequent generations of daughters cells after a shift from zinc replete to zinc deficient medium as other cellular compartments do not significantly contribute to zinc stores (Simm et al. 2007).

Some controversy exists in the identification of the mechanism of intracellular zinc trafficking. One group suggests that there is a metallochaperone system akin to that for copper; this, an idea that is supported by evidence indicating that there is less than one free atom of zinc in a cell (Outten and O'Halloran 2001). Conversely, the idea of intracellular metallochaperones has been discounted by other groups citing the dependence on protein–protein interactions for the delivery of metal ions to target proteins by chaperone and the wide variety of zinc proteins in the cell make this an unlikely situation as there would need to be almost as many chaperones as there were zinc proteins. There is also a lack of sequence similarity among zinc proteins to suggest a common protein–protein interaction that would allow for a small number of chaperones to deliver zinc to an array of

target proteins. As an alternative, it has been suggested that intracellular zinc levels cycle between deficient and replete allowing for regulation through transcription factors (Eide 2006).

Zinc in human disease

Adult humans contain about 2 g of zinc that is evenly distributed throughout the body (Hambidge and Krebs 2007). Zinc deficiency in humans was first hypothesized in the 1960s as a cause of nutritional dwarfism in the Middle East (Halsted et al. 1972; Prasad et al. 1990). Since then, zinc deficiency has been better recognized and it is now estimated that between 20 and 25% of the world is at risk of becoming zinc deficient (Wuehler et al. 2005; Maret and Sandstead 2006). Causes of zinc deficiency range from insufficient dietary zinc uptake which can be exacerbated by consumption of foods such as cereal grains which are rich in phytates (compounds that inhibit dietary absorbtion of zinc (Fischer Walker et al. 2009)) to excessive alcohol use to the rare genetic disorder acroermatitis enteropathica where mutations in the *SLC39A4* gene disrupt the function of the Zip4 zinc transporter in the gut (Wang et al. 2004). Because zinc is involved in so many physiological processes, the symptoms of zinc deficiency are varied but include alopecia, diarrhea, psychological impairment, male hypogonadism and neurosensory disorders (Turek and Fazel 2009). Zinc is essential for the development of many aspects of the immune system including neutrophils, macrophages and natural killer cells along with growth and function of both T and B cells (Prasad 2009). In model organisms, zinc deficiency led to atrophy of the thymus and lymphoid tissue and they have a decreased response to both T-cell dependent and independent antigens (Fraker et al. 1977, 1978). The effects of zinc deficiency on both the innate and specific immune systems drastically alter host resistance to infection by a multitude of bacteria and viruses (Shankar and Prasad 1998). The drastic effects of zinc deficiency are manifested in the estimated 800,000 deaths annually among children under 5 years of age where zinc deficiency is a contributing factor (Hambidge and Krebs 2007).

Zinc toxicity can be observed in both acute and chronic forms. Acute zinc toxicity is rare and usually results from consumption of contaminated food and drinks from galvanized containers and can cause gastrointestinal irritation, fatigue, muscle pain and fever. More common is the chronic zinc toxicity that usually occurs as a result of over use or prolonged use of oral zinc supplements. Excess zinc can also interfere with iron and copper levels (Valko et al. 2005).

In contrast to iron and copper which promote accumulation of amyloid-β peptide (Aβ) in the formation of plaques in Alzheimer's disease, zinc is though to play a protective role. Binding of zinc to Aβ causes a conformational change in the peptide that prevents the association of other metals with Aβ and thus prevents the oxidative damage that is associated with the metal-Aβ complex (Cuajungco et al. 2000). Oxidative and nitrosative stress lead to a release of zinc from the vesicular zinc pool and the excess zinc promotes aggregation of Aβ (Cuajungco and Lees 1997; Miller et al. 2010). Zinc at normal physiological concentrations protects against the contributions of other metals to Alzheimer's disease but if the concentration of zinc ions is raised zinc becomes a contributing factor to the formation of plaques.

Manganese

Manganese is found in three major biologically-relevant oxidation states: Mn(II), Mn(III) and Mn(IV). Manganese differs from other metals such as iron and copper in that the middle oxidation state, Mn(III) readily disproportionates to Mn(II) and Mn(IV) where as Fe(III) and Cu(II), the middle oxidation states for the respective metals, are the most stable. Mn^{2+} is stable in acidic environments whereas MnO_2 is more stable in alkaline and oxygenated environments. Biochemically, Mn(II) is thought to be an intermediate between Ca(II), Mg(II) and Zn(II) and can replace these ions in specific systems. Because manganese is present at much lower concentrations in cells than other metals, it has become apparent that manganese is incorporated into biochemical systems under special conditions or used preferentially in cellular compartments such as mitochondria, the Golgi and vesicles. Manganese is readily bioavailable and is soluble in the presence of oxygen. The solubility of manganese sulphides lead to the hypothesis that manganese must have been utilized extensively in early life as it would have been more available than other transition metal salts which is also supported by the presence of Mn^{2+} in the cytoplasm of unicellular anaerobes. Like other redox-active transition metals,

manganese has the ability to generate reactive oxygen species when unregulated (Ali et al. 1995).

Manganese in biology

The function of manganese in biology ranges from a Lewis acid catalyst to crossing a number of oxidation states to carry out the oxidation of water (Yocum and Pecoraro 1999). Manganese has long been known to be important for photosynthesis and has since been shown to function in photosystem II in the oxidation of water to O_2 (Tommos et al. 1998). Although manganese is capable on contributing to oxidative stress, many of the enzymes with which it is associated function in protecting cells against oxidative damage. Catalases, peroxidases and a mitochondrial form of superoxide dismutase depend on the incorporation of manganese ions to break down oxidative molecules in the cell (Horsburgh et al. 2002). Manganese itself can also play a direct role in oxidative stress protection in the absence of Cu/Zn SOD (Reddi et al. 2009b). Many manganese proteins are localized extracellularly or in vesicles and the function of many of these proteins in mirrored by copper enzymes also found in vesicles. This has led to the hypothesis that when copper became bioavailable, it replaced manganese in these systems later in evolution (Frausto da Silva and Williams 2001b). Coordination of manganese into proteins does not occur through common mechanisms between proteins that have been grouped as with copper. Proteins containing manganese ions include DNA and RNA polymerases, kinases, decarboxylases, sugar transferases, phosphatases and arginases (Frausto da Silva and Williams 2001b; Reddi et al. 2009b). The roles of these and other proteins make manganese essential for skeletal development, reproductive hormone production, nervous system function and a multitude of other cellular functions (Santamaria and Sulsky 2010).

Manganese homeostasis in *S. cerevisiae*

Uptake of manganese by yeast is somewhat different from the uptake of other transition metals (Fig. 4). Smf1p, the broad spectrum divalent metal transporter of the Nramp family, has been shown to be a proton-dependent, high affinity manganese transporter localized to the plasma membrane (Supek et al. 1996) and its over-expression leads to accumulation of manganese

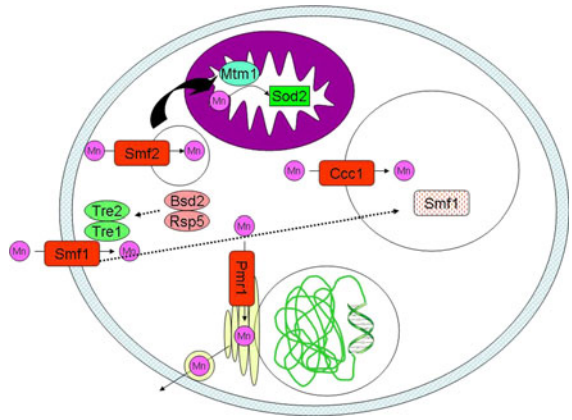

Fig. 4 Manganese homeostasis in *S. cerevisiae*. See text for details of the proteins in this figure. Transporters are shown in *red*, the other proteins in the figure did not fit into the categories used throughout this chapter and have been colored individually

along with other metals including copper and iron (Cohen et al. 2000). However, a *smf1Δ* strain shows no significant difference in cellular manganese levels to those found in a wild type strain and consequently, no defect in manganese proteins was detected in the deletion strain (Luk and Culotta 2001). Despite the affinity of Smf1p for manganese, this data suggested that Smf1p could not be the major plasma membrane transport protein for manganese uptake. More recently Smf1p has been shown to function in oxidative stress resistance where manganese itself acts as an antioxidant and it is brought into the cell by Smf1p (Reddi et al. 2009a). Although Smf2p is closely related to Smf1p the function of Smf2p in manganese homeostasis is drastically different. Strains lacking Smf2p have a defect in manganese accumulation and there is a lack of activity observed for manganese-dependent enzymes (Luk and Culotta 2001). Although this is consistent with the expected phenotype for a membrane transporter that functions in uptake based on known transporters for other transition metals, no Smf2p is detected at the cell membrane in localization experiments. Most of the Smf2p localizes to Golgi-like vesicles which have been proposed to function as transient stores for manganese with Smf2p functioning in release (Culotta et al. 2005). Smf3p has no known role in manganese homeostasis (Reddi et al. 2009a). Manganese uptake in yeast has been shown to be much slower than know rates of transport for Nramp proteins in other organisms which led to the suggestion that manganese uptake is facilitated by a very small number

of membrane localized transporters (Stimpson et al. 2006) and that Smf2p may be the transporter that exists at sub detectable levels (Culotta et al. 2005). Pho84p, a high affinity phosphate transporter (Bun-Ya et al. 1991), also functions as a low affinity manganese transporter that transports manganese as manganese phosphate and only functions in manganese surplus conditions (Jensen et al. 2003). Manganese taken up through Pho84p acts as a toxin rather than as an essential nutrient or a protectant against oxidative stress (Reddi et al. 2009a). One explanation is that Pho84p normally functions in uptake of $MgHPO_4$ but in conditions of manganese excess, Pho84p can use $MnHPO_4$ as a substrate (Reddi et al. 2009a) (Fig. 4).

Regulation of transporters for other transition metals is mostly accomplished through the activity of metal-responsive transcription factors (Rutherford and Bird 2004). No manganese-responsive transcription factor has been identified and levels of Smf1/2p are regulated post-translationally. Under manganese replete conditions, the majority of protein molecules are targeted to the vacuole for degradation. When manganese becomes limiting, this process is turned off and the transporters become prevalent at the membrane; under conditions of manganese excess, the process is enhanced and there is essentially no transporter localized to the membrane (Reddi et al. 2009a). Targeting of Smf1/2p to the vacuole occurs through ubiquitination by Rsp5p in a process facilitated by Bsd2p (Sullivan et al. 2007) that also requires the transferrin-receptor like adaptor proteins Tre1p and Tre2p (Stimpson et al. 2006). The current model has Bsd2p forming a complex with Rsp5p and this complex binds to the Tre proteins that have associated with Smf1p leading to ubiquitination and targeting to the vacuole for degradation (Sullivan et al. 2007; Reddi et al. 2009a). The exact mechanism by which Smf1/2p are redirected to the cell surface under manganese limiting conditions is still unknown. There is a complete loss of control by Bsd2p and Tre1/2p and low Golgi manganese levels are known to correspond to an increase in Smf1p in the plasma membrane which suggests that Golgi-sensing controls the manganese starvation response (Jensen et al. 2009). Under high manganese conditions, there is an Rsp5p independent targeting of Smf1p to the vacuole which occurs at a much slower rate than the ubiquitin mediated process that is proposed to reflect the anti-oxidant role of manganese and Smf1p transport (Reddi et al. 2009a).

A key target for cellular manganese is the Golgi apparatus where manganese is incorporated into sugar transferases (STases) which glycosylate proteins in the secretory pathway (Luk and Culotta 2001). Transport of manganese (and calcium) into the Golgi from the cytoplasm occurs via Pmr1p a P-type ATPase (Durr et al. 1998), a member of the SPCA (secretory pathway Ca^{2+} ATPases) family, members of which can be found from fungi to humans (Van Baelen et al. 2004). Hyper-accumulation of manganese in a *Δpmr1* strain and sensitivity to elevated manganese concentrations (Lapinskas et al. 1995) led to the identification of a role for Pmr1p in manganese detoxification by pumping excess metal out of the cell through the secretory pathway. In combination with Smf1p, Pmr1p also provides antioxidant manganese for protection against oxidative stress in a *sod1Δ* strain through a mechanism independent of the manganese dependent Sod2p (Reddi et al. 2009a).

Mitochondria contain the machinery for oxidative respiration and thus generate a large amount of oxidative molecules. Sod2p, a manganese superoxide dismutase, is localized to the mitochondrial matrix and protects against the damaging effects of oxygen radicals (van Loon et al. 1986). Each of the four subunits of Sod2p bind one manganese atom (Ravindranath and Fridovich 1975). Mtm1p, a member of the mitochondrial carrier family (MCF), facilitates manganese activation of Sod2p but *Δmtm1* yeast accumulate slightly higher concentrations of mitochondrial manganese than wild type indicating that Mtm1p is likely not a mitochondrial manganese transporter (Luk et al. 2003). Mitochondrial manganese uptake is thought to occur through a process mediated by Smf2p containing vesicles as an *Δsmf2* strain has diminished Sod2p activity (Luk and Culotta 2001). Based on the accumulation of mitochondrial iron in an *mtm1Δ* strain and the fact that iron can inactivate manganese superoxide dismutases, it has been proposed that Mtm1p ensures that Sod2p is loaded with manganese as opposed to the more prevalent iron (Culotta et al. 2005).

Excess cellular manganese is concentrated in the vacuole. Vacuolar uptake occurs through the transport protein Ccc1p (Li et al. 2001), which also functions in vacuolar iron accumulation. No vacuolar export mechanism specific for manganese has been identified but it is possible that the iron export pathways can also use manganese as a substrate in the

same manner as Ccc1p. Alternatively vacuolar manganese accumulation could function only in detoxification and not in storage as seen for iron and other metal ions.

Intracellular trafficking of manganese occurs through an unknown mechanism but two systems have been hypothesized (Culotta et al. 2005). Chaperones similar to those that function in copper homeostasis have been proposed to exist to transport manganese from transporters to cellular locales such as the mitochondrial and Golgi. Alternatively, a vesicle-mediated system employing Smf2p for release of manganese from the vesicle at targets that is analogous to the suggested system that exists for trafficking iron to mitochondria in erythroid cells of higher eukaryotes (Ponka et al. 2002; Zhang et al. 2005a).

Manganese in human disease

On average, an adult human body contains 10–12 mg of manganese. Manganese deficiency is not known to occur other than in experimentally induced cases (Hardy 2009). Overexposure to manganese from environmental or industrial sources can lead to the neurological condition know as manganism, symptoms of which are similar to Parkinson's disease (Roth 2006). Manganism differs from Parkinson's in that patients do not respond to dopamine replacement therapy (Martin 2006). Despite the fact that this condition was first described in 1837, little is know about the exact mechanism of neurotoxicity; however, lesions in the globus pallidus have been observed with some consistency in post mortem examinations of brains effected by manganism (Perl and Olanow 2007).

Hailey–Hailey disease is an autosomal dominant condition that involves mutations in the gene for the human homolog of the Golgi Ca/Mn transporter *PMR1*, *ATP2C1* (Hu et al. 2000; Sudbrak et al. 2000). The disease is characterized by fluctuations of itchy painful skin lesions that occur in middle age and are brought on by friction, sweating and UV radiation with the cause related to a loss of cell–cell adhesion in the suprabasal layer of the epidermis (Kellermayer 2005). More than 82 mutations have been identified as contributing to the disease and most of the pathology on a molecular level has been attributed to calcium homeostasis; as a result, it is unclear what if any role manganese plays in the disease (Missiaen et al. 2004).

Prion proteins have been shown to be involved in the link between manganese and neurotoxicity. Cells expressing normal prion protein were found to be less susceptible to manganese-induced cytotoxicity compared to cells lacking the protein. Further results from this study showed that the prion protein interferes with manganese uptake and protects against manganese induced oxidative stress and subsequent apoptotic cell death (Choi et al. 2007). This provides the basis for the implication of altered manganese homeostasis in prion diseases.

Cobalt and nickel

Cobalt and nickel are both elements with limited function in higher eukaryotes. The only known function of cobalt is as a component of vitamin B12 which is incorporated into three classes of enzymes: isomerases, methyl transferases and reductive dehalogenases. These enzymes participate in reactions essential to DNA synthesis, fatty acid synthesis and energy production among other biological processes (Banerjee and Ragsdale 2003). The role of nickel is more prevalent in bacteria (Kaluarachchi et al. 2010) and plants (Tejada-Jimenez et al. 2009). Concentrations of cobalt and nickel are sufficiently higher in anaerobic prokaryotes; this is thought to represent the extensive use of cobalt and nickel in the early evolution of life in an atmosphere lacking oxygen. Upon the oxygenation of the atmosphere, zinc and copper became bioavailable and have a number of chemical advantages over nickel and cobalt which led to the evolution of systems where copper and zinc replaced cobalt and nickel (Frausto da Silva and Williams 2001c).

Molecular details of cobalt and nickel uptake in *S. cerevisiae* are not well known but it is likely that they are transported into the cell by broad-specificity divalent metal transporters as cobalt is known to competitively inhibit iron uptake (Lesuisse et al. 1987) and mutant strains with reduced magnesium uptake are resistant to both nickel and cobalt (Joho et al. 1991). Excess cobalt is transported into the vacuole by the vacuolar transporter Cot1p that functions in cobalt accumulation and detoxification (Conklin et al. 1992). Similarly, nickel detoxification also involves vacuolar sequestration of the ion (Joho et al. 1995) but the mechanism by which this occurs

is unknown but is dependent on a proton gradient (Nishimura et al. 1998).

Cobalt and nickel may have a minimal role in normal biological function of cells but both metals are toxic in excess—a situation that often occurs as a result of overexposure often in industrial occupations. Overexposure to cobalt has been shown to result in asthma, pneumonia and wheezing and has been associated with a higher risk of lung cancer when the cobalt is inhaled (Valko et al. 2005). Cobalt ions can directly induce DNA damage, interfere with DNA repair, DNA–protein crosslinking and sister chromatid exchange (Leonard et al. 1998; Hengstler et al. 2003). The exact mechanism for cobalt carcinogenicity remains to be elucidated but it does involve the generation of reactive oxygen species (De Boeck et al. 2003). Nickel exposure can result in dermatitis, cardiovascular and kidney diseases and cancer. The molecular mechanism of nickel carcinogenesis is not fully understood but does not seem to be entirely dependent on oxidative stress (Gilman 1962). Nickel is also not known to interact directly with DNA, current hypotheses on the mechanisms of nickel toxicity involve nickel binding to other molecules in the cell and exerting downstream effects (Kasprzak et al. 2003).

Conclusion

As reflected by the human diseases that result from nutritional deficiencies, transition metal ions play a central role in a variety of biological processes. However, when not properly regulated, transition metal ions can be extremely toxic as shown by both the symptoms of over-exposure to a metal and inherited gene mutations that inhibit regulatory processes. Although much is known about the uptake mechanisms for these elements and how they are employed biologically, there is still much to be learned about the mechanisms by which their levels are controlled intracellularly to maintain the balance between necessary and toxic levels of metal ions. One of the most useful tools in further elucidating such mechanisms is the model organism *S. cerevisiae*. The life cycle and molecular biology of yeast make identification and characterization of gene products easier and more cost effective. As technology progresses, the tools for high-throughput screening of yeast libraries will continue to advance with the resulting identification of novel genes that function in transition metal homeostasis. As misregulation of transition metals is becoming increasingly implicated in neurodegenerative disorders, identification of novel genes and pathways that protect cells against the potential damaging effects will be of increasing importance in diagnosis and treatment of such diseases.

Acknowledgments This work was supported in part by a grant from the Canadian Institute for Health Research (to RTAM). MRB was supported by a graduate fellowship from the Strategic Training Program in Transfusion Science funded by CIHR and the Heart & Stroke Foundation of Canada.

References

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

Ala A, Walker AP et al (2007) Wilson's disease. Lancet 369(9559):397–408

Ali SF, Duhart HM et al (1995) Manganese-induced reactive oxygen species: comparison between Mn+2 and Mn+3. Neurodegeneration 4(3):329–334

Altamura S, Muckenthaler MU (2009) Iron toxicity in diseases of aging: Alzheimer's disease, Parkinson's disease and atherosclerosis. J Alzheimers Dis 16(4):879–895

Amalinei C, Caruntu ID et al (2007) Biology of metalloproteinases. Rom J Morphol Embryol 48(4):323–334

Andrews NC (1999) Disorders of iron metabolism. N Engl J Med 341(26):1986–1995

Andrews NC, Schmidt PJ (2007) Iron homeostasis. Annu Rev Physiol 69:69–85

Arita A, Zhou X et al (2009) A genome-wide deletion mutant screen identifies pathways affected by nickel sulfate in *Saccharomyces cerevisiae*. BMC Genomics 10:524

Askwith C, Eide D et al (1994) The FET3 gene of *S. cerevisiae* encodes a multicopper oxidase required for ferrous iron uptake. Cell 76(2):403–410

Atwood CS, Obrenovich ME et al (2003) Amyloid-beta: a chameleon walking in two worlds: a review of the trophic and toxic properties of amyloid-beta. Brain Res Brain Res Rev 43(1):1–16

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

Banerjee R, Ragsdale SW (2003) The many faces of vitamin B12: catalysis by cobalamin-dependent enzymes. Annu Rev Biochem 72:209–247

Beinert H (2000) Iron-sulfur proteins: ancient structures, still full of surprises. J Biol Inorg Chem 5(1):2–15

Bermingham-McDonogh O, Gralla EB et al (1988) The copper, zinc-superoxide dismutase gene of *Saccharomyces cerevisiae*: cloning, sequencing, and biological activity. Proc Natl Acad Sci USA 85(13):4789–4793

Berthelet S, Usher J et al (2010) Functional genomics analysis of the *Saccharomyces cerevisiae* iron responsive

transcription factor Aft1 reveals iron-independent functions. Genetics 185(3):1111–1128
Bird A, Evans-Galea MV et al (2000) Mapping the DNA binding domain of the Zap1 zinc-responsive transcriptional activator. J Biol Chem 275(21):16160–16166
Bird AJ, McCall K et al (2003) Zinc fingers can act as Zn2+ sensors to regulate transcriptional activation domain function. EMBO J 22(19):5137–5146
Bird AJ, Blankman E et al (2004) The Zap1 transcriptional activator also acts as a repressor by binding downstream of the TATA box in ZRT2. EMBO J 23(5):1123–1132
Bleackley MR, Wong AY et al (2009) Blood iron homeostasis: newly discovered proteins and iron imbalance. Transfus Med Rev 23(2):103–123
Bleackley MR, Young BP et al (2011) High density array screening to identify the genetic requirements for transition metal tolerance in *Saccharomyces cerevisiae*. Metallomics 3(2):195–205
Blindauer CA, Leszczyszyn OI (2010) Metallothioneins: unparalleled diversity in structures and functions for metal ion homeostasis and more. Nat Prod Rep 27(5):720–741
Bode HP, Dumschat M et al (1995) Iron sequestration by the yeast vacuole. A study with vacuolar mutants of *Saccharomyces cerevisiae*. Eur J Biochem 228(2):337–342
Bonaccorsi di Patti MC, Miele R et al (2005) The yeast multicopper oxidase Fet3p and the iron permease Ftr1p physically interact. Biochem Biophys Res Commun 333(2): 432–437
Borden KL, Freemont PS (1996) The RING finger domain: a recent example of a sequence-structure family. Curr Opin Struct Biol 6(3):395–401
Bovallius A, Zacharias B (1971) Variations in the metal content of some commercial media and their effect on microbial growth. Appl Microbiol 22(3):260–262
Braun NA, Morgan B et al (2010) The yeast CLC protein counteracts vesicular acidification during iron starvation. J Cell Sci 123(Pt 13):2342–2350
Bruijnincx PC, van Koten G et al (2008) Mononuclear nonheme iron enzymes with the 2-His-1-carboxylate facial triad: recent developments in enzymology and modeling studies. Chem Soc Rev 37(12):2716–2744
Bull PC, Thomas GR et al (1993) The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat Genet 5(4):327–337
Bulteau AL, O'Neill HA et al (2004) Frataxin acts as an iron chaperone protein to modulate mitochondrial aconitase activity. Science 305(5681):242–245
Bun-Ya M, Nishimura M et al (1991) The PHO84 gene of *Saccharomyces cerevisiae* encodes an inorganic phosphate transporter. Mol Cell Biol 11(6):3229–3238
Camaschella C (2009) Hereditary sideroblastic anemias: pathophysiology, diagnosis, and treatment. Semin Hematol 46(4):371–377
Campuzano V, Montermini L et al (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 271(5254):1423–1427
Chen XZ, Peng JB et al (1999) Yeast SMF1 mediates H(+)-coupled iron uptake with concomitant uncoupled cation currents. J Biol Chem 274(49):35089–35094
Chen OS, Crisp RJ et al (2004) Transcription of the yeast iron regulon does not respond directly to iron but rather to iron-sulfur cluster biosynthesis. J Biol Chem 279(28): 29513–29518
Choi CJ, Anantharam V et al (2007) Normal cellular prion protein protects against manganese-induced oxidative stress and apoptotic cell death. Toxicol Sci 98(2):495–509
Cohen A, Nelson H et al (2000) The family of SMF metal ion transporters in yeast cells. J Biol Chem 275(43):33388–33394
Conklin DS, McMaster JA et al (1992) COT1, a gene involved in cobalt accumulation in *Saccharomyces cerevisiae*. Mol Cell Biol 12(9):3678–3688
Cooper CE, Nicholls P et al (1991) Cytochrome c oxidase: structure, function, and membrane topology of the polypeptide subunits. Biochem Cell Biol 69(9):586–607
Courel M, Lallet S et al (2005) Direct activation of genes involved in intracellular iron use by the yeast iron-responsive transcription factor Aft2 without its paralog Aft1. Mol Cell Biol 25(15):6760–6771
Crichton RR, Pierre JL (2001) Old iron, young copper: from Mars to Venus. Biometals 14(2):99–112
Crow JP, Beckman JS et al (1995) Sensitivity of the essential zinc-thiolate moiety of yeast alcohol dehydrogenase to hypochlorite and peroxynitrite. Biochemistry 34(11):3544–3552
Cuajungco MP, Lees GJ (1997) Zinc and Alzheimer's disease: is there a direct link? Brain Res Brain Res Rev 23(3): 219–236
Cuajungco MP, Goldstein LE et al (2000) Evidence that the beta-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of abeta by zinc. J Biol Chem 275(26):19439–19442
Culotta V (2010) Cell biology of copper. J Biol Inorg Chem 15(1):1–2
Culotta VC, Yang M et al (2005) Manganese transport and trafficking: lessons learned from *Saccharomyces cerevisiae*. Eukaryot Cell 4(7):1159–1165
Curtis AR, Fey C et al (2001) Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. Nat Genet 28(4):350–354
Dancis A, Haile D et al (1994a) The *Saccharomyces cerevisiae* copper transport protein (Ctr1p). Biochemical characterization, regulation by copper, and physiologic role in copper uptake. J Biol Chem 269(41):25660–25667
Dancis A, Yuan DS et al (1994b) Molecular characterization of a copper transport protein in *S. cerevisiae*: an unexpected role for copper in iron transport. Cell 76(2):393–402
Davis-Kaplan 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 Bie P, Muller P et al (2007) Molecular pathogenesis of Wilson and Menkes disease: correlation of mutations with molecular defects and disease phenotypes. J Med Genet 44(11):673–688
De Boeck M, Kirsch-Volders M et al (2003) Cobalt and antimony: genotoxicity and carcinogenicity. Mutat Res 533(1–2): 135–152
Deng HX, Hentati A et al (1993) Amyotrophic lateral sclerosis and structural defects in Cu, Zn superoxide dismutase. Science 261(5124):1047–1051
Desai V, Kaler SG (2008) Role of copper in human neurological disorders. Am J Clin Nutr 88(3):855S–858S

Devireddy LR, Hart DO et al (2010) A mammalian siderophore synthesized by an enzyme with a bacterial homolog involved in enterobactin production. Cell 141(6):1006–1017

Dexter DT, Carayon A et al (1991) Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. Brain 114(Pt 4):1953–1975

Dingwall C (2007) A copper-binding site in the cytoplasmic domain of BACE1 identifies a possible link to metal homoeostasis and oxidative stress in Alzheimer's disease. Biochem Soc Trans 35(Pt 3):571–573

Dix DR, Bridgham JT et al (1994) The FET4 gene encodes the low affinity Fe(II) transport protein of *Saccharomyces cerevisiae*. J Biol Chem 269(42):26092–26099

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

Dujon B (1996) The yeast genome project: what did we learn? Trends Genet 12(7):263–270

Durai S, Mani M et al (2005) Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells. Nucleic Acids Res 33(18): 5978–5990

Durr G, Strayle J et al (1998) The medial-Golgi ion pump Pmr1 supplies the yeast secretory pathway with Ca2+ and Mn2+ required for glycosylation, sorting, and endoplasmic reticulum-associated protein degradation. Mol Biol Cell 9(5):1149–1162

Eide DJ (2003) Multiple regulatory mechanisms maintain zinc homeostasis in *Saccharomyces cerevisiae*. J Nutr 133(5 Suppl 1):1532S–1535S

Eide DJ (2004) The SLC39 family of metal ion transporters. Pflugers Arch 447(5):796–800

Eide DJ (2006) Zinc transporters and the cellular trafficking of zinc. Biochim Biophys Acta 1763(7):711–722

Eide DJ (2009) Homeostatic and adaptive responses to zinc deficiency in *Saccharomyces cerevisiae*. J Biol Chem 284(28):18565–18569

Eklund H, Uhlin U et al (2001) Structure and function of the radical enzyme ribonucleotide reductase. Prog Biophys Mol Biol 77(3):177–268

Emdin SO, Dodson GG et al (1980) Role of zinc in insulin biosynthesis. Some possible zinc-insulin interactions in the pancreatic B-cell. Diabetologia 19(3):174–182

Eng BH, Guerinot ML et al (1998) Sequence analyses and phylogenetic characterization of the ZIP family of metal ion transport proteins. J Membr Biol 166(1):1–7

Ferenci P (2006) Regional distribution of mutations of the ATP7B gene in patients with Wilson disease: impact on genetic testing. Hum Genet 120(2):151–159

Ferreira GC, Franco R et al (1995) Structure and function of ferrochelatase. J Bioenerg Biomembr 27(2):221–229

Fischer Walker CL, Ezzati M et al (2009) Global and regional child mortality and burden of disease attributable to zinc deficiency. Eur J Clin Nutr 63(5):591–597

Fox JH, Kama JA et al (2007) Mechanisms of copper ion mediated Huntington's disease progression. PLoS One 2(3):e334

Fraker PJ, Haas SM et al (1977) Effect of zinc deficiency on the immune response of the young adult A/J mouse. J Nutr 107(10):1889–1895

Fraker PJ, DePasquale-Jardieu P et al (1978) Regeneration of T-cell helper function in zinc-deficient adult mice. Proc Natl Acad Sci USA 75(11):5660–5664

Frausto da Silva JJR, Williams RJP (2001a) Copper: extracytoplasmic oxidases and matrix formation. In: Frausto da Silva JJR, Williams RJP (eds) The biological chemistry of the elements, 2nd edn. Oxford University Press, Oxford

Frausto da Silva JJR, Williams RJP (2001b) Manganese: dioxygen evolution and glycosylation. In: Frausto da Silva JJR, Williams RJP (eds) The biological chemistry of the elements, 2nd edn. Oxford University Press, Oxford

Frausto da Silva JJR, Williams RJP (2001c) Nickel and cobalt: remnants of early life? In: Frausto da Silva JJR, Williams RJP (eds) The biological chemistry of the elements, 2nd edn. Oxford University Press, Oxford

Frei R, Gaucher C et al (2009) Fluctuations in Precambrian atmospheric oxygenation recorded by chromium isotopes. Nature 461(7261):250–253

Friedlich AL, Tanzi RE et al (2007) The 5′-untranslated region of Parkinson's disease alpha-synuclein messengerRNA contains a predicted iron responsive element. Mol Psychiatry 12(3):222–223

Fu D, Beeler TJ et al (1995) Sequence, mapping and disruption of CCC2, a gene that cross-complements the Ca(2+)-sensitive phenotype of csg1 mutants and encodes a P-type ATPase belonging to the Cu(2+)-ATPase subfamily. Yeast 11(3):283–292

Geier BM, Schagger H et al (1995) Kinetic properties and ligand binding of the eleven-subunit cytochrome-c oxidase from *Saccharomyces cerevisiae* isolated with a novel large-scale purification method. Eur J Biochem 227(1–2): 296–302

Georgatsou E, Alexandraki D (1994) Two distinctly regulated genes are required for ferric reduction, the first step of iron uptake in *Saccharomyces cerevisiae*. Mol Cell Biol 14(5):3065–3073

Gerber J, Muhlenhoff U et al (2003) An interaction between frataxin and Isu1/Nfs1 that is crucial for Fe/S cluster synthesis on Isu1. EMBO Rep 4(9):906–911

Giaever G, Chu AM et al (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418(6896): 387–391

Gilman JP (1962) Metal carcinogenesis. II. A study on the carcinogenic activity of cobalt, copper, iron, and nickel compounds. Cancer Res 22:158–162

Gitan RS, Luo H et al (1998) Zinc-induced inactivation of the yeast ZRT1 zinc transporter occurs through endocytosis and vacuolar degradation. J Biol Chem 273(44):28617–28624

Goffeau A, Barrell BG et al (1996) Life with 6000 genes. Science 274(5287):546, 563–567

Gola S, Martin R et al (2003) New modules for PCR-based gene targeting in Candida albicans: rapid and efficient gene targeting using 100 bp of flanking homology region. Yeast 20(16):1339–1347

Graham RK, Deng Y et al (2006) Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. Cell 125(6):1179–1191

Guldener U, Munsterkotter M et al (2005) CYGD: the comprehensive yeast genome database. Nucleic Acids Res 33(Database issue):D364–D368

Gurney ME, Pu H et al (1994) Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science 264(5166):1772–1775

Halsted JA, Ronaghy HA et al (1972) Zinc deficiency in man. The Shiraz experiment. Am J Med 53(3):277–284

Hambidge KM, Krebs NF (2007) Zinc deficiency: a special challenge. J Nutr 137(4):1101–1105

Hardy G (2009) Manganese in parenteral nutrition: who, when, and why should we supplement? Gastroenterology 137(5 Suppl):S29–S35

Harris WR (2002) Molecular and cellular iron transport. New York, Marcel Dekker Inc

Harris ZL, Klomp LW et al (1998) Aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis. Am J Clin Nutr 67(5 Suppl):972S–977S

Harrison MD, Jones CE et al (1999) Copper chaperones: function, structure and copper-binding properties. J Biol Inorg Chem 4(2):145–153

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(Pt 2):477–484

Hengstler JG, Bolm-Audorff U et al (2003) Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. Carcinogenesis 24(1):63–73

Herbig A, Bird AJ et al (2005) Zap1 activation domain 1 and its role in controlling gene expression in response to cellular zinc status. Mol Microbiol 57(3):834–846

Hooper NM (1994) Families of zinc metalloproteases. FEBS Lett 354(1):1–6

Horng YC, Cobine PA et al (2004) Specific copper transfer from the Cox17 metallochaperone to both Sco1 and Cox11 in the assembly of yeast cytochrome C oxidase. J Biol Chem 279(34):35334–35340

Horsburgh MJ, Wharton SJ et al (2002) Manganese: elemental defence for a life with oxygen. Trends Microbiol 10(11): 496–501

Howitt J, Putz U et al (2009) Divalent metal transporter 1 (DMT1) regulation by Ndfip1 prevents metal toxicity in human neurons. Proc Natl Acad Sci USA 106(36): 15489–15494

Hu Z, Bonifas JM et al (2000) Mutations in ATP2C1, encoding a calcium pump, cause Hailey-Hailey disease. Nat Genet 24(1):61–65

Iolascon A, De Falco L et al (2009) Molecular basis of inherited microcytic anemia due to defects in iron acquisition or heme synthesis. Haematologica 94(3):395–408

Irazusta V, Cabiscol E et al (2006) Manganese is the link between frataxin and iron-sulfur deficiency in the yeast model of Friedreich ataxia. J Biol Chem 281(18):12227–12232

Jensen LT, Ajua-Alemanji M et al (2003) The *Saccharomyces cerevisiae* high affinity phosphate transporter encoded by PHO84 also functions in manganese homeostasis. J Biol Chem 278(43):42036–42040

Jensen LT, Carroll MC et al (2009) Down-regulation of a manganese transporter in the face of metal toxicity. Mol Biol Cell 20(12):2810–2819

Jo WJ, Loguinov A et al (2008) Identification of genes involved in the toxic response of *Saccharomyces cerevisiae* against iron and copper overload by parallel analysis of deletion mutants. Toxicol Sci 101(1):140–151

Joho M, Tarumi K et al (1991) Co2+ and Ni2+ resistance in *Saccharomyces cerevisiae* associated with a reduction in the accumulation of Mg2+. Microbios 67(272–273): 177–186

Joho M, Inouhe M et al (1995) Nickel resistance mechanisms in yeasts and other fungi. J Ind Microbiol 14(2):164–168

Kaluarachchi H, Chan Chung KC et al (2010) Microbial nickel proteins. Nat Prod Rep 27(5):681–694

Kasprzak KS, Sunderman FW Jr et al (2003) Nickel carcinogenesis. Mutat Res 533(1–2):67–97

Kellermayer R (2005) Hailey-Hailey disease as an orthodisease of PMR1 deficiency in *Saccharomyces cerevisiae*. FEBS Lett 579(10):2021–2025

Klomp LW, Lin SJ et al (1997) Identification and functional expression of HAH1, a novel human gene involved in copper homeostasis. J Biol Chem 272(14):9221–9226

Klug A (2010) The discovery of zinc fingers and their applications in gene regulation and genome manipulation. Annu Rev Biochem 79:213–231

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

Kosman DJ (2003) Molecular mechanisms of iron uptake in fungi. Mol Microbiol 47(5):1185–1197

Krishna SS, Majumdar I et al (2003) Structural classification of zinc fingers: survey and summary. Nucleic Acids Res 31(2): 532–550

Kumanovics A, Chen OS et al (2008) Identification of FRA1 and FRA2 as genes involved in regulating the yeast iron regulon in response to decreased mitochondrial iron-sulfur cluster synthesis. J Biol Chem 283(16):10276–10286

Kumar N (2006) Copper deficiency myelopathy (human swayback). Mayo Clin Proc 81(10):1371–1384

Kwok EY, Severance S et al (2006) Evidence for iron channeling in the Fet3p-Ftr1p high-affinity iron uptake complex in the yeast plasma membrane. Biochemistry 45(20): 6317–6327

La Fontaine S, Mercer JF (2007) Trafficking of the copper-ATPases, ATP7A and ATP7B: role in copper homeostasis. Arch Biochem Biophys 463(2):149–167

Labbe S, Zhu Z 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

Laity JH, Lee BM et al (2001) Zinc finger proteins: new insights into structural and functional diversity. Curr Opin Struct Biol 11(1):39–46

Lander ES, Linton LM et al (2001) Initial sequencing and analysis of the human genome. Nature 409(6822):860–921

Lapinskas PJ, Cunningham KW et al (1995) Mutations in PMR1 suppress oxidative damage in yeast cells lacking superoxide dismutase. Mol Cell Biol 15(3):1382–1388

Larsson S, Kallebring B et al (1995) The CuA center of cytochrome-c oxidase: electronic structure and spectra of models compared to the properties of CuA domains. Proc Natl Acad Sci USA 92(16):7167–7171

Leonard S, Gannett PM et al (1998) Cobalt-mediated generation of reactive oxygen species and its possible mechanism. J Inorg Biochem 70(3–4):239–244

Lesuisse E, Raguzzi F et al (1987) Iron uptake by the yeast *Saccharomyces cerevisiae*: involvement of a reduction step. J Gen Microbiol 133(11):3229–3236

Lesuisse E, Santos R et al (2003) Iron use for haeme synthesis is under control of the yeast frataxin homologue (Yfh1). Hum Mol Genet 12(8):879–889

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

Lill R, Muhlenhoff U (2006) Iron-sulfur protein biogenesis in eukaryotes: components and mechanisms. Annu Rev Cell Dev Biol 22:457–486

Lin SJ, Culotta VC (1995) The ATX1 gene of *Saccharomyces cerevisiae* encodes a small metal homeostasis factor that protects cells against reactive oxygen toxicity. Proc Natl Acad Sci USA 92(9):3784–3788

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

Lin H, Kumanovics A et al (2008) A single amino acid change in the yeast vacuolar metal transporters ZRC1 and COT1 alters their substrate specificity. J Biol Chem 283(49):33865–33873

Lindskog S (1997) Structure and mechanism of carbonic anhydrase. Pharmacol Ther 74(1):1–20

Lippard SJ, Berg JM (1994) Principles of bioinorganic chemistry. University Science Books, Mill Valley

Luk EE, Culotta VC (2001) Manganese superoxide dismutase in *Saccharomyces cerevisiae* acquires its metal co-factor through a pathway involving the Nramp metal transporter, Smf2p. J Biol Chem 276(50):47556–47562

Luk E, Carroll M et al (2003) Manganese activation of superoxide dismutase 2 in *Saccharomyces cerevisiae* requires MTM1, a member of the mitochondrial carrier family. Proc Natl Acad Sci USA 100(18):10353–10357

Lyons TJ, Gasch AP et al (2000) Genome-wide characterization of the Zap1p zinc-responsive regulon in yeast. Proc Natl Acad Sci USA 97(14):7957–7962

MacDiarmid CW, Gaither LA et al (2000) Zinc transporters that regulate vacuolar zinc storage in *Saccharomyces cerevisiae*. EMBO J 19(12):2845–2855

MacDiarmid CW, Milanick MA et al (2002) Biochemical properties of vacuolar zinc transport systems of *Saccharomyces cerevisiae*. J Biol Chem 277(42):39187–39194

MacDiarmid CW, Milanick MA et al (2003) Induction of the ZRC1 metal tolerance gene in zinc-limited yeast confers resistance to zinc shock. J Biol Chem 278(17):15065–15072

MacPherson IS, Murphy ME (2007) Type-2 copper-containing enzymes. Cell Mol Life Sci 64(22):2887–2899

Makeyev AV, Liebhaber SA (2002) The poly(C)-binding proteins: a multiplicity of functions and a search for mechanisms. RNA 8(3):265–278

Maret W, Sandstead HH (2006) Zinc requirements and the risks and benefits of zinc supplementation. J Trace Elem Med Biol 20(1):3–18

Martin CJ (2006) Manganese neurotoxicity: connecting the dots along the continuum of dysfunction. Neurotoxicology 27(3):347–349

Martins LJ, Jensen LT et al (1998) Metalloregulation of FRE1 and FRE2 homologs in *Saccharomyces cerevisiae*. J Biol Chem 273(37):23716–23721

Matile P, Wiemken A (1967) The vacuole as the lysosome of the yeast cell. Arch Mikrobiol 56(2):148–155

Matthews JM, Sunde M (2002) Zinc fingers—folds for many occasions. IUBMB Life 54(6):351–355

McCall KA, Huang C et al (2000) Function and mechanism of zinc metalloenzymes. J Nutr 130(5S Suppl):1437S–1446S

McGregor JA, Shayeghi M et al (2005) Impaired iron transport activity of ferroportin 1 in hereditary iron overload. J Membr Biol 206(1):3–7

Miller Y, Ma B et al (2010) Zinc ions promote Alzheimer Abeta aggregation via population shift of polymorphic states. Proc Natl Acad Sci USA 107(21):9490–9495

Missiaen L, Raeymaekers L et al (2004) SPCA1 pumps and Hailey-Hailey disease. Biochem Biophys Res Commun 322(4):1204–1213

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

Muhlenhoff U, Stadler JA et al (2003) A specific role of the yeast mitochondrial carriers MRS3/4p in mitochondrial iron acquisition under iron-limiting conditions. J Biol Chem 278(42):40612–40620

Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270(45): 26723–26726

Nishida CR, Gralla EB et al (1994) Characterization of three yeast copper-zinc superoxide dismutase mutants analogous to those coded for in familial amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 91(21):9906–9910

Nishimura K, Igarashi K et al (1998) Proton gradient-driven nickel uptake by vacuolar membrane vesicles of *Saccharomyces cerevisiae*. J Bacteriol 180(7):1962–1964

Ono Y, Fujii T et al (1989) Phorbol ester binding to protein kinase C requires a cysteine-rich zinc-finger-like sequence. Proc Natl Acad Sci USA 86(13):4868–4871

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

Ostrerova-Golts N, Petrucelli L et al (2000) The A53T alpha-synuclein mutation increases iron-dependent aggregation and toxicity. J Neurosci 20(16):6048–6054

Ottolenghi AC (1965) Phospholipase C from *Bacillus cereus*, a zinc-requiring metalloenzyme. Biochim Biophys Acta 106(3):510–518

Outten CE, O'Halloran TV (2001) Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. Science 292(5526):2488–2492

Pagani MA, Casamayor A et al (2007) Disruption of iron homeostasis in *Saccharomyces cerevisiae* by high zinc levels: a genome-wide study. Mol Microbiol 65(2):521–537

Pan KM, Stahl N et al (1992) Purification and properties of the cellular prion protein from Syrian hamster brain. Protein Sci 1(10):1343–1352

Pantopoulos K (2004) Iron metabolism and the IRE/IRP regulatory system: an update. Ann N Y Acad Sci 1012:1–13

Pavletich NP, Pabo CO (1991) Zinc finger-DNA recognition: crystal structure of a Zif268-DNA complex at 2.1 A. Science 252(5007):809–817

Perl DP, Olanow CW (2007) The neuropathology of manganese-induced Parkinsonism. J Neuropathol Exp Neurol 66(8):675–682

Philpott CC, Protchenko O et al (2002) The response to iron deprivation in *Saccharomyces cerevisiae*: expression of siderophore-based systems of iron uptake. Biochem Soc Trans 30(4):698–702

Pierre JL, Fontecave M (1999) Iron and activated oxygen species in biology: the basic chemistry. Biometals 12(3): 195–199

Pietrangelo A (2004) Hereditary hemochromatosis—a new look at an old disease. N Engl J Med 350(23):2383–2397

Plocke DJ, Levinthal C et al (1962) Alkaline phosphatase of *Escherichia coli*: a zinc metalloenzyme. Biochemistry 1: 373–378

Ponka P (1999) Cell biology of heme. Am J Med Sci 318(4): 241–256

Ponka P, Sheftel AD et al (2002) Iron targeting to mitochondria in erythroid cells. Biochem Soc Trans 30(4):735–738

Portnoy ME, Liu XF et al (2000) *Saccharomyces cerevisiae* expresses three functionally distinct homologues of the nramp family of metal transporters. Mol Cell Biol 20(21): 7893–7902

Portnoy ME, Jensen LT et al (2002) The distinct methods by which manganese and iron regulate the Nramp transporters in yeast. Biochem J 362(Pt 1):119–124

Prasad AS (2009) Zinc: role in immunity, oxidative stress and chronic inflammation. Curr Opin Clin Nutr Metab Care 12(6):646–652

Prasad AS, Miale A Jr et al (1990) Clinical and experimental. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. 1963. J Lab Clin Med 116(5):737–749

Puig S, Lee J et al (2002) Biochemical and genetic analyses of yeast and human high affinity copper transporters suggest a conserved mechanism for copper uptake. J Biol Chem 277(29):26021–26030

Radisky D, Kaplan J (1999) Regulation of transition metal transport across the yeast plasma membrane. J Biol Chem 274(8):4481–4484

Rana A, Gnaneswari D et al (2009) Prion metal interaction: is prion pathogenesis a cause or a consequence of metal imbalance? Chem Biol Interact 181(3):282–291

Rasia RM, Bertoncini CW et al (2005) Structural characterization of copper(II) binding to alpha-synuclein: insights into the bioinorganic chemistry of Parkinson's disease. Proc Natl Acad Sci USA 102(12):4294–4299

Ravindranath SD, Fridovich I (1975) Isolation and characterization of a manganese-containing superoxide dismutase from yeast. J Biol Chem 250(15):6107–6112

Reddi AR, Jensen LT et al (2009a) Manganese homeostasis in *Saccharomyces cerevisiae*. Chem Rev 109(10):4722–4732

Reddi AR, Jensen LT et al (2009b) The overlapping roles of manganese and Cu/Zn SOD in oxidative stress protection. Free Radic Biol Med 46(2):154–162

Rees EM, Lee J et al (2004) Mobilization of intracellular copper stores by the ctr2 vacuolar copper transporter. J Biol Chem 279(52):54221–54229

Robinson NJ, Winge DR (2010) Copper metallochaperones. Annu Rev Biochem 79:537–562

Robson KJ, Merryweather-Clarke AT et al (2004) Recent advances in understanding haemochromatosis: a transition state. J Med Genet 41(10):721–730

Roetto A, Totaro A et al (2001) New mutations inactivating transferrin receptor 2 in hemochromatosis type 3. Blood 97(9):2555–2560

Rogers JT, Randall JD et al (2002) An iron-responsive element type II in the 5′-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem 277(47): 45518–45528

Rosen DR, Siddique T et al (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362(6415):59–62

Roth JA (2006) Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. Biol Res 39(1):45–57

Rund D, Fucharoen S (2008) Genetic modifiers in hemoglobinopathies. Curr Mol Med 8(7):600–608

Rutherford JC, Bird AJ (2004) Metal-responsive transcription factors that regulate iron, zinc, and copper homeostasis in eukaryotic cells. Eukaryot Cell 3(1):1–13

Rutherford JC, Jaron S et al (2001) A second iron-regulatory system in yeast independent of Aft1p. Proc Natl Acad Sci USA 98(25):14322–14327

Santamaria AB, Sulsky SI (2010) Risk assessment of an essential element: manganese. J Toxicol Environ Health A 73(2):128–155

Saurin AJ, Borden KL et al (1996) Does this have a familiar RING? Trends Biochem Sci 21(6):208–214

Schmidt PJ, Ramos-Gomez M et al (1999) A gain of superoxide dismutase (SOD) activity obtained with CCS, the copper metallochaperone for SOD1. J Biol Chem 274(52): 36952–36956

Seetharaman SV, Prudencio M et al (2009) Immature copper-zinc superoxide dismutase and familial amyotrophic lateral sclerosis. Exp Biol Med (Maywood) 234(10):1140–1154

Sera T (2009) Zinc-finger-based artificial transcription factors and their applications. Adv Drug Deliv Rev 61(7–8): 513–526

Shakoury-Elizeh M, Tiedeman J et al (2004) Transcriptional remodeling in response to iron deprivation in *Saccharomyces cerevisiae*. Mol Biol Cell 15(3):1233–1243

Shakoury-Elizeh M, Protchenko O et al (2010) Metabolic response to iron deficiency in *Saccharomyces cerevisiae*. J Biol Chem 285(19):14823–14833

Shankar AH, Prasad AS (1998) Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68(2 Suppl):447S–463S

Shaw GC, Cope JJ et al (2006) Mitoferrin is essential for erythroid iron assimilation. Nature 440(7080):96–100

Shi W, Zhan C et al (2005) Metalloproteomics: high-throughput structural and functional annotation of proteins in structural genomics. Structure 13(10):1473–1486

Shi H, Bencze KZ et al (2008) A cytosolic iron chaperone that delivers iron to ferritin. Science 320(5880):1207–1210

Simm C, Lahner B et al (2007) *Saccharomyces cerevisiae* vacuole in zinc storage and intracellular zinc distribution. Eukaryot Cell 6(7):1166–1177

Singh A, Severance S et al (2006) Assembly, activation, and trafficking of the Fet3p.Ftr1p high affinity iron permease

complex in *Saccharomyces cerevisiae*. J Biol Chem 281(19):13355–13364
Singh A, Kaur N et al (2007) The metalloreductase Fre6p in Fe-efflux from the yeast vacuole. J Biol Chem 282(39): 28619–28626
Stadler JA, Schweyen RJ (2002) The yeast iron regulon is induced upon cobalt stress and crucial for cobalt tolerance. J Biol Chem 277(42):39649–39654
Stearman R, Yuan DS et al (1996) A permease-oxidase complex involved in high-affinity iron uptake in yeast. Science 271(5255):1552–1557
Stimpson HE, Lewis MJ et al (2006) Transferrin receptor-like proteins control the degradation of a yeast metal transporter. EMBO J 25(4):662–672
Sudbrak R, Brown J et al (2000) Hailey-Hailey disease is caused by mutations in ATP2C1 encoding a novel Ca(2+) pump. Hum Mol Genet 9(7):1131–1140
Sullivan JA, Lewis MJ et al (2007) Multiple interactions drive adaptor-mediated recruitment of the ubiquitin ligase rsp5 to membrane proteins in vivo and in vitro. Mol Biol Cell 18(7):2429–2440
Supek F, Supekova L et al (1996) A yeast manganese transporter related to the macrophage protein involved in conferring resistance to mycobacteria. Proc Natl Acad Sci USA 93(10):5105–5110
Takumi S, Kimura H et al (2010) DNA microarray analysis of genomic responses of yeast *Saccharomyces cerevisiae* to nickel chloride. J Toxicol Sci 35(1):125–129
Tapiero H, Tew KD (2003) Trace elements in human physiology and pathology: zinc and metallothioneins. Biomed Pharmacother 57(9):399–411
Tejada-Jimenez M, Galvan A et al (2009) Homeostasis of the micronutrients Ni, Mo and Cl with specific biochemical functions. Curr Opin Plant Biol 12(3):358–363
Tommos C, Hoganson CW et al (1998) Manganese and tyrosyl radical function in photosynthetic oxygen evolution. Curr Opin Chem Biol 2(2):244–252
Ton VK, Mandal D et al (2002) Functional expression in yeast of the human secretory pathway Ca(2+), Mn(2+)-ATPase defective in Hailey-Hailey disease. J Biol Chem 277(8): 6422–6427
Torti FM, Torti SV (2002) Regulation of ferritin genes and protein. Blood 99(10):3505–3516
Tumer Z, Moller LB et al (1999) Mutation spectrum of ATP7A, the gene defective in Menkes disease. Adv Exp Med Biol 448:83–95
Turek MJ, Fazel N (2009) Zinc deficiency. Curr Opin Gastroenterol 25:136–143
Ueta R, Fujiwara N et al (2007) Mechanism underlying the iron-dependent nuclear export of the iron-responsive transcription factor Aft1p in *Saccharomyces cerevisiae*. Mol Biol Cell 18(8):2980–2990
Umbreit J (2005) Iron deficiency: a concise review. Am J Hematol 78(3):225–231
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
Uriu-Adams JY, Scherr RE et al (2010) Influence of copper on early development: prenatal and postnatal considerations. Biofactors 36(2):136–152
Valko M, Morris H et al (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12(10):1161–1208
Van Baelen K, Dode L et al (2004) The Ca2+/Mn2+ pumps in the Golgi apparatus. Biochim Biophys Acta 1742(1–3): 103–112
van Loon AP, Pesold-Hurt B et al (1986) A yeast mutant lacking mitochondrial manganese-superoxide dismutase is hypersensitive to oxygen. Proc Natl Acad Sci USA 83(11):3820–3824
Vidal R, Ghetti B et al (2004) Intracellular ferritin accumulation in neural and extraneural tissue characterizes a neurodegenerative disease associated with a mutation in the ferritin light polypeptide gene. J Neuropathol Exp Neurol 63(4):363–380
Wang J, Slunt H et al (2003) Copper-binding-site-null SOD1 causes ALS in transgenic mice: aggregates of non-native SOD1 delineate a common feature. Hum Mol Genet 12(21): 2753–2764
Wang F, Kim BE et al (2004) Acrodermatitis enteropathica mutations affect transport activity, localization and zinc-responsive trafficking of the mouse ZIP4 zinc transporter. Hum Mol Genet 13(5):563–571
Wang J, Xu G et al (2005) Somatodendritic accumulation of misfolded SOD1-L126Z in motor neurons mediates degeneration: alphaB-crystallin modulates aggregation. Hum Mol Genet 14(16):2335–2347
Wang Z, Feng LS et al (2006) Solution structure of a Zap1 zinc-responsive domain provides insights into metalloregulatory transcriptional repression in Saccharomyces cerevisiae. J Mol Biol 357(4):1167–1183
Waters BM, Eide DJ (2002) Combinatorial control of yeast FET4 gene expression by iron, zinc, and oxygen. J Biol Chem 277(37):33749–33757
West AP Jr, Giannetti AM et al (2001) Mutational analysis of the transferrin receptor reveals overlapping HFE and transferrin binding sites. J Mol Biol 313(2):385–397
West AR, Thomas C et al (2006) Haemochromatosis protein is expressed on the terminal web of enterocytes in proximal small intestine of the rat. Histochem Cell Biol 125(3): 283–292
Westergard L, Christensen HM et al (2007) The cellular prion protein (PrP(C)): its physiological function and role in disease. Biochim Biophys Acta 1772(6):629–644
Williams RJP (1987) The biochemistry of zinc. Polyhedron 6(1):61–69
Wilson RB, Roof DM (1997) Respiratory deficiency due to loss of mitochondrial DNA in yeast lacking the frataxin homologue. Nat Genet 16(4):352–357
Winge DR, Nielson KB et al (1985) Yeast metallothionein. Sequence and metal-binding properties. J Biol Chem 260(27):14464–14470
Winzeler EA, Shoemaker DD et al (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285(5429):901–906
Wong BS, Chen SG et al (2001) Aberrant metal binding by prion protein in human prion disease. J Neurochem 78(6): 1400–1408
Wu CY, Bird AJ et al (2008) Differential control of Zap1-regulated genes in response to zinc deficiency in *Saccharomyces cerevisiae*. BMC Genomics 9:370

Wuehler SE, Peerson JM et al (2005) Use of national food balance data to estimate the adequacy of zinc in national food supplies: methodology and regional estimates. Public Health Nutr 8(7):812–819

Yamaguchi-Iwai Y, Dancis A et al (1995) AFT1: a mediator of iron regulated transcriptional control in *Saccharomyces cerevisiae*. EMBO J 14(6):1231–1239

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

Yamasaki S, Sakata-Sogawa K et al (2007) Zinc is a novel intracellular second messenger. J Cell Biol 177(4):637–645

Yocum CF, Pecoraro VL (1999) Recent advances in the understanding of the biological chemistry of manganese. Curr Opin Chem Biol 3(2):182–187

Yonkovich J, McKenndry R et al (2002) Copper ion-sensing transcription factor Mac1p post-translationally controls the degradation of its target gene product Ctr1p. J Biol Chem 277(27):23981–23984

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

Zhang Y, Gladyshev VN (2009) Comparative genomics of trace elements: emerging dynamic view of trace element utilization and function. Chem Rev 109(10):4828–4861

Zhang AS, Sheftel AD et al (2005a) Intracellular kinetics of iron in reticulocytes: evidence for endosome involvement in iron targeting to mitochondria. Blood 105(1):368–375

Zhang Y, Lyver ER et al (2005b) Frataxin and mitochondrial carrier proteins, Mrs3p and Mrs4p, cooperate in providing iron for heme synthesis. J Biol Chem 280(20):19794–19807

Zhang Y, Lyver ER et al (2006) Mrs3p, Mrs4p, and frataxin provide iron for Fe-S cluster synthesis in mitochondria. J Biol Chem 281(32):22493–22502

Zhao H, Eide D (1996a) The yeast ZRT1 gene encodes the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. Proc Natl Acad Sci USA 93(6): 2454–2458

Zhao H, Eide D (1996b) The ZRT2 gene encodes the low affinity zinc transporter in *Saccharomyces cerevisiae*. J Biol Chem 271(38):23203–23210

Zhao H, Eide DJ (1997) Zap1p, a metalloregulatory protein involved in zinc-responsive transcriptional regulation in *Saccharomyces cerevisiae*. Mol Cell Biol 17(9):5044–5052

Zhao H, Butler E et al (1998) Regulation of zinc homeostasis in yeast by binding of the ZAP1 transcriptional activator to zinc-responsive promoter elements. J Biol Chem 273(44):28713–28720