Comparative Analysis of Copper and Iron Metabolism in Photosynthetic Eukaryotes vs Yeast and Mammals

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1. INTRODUCTION

Copper and iron are essential micronutrients for all organisms because of their function as cofactors in enzymes that catalyze redox reactions in fundamental metabolic processes. Prominent examples of such enzymes include cytochrome oxidase in respiration, plastocyanin in photosynthesis, superoxide dismutase in oxidative stress, and ceruloplasmin in iron metabolism. Copper and iron carry out very similar functions in biology because both exhibit stable, redox-interchangeable ionic states with the potential to generate less stable electron-deficient intermediates during multielectron redox reactions involving oxygen chemistry. The major difference between copper and iron in biological systems derives from their individual ligand preferences and coordination geometries. The bioavailability of copper and iron is low so that organisms are faced with the challenge of acquiring sufficient copper and iron for cellular requirements while avoiding the buildup of levels that could lead to cellular toxicity. Over the last decade, it has become apparent that organisms have developed a suite of strategies to combat such challenges, so that an intricate balance between uptake, utilization, storage and detoxification, and efflux pathways for copper and iron exists.

A paradigm for copper and iron metabolism in eukaryotic cells has been developed through studies with the baker’s yeast *Saccharomyces cerevisiae* (reviewed in refs. 1 and 2). Genetic analysis of iron transport in *S. cerevisiae* has revealed a dependence of iron metabolism on adequate copper nutrition and this molecular connection has provided valuable insight into the basis for the well-established link between copper and iron nutrition in mammals. With the objective of comparing copper and iron metabolism in photosynthetic eukaryotes with that in yeast and mammals, this chapter summarizes the present state of knowledge of the relevant pathways in yeast and mammals, especially the basis for the copper–iron link, followed by a discussion of copper and iron metabolism in the model photosynthetic organisms *Arabidopsis thaliana* and *Chlamydomonas reinhardtii* and concludes with evidence for a copper–iron link in Chlamydomonas. The relevant pathways in other model organisms such as *Drosophila* and *Caenorhabditis elegans* will not be covered in this chapter, as our objective is to provide a background for comparison to plants rather than to embark on a comprehensive review of these processes in all model organisms.
2. YEAST COPPER–IRON LINK

2.1. Copper

2.1.1. Uptake

In *S. cerevisiae*, high-affinity copper uptake is mediated by the *CTR1* and *CTR3* gene products (Ctnp1 and Ctnp3, respectively) (2–5) and is associated with copper reductase activity of Fts3p and Fre2p to reduce Cu^{2+} to Cu^{1+} prior to uptake (6,7). *CTR1*, *CTR3*, and *FRE1* are transcriptionally activated in response to copper deficiency by the copper-dependent transcriptional activator Mac1p (2,8–10). Copper-dependent endocytosis of Ctnp1 at low copper concentrations and degradation of Ctnp1 in copper-replete cells provides an additional posttranslational level of regulation (11). Three putative low-affinity copper-transport systems also have been described: Fet4p (12), Smf1p/Smf2 (13,14), and Ctr2 (15).

2.1.2. Distribution, Storage, and Regulation

Inside the cell, copper is bound by cytoplasmic copper chaperones and delivered to specific destinations within the cell. These chaperones include Cox17p, Lys7p, and Atx1p, for copper delivery to cytochrome oxidase in the mitochondria, cytosolic and mitochondrial superoxide dismutase, and the copper transporter Ccc2p in the secretory pathway, respectively (2,16–20). Ccc2p is required for loading copper onto Fet3p, a multicopper oxidase, required for high-affinity iron transport across the plasma membrane (21). The chloride-ion channel Gsf1p, located in the same compartment, is also required for copper loading onto Fet3p (22,23). Sco1p and Sco2p were identified as integral membrane proteins within the inner mitochondrial membrane and may be involved in the transfer of copper from Cox17p to cytochrome oxidase (5,24,25).

With increased intracellular copper levels, copper-storage proteins such as the metallothioneins encoded by *CUP1* and *CRS5* are induced and sequester the copper (8). Storage of copper in the vacuole may be an additional mechanism of preventing copper toxicity (5).

Mac1p and Ace1p are the two copper-responsive transcriptional activators in *S. cerevisiae* (2,9,10). Under copper-deficient conditions, Mac1p activates the expression of six genes, *CTR1*, *CTR3*, *FRE1*, *FRE7*, *YFR055w*, and *YJL217w*. With elevated copper levels, Ace1p activates the expression of copper-detoxification proteins, Cup1p and Crs5p.

2.2. Iron

2.2.1. Uptake

Iron uptake into *S. cerevisiae* involves several different assimilatory pathways depending on the chemical source of iron and its concentration. In general, all of the pathways require reduction of Fe^{3+} to Fe^{2+} by one or more products of the *FRE1*–*FRE7* genes (14,26). Under iron-limiting conditions, high-affinity uptake is mediated by the inducible Fts3p/Ftr1p complex in the plasma membrane (1,14,27). Fts3p is a multicopper oxidase (28) whose active sites are related to multicopper oxidases such as laccase, ascorbate oxidase, and ceruloplasmin. Fts3p functions to oxidize Fe^{3+} to Fe^{2+} at the cell surface; Fe^{2+} is then delivered from Fts3p to the associated Fe^{2+} permease, Ftr1p, for transport into the cell (14,27). Highly related pathways operate in other fungi. In *Schizosaccharomyces pombe*, Frp1 is related to the Fre reductases, and Fio1/Fip1 forms an iron-uptake complex related to Fts3p/Ftr1p (29). The fungal pathogen *Candida albicans* also uses a similar pathway, involving a surface-associated reductase, Cfl1p (30), two Ftr1p-related permeases, CaFr1 and CaFr2 (31), and a Fet3p-like component (30). Expression of the permeases and their associated oxidases is regulated by iron nutritional status (27,29), whereas some reductases are regulated by iron status and others by both copper and iron—the latter category functioning in both copper and iron assimilation (10,14). Under iron-replete conditions, low-affinity systems, such as Fet4p,
with broad transition metal specificity (12,32), or Smf1/Smf2, which were originally identified as manganese transporters (13), operate. When iron is available in complex with siderophores, it can enter cells either through the Fet3p/Ftr1p complex after reduction by the Fre proteins and subsequent release of the iron from the siderophore, or if the Fet3p/Ftr1p is inoperable, by the ARN family of siderophore transporters through an endocytic pathway (33–38).

2.2.2. Distribution, Storage, and Regulation

Whereas the Fet3p component of iron assimilation is conserved from yeast to man, the mechanism of iron storage appears diverse. S. cerevisiae lacks genetic information for ferritin (8); rather, iron (with other ions) is stored in the vacuole (39,40). A homolog of the oxidase–permease complex Fet3p/Ftr1p, designated Fet5p/Fth1, was localized to the vacuole and proposed to function under iron starvation conditions to mobilize stored iron from the vacuole (41).

Another key organelle in iron metabolism is the mitochondrion. Not only is it an important target site for iron utilization, but it is also a major player in the maintenance of cellular iron homeostasis. Yfh1p regulates mitochondrial iron accumulation, possibly at the level of efflux (42,43), Ccc1p limits mitochondrial iron uptake (44), Nfs1p is involved in maintaining or synthesizing cytosolic Fe–S proteins (45,46), Atm1p is a transporter of the inner mitochondrial membrane (47), and Ssc2p is a protein of the Hsp70 class of chaperones with a role in Yfh1p maturation (48). Although there are as yet no defined chaperones for the delivery of iron to specific intracellular targets, the IscA family of proteins are candidates for iron delivery to sites of iron–sulfur cluster assembly (49).

Iron uptake in S. cerevisiae is regulated in response to cellular iron levels via the iron-responsive transcriptional activator Aft1p. Under iron-limiting conditions, Aft1p-mediated activation leads to induction of FRE1, FRE2, FTR1, FET3, FET5, and FTH1, as well as the induction of expression of the genes involved in copper transport, CCC2 and ATX1 (8). A further level of regulation of iron uptake exists that is mediated through Tpk2, a catalytic subunit of the yeast A kinases (PKA) (50). According to the proposed model, during fermentative growth on glucose Tpk2, activated by cAMP, represses genes involved in iron uptake, but as the glucose is depleted, Tpk2 activity is inhibited, thus relieving the repression of the iron-transport genes. This derepression allows iron transport into the cell for incorporation into respiratory enzymes and permits growth on nonfermentable carbon sources.

2.3. Copper–Iron Link

In S. cerevisiae, the copper–iron link is evident at both the level of protein biosynthesis and function and at the level of gene regulation. Copper is required for the assembly and function of the high-affinity Fet3p/Ftr1p iron-uptake complex, and thus for high-affinity iron uptake. Therefore, S. cerevisiae cells that are copper deficient are also iron deficient. The iron-uptake genes are regulated as a function of iron availability, so that the expression of these genes is activated in cells that are starved for iron. Because the Fre1 reductase functions in copper and iron uptake, its expression is induced by both copper and iron deficiency, whereas the copper-transport genes (CCC2 and ATX1), whose activity is critical for the production of functional iron-uptake proteins, are induced by iron deficiency, but not by copper deficiency (51–54). In addition, in S. pombe, a copper-responsive transcription factor, Cuf1, activates CTR4 gene expression for copper uptake under copper-starvation conditions while mediating repression of the iron-uptake genes under these same conditions to prevent futile expression of these genes when there is insufficient copper cofactor available to produce functional iron-uptake proteins (55). Therefore, an intricate system of interplay among transport, storage, chaperone and regulatory proteins exists to maintain copper and iron homeostasis in S. cerevisiae and S. pombe.

3. MAMMALIAN COPPER–IRON LINK

Although evidence for a physiological link between copper and iron metabolism in mammals was elucidated approximately 75 yr ago (56,57), the molecular basis for such a link is only now beginning
to unfold. The discovery of ceruloplasmin (58), the ceruloplasmin homologs Fet3p in yeast (28) and Hephaestin in mice (60) and a role for all of them in iron metabolism have been pivotal in furthering our understanding of the copper–iron connection in mammals (1,59).

3.1. Copper

3.1.1. Absorption

The first step in the proposed scheme for copper transport through the body is the absorption of dietary copper through the intestinal mucosa (61,62), although the precise mechanism of copper absorption is not known. A candidate protein for copper uptake into intestinal cells is hCtrl, a homolog of the yeast high-affinity copper transporter Ctr1p (63). Recently, the mouse homolog was shown to be essential (64). Inside the cell, copper is likely to be bound by the human counterparts of the yeast intracellular copper chaperones, Ato1 (Hah1), hCox17, and hCCS, for copper delivery to the secretory pathway, mitochondria, and Cu/Zn-superoxide dismutase (SOD), respectively (5,17,18,65–67). In intestinal enterocytes and the majority of nonhepatic cells, Atol1 delivers copper to the Ccc2p homolog, the Menkes protein (MNK) located at the trans-Golgi network (TGN) (66). MNK, in turn, transports copper into the TGN lumen for incorporation into copper-dependent enzymes that are transported by the secretory pathway, such as lysyl oxidase (68). An additional role of MNK is in the efflux of excess copper from the cell, which is achieved through a mechanism whereby copper concentrations above a certain threshold level stimulate a steady-state shift in the distribution of MNK from the TGN to the plasma membrane (69,70). Under normal steady-state conditions, MNK continually recycles between the TGN and plasma membrane (71,72). Therefore, MNK located at the basolateral surface of intestinal epithelial cells may also serve to transport copper across the basolateral membrane into the bloodstream.

3.1.2. Distribution

Albumin, the major copper carrier in the circulation, transports copper via the portal blood to the liver (73). Here, the MNK counterpart and homolog of Ccc2p, the Wilson protein (WND), is also located at the TGN and relocalizes in elevated copper to an unidentified vesicular compartment, possibly en route to the biliary canalicular membrane (74–76). In the liver, WND functions to excrete the excess copper into the bile (77) and to transport much of the copper that enters hepatic cells into the TGN lumen for incorporation into ceruloplasmin (78). The ceruloplasmin holoenzyme, which contains six copper atoms (79), is secreted from the liver and carries > 95% of total serum copper for delivery to tissues (80). In addition, ceruloplasmin, via its ferroxidase activity, mediates iron release from the liver, also for delivery to tissues (81).

3.1.3. Regulation

In mammals at a whole-body level, copper absorption from the small intestine is, to some extent, regulated, with increased uptake when the animal is copper deficient and decreased uptake when the diet is adequate in copper (82). Details of the molecular mechanisms regulating absorption remain to be elucidated. The control of copper excretion, which is also an important part of the protection against excess copper exposure, is regulated by the liver, with excess copper being disposed of through the bile (83). Within the cell, excess copper is detoxified by at least two mechanisms; sequestration by metal-binding proteins such as metallothioneins (MT) and enhanced export by the copper ATPases. MNK, and WND (84). Although a definitive biological role for MTs remains elusive despite years of research, MTs are known to be small cysteine-rich proteins that bind heavy metals, including copper, zinc, and cadmium, through cysteine thiolates (85,86). There are 17 genes encoding four isoforms of mammalian MTs (MT-1 to MT-4), each of which have subtypes. Induction of MT synthesis occurs at the level of transcription by heavy metals themselves (Zn²⁺, Cu²⁺, and Cd²⁺) through metal-regulatory elements (MREs) tandemly arranged within the 5' flanking regions of MT genes (87,88). In contrast, the regulation of copper export by MNK and WND occurs at a post-translational and enzymatic level that couples copper efflux to the intracellular copper concentration (69,74,75,77).
3.2. Iron

3.2.1. Absorption

The current model of iron uptake and utilization begins with the absorption of iron from nutritional sources across the intestinal epithelium. Iron, taken into the body as Fe$^{3+}$, is reduced to Fe$^{2+}$ by DcYtb and perhaps other as yet unidentifiedферrireductases and transported across the apical surface of enterocytes lining the gut by the divalent metal-ion transporter DMT1 (DCT1, Nramp2) (89–92). The HFE protein has a role in regulating intestinal iron absorption (91,93,94). HFE associates at the cell surface with β2-microglobulin and the transferrin receptor, but the significance of these associations remains to be fully elucidated (91). However, the discovery of a reciprocal regulation of HFE and DMT1 (95) led to the proposal that HFE regulates trafficking of DMT1 to the plasma membrane and therefore iron uptake, depending on cellular iron concentrations (96). Inside the cell, Fe$^{2+}$ is incorporated into iron-containing proteins, bound to ferritin for storage and detoxification, or it is directed to the mitochondria for heme synthesis and assembly of nonheme iron proteins (97). The Frataxin protein regulates mitochondrial iron accumulation although its precise function remains to be defined (1). Although chaperones for delivering iron to intracellular targets are believed to exist, these putative chaperones have not been identified (98).

3.2.2. Distribution

Iron also is transported across the basolateral surface of enterocytes into the blood for transport throughout the body. This export of iron from the enterocyte is mediated by a basolateral transporter Ferroportin1 (99)/IREG1 (100,101), although the species of iron transported has not been established. Iron is carried throughout the body, fulfilling the physiological iron requirements of cells and tissues, bound to the plasma protein transferrin. The loading of iron onto transferrin requires oxidation from Fe$^{2+}$ to Fe$^{3+}$, which may be mediated by either or both of the multicopper ferroxidases, Hephaestin and ceruloplasmin. Hephaestin may act together with Ferroportin1 at the basolateral surface of enterocytes to oxidize Fe$^{2+}$ to Fe$^{3+}$ prior to export into the plasma (102). Alternatively or in addition, plasma ceruloplasmin oxidizes Fe$^{2+}$ to Fe$^{3+}$ subsequent to export into blood plasma for loading onto transferrin. An additional important role of ceruloplasmin is the mobilization of iron from tissues such as the liver where ceruloplasmin is synthesized (59). In cells other than those of the intestine, iron is taken up bound to transferrin. The transferrin receptor at the cell surface binds diferric transferrin and is internalized and taken up into endosomes (103). The Fe$^{3+}$ is released from transferrin into the acidified endosome and reduced to Fe$^{2+}$ (97), followed by transport into the cytoplasm by DMT1 (89,104).

3.2.3. Regulation

The regulation of intestinal iron uptake is mediated through iron-dependent transcriptional and translational control of DCT1 (105). The iron concentration within the cell is controlled by reciprocal regulation of ferritin and the transferrin receptor at the posttranscriptional level. This regulation is mediated by iron-regulatory proteins (IRPs) 1 and 2 that bind iron-responsive elements (IREs) located in the 5' and 3' untranslated regions of ferritin and transferrin receptor mRNAs, respectively, to modulate the amount of each protein that is produced based on the cellular iron status (90,91,97,106–108). IREs or IRE-like elements have been identified in the UTRs of other proteins, including an IRE-like element within the 5' UTR of the IREG1 mRNA and the 3' UTR of DMT1 mRNA (90,91,106), but regulation of DMT1 and IREG1 by mechanisms involving this element have not been characterized.

3.3. Copper–Iron Link

Although the existence of a copper–iron link was documented in nutritional studies many decades ago, the discovery of the molecular basis for this link is relatively recent. In particular, the multicopper ferroxidases form the basis of the copper–iron connection. Ceruloplasmin is central to interorgan copper and iron metabolism. The copper atoms carried by ceruloplasmin not only serve to satisfy the
copper requirements of body tissues but are also critical for its catalytic function as a ferroxidase for iron mobilization and release from the liver for transport around the body. The assumption is that copper is also critical for the ferroxidase function of Hephaestin, mediating iron absorption from the small intestine into the bloodstream. Hence, iron metabolism is critically dependent on copper both at the level of absorption from dietary sources and transport around the body.

Clearly, there are parallels between the yeast and human copper–iron transport systems that extend to the fact that the dependence of iron metabolism on copper has been maintained over a vast evolutionary distance. Many of the metabolic catalysts are conserved, such as Atx1p/Atox1, Ccc2p/Menkes–Wilson ATPases, Fet3p/ceruloplasmin–Hephaestin, Yfh1p/Frataxin, Ctr1p/hCtrl, Cox17p/hCox17, Lys7/CCS, and the Sco proteins; yet others are distinct between yeast and man, such as Ftr1p vs IREG1 or DMT1, and Dcytb vs the Fre reductases.

4. COPPER–IRON LINK IN PHOTOSYNTHETIC ORGANISMS

Besides having the same copper and iron requirements as human and yeast cells, photosynthetic organisms have additional requirements for these metals in the plastid, where they are utilized as the redox active cofactors in many metabolic processes, including, in particular, the proteins involved in photosynthetic electron transfer in the chloroplast. Iron-containing cytochromes, Fe–S centers (>20 atoms per PSII/PSI unit), and copper-requiring plastocyanin are abundant proteins in photosynthetic organisms. Metal metabolism in this context presumably requires a completely separate set of factors in addition to those required for cellular and mitochondrial iron and copper metabolism. A further challenge for photosynthetic organisms is that biologically useful levels of soil copper and iron are low. The variability in the mineral composition of soils means that for plants to survive and grow they must adapt to the variation in supply of copper and iron. As with yeast and humans, the essential elements of copper and iron metabolism include transport, storage and detoxification, and utilization. The transport process can be further subdivided into reduction, uptake, and intraorganelar and intracellular transport. The storage and detoxification of copper and iron may involve not only sequestration by cellular proteins but also compartmentalization into vacuoles and plastids, which, in turn, requires proteins for transport into these compartments. Most of our knowledge about copper and iron metabolism in photosynthetic organisms comes from studies on the vascular plant Arabidopsis thaliana, the unicellular green alga Chlamydomonas reinhardtii, and the cyanobacterium Synechocystis 6803, which serves as a model for chloroplast biology. The biology of metal metabolism in these organisms is ripe for study because the mechanisms of copper and iron homeostasis in photosynthetic organisms are less well characterized than in humans and yeast.

4.1. Arabidopsis

Arabidopsis thaliana is a small weed in the mustard family, formally known as the family Brassicaceae. It has become an invaluable tool for studying plant physiology, biochemistry, development, and molecular genetics, and joins organisms such as Escherichia coli, yeast, Drosophila, Caenorhabditis elegans, and mice as vehicles for providing insight into fundamental biological processes.

4.1.1. Copper: Transport and Distribution

Copper assimilation and its regulation in plants is largely uncharacterized. Two approaches are commonly used: the identification of genes or expressed sequence tags (ESTs) encoding metal transporters based on sequence relationships, and functional complementation of well-characterized S. cerevisiae mutants.

4.1.1.1. COPT1

One of the first Arabidopsis genes encoding a copper transporter to be identified by the latter approach was COPT1, which encodes a highly hydrophilic protein that was capable of rescuing a S. cerevisiae ctrl1 strain defective in high-affinity copper uptake (15). In copper-replete plants, COPT1
is expressed in flowers, stems, and leaves, but not in roots (109), suggesting that the gene product might function in distribution rather than in assimilation. The expression of COPT1 in copper-deficient plants has not yet been analyzed. Two sequences, highly related to COPT1, are evident in the complete genome of Arabidopsis; the genes seemed to be expressed, but their function in metal metabolism has not been determined (Table 1). Perhaps one of these may represent an assimilatory pathway component. Two less closely related sequences that are not known to be expressed also were identified.

4.1.1.2. RAN1 and the CPX Family of Metal Transporters

The family of ethylene receptors comprises an important function for copper in plants. These molecules use copper as a cofactor at the binding site for this gaseous plant signaling molecule (117). Genetic analysis of the ethylene signaling pathway in Arabidopsis led to the identification of the RAN1 locus, which encodes a copper transporter related in sequence and in function to CCC2 of S.
cerevisiae (118,119). In plants, RAN1 is proposed to function by analogy to Ccc2p to load copper proteins such as the ethylene receptors. Phenotypic analysis of a strong ran1⁻ allele suggests that RAN1 may function also to load copper proteins in addition to the ethylene receptors.

A homolog of RAN1 has been annotated in the Arabidopsis genome as a copper-transporting ATPase, but its functional relationship to RAN1 is not known (Table 1). The occurrence of highly related sequences in the EST database for other plant species suggests that the RAN1 homolog is expressed. Williams and co-workers (120) have noted that the Arabidopsis genome encodes additional CPx-type metal-transporting ATPases, including PAA1 identified previously as a putative plastid-localized copper transporter (111), HMA1 and HMA4, and more recent database analyses reveal yet more sequences in this family. A reverse genetic approach to their study should add much to our knowledge of plant metal-transport mechanisms.

In the meantime, cyanobacteria, which are useful models for chloroplast biology, have been used to deduce a role for CPx-type transporters in metal delivery to the plastid (127-125). Two such proteins, PacS located in the thylakoid membrane (121) and CtaA (122), were found to be required for copper delivery to plastocyanin in the thylakoid lumen, and by analogy, these authors proposed that similar proteins might function in the plastid thylakoid membrane and envelope, respectively, for delivery to plastid copper enzymes (125). PAA1 is one good candidate for a plastid-localized copper transporter (111). Synechocystis mutants disrupted for PacS and CtaA function displayed an increased requirement for iron (125), suggesting a connection between copper and iron metabolism in this organism and, perhaps by extension, in plastids as well (see Section 4.2.).

4.1.1.3. Copper Chaperones

A copper metallochaperone, designated Atx1, was recently identified in Synechocystis PCC6803 and shown to interact with the CtaA copper importer to acquire and then deliver copper to the PacS copper ATPase (125a). An Arabidopsis homolog of a copper chaperone (mammalian ATOX1 and Saccharomyces ATX1) was identified as a gene that was upregulated during senescence (Table 1). This gene, called CCH, can functionally complement an atx1 mutant yeast strain for both antioxidant and copper-chaperone activity (115), and the encoded protein may also function in intercellular copper transport in plants (126). Because CCH is induced during senescence, a role in sequestering copper following degradation of copper-containing proteins has also been proposed. RAN1 is upregulated during leaf senescence as well, suggesting that CCH and RAN1 may function together to recycle copper during senescence (127). Genes encoding functional metallothioneins had been identified previously in A. thaliana (128). One of these, MT1, is induced during leaf senescence and also in leaves with higher than normal copper concentrations, whereas another, MT2, is expressed in leaves prior to senescence. It is suspected that these metallothionein-like proteins may function in copper homeostasis to prevent copper toxicity from copper released during degradation of senescing leaf proteins (127).

Homologs of two other copper chaperones, Cox17p and Lys7p/CCS1, also are found in the Arabidopsis genome (Table 1). CCS1, which binds metal (114), is proposed to be targeted to the plastid as is appropriate because Cu,Zn-SOD is present in this organelle in plants. These homologs can complement the appropriate Saccharomyces mutant, suggesting that the pathways for copper delivery are conserved between plants, mammals, and fungi, but it should be noted that their function and subcellular localization in plants has not yet been studied. It is certainly possible that the plant homologs have analogous rather than homologous functions. Genome analysis has revealed Sco1 and Cox11 homologs as well (Table 1), but these have not been subject to experimental analysis at any level.

A number of other sequences and putative open reading frames with the chaperone-like metal-binding site Mx CXGC (or simply a CxxC metal-binding site) can be found in the Arabidopsis genome. One family of these proteins has been characterized and consists of C-terminal prenylated proteins that bind transition metals (129). Because these proteins might be membrane associateable, one hypoth-
esis is that they may function in copper delivery to extracellular plasma membrane or secreted copper proteins. Another possibility is that they might be involved in some other aspect of metal homeostasis, such as metal sequestration for resistance.

4.1.2. Iron: Acquisition and Storage

4.1.2.1. Copper-Independent Acquisition

All plants must extract iron from insoluble Fe\textsuperscript{3+} complexes in the soil and convert it to the more soluble Fe\textsuperscript{2+} form for transport into the cell. Two types of iron acquisition mechanisms have been described for plants: Strategy I and Strategy II. Neither pathway seems to require a copper-containing ferroxidase as noted for animals, fungi (see Sections 2.2. and 3.2.) and algae (see Section 4.2.2.). Nevertheless, the mechanisms of iron movement within the plant, from the roots to the shoots, is not well characterized at the molecular level, and there is some indication that copper deficiency can cause a localized iron deficiency. Therefore, a connection with copper may yet be discovered.

In Strategy I plants (dicotyledons and nongraminaceous monocotyledons), Fe\textsuperscript{3+} is released from chelates by reduction and Fe\textsuperscript{2+} is taken up by a Fe\textsuperscript{2+}-specific transporter. A putative ferric chelate reductase-encoding gene, FRO2, was identified on the basis of its relationship to yeast (FRE) and mammalian (gp91phox) homologs (130). FRO2 is upregulated in iron deficiency and complements fdl1 mutants. Roots of the fdl1 mutants display impaired ferric chelate reductase activity and iron uptake (131, 132); hence FRO2 is a key component of iron assimilation. Several homologs of FRO2 are found in the Arabidopsis genome (Table 2); their function is not known yet but it is assumed that they participate in iron assimilation or distribution within the plant. A cytochrome-b\textsubscript{5} reductase, encoded by NFR, with Fe\textsuperscript{3+}-chelate reductase activity also was cloned from maize and a homolog was identified in Arabidopsis (138). Because the protein is localized to the tonoplast, its role in assimilation is questioned, but a role in intracellular iron or copper homeostasis is still open (140). Nevertheless, genetic evidence for NFR function in metal metabolism is not yet available. In addition to iron reduction, iron solubility in the rhizosphere is increased through activation of a specific H\textsuperscript{+}-ATPase. The gene AHA2 encodes one member of a large family of H\textsuperscript{+}-ATPases in Arabidopsis; it is upregulated under iron deficiency and therefore assigned function in iron assimilation (109).

Two types of iron transporters are known in plants: IRT1 and the NRAMP family (Table 2). IRT1 was identified independently by functional complementation of S. cerevisiae fet3/fe3 or smf1 mutants (133–135), whereas the NRAMP family from plants was identified on the basis of their homology to fungal and mammalian NRAMPs and tested for function by complementation of fet3/fe3 or smf1 S. cerevisiae mutants (136, 137). In Arabidopsis, IRT1 is expressed in roots and is upregulated under iron-deficient growth conditions, implying a role in Fe uptake (133). IRT1 is one member of a large family of divalent cation transporters called the ZIP family that is distributed widely in nature (135). The NRAMP family of transporters (120), including NRAMP 1, 3, and 4, is also implicated in iron assimilation by virtue of their increased expression in iron-deficient plants and their ability to rescue fet3/fe4 mutants (136, 137). Both the IRT1 and NRAMP type of transporters seem to have broad metal specificity for divalent, cations but their physiological specificity may well be more narrow. Because both types of transporters take up Fe\textsuperscript{2+}, a role for a ferroxidase (as in mammals, fungi, and algae) is not evident.

The Strategy II iron acquisition mechanism is found in grasses (graminaceous monocotyledons). The roots of these plants secrete phytosiderophores which chelate Fe\textsuperscript{3+}, and a siderophore transporter, YS1, takes up the resulting complex (139, 141). YS1, which was cloned from an Ac-tagged ysl allele, belongs to the major facilitator superfamily. The mutants display interveinal chlorosis that can be rescued by direct application of iron to the leaves or via the xylem after the roots are excised (142). Like the enzymes involved in phytosiderophore biosynthesis, iron–phytosiderophore uptake is induced under iron-deficient conditions through increased expression of the YSI gene. Arabidopsis contains 8 YSI-like genes, designated YSL1 through YSL8, but whether they all function in siderophore metabolism remains to be determined (139).
Table 2
Iron Assimilation Components in Arabidopsis thaliana

<table>
<thead>
<tr>
<th>Gene</th>
<th>Homolog</th>
<th>Function</th>
<th>Experimental evidence</th>
<th>Reference</th>
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<tr>
<td>IRT1</td>
<td>ZIP family</td>
<td>Iron transporter</td>
<td>Rescues <em>S. cerevisiae</em> ftr3/4 double mutant; expressed in roots; induced in Fe deficiency</td>
<td>133–135</td>
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<tr>
<td>NRAMP1, NRAMP3, NRAMP4</td>
<td></td>
<td>Iron transporters</td>
<td>Rescues <em>S. cerevisiae</em> ftr3/4 double mutant; increase in mRNA accumulation in Fe deficiency</td>
<td>136,137</td>
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<td>FRO2</td>
<td><em>S. cerevisiae</em> FRI, <em>S. pombe</em> FPR1, and mammalian gp91phox</td>
<td>Iron-chelate reductase</td>
<td>Increased accumulation of FRO2 mRNA in Fe deficiency; complements frd1 mutant</td>
<td>130</td>
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<tr>
<td>FRO2</td>
<td>FRO2</td>
<td>Iron-chelate reductases</td>
<td>Based on similarity to FRO2</td>
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<td>Based on sequence similarity</td>
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<tr>
<td>YSL1-8</td>
<td>Maize YSI</td>
<td>Siderophore uptake</td>
<td>Based on sequence similarity</td>
<td>139</td>
</tr>
</tbody>
</table>

4.1.2.2. Iron Storage

Iron is stored within plants in ferritin, which is localized in the plastids in the stroma (143). Ferritin is found primarily in roots and leaves of young plants with much lower levels in mature plants (144). The native molecule consists of 24 subunits of a single type of chain that is related to animal ferritin chains with an additional plant-specific N-terminal sequence in the mature protein known as the extension peptide. The plant ferritin chain has the iron oxidation site found in animal ferritin H chains and also the carboxylates of the L chain that are responsible for efficient mineralization of the core.

Ferritin has a storage function; it accumulates in seeds during maturation and is mobilized by degradation of the protein during germination (141). The abundance of ferritin in the stroma of the plastid decreases as chloroplasts develop, and this corresponds to the movement of iron from the stroma to the thylakoid membrane where iron-containing electron-transfer proteins reside. Ferritin is found only at low levels in mature plants. When ferritin is incorrectly expressed in mature plants, it results in iron-deficiency chlorosis, confirming that ferritin sequesters iron and supporting the model that ferritin serves as an iron reservoir for iron supply to the photosynthetic apparatus in developing leaves (145). Ferritins probably function also for transient iron storage (e.g., as is necessary during senescence or other situations where iron proteins are degraded) (144). This might explain the anomalous finding that mRNAs encoding ferritin are upregulated during Fe deficiency; in Fe-deficiency-induced chlorosis, Fe might be released as PSI is degraded (see Section 4.2.2.2.). Thus, plant ferritin accumulation is regulated at the level of transcription in response to iron levels among other stimuli (146). In soybean, transcriptional regulation is mediated by an iron-regulatory element (FRE) and iron-sensing trans-acting factor (147), whereas in *A. thaliana* and maize, an iron-dependent regulatory sequence (IDRS) was recently identified within the promoter region of the respective ferritin genes (*AtFer1* and *ZmFer1*) and shown to be involved in iron-responsive transcriptional control (148).
4.1.3. Copper–Iron Link

Evidence in support of a definitive link between copper and iron metabolism in vascular plants remains to be firmly established. It is well accepted that ferric reductase is specifically induced in response to iron deficiency. However, in pea plants, copper deficiency also stimulates elevated root ferric reductase activity, as well as rhizosphere acidification, another response that is induced by iron deficiency (149, 150). Cohen et al. (149) suggested that the copper–iron-deficiency-induced activity derives from a single ferric reductase enzyme and, furthermore, that the copper deficiency causes a localized iron deficiency in the plant, which consequently leads to induction of the ferric reductase activity, a scenario that parallels the human and yeast copper–iron link.

In Arabidopsis, iron deficiency leads to an increase in both ferric reductase activity and copper-chelate reductase activity at the root surface (131). Both activities are absent in frd1-1 mutants (131) and are restored in transgenic plants expressing FRO2 (130). Although these data suggested that the ferric reductase can also reduce Cu$^{2+}$ to Cu$^{+}$, the accumulation of copper (as well as manganese and zinc) was not inhibited in frd1-1 mutants, leading to the conclusion that other factors must be involved in regulating divalent cation influx (131).

Much is still to be learned about the complex mechanisms underlying copper and iron homeostasis in plants, from uptake by the roots to transport into, and storage and utilization by the cells of the various plant tissues. Although molecular approaches to identify and functionally characterize plant genes and proteins are proceeding at a rapid pace, significant insights into plant metal metabolism also can be gained from studying other model photosynthetic organisms such as the unicellular green alga, *Chlamydomonas reinhardtii*.

4.2. Chlamydomonas

The unicellular green alga *Chlamydomonas reinhardtii* is also a genetically tractable organism that can be grown on very highly defined media consisting of simple salts, which facilitates studies involving trace micronutrients such as copper. Therefore, it has served as a model for the analysis of copper metabolism in the context of the photosynthetic apparatus. The organism grows heterotrophically as well as photoautotrophically, so that mutants affected in metal supply to the photosynthetic apparatus can be maintained and analyzed (151). Under photoautotrophic conditions, the chloroplast is a sink organelle for metal micronutrients like copper and iron, whereas under heterotrophic conditions, copper and iron are distributed to both the mitochondrion as well as the plastid. The mechanisms for metal homeostasis involving the plastid are only beginning to be studied. The recent generation of an EST database for *C. reinhardtii* has provided an opportunity for comparative analysis of metal metabolism in this organism relative to plants and animals.

4.2.1. Copper: Adaptation to Deficiency

4.2.1.1. Plastocyanin vs Cytochrome-c$_6$

*Chlamydomonas reinhardtii* has provided a fascinating system for the study of biological compensation for copper deficiency since Wood (1978) (152) recognized that the organism remains photosynthetically competent in copper-deficient medium by replacing an abundant copper protein, plastocyanin, with an iron-containing cytochrome, cytochrome-c$_6$. Our laboratory has since discovered that this occurs through regulated proteolysis of apoplastocyanin (so that the protein is degraded in −Cu cells but accumulates as the holoprotein in +Cu cells), and transcriptional activation of the Cyc6 gene (encoding cytochrome-c$_6$) (153, 154). Interestingly, the degree of transcriptional activation is proportional to the perceived copper deficiency (155). In a fully copper-supplemented medium (> 400 nM for a late log-phase culture), the photosynthetic apparatus maintains several million molecules of plastocyanin per cell. When the medium copper concentration falls below the amount necessary to provide copper at this stoichiometry, the Cyc6 gene is activated to the extent necessary to compensate for the loss of plastocyanin. Genetic analysis of plastocyanin mutants (pco strains) demonstrated that the activation of the Cyc6 gene responded to perception of copper levels rather than to the perception of holoplastocyanin deficiency because strains lacking plastocyanin (e.g., frame shift
or stop codon in the structural gene for preapoplastocyanin) still display copper-responsive Cyc6 expression (156,157). Therefore, a copper sensor must exist in this organism.

4.2.1.2. COPPER ASSIMILATION

In addition to the regulation of alternate (Cu vs Fe) carriers for the photosynthetic apparatus, there are other adaptations to copper deficiency. As is the case generally for nutrient deficiencies, an assimilatory pathway is activated in copper deficiency (158). This pathway involves a reductase component and a transporter. Because the pathway is activated coordinately (with respect to medium copper concentration) with Cyc6 expression, it is likely that the assimilatory components are downstream targets of the same signal transduction pathway. When medium copper content is measured in Chlamydomonas cultures grown in copper-deficient versus copper-sufficient or copper-excess conditions, one notes that there is no residual copper in the medium as long as this nutrient is below that required to fill the active site of all copper enzymes. Once the copper content of the medium exceeds that required to saturate the copper enzymes, net copper uptake ceases and the excess copper remains in the medium. The mechanism underlying this tight homeostatic control is not known. Is there a balance between an influx versus efflux transporter? Or is the uptake transporter regulated at multiple points (e.g., posttranslationally and also transcriptionally) to achieve this level of control? In this context it should be noted that activation of assimilatory pathways puts the organism at risk for toxicity when the nutrient is resupplied. Hence, it is reasonable to imagine multiple levels of control operating to balance intracellular copper levels in the face of deficiency on the one hand and overload on the other.

Chlamydomonas does not seem to have genetic information for metallothioneins and probably uses only phytochelatins for detoxification of copper, cadmium, mercury, and other heavy metals (159–161), although it is possible that the metallothioneins of Chlamydomonas have not yet been discovered. Perhaps genomics will provide the answer to this question.

4.2.1.3 OTHER TARGETS

Three other plastid-localized enzymes are regulated by copper availability in Chlamydomonas: coproporphyrinogen oxidase (Cpx1) encoded by Cpx1 (161), and two putative diiron enzymes encoded by Crdl and Cthl (162). The function of the latter is not known, but one possibility is that they are required for iron metabolism in the plastid. Because Chlamydomonas uses a multicopper oxidase for iron assimilation (see Section 4.2.2.), copper-deficient cells probably have to devise mechanisms for bypassing this step in order to remain iron replete. The existence of such mechanisms is evident from the finding that copper-deficient cells are not iron deficient (158). Indeed, crdl mutants behave as if they exhibit a localized iron deficiency in the plastid. Crdl and Cthl are hypothesized to have similar functions, whereby Cthl is used in copper-replete cells that are not iron challenged, whereas Crdl is used in copper-deficient cells that may be adapting to a less effective iron-assimilating pathway owing to reduced plasma membrane ferroxidase activity.

Coprogen oxidase catalyzes a step in tetrapyrrole biosynthesis, leading to heme and chlorophyll in the plastid. The increased activity of coprogen oxidase in −Cu cells has been attributed to an increased demand for heme (e.g., for cyt-c4 synthesis) in this situation. Nevertheless, this remains an unsatisfactory explanation because the fraction of the tetrapyrrole pool (various chlorophylls and hemes) in cyt-c4 is very small. With the discovery of a copper-requiring enzyme in iron assimilation and the logical corollary of a copper-independent backup pathway operating in copper-deficient cells, one must re-evaluate the function of Cpx1 in −Cu cells. Increased expression of the gene does not result in relocation to a different compartment, such as the mitochondrion (163). One possibility is that increased flux through the tetrapyrrole pathway draws iron into the plastid.

The EST database has revealed several other copper metabolizing proteins (Table 3). For some of these, the function can be predicted by analogy to the function of the homologs, but for others, such as CucA and CucC homologs, genetic analysis is required. In the meantime, expression studies and subcellular localization will provide some clues.
Table 3
Homologs of Copper Transporters, Chaperones and Other Copper-Metabolizing Factors in Chlamydomonas reinhardtii

<table>
<thead>
<tr>
<th>Gene</th>
<th>Homolog</th>
<th>Putative function</th>
<th>Experimental evidence</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccc2</td>
<td>Arabidopsis Ran1</td>
<td>Copper transport</td>
<td>Based on sequence similarity.</td>
<td>BE761354</td>
</tr>
<tr>
<td></td>
<td>vs A. thaliana RAN1 = 1e-8; vs Menkes protein = 2e-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atxl</td>
<td>Arabidopsis CCH and S. cerevisiae Atxl</td>
<td>Copper chaperone/delivery for Ran1/Ccc2</td>
<td>Rescues S. cerevisiae atxl mutant; transcripts induced up to 6.5x in Fe deficiency.</td>
<td>AF280056</td>
</tr>
<tr>
<td></td>
<td>vs A. thaliana CCH = 3e-13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>vs S. cerevisiae Atxl = 8e-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cox17</td>
<td>Human and Arabidopsis Cox17 homologs</td>
<td>Copper chaperone for cytochrome oxidase assembly</td>
<td>Rescues cox17 mutant weakly.</td>
<td>AF280543</td>
</tr>
<tr>
<td></td>
<td>vs human Cox17 = 1e-11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>vs A. thaliana = 2e-11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>vs S. cerevisiae = 2e-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sco1</td>
<td>Related to S. cerevisiae Sco1 and its Arabidopsis homolog</td>
<td>Copper delivery to cytochrome oxidase</td>
<td>Based on sequence similarity.</td>
<td>AV620545</td>
</tr>
<tr>
<td></td>
<td>vs A. thaliana = 2e-30; vs S. cerevisiae/human = 5e-22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cox11</td>
<td>Related to S. cerevisiae Cox11 and its Arabidopsis homolog</td>
<td>Cytochrome oxidase Cu₉ center assembly factor</td>
<td>Based on sequence similarity.</td>
<td>BE129228</td>
</tr>
<tr>
<td></td>
<td>Blast score (BE129228): vs A. thaliana = 9e-29; vs human = 5e-31; vs S. cerevisiae = 2e-29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CutA</td>
<td>Arabidopsis, human CutA</td>
<td>Copper homeostasis protein</td>
<td>Based on sequence similarity.</td>
<td>BE237847</td>
</tr>
<tr>
<td></td>
<td>Blast score (EST): vs A. thaliana = 3e-36; vs human = 6e-26; vs E. coli CutA = 2e-19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CutC</td>
<td>Human, E. coli CutC</td>
<td>Copper homeostasis protein</td>
<td>Based on sequence similarity.</td>
<td>AV387727</td>
</tr>
<tr>
<td></td>
<td>Blast score (EST): vs human homolog = 2e-37; vs E. coli CutA = 2e-28</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2.1.4. Hypoxic Expression of Copper-Deficiency Targets

Cyc6, Cpx1, Crd1, and Cth1 are each regulated also by hypoxia (162, 164). A simple model to explain this observation is that hypoxic cells are copper deficient, but this was ruled out by demonstrating that hypoxic cells make holoplasmodocyanin (165). Further, the expression pattern of these genes in response to –Cu vs –O₂ is not the same; Cpx1 and Crd1 are more strongly activated by hypoxia
than by copper deficiency, whereas the converse is true for Cyc6. Promoter analysis of Cpx1 identified a hypoxia-responsive element that is not required for the nutritional copper response, strengthening the argument for separation of the two responses. For the Crd1 gene, the hypoxic response is shown to be physiologically significant because the crd1 mutant recapitulates the copper-deficiency chlorotic phenotype, indicating that Crd1 function is required for adaptation to hypoxia. The mechanism of hypoxia perception in Chlamydomonas and its relationship to copper sensing is not known, but insights may emerge when the molecular details of Ctrl (the copper response regulator, see below) are revealed. Because copper deficiency and hypoxia probably co-occur in nature, it may be that the ability to respond to either signal allows the organism to anticipate and, hence, adapt more rapidly to copper deficiency.

4.2.1.5. A Master Regulator

Genetic analysis of the nutritional copper sensor led to the identification of two mutants at the CRR1 locus that are blocked in activation of all of the above genes (Eriksson and Merchant, unpublished). Because these genes are not activated by copper deficiency or by hypoxia in these mutants, it was concluded that Crr1 must be involved in both signal transduction pathways. Whether Crr1 functions also in mediating the response to copper toxicity has not been tested.

4.2.2. Iron: Assimilation and Deficiency

4.2.2.1. Iron Assimilation

As in other organisms, iron uptake by Chlamydomonas involves reductases (158, 166–168). The reductases are induced in iron deficiency and may be the same enzyme as that induced in Cu cells (158). The EST database does not reveal homologs corresponding to the Saccharomyces FRE genes nor to the Arabidopsis FRO genes, but a putative homolog of NFR has been identified (Table 4). It should be a simple matter to assess its role in metal metabolism by testing its expression in copper- or iron-deficient cells.

The discovery of a multicopper oxidase encoded by Fox1 in Chlamydomonas with significant similarity to mammalian ceruloplasmin and hephaestin was startling (169, 170). First, it had been assumed that iron assimilation in Chlamydomonas was independent of copper because copper-deficient cells do not appear iron deficient. Second, multicopper oxidases have not previously been implicated in iron metabolism in plants. A role for the Fox1 gene product in iron assimilation is suggested by its pattern of expression, which shows greater than 100-fold induction under iron-deficient conditions, the parallel expression of an Ftr1-like permease (169), and the localization of Fox1 to the plasma membrane by Buckhout and co-workers (170). Components potentially involved in loading the oxidase with copper, such as the Ccc2 transporter and the Atx1 metallochaperone homologs (Table 3), also are found in Chlamydomonas. The latter is induced under Fe conditions, albeit not as strongly as Fox1 and Ftr1. Ccc2 and Atx1 homologs of Chlamydomonas are more related to the plant homologs RAN1 and CCH, respectively, than to the human or Saccharomyces counterparts (Table 3). Ccc2 and Atx1 of Chlamydomonas and plants probably function to load a variety of copper-containing enzymes in the secretory pathway in addition to Fox1. In plants, RAN1 is required for loading copper into the active site of the ethylene receptor (118) and perhaps other enzymes as well. Loss of RAN1 is not lethal in the plant, probably because of the occurrence of a homolog (Table 1). In Chlamydomonas, Ccc2/Ran1 is likely to be required for loading Fox1, but there may also be other copper proteins that are substrates for Ccc2/Ran1.

4.2.2.2. Iron Deficiency

One of the characteristic symptoms of iron deficiency in a photosynthetic organism is the development of chlorosis (chlorophyll deficiency). When heterotrophic Chlamydomonas cells are transferred from the usual medium (18 μM iron-EDTA) to iron-deficient medium (0.1 μM iron-EDTA), photosystem I and light harvesting proteins are lost (Moseley, et al., unpublished). Iron assimilation components are activated prior to development of chlorosis, which occurs only if assimilatory mechanisms remain
## Table 4
Homologs of Iron Transporters and Iron-Metabolizing Enzymes in *Chlamydomonas reinhardtii*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Homolog</th>
<th>Putative function</th>
<th>Experimental evidence</th>
<th>Reference/accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fox1</td>
<td>Related to mammalian ceruloplasmin and Hephaestin</td>
<td>Ferroxidase required for iron assimilation or distribution</td>
<td>Transcripts induced up to 400× in Fe deficiency; plasma membrane localized Blast score: vs hephaestin = 1e-131 vs ceruloplasmin = 1e-113</td>
<td>Unpublished; 170</td>
</tr>
<tr>
<td>Ftr1</td>
<td>Related to <em>C. albicans</em> CaFtr1</td>
<td>Fe³⁺ transporter</td>
<td>Transcripts induced up to 230× in Fe deficiency; 2× RGExE motifs in cDNA Blast score (partial cDNA sequence): vs <em>C. albicans</em> CaFtr1 = 4e-10</td>
<td>Unpublished</td>
</tr>
<tr>
<td>Fer1</td>
<td>Related to plant and animal ferritins</td>
<td>Iron storage in plastid</td>
<td>Based on sequence similarity; increased expression in Fe deficiency. Blast score (assembled sequence): vs <em>A. thaliana</em> ferritin 1 = 7e-47 vs Xenopus ferritin H chain 2 = 4e-30</td>
<td>AV632653 AV619384 BE227588 AV623127 AV395232</td>
</tr>
<tr>
<td>Nramp</td>
<td>Arabidopsis <em>Nramp</em> homolog</td>
<td>Divalent metal ion transport</td>
<td>Based on sequence similarity. Blast score (assembled sequence): vs <em>A. thaliana</em> NRAMP1 = 4e-22</td>
<td>AV625564 AV389837 BE452312 BE452998</td>
</tr>
<tr>
<td>IscA2</td>
<td><em>Azotobacter vinelandii</em> IscA</td>
<td>Iron–sulfur cluster assembly</td>
<td>Based on sequence similarity. Blast score (assembled sequence): vs <em>A. vinelandii</em> IscA = 9e-94</td>
<td>AV636381 AV633541 AV386551 BE725609</td>
</tr>
<tr>
<td>IscU</td>
<td><em>Pseudomonas aeruginosa</em> IscU</td>
<td>Iron–sulfur cluster assembly</td>
<td>Based on sequence similarity. Blast score (assembled sequence): vs <em>P. aeruginosa</em> IscU = 2e-46 vs <em>A. thaliana</em> homolog = 1e-55</td>
<td>AV397853 AV619693 AV620447 AV624765 AV623500 AV623764 AV623473 AV638761 AV621762 AV625355</td>
</tr>
</tbody>
</table>
inadequate because of limited iron nutrition. It is possible that the threshold for cellular responses (such as activation of Fox1) and organelle responses (such as degradation of chlorophyll proteins) is different and/or that separate iron sensors occur in each compartment.

Paradoxically, the Fer1 gene, encoding a plastid ferritin, is also induced in iron-deficient cells. Because ferritin has an iron-storage function, the increase in its mRNA in Fe cells is counterintuitive. Two models have been offered to explain this observation; first, that increased ferritin might allow the cell to handle overload when iron is resupplied to the deficient cells. However, this model does not sit well when one considers that ferritin overexpression causes iron deficiency. It is more likely that ferritin may serve to buffer iron as it is released from degrading PSI and recycled to other compartments (such as the mitochondrion). This model is supported by the finding that iron deficiency impacts the chloroplast more severely than it does respiration. It also is compatible with the increase in Fer1 gene expression in degreening N-starved Chlorella cells (171).

4.2.2.3. Iron Metabolism Components

Based solely on BLAST searches, other candidates for iron-metabolizing components have been identified in Chlamydomonas (Table 4). These include components required for assembly of iron-sulfur clusters (Isc genes) and candidate metal transporters (Nram). Comparison of Chlamydomonas metal-metabolizing components with those of plants and animals leads us to wonder whether Chlamydomonas is half beast/half plant. Some components have their closest homologs in mammals, such as the ferroxidase, whereas others have their closest homolog in plants, such as Ccc2/Ran1.

4.2.3. Copper–Iron Link

Unlike yeast cells, wild-type copper-deficient Chlamydomonas cells do not exhibit symptoms of iron deficiency (chlorosis in the case of photosynthetic organisms) when grown under copper-deficient conditions. In fact, copper-deficient and copper-supplemented Chlamydomonas cells grow equally well (162,169). This suggests that either a copper–iron link equivalent to that established for yeast and mammals does not exist in Chlamydomonas or, alternatively, that this organism has backup systems that function when the copper-dependent iron uptake/metabolism is compromised. Our recent results (Section 4.2.2.) with iron-responsive expression of putative iron metabolizing proteins further supports the idea that copper-requiring components are required for iron metabolism in Chlamydomonas.

A survey of copper and iron proteins reveals several reactions for which both copper- and iron-containing proteins are known. Key examples are hemocyanin/hemoglobin, cytochrome oxidase/alternative oxidase, Cu,Zn-SOD/Fe-SOD, copper/heme nitrite reductases, membrane–methane monoxygenase/soluble methane monoxygenase, plastocyanin/cytochrome-c6. The copper enzymes are believed to be more recent additions to the biochemical repertoire relative to iron enzymes (172). The similarity in catalytic ability between copper and iron enzymes raises the question of whether the substitution of a copper enzyme with a “backup” iron version might represent a general metabolic adaptation to copper deficiency. Azurin and cytochrome-c551 in Pseudomonas aeruginosa form a pair analogous to plastocyanin and cytochrome-c6, and it has been suggested, although not documented in vivo, that they might function interchangeably. A fascinating example of another pair of iron and copper proteins became known more recently following the discovery that a membrane-bound methane monoxygenase is a multicopper oxidase (173). The enzyme catalyzes the hydroxylation of methane to methanol using O2 as a substrate. A soluble version of this enzyme is well characterized and contains a diiron active site (174). Some methanotrophic bacteria have both types of enzymes, and which one is used depends on copper availability in the medium, with control being effected by transcriptional regulation (175). When copper is available, transcription of the gene encoding the diiron-hydroxylase is repressed, whereas synthesis of the copper enzyme is stimulated. The copper enzyme is the protein of choice when both copper and iron are available. The retention of genetic information for the diiron enzyme implies that both must be required in the natural environment; in a hypoxic environment, Cu2+ might be lost in insoluble precipitates, but Fe2+ might be more
readily available. As mentioned earlier, in Chlamydomonas, the copper-deficiency responses can be mimicked by hypoxia (164). One explanation for this is that oxygen deficiency signals incipient copper deficiency and the response to hypoxia allows the organism to anticipate and, hence, survive copper-deficient conditions.

5. CONCLUDING REMARKS

Studies of copper–iron crosstalk in photosynthetic organisms are still in their infancy and many issues remain to be addressed. The full extent of the putative backup system for iron metabolism in copper-deficient Chlamydomonas must be elucidated. In an organism designed to undertake two fundamental metabolic processes (respiration and photosynthesis), the existence of such a backup system would appear to represent a shrewd evolutionary development. Does such a system also exist in vascular plants? With the current data explosion derived from complete genome sequences, EST databases, and microarray expression analysis, the resolution of such fundamental issues ought to be attainable in the very near future.

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