Iron and copper transport in yeast and its relevance to human disease

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Recent progress in the field of copper and iron metabolism has resulted from a convergence of human and yeast genetics. The mechanisms of iron and copper transport are remarkably conserved between yeast and humans. Studies of the yeast homologs of human disease genes involved in metal homeostasis have shed light on the pathophysiology of these disorders.

TRANSITION METALS are essential elements for all eukaryotes. Metals, such as copper and iron, are vital components of redox reactions because they readily gain and lose electrons. This same property, however, also makes them potentially toxic. Cells use several mechanisms to manage metal toxicity, including enzymes that neutralize radicals, intracellular metal chelators, and strict control of transmembrane metal transport. Transmembrane transport maintains a balance between the amount of metal required for biological processes and the amount that might be toxic. Most metal-transport systems have narrow specificity and transport one or a few metals. Cells, therefore, contain multiple, genetically separable transport systems with sometimes overlapping specificity. Although deficiency of transition-metal transport results in disease, the molecular details of transmembrane metal transport have remained elusive. Recent investigations using the model eukaryote *Saccharomyces cerevisiae* have rapidly advanced the field of metal metabolism (Table I). The elucidation of human disease genes that affect transition-metal metabolism has also contributed towards our understanding of transition-metal transport. While analysis of yeast genes has illuminated aspects of transition-metal homeostasis in humans, it is ironic (pun intended) that studies of mammalian transition-metal physiology have led to insights into yeast physiology.

Plasma-membrane iron transport

Studies on plasma-membrane iron transport in yeast were begun assuming that any iron transporters identified would possess human homologs. The search for iron transporters in yeast, however, has not led to the direct discovery of a human transmembrane iron transporter. Instead, the study of iron transport in yeast has been surprisingly fruitful in identifying and characterizing human copper transporters. This is because high-affinity iron transport in yeast depends upon a copper-containing protein, Fet3p. The high-affinity iron transport system is bipartite, consisting of Ftr1p, a transmembrane iron permease, and Fet3p, an integral membrane protein with an extracellular multicopper oxidase domain[1,2]. Multicopper oxidases comprise a small family of copper-containing enzymes that oxidize substrate with the concomitant reduction of molecular oxygen to water. Fet3p mediates iron transport by acting as a ferroxidase, converting ferrous iron to ferric iron, which is then transported by Ftr1p (Refs 1, 3). The combined action of the ferroxidase and the permease could be required to impart specificity to the high-affinity iron transport system[4]. The substrate for Fet3p is ferrous iron that is produced by cell surface ferrireductases[5–7]. These proteins mediate transmembrane electron transport that reduces ferric iron and cupric copper. The activity of the ferrireductase is required, because it solubilizes extracellular iron and copper chelates, making the metals bioavailable for transport. Although physiological data suggest that a ferrireductase might be involved in mammalian iron transport, the molecular identifcation of this activity is lacking[8,9].

The first physiological link between iron transport and copper was made over 30 years ago in swine that were made copper-deficient by dietary restriction. These animals showed very low activity of the multicopper oxidase ceruloplasmin and profound alterations in iron metabolism[10]. Iron could not be exported into plasma, resulting in its accumulation in parenchymal tissue as well as anemia. The anemia was thought to be due to a deficiency of iron loading into the plasma glycoprotein transferrin[11]. Recently, the role of ceruloplasmin in iron metabolism was confirmed through analysis of the human genetic disease aceruloplasminemia[12]. Affected individuals have no ceruloplasmin and develop defects in iron homeostasis (including iron overload within parenchymal tissues) similar to those observed in copper-deficient swine. However, the anemia observed in aceruloplasmic humans is not as severe as that observed in the copper-deficient swine. In addition, aceruloplasmic humans develop severe iron loading within the brain, resulting in neurological symptoms, suggesting that ceruloplasmin plays a pivotal role in iron export from the human brain[12,13].

Fet3p and ceruloplasmin have analogous functions, although they share low overall sequence identity. Ceruloplasmin functions enzymatically to mediate iron mobilization by acting as a ferroxidase, converting ferrous iron to ferric iron, which is then bound by transferrin. While ferrous iron can be oxidized spontaneously to ferric iron, the available evidence suggests that the presence of ceruloplasmin accelerates the rate. It

Table I. Homologous genes that affect iron metabolism in humans and yeast

Phenotype of yeast disruption	Protein	Human homolog	Phenotype of mammalian disruption
Deficient growth on low Fe	Fet3p	Ceruloplasmin	Deficient iron mobilization
Deficient growth on low Fe/Cu	Ctr1p	hCtr1p	?
Deficient growth on low Fe/Cu	Atx1p	Hah1p	?
Deficient growth on low Fe/Cu	Ccc2p	Menkes (Atp7ap)	Severe copper deficiency
		Wilson (Atp7bp)	Copper overload
Deficient growth on low Mn	Smf1p/Smf2p	Nramp2p	Microcytic anemia due to deficient iron transport
Deficient growth on non-fermentable carbon sources due to mitochondrial iron overload	Yfh1p	Frataxin	Decreased amounts of protein cause Friedreich's ataxia

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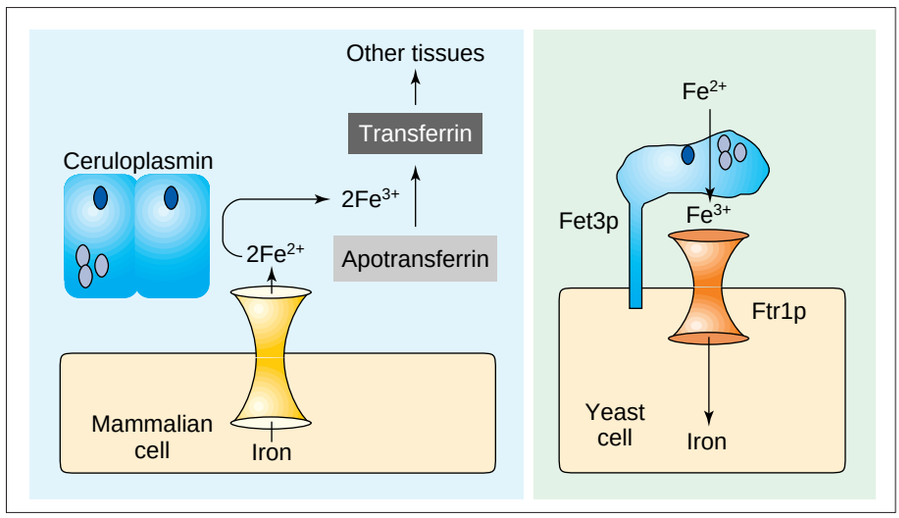

Figure 1

Comparison of oxidase-dependent iron transport in yeast and mammals. In mammals, the plasma glycoprotein ceruloplasmin mediates iron oxidation, facilitating iron export from cells and delivery throughout the body. In yeast, Fet3p is an integral membrane protein that mediates iron oxidation, resulting in plasma-membrane iron transport through the permease Ftr1p. A recent report suggests that ceruloplasmin could also behave like Fet3p and play a role in plasma-membrane iron transport into mammalian cells (see text). Small dark blue and violet circles represent copper molecules within the multicopper oxidases.

is clear, however, that Fet3p acts as a ferroxidase to facilitate iron transport through the iron permease, Ftr1p. Thus, the yeast multicopper ferroxidase mediates iron transport into cells, whereas the human multicopper ferroxidase mediates cellular iron release and iron transport throughout the body (Fig. 1). A recent paper presents *in vitro* data suggesting that ceruloplasmin also increases cellular iron accumulation[14]. These results, however, do not explain the older physiological data indicating that ceruloplasmin effects iron egress, rather than iron uptake.

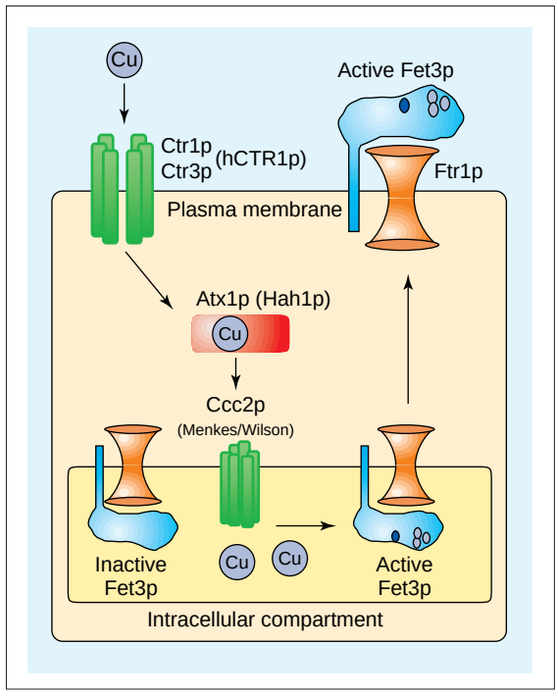

Figure 2

Fet3p and copper homeostasis. Defects in the plasma-membrane copper transporters Ctr1p and Ctr3p, cytosolic copper chaperone Atx1p, or vesicular copper transporter Ccc2p affect Fet3p activity. The genes encoding these also have mammalian partners (in parenthesis) that can substitute for their yeast counterparts to mediate Fet3p copper loading. Small dark blue and violet circles represent copper molecules within Fet3p.

The first mammalian transmembrane iron transporter was recently identified simultaneously by two different laboratories. One group identified *NRAMP2* as the gene responsible for the microcytic anemia (*mk*) disease in mice[15]. These mice have defects in intestinal iron transport and reticulocyte iron uptake. Another group isolated *NRAMP2* based on its ability to mediate plasma membrane iron transport in oocytes[16]. *NRAMP2* was initially identified by its homology to *NRAMP1*, a gene involved in antimicrobial killing in macrophages[17]. The Nramp2 protein shows no similarity to either the yeast high-affinity (Ftr1p) or low-affinity (Fet4p) iron transporters, but does show strong homology to the yeast manganese transporters, Smf1p and Smf2p[18]. In fact, Nramp2p transports manganese as well as iron in oocytes, and can substitute for Smf1p or Smf2p in yeast[16,19]. Thus, the physiological role of these transporters is different between yeast and humans, even though their protein sequences are conserved. Alteration of metal specificity between similar transporters in different species has been observed before: for example, an *Arabidopsis* iron transporter, Irt1p, was identified based on its ability to transport iron in yeast[20]. However, the yeast protein most similar to this plant iron transporter is a zinc transporter, *ZRT1*. These data suggest that certain polypeptide motifs might be conserved and shared for the purpose of transporting transition metals, but with a different metal specificity and physiological role. Thus, sequence similarity between transporters of different species does not necessarily mean they transport the same metal.

Copper homeostasis in yeast and human

The search for yeast genes involved in iron transport has led to a convergence of copper and iron metabolism (Fig. 2). Many of the copper transporters identified affect copper loading to the multicopper oxidase Fet3p and, thus, affect high-affinity iron transport (Table I). High-affinity copper transport across the yeast plasma membrane is mediated by Ctr1p and Ctr3p (Refs 21, 22). Once copper enters the cell, it is bound by cytosolic copper chaperones, which effect subcellular copper delivery to specific destinations. Cox17p mediates copper delivery to the mitochondria and Lys7p delivers copper to the cytosolic superoxide dismutase[23,24]. Atx1p mediates copper delivery to the vesicular copper transporter Ccc2p, which is responsible for Fet3p copper loading[25–27] (Fig. 2). Disruption of any one of the genes affecting the cellular copper-transport system composed of Ctr1p, Atx1p, Ccc2p results in a deficiency of Fet3p activity and high-affinity iron transport[21,26–28].

All the genes involved in copper loading of Fet3p in yeast have, to date, human homologues with high degrees of sequence conservation. A putative human plasma-membrane copper transporter, hCtr1p, with homology to Ctr1p/Ctr3p, has been identified[29]. Hah1p is the human homologue of the yeast cytosolic transporter Atx1p (Ref. 30). Ccc2p is homologous to the Menkes and Wilson disease proteins[27]. Menkes and Wilson

diseases result from disruption of copper homeostasis. The Menkes disease gene (*ATP7A*) is expressed in most tissues except the liver[31–33]. In this disease, intestinal copper transport is defective, and what little copper is absorbed cannot be effectively mobilized from tissues, resulting in copper deficiency. In Wilson disease, the tissues that normally express the gene product (*ATP7B*), are unable to export copper effectively and accumulate it[34–36], which causes tissue damage, particularly to the brain and liver. In addition, loss of *ATP7B* results in defective copper transport into the intracellular vesicle where ceruloplasmin normally becomes copper loaded. Because the liver is the primary site of ceruloplasmin biosynthesis, Wilson patients show low levels of active ceruloplasmin[37]; in some individuals, this level is so low that anemia is observed, which is similar to the copper-deficient swine. Thus, the genes involved in the transport of copper to the multicopper oxidases are completely conserved between yeast and human. In fact, the human genes involved in copper delivery can substitute for their yeast counterparts[29,30,38]. This has allowed the study of the structure and function of these human genes within the easily malleable yeast system.

Intracellular iron homeostasis

Based on the recent advances of intracellular copper transport, it is likely that similar mechanisms exist for the intracellular transport of iron. These mechanisms are just beginning to be discovered. Recently, the search for iron transporters has uncovered a gene involved in mitochondrial iron accumulation. The characterization of this yeast gene, *YFH1*, has contributed towards the understanding of human disease, because its product, Yfh1p, is the yeast homolog of the human protein frataxin[39]. Due to an intronic triplet expansion, low levels of frataxin cause the genetic disease Friedreich's ataxia[40]. This disease is a lethal disorder that manifests initially in coordination and gait problems (ataxia) resulting from a progressive sensory neuropathy. The major cause of death in affected individuals, however, is cardiac myopathy[41,42]. Even though Friedreich's is the most common of the inherited ataxias, the pathophysiology of the disease is unclear. The sequence of frataxin provided little information as to its function[43]. Studies of *YFH1*, the yeast homolog of frataxin, however, suggest a reasonable explanation for the pathophysiology of this disorder.

The *YFH1* gene was identified as a high-copy suppresser of a mutant yeast strain that could not grow on iron-limited media[39]. Overexpression of the non-allelic gene *YFH1* allowed this strain to grow under iron-limited conditions, suggesting that *YFH1* affected intracellular iron homeostasis. *YFH1* was found to be a nuclear gene encoding a mitochondrial protein, Yfh1p, which affects mitochondrial iron efflux, although it does not appear to be a transporter (Fig. 3). Disruption of *YFH1* results in excessive mitochondrial iron accumulation and permanent respiratory incompetence, owing to deletions in the mitochondrial genome[39,44,45]. The iron-induced damage can be attenuated by limiting cellular iron accumulation.

The human protein, frataxin, is also localized within mitochondria[39,46]. Individuals with Friedreich's ataxia possess low levels of frataxin and homozygous null mutations have not been identified. If frataxin behaves as Yfh1p, then reduction of frataxin levels will cause mitochondrial iron accumulation, eventually leading to excessive deposition of mitochondrial iron. Radicals will damage mitochondrial proteins and DNA, resulting in compromised mitochondrial function. Yeast can survive such damage because they are able to rely on glycolysis for their energy requirements. Compromised mitochondrial function in mammalian cells, however, can lead to apoptosis or decreased cellular activity. Evidence supports the view that Friedreich's ataxia results from mitochondrial iron overload. For example, excessive iron deposition is observed in heart biopsies of Friedreich's patients[42]. In addition, patients show decreased activity of mitochondrial iron–sulfur enzymes[47].

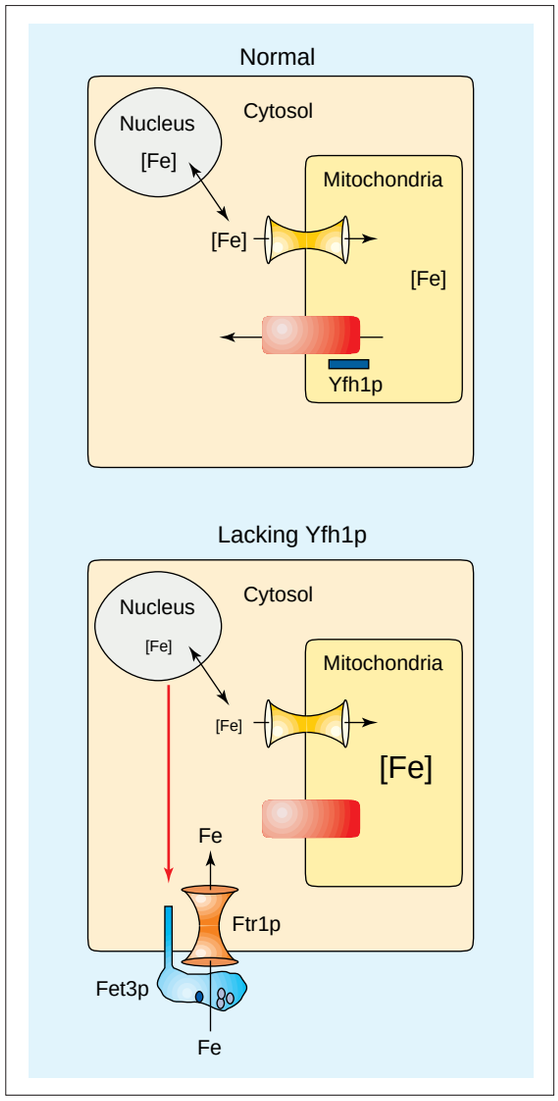

Figure 3

A model of how the loss of Yfh1p (dark blue rectangle) causes mitochondrial iron accumulation. Under normal conditions, mitochondrial iron transport is regulated, such that iron does not accumulate within mitochondria. In the absence of Yfh1p, mitochondrial iron export is defective, resulting in iron accumulation (large [Fe]), which causes mitochondrial-DNA damage and respiratory incompetence. Because iron accumulates in the mitochondria, the cytosol becomes depleted (small [Fe]), resulting in the induction of the plasma-membrane high-affinity iron transport system (red arrow). More iron is transported into the cell, effecting in an even greater amount of mitochondrial iron accumulation and damage.

Perspectives

These studies highlight the evolutionary conservation of proteins that are responsible for transition-metal homeostasis, an essential biochemical process. They also demonstrate that these homologous proteins can undergo subtle changes in physiological function and specificity. The study of yeast transition-metal metabolism has been successful, not only in discerning the genes and pathways functioning in yeast, but also the genes and pathways that are active in humans. In addition, the large reservoir of information on human physiology and disease can be applied profitably to simple eukaryotes. This interactive approach to physiology and biochemistry

has been successful in beginning to define the physiology of yeast as well as the pathophysiology of human disease.

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The ins and outs of a molecular chaperone machine

Alexandra Richardson, Samuel J. Landry and Costa Georgopoulos

Genetic and biochemical work has highlighted the biological importance of the GroEL/GroES (Hsp60/Hsp10; cpn60/cpn10) chaperone machine in protein folding. GroEL's donut-shaped structure has attracted the attention of structural biologists because of its elegance as well as the secrets (substrates) it can hide. The recent determination of the GroES and GroEL/GroES structures provides a glimpse of their plasticity, revealing dramatic conformational changes that point to an elaborate mechanism, coupling ATP hydrolysis to substrate release by GroEL.

SINCE THE LATE 1980s, it has become clear that many proteins require assistance for folding *in vivo*. The process of assisted protein folding is carried out by chaperones, a universally conserved class of proteins. Many chaperones are also stress or heat-shock proteins, whose rate of synthesis accelerates under various protein-damaging conditions.

This review will concentrate on the GroE chaperone machine, originally identified by genetic studies of bacteriophages (reviewed in Ref. 1), with special emphasis on the lessons derived from recent structural work.

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A new twist: GroE machine structure and function

Previous work had established that the GroE machine is composed of two members, GroEL and GroES, both essential proteins for *Escherichia coli* under all conditions tested[2]. Extensive structural work revealed that both chaperonins are organized into rings with a seven-fold rotational axis[3,4]. GroEL is composed of 14 subunits of 58 kDa each, arranged in two head-to-head rings in whose central, non-connected cavities various substrate proteins can be transiently sequestered from the medium and allowed to mature in solitary confinement. GroES is composed of seven subunits of 10.5 kDa each that form a dome capping GroEL's central cavity in the presence of nucleotides, thereby providing additional space and protection for the substrate. ATP binding and hydrolysis in the equatorial domain of GroEL plays a key role in GroES and substrate binding to, and release from, GroEL (reviewed in Refs 5, 6; see below).

The latest model of substrate maturation by the GroE chaperone machine is shown in Fig. 1 (adapted from Refs 7, 8). It should be emphasized here that many of the details of this folding pathway have not been completely ironed out. For example, the question of American footballs vs. bullets, that is, whether one or two GroES molecules bind GroEL

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