Iron Cofactor Assembly in Plants

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Abstract

Iron is an essential element for all photosynthetic organisms. The biological use of this transition metal is as an enzyme cofactor, predominantly in electron transfer and catalysis. The main forms of iron cofactor are, in order of decreasing abundance, iron-sulfur clusters, heme, and di-iron or mononuclear iron, with a wide functional range. In plants and algae, iron-sulfur cluster assembly pathways of bacterial origin are localized in the mitochondria and plastids, where there is a high demand for these cofactors. A third iron-sulfur cluster assembly pathway is present in the cytosol that depends on the mitochondria but not on plastid assembly proteins. The biosynthesis of heme takes place mainly in the plastids. The importance of iron-sulfur cofactors beyond photosynthesis and respiration has become evident with recent discoveries of novel iron-sulfur proteins involved in epigenetics and DNA metabolism. In addition, increased understanding of intracellular iron trafficking is opening up research into how iron is distributed between iron cofactor assembly pathways and how this distribution is regulated.

Contents

INTRODUCTION

The iron (Fe) content in food plants generally lies in the range of 0.3 mg per 100 g (in starchy tubers) to 3 mg per 100 g (for broccoli and peas), and it can be as high as 11 mg per 100 g (in dried lentils) (30). Vegetables are therefore an important source of nutritional Fe. The amount of Fe in plants is 5–100-fold higher than that of any other transition metal required for plant life—namely, molybdenum, manganese, cobalt, nickel, and copper. However, Fe is difficult to extract from the environment, being insoluble in its oxidized form. Moreover, as a redox-active element, Fe is potentially very toxic, and its intracellular concentration therefore needs to be tightly regulated. Clearly, these disadvantages are outweighed by the benefits of Fe for (plant) life, which lie in its extreme versatility as an enzyme cofactor.

In photosynthetic organisms, iron-sulfur (Fe-S) clusters are arguably the most abundant use of Fe as a cofactor, much more so than heme. However, no precise figures can be given because the labile Fe-S clusters are not easy to quantify, and the abundances of Fe-S and heme proteins vary in different plant tissues. The key role of Fe-S clusters in photosynthesis sparked early interest and research into these cofactors (27, 108, 115) and raised the question of how they are assembled (61, 109, 110). Despite these efforts, the principal genes involved in Fe-S cluster assembly were identified in bacteria and yeast only in the last 15 years, almost 50 years after the clusters were discovered (64, 111, 139). By contrast, heme biosynthesis pathways were unraveled several decades ago, and research is currently directed to the cell biology and regulation of heme biosynthesis (74, 112).

In this review, we focus on the recently characterized assembly pathways of Fe-S cofactors in plants, mainly in *Arabidopsis*. (For more details on heme biosynthesis, see the reviews provided by References 74 and 112.) We also discuss the intersections of the assembly pathways of Fe-S clusters, heme, and molybdenum cofactor (Moco). The pathways are placed in a cell-biological context, which is particularly relevant in plant cells because of the compartmentalization in plastids, mitochondria, and cytosol. We mainly provide outlines of the Fe-S cluster assembly pathways;

Moco: molybdenum cofactor

for more biochemical and biophysical details on the individual proteins involved, we refer readers to several excellent reviews (23, 65, 97). In addition, we summarize how mutants in Fe-S cluster assembly affect specific processes in plant growth and development. We also include data from large-scale systems studies to discuss how the biosynthesis of Fe cofactors may be regulated.

Fenton reaction: reaction of ferrous iron (Fe^{2+}) with hydrogen peroxide to produce ferric iron (Fe^{3+}) and a highly reactive hydroxyl radical

THE CHEMISTRY OF FE, HEME, AND FE-S CLUSTERS

Before we can discuss the cell biology of Fe cofactors, it is necessary to briefly go over the chemical properties of Fe, either on its own or in complex with sulfur (S) or organic structures. Fe and S are abundant elements that occurred as soluble Fe^{2+} and S^{2-} (sulfide) in the early anoxic existence of our planet. Mineral surfaces of FeS are naturally good catalysts. In some minerals, Fe and sulfide form rhombs or cubanes similar to those found in biological Fe-S clusters (87). Upon the dramatic rise in atmospheric oxygen, Fe was oxidized, and it now exists mainly as insoluble Fe^{3+}. S is present in the environment predominantly as soluble SO_4^{2-}, and eight reducing equivalents are required to incorporate S into the amino acids cysteine and methionine, or, via cysteine, into Fe-S clusters. Fe-S clusters are easily destroyed by O_2 or reactive oxygen species, releasing free Fe^{2+} that catalyzes the Fenton reaction, generating more reactive oxygen species. Nevertheless, Fe, heme, and Fe-S-dependent enzymes are widespread in the aerobic world and play many critical roles in essential metabolic pathways. Only a handful of examples exist in which Fe has been replaced by copper as a redox center (72) or by different chemical structures altogether. Clearly, the properties of Fe are unique and irreplaceable, and cells spend a large amount of energy to acquire Fe from the environment and handle it safely inside the cell.

Fe belongs to the group of transition metals, which can give rise to cations with an incomplete *d* subshell of electrons. The more exposed electrons can form π bonds with organic ligands. The accessible oxidation states are Fe^{2+} with six *d* electrons and Fe^{3+} with five *d* electrons. Organic structures, ligands, and protein folds can extend the range of redox potentials of Fe cofactors from −650 mV to +450 mV. Generally, Fe-S enzymes tend to have more negative redox potentials, and heme and other Fe proteins tend to have more positive redox potentials (11).

In heme, Fe is coordinated in a tetrapyrrole ring with four nitrogens as ligands. Heme is a flat, rigid structure that is highly stable (**Figure 1*a***). The function of Fe in heme depends on the axial ligands perpendicular to the tetrapyrrole ring. If one axial ligand is unoccupied, Fe can bind oxygen and serve as an oxygen carrier, as in hemoglobin and myoglobin. In cytochrome P450 enzymes, the free site binds water to catalyze hydroxylations and other reactions. In cytochromes with an electron transfer function, the axial ligands are commonly two histidines or one histidine and one methionine. In the siroheme proteins sulfite reductase and nitrite reductase, a cysteine ligand bridges to an Fe_4S_4 cluster (**Figure 1*b***). Heme can occur as free heme, but it is highly insoluble and its concentration inside the cell is extremely low. Heme is transported across membranes, but the current knowledge of heme transporters is fragmented and in some cases ambiguous (39).

In Fe-S clusters, $Fe^{2+/3+}$ is combined with S^{2-}, which is inserted in the sulfane (S^0) form and subsequently reduced. Once incorporated in a cluster, the S^{2-} does not undergo redox transitions, but the *d* electrons of Fe become delocalized. Therefore, the valency of the cluster as a whole is indicated using square brackets: $[2Fe\text{-}2S]^{2+} + e \leftrightarrow [2Fe\text{-}2S]^{+}$. The Fe atoms are usually bound by cysteinyl ligands of the protein. Other ligands also occur, such as histidine in the Rieske-type cluster, increasing the redox potential to +300 mV. Most common are rhombic Fe_2S_2 and cubane Fe_4S_4 clusters (**Figure 1*c***). Interconversions of cluster types have been demonstrated in vitro and in vivo, illustrating the modular nature of the clusters (12, 138). The Fe_4S_4 cluster is less stable than Fe_2S_2. Although, as mentioned above, Fe-S clusters are easily destabilized by oxygen, it should be

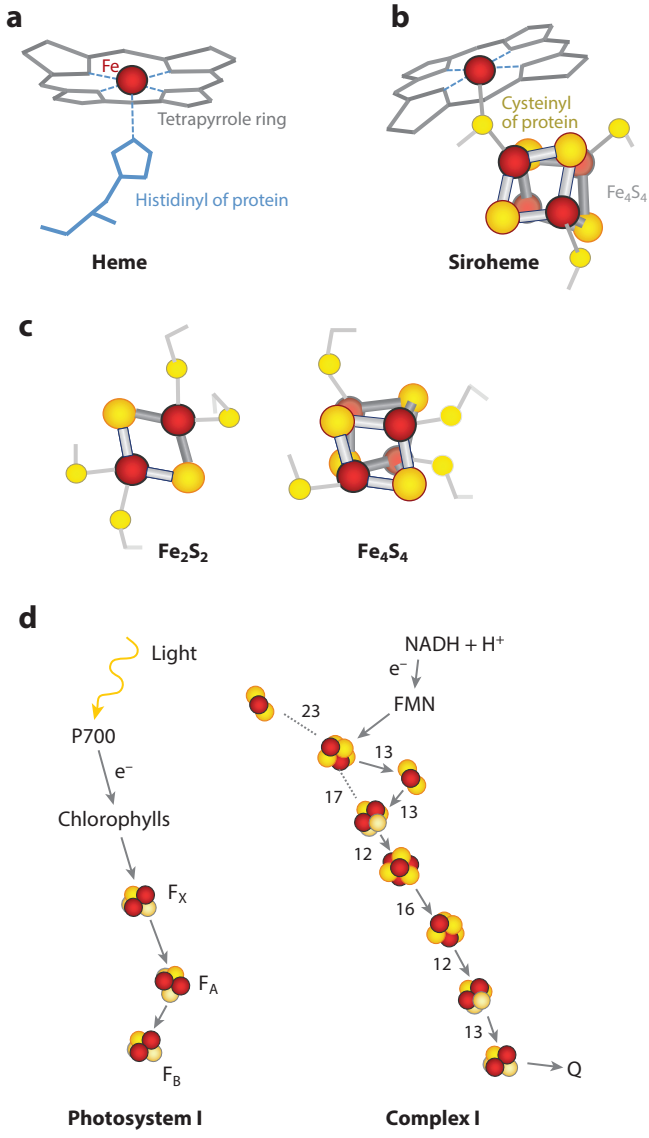

Figure 1

Structures of Fe cofactors. (*a*) Heme is a planar structure consisting of a tetrapyrrole ring (*gray*) and Fe (*red*). Fe is inserted in the center and bound by four nitrogen ligands. The axial ligands of heme bind the cofactor to the protein backbone (histidine residue in *blue*) or, when unoccupied, are involved in substrate binding. (*b*) In siroheme, a protein-bound cysteinyl residue forms a bridge between heme Fe and an Fe_4S_4 cluster. (*c*) Fe-S clusters are modular structures. Two Fe_2S_2 clusters (*left*) can be converted into an Fe_4S_4 cluster (*right*) by reductive coupling. Fe_4S_4 clusters are the most common form of Fe-S cluster. The Fe atoms bind the protein cysteinyl groups as depicted here, but other amino acid residues are also found as ligands, for instance, histidine in Rieske protein. (*d*) Strings of Fe-S clusters channel electrons across protein complexes, such as in photosystem I and respiratory complex I. Indicated are the P700 reaction center of photosystem I and the organic cofactors NADH, flavin mononucleotide (FMN), and ubiquinone (Q) for complex I. The distances between clusters (in angstroms) are indicated for complex I. Panels were drawn manually from available 3D models based on crystal structures.

noted that Fe_2S_2 ferredoxins are remarkably stable when purified in the presence of atmospheric oxygen levels.

Most Fe-S clusters have an electron transfer function (11). A string of clusters with individual distances of less than 14 Å can transfer electrons across a protein complex, such as in photosystem I and respiratory complex I (**Figure 1*d***). Electron transfer chains often bridge flavin cofactors with ubiquinone or hemes.

Fe-S clusters can also have catalytic functions. For example, the Fe_4S_4 cluster in aconitase acts as a Lewis acid: One of the nonliganded Fe atoms of the cluster binds the substrate citrate and removes a proton and a hydroxyl group, which is followed by rehydration of the substrate to form isocitrate. In radical *S*-adenosyl methionine enzymes such as biotin synthase and lipoate synthase, an Fe_4S_4 cluster is employed to stabilize an *S*-adenosyl radical, and a second cluster serves as an S donor. In helicases and DNA-binding proteins, Fe_4S_4 clusters appear to provide stability to a protein fold (129). However, why Fe-S clusters are chosen over zinc centers in certain proteins remains to be established. The labile nature of Fe_4S_4 clusters has been exploited in several regulatory proteins, mainly in bacteria—for example, in redox-sensitive transcription factors (29). Thus far, no examples of regulatory Fe-S proteins have been found in plants or algae.

Holoprotein: a protein combined with its prosthetic group, including cofactors or metal ions (cf. apoprotein, defined below)

TAT: twin-arginine transport

Iron-sulfur cluster (ISC): an assembly pathway in mitochondria or bacteria

Sulfur mobilization (SUF): an Fe-S assembly pathway in the plastids or (cyano)bacteria

COMPARTMENTALIZATION OF FE COFACTOR ASSEMBLY IN PLANT CELLS

Mitochondria and chloroplasts harbor many abundant Fe proteins and are the principal sites of Fe cofactor assembly. Heme biosynthesis, in particular tetrapyrrole biosynthesis, is localized in the plastids, but some reports have indicated that the last step, Fe insertion by ferrochelatase, may also take place in the mitochondria (74, 112). By contrast, Fe-S cluster assembly is mediated by distinct pathways in different cell compartments, in line with the cellular distribution of Fe-S proteins and the instability of Fe-S clusters. In addition to being in the chloroplasts and mitochondria, Fe-S proteins are found in the cytosol and nucleus, but they are likely to be absent from the endomembrane system (endoplasmic reticulum) and peroxisomes. In the endoplasmic reticulum, redox reactions and hydroxylations are carried out by thiol oxidases and heme enzymes. In the case of peroxisomes, it is thought that the H_2O_2 generated in this compartment would destroy Fe-S clusters. As a consequence, one step of the glyoxylate cycle, the conversion of citrate to isocitrate by aconitase, is located in the cytosol, necessitating the transport of citrate out of the peroxisome and isocitrate back into it (42).

Protein localization data showed that proteins involved in the assembly of Fe-S clusters are present in the plastid stroma, mitochondrial matrix, and cytosol (**Figure 2*a***). Those Fe-S proteins with a nuclear destination are likely to be transported as holoproteins across the nuclear envelopes through the nuclear pores. Within the organelles, the Rieske proteins are found in subcompartments and are therefore separated from the assembly machinery by a membrane. The Rieske proteins in the thylakoids of plastids are thought to be transported by the twin-arginine transport (TAT) system (38). The TAT system is well studied in bacteria and mediates the membrane transport of completely folded proteins with their cofactors (91). The universal occurrence of a TatC homolog in plant mitochondrial genomes and a twin arginine in the N terminus of the Rieske protein of cytochrome bc_1 is suggestive of TAT-mediated transport of this protein in plant mitochondria, but this remains to be investigated.

The Fe-S cluster assembly pathways in plant mitochondria and chloroplasts are similar to those found in bacteria. In *Escherichia coli*, two different genetic systems for Fe-S cluster assembly have been identified and well characterized: the *iron-sulfur cluster* (*isc*) gene cluster (comprising seven core genes) and the *sulfur mobilization* (*suf*) operon (comprising six genes) (97). Systems in other

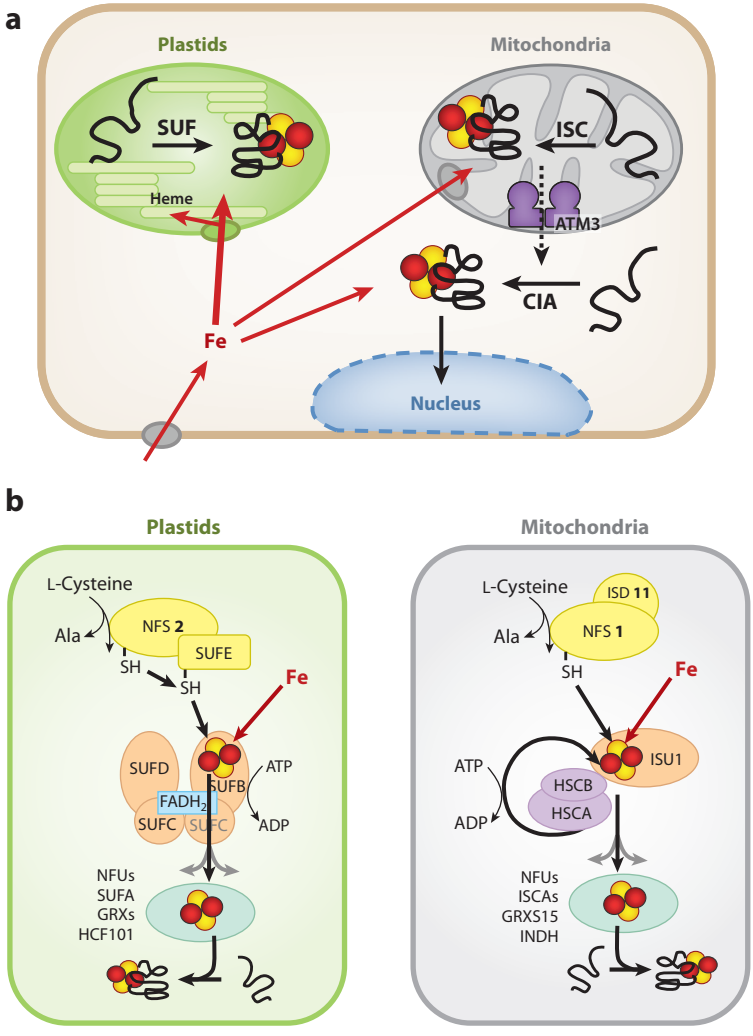

Figure 2

Outline of Fe-S cluster assembly pathways in plant cells. (*a*) Localization of the sulfur mobilization (SUF) pathway in plastids (stroma), the iron-sulfur cluster (ISC) pathway in mitochondria (matrix), and cytosolic iron-sulfur protein assembly (CIA) proteins in the cytosol. Iron (*red*) enters the cell via the plasma membrane and is then distributed between the different Fe cofactor assembly pathways. The amount of iron allocated to each assembly pathway depends on requirements in the cell, but, especially in leaves, most of the iron goes to the plastids (*thicker arrow*). Assembly of Fe-S proteins in the cytosol depends on the ATP-binding cassette (ABC) transporter ATM3, which transports an unknown compound. (*b*) The basic steps in the SUF and ISC pathways. After cluster assembly on the scaffold (SUFBCD and ISU1, respectively), the cluster can be transferred to different carrier proteins (NFU, etc.), indicated by gray arrows. Both systems also need electrons and electron donors, but for simplicity, these are not depicted.

organisms bear significant similarity to one of these two systems. For example, the *nifS* and *nifU* genes in nitrogen-fixing bacteria have clear homology to the *iscS*, *iscU*, and *fdx* genes of the *isc* gene cluster in *E. coli* (139). Gram-positive bacteria and cyanobacteria have *suf*-like systems, whereas archaea have homologs of at least one *sufB* and one *sufC* gene (111).

KEY DIFFERENCES IN FE-S CLUSTER ASSEMBLY PATHWAYS BETWEEN PLANTS AND FUNGI/METAZOA

Studies of *Arabidopsis* genes and mutant alleles involved in Fe-S cluster assembly have identified several key differences compared with fungi and metazoa:

- Plants have an additional Fe-S cluster assembly pathway in the plastids, including a complete set of SUF proteins and additional NFU proteins, similar to the pathway in cyanobacteria.
- Specialized SUFE proteins characterized by different C-terminal domains are present in higher plants but not in algae.
- The ABC transporter of the mitochondria (ATM3 in *Arabidopsis*) is required for Fe-S protein assembly in the cytosol but plays no role in Fe signaling. This suggests that the Fe sensor, or the Fe-sensing mechanism in plants, is different from that in yeast or mammals (see sidebar Fe-Sensing Mechanisms in Yeast and Mammals, below).
- Plants and algae do not have a CFD1 homolog in the cytosolic assembly pathway. The NBP35 protein is thought to function as a dimer, in contrast to the Nbp35-Cfd1 heterotetrameric complex in yeast and mammals.

From the *Arabidopsis* genome sequence released in 2000 (4) it became clear that plants have complete sets of homologs for both the ISC and SUF systems (6, 48). All ISC and SUF homologs are encoded by the nuclear genome, but most have N-terminal sequences with signatures for organellar targeting. Bioinformatics algorithms predict that all SUF homologs are targeted to the plastids and all ISC homologs are targeted to the mitochondria (**Figure 2*a***, **Table 1**). A decade of gene characterization studies using fluorescent protein fusions or immunolabeling has indeed confirmed the predicted localization of most key components. Large-scale proteomics studies support these results (**Table 2**).

The presence of essential Fe-S proteins in the cytosol and nucleus indicates that the cytosol has the capacity to assemble and repair Fe-S clusters. Proteins involved in cytosolic/nuclear Fe-S cluster assembly have been identified in yeast and placed together in the so-called cytosolic iron-sulfur protein assembly (CIA) pathway (64). Five of the six CIA protein members are highly conserved in all eukaryotes, but they bear no sequence similarity to proteins of the ISC and SUF pathways. Nevertheless, functional parallels (electron transfer, scaffolds) can be drawn to some extent with the ISC system (**Table 1**). The cytosolic pathway appears to depend on the mitochondrial assembly pathway and an ATP-binding cassette (ABC) transporter protein in the inner mitochondrial membrane (see below and **Figure 2*a***).

Genome analysis of *Chlamydomonas* (37), rice (63), and alveolates with secondary plastids such as diatoms (17) and *Plasmodium* (41) suggests that the pathways of Fe-S cluster assembly are highly conserved in the green lineage. The main differences compared with fungi and metazoa are summarized in the sidebar Key Differences in Fe-S Cluster Assembly Pathways Between Plants and Fungi/Metazoa.

CIA: cytosolic iron-sulfur protein assembly

ABC transporter: ATP-binding cassette transporter

MOLECULAR STEPS OF THE ISC AND SUF ASSEMBLY PATHWAYS

Functional analyses of the ISC and SUF proteins in *Arabidopsis* indicate that the pathways operate similarly to the well-studied systems in bacteria (ISC and SUF) and yeast mitochondria (ISC). Therefore, we provide a brief overview of the key steps in the assembly process here, and refer readers to other excellent reviews for further details (23, 65, 97).

Table 1 Proteins involved in Fe-S cluster assembly and the last step of heme biosynthesis

Plastids	Mitochondria	Cytosol	Proposed function	
Fe-S cluster assembly				
SUF pathway	**ISC pathway**	**CIA pathway**		
NFS2/CpNifS/SUFS (AT1G08490)	**NFS1** (AT5G65720)	Donated by NFS1 via **ATM3**/STA1/ABCB25 (AT5G58270)?	Cysteine desulfurase (Persulfide generation)	
SUFE1-BolA[a] (AT4G26500) **SUFE2** (AT1G67810) **SUFE3**-NADA[a] (AT5G50210)	**ISD11** (AT5G61220) SUFE1-BolA[b] (AT4G26500)		Necessary for NFS2 and NFS1 activity, respectively	
FAD cofactor of the $SUFBC_2D$ complex	**ADXR**/MFDR (AT4G32360)	**TAH18**/ATR3 (AT3G02280)	Reductase	Electron donation
	ADX1/MDFX2 (AT4G21090) ADX2/MDFX1 (AT4G05450)	**DRE2**/CIAPIN (AT5G18400)	Electron transfer	
$SUFBC_2D$ complex (AT4G04770, AT3G10670, AT1G32500)	**ISU1** (AT4G22220) ISU2 (AT3G01020) ISU3 (AT4G04080)	**NBP35** (AT5G50960)	Fe-S cluster scaffold	
	FH/frataxin (AT4G03240)		Putative Fe donor, regulator of NFS1	
	HSCA1 (AT4G37910) HSCA2 (AT5G09590)		Chaperone	
	HSCB (AT5G06410)		Cochaperone	
NFU1 (AT4G01940) **NFU2** (AT5G49940) **NFU3** (AT4G25910)	**NFU4** (AT3G20970) NFU5 (AT1G51390)		Fe-S carrier	
SUFA/CpIscA (AT1G10500)	ISA1/ISCA2 (AT2G16710) ISCA3 (AT2G36260) ISCA4 (AT5G03905)		Fe-S carrier (A-type), Fe donor?	
		CIA1 (AT2G26060) **AE7**/CIA2 (AT1G68310) **MET18**/MMS19 (AT3G02280) **NAR1**/GOLLUM (AT4G16440)	Formation of a complex and association with target Fe-S proteins	
GRXS14 (AT3G54900) GRXS16 (AT2G38270)	GRXS15 (AT3G15660)	GRX?[c]	Glutathione-dependent redoxins	
BolA domain of SUFE1	BolA?[c]	BolA?[c]	Interaction with glutaredoxins	
HCF101 (AT3G24430)	INDH (AT4G19540)		Assembly of Fe-S clusters on selected proteins, ATP dependent	
	IBA57 (AT4G12130)		Assembly of Fe-S clusters on aconitase and radical SAM proteins in yeast	
HydEF, HydG (*Chlamydomonas*)			Assembly of H-type Fe-S clusters in hydrogenase	

(*Continued*)

Table 1 (*Continued*)

Plastids	Mitochondria	Cytosol	Proposed function
Heme biosynthesis			
Ferrochelatase I (AT5G26030) Ferrochelatase II (AT2G30390)	Ferrochelatase I? (AT5G26030)		Insertion of Fe in heme
SIRB (AT1G50170)			Insertion of Fe in siroheme

The principal Fe cofactor assembly proteins found in higher-plant species, based on *Arabidopsis* studies and genome analyses of other species, are in bold. Paralogs present in the *Arabidopsis* genome are also listed. The *Arabidopsis* AGI numbers are in parentheses. Because of space limitations, not all proteins are discussed and referenced in this review, but further details can be found in References 9 and 23. The colors match those used in **Figure 2*b***.

[a]BolA and NADA are protein domains of SUFE1 and SUFE3, respectively.

[b]Mitochondrial localization of SUFE1 is reported in References 100 and 134 (see also **Table 2**).

[c]The existence of this putative assembly protein is based on studies in yeast or human cells and the presence of candidate genes in the *Arabidopsis* genome.

The ISC and SUF systems have clear homologies with respect to the proteins involved in S transfer and those involved in cluster transfer to target proteins. However, the scaffolds for cluster assembly of each system consist of a different set of proteins (**Figure 2*b***, **Table 1**).

Persulfide Transfer

S is provided by a cysteine desulfurase (EC 2.8.1.7), a pyridoxal phosphate–dependent enzyme that catalyzes the removal of S from L-cysteine. The sulfane S (S^0) is bound to the active site of the enzyme as a persulfide (R-S-S^0H) and transferred in this form to the accepting scaffold protein. Although S^{2-} is the form of S in the clusters, H_2S or other forms of S^{2-} cannot be used effectively for Fe-S cluster assembly in vivo. For instance, mutants in sulfite reductase do not have defects in Fe-S protein biogenesis (**http://www.yeastgenome.org**).

Plants have three cysteine desulfurases, NFS1, NFS2, and ABA3, which have been characterized in vivo and in vitro in *Arabidopsis* (reviewed in 9, 23). NFS1 has extensive sequence homology with bacterial IscS and is localized in the mitochondria. NFS2 is more closely related to SufS in (cyano)bacteria and functions in the plastids. ABA3 consists of a sulfurtransferase domain and a C-terminal Moco-binding domain (127). The function of ABA3 is dedicated to the sulfuration of Moco in the cytosol and is not involved in Fe-S cluster assembly (14).

NFS1 and NFS2 differ in various aspects, such as their partner proteins, oxygen sensitivity, and enzyme kinetics. Yeast NFS1 requires the ISD11 protein for its stability and activity (2, 92, 126). ISD11 is highly conserved in all eukaryotes, including *Arabidopsis*, but is absent from bacteria. Several attempts to express *Arabidopsis* NFS1 in *E. coli* resulted in insoluble protein, but refolding of purified inclusion bodies yielded small amounts of functional enzyme with low activity (32, 94, 117, 134). Coexpression of ISD11 may improve stability of *Arabidopsis* NFS1, but this has not been tested.

Arabidopsis NFS2 (also named CpNifS) has been expressed at high levels in *E. coli* and purified. The cysteine desulfurase activity is >40-fold stimulated by SUFE1 (134, 137), similar to the activation of SufS by SufE in *E. coli* (90). Three forms of the SUFE protein are encoded in higher-plant genomes, compared with one form in cyanobacteria and green algae (23, 37, 81). SUFE1 has an additional C-terminal domain with sequence homology to bacterial BolA proteins, so called because overexpression of BolA in *E. coli* influences the stability of the bacterial cytoskeleton and induces the bacteria to become ball-shaped (33). SUFE2 has no extra C-terminal domain, whereas

Table 2 Proteomics data for Fe cofactor assembly proteins in *Arabidopsis* and potato mitochondria

Protein names	AGI number	*Arabidopsis* mitochondria (SUBA3, MS/MS)[a]	Potato mitochondria[b]
Mitochondrial proteins required for mitochondrial Fe-S cluster assembly			
NFS1	AT5G65720	Yes (4 independent studies)	Yes
ISD11	AT5G61220		Yes
SUFE1	AT4G26500		Yes (!)
ADXR/MFDR	AT4G32360	Yes (52)	Yes
ADX1/MDFX2	AT4G21090		Yes
ADX2/MDFX1	AT4G05450		Two other Fdx proteins with homology to AT3G07480 were found
ISU1	AT4G22220		Yes
ISU2	AT3G01020		
ISU3	AT4G04080		
FH	AT4G03240		Yes
NFU4	AT3G20970	Yes (52)	Yes
NFU5	AT1G51390		
HSCA1	AT4G37910	Yes (7 independent studies)	
HSCA2	AT5G09590	Yes (6 independent studies)	Yes
HSCB	AT5G06410		Yes
INDH	AT4G19540	Yes (52)	Yes
ISA	AT2G16710		Yes (2 homologs)
	AT2G36260		
	AT5G03905		Yes
IBA57	AT4G12130	Yes (113)	Yes
GRXS15	AT3G15660	Yes (5 independent studies)	Yes
Ferritins			Yes
Mitochondrial proteins required for cytosolic Fe-S cluster assembly			
ATM3/STA1/ABCB25	AT5G58270	Yes (3 independent studies)[c]	Yes
ERV1	AT1G49880		
Cytosolic proteins (CIA pathway)			
TAH18/ATR3	AT3G02280		No CIA proteins were found in purified potato mitochondria
DRE2/CIAPIN1	AT5G18400		
NBP35	AT5G50960		
NAR1	AT4G16440	Yes, in cytosol (47)	
CIA1	AT2G26060		
AE7/CIA2	AT1G68310		
	AT3G50845		
HSCB	AT5G06410		

(*Continued*)

Table 2 (*Continued*)

Protein names	AGI number	*Arabidopsis* plastids (SUBA3, MS/MS)[a]	Potato mitochondria[b]
Plastid proteins (SUF pathway)			
NFS2/CpNifS/CpSufS	AT1G08490	Yes (2 independent studies)	
SUFE1	AT4G26500	Yes (4 independent studies)	Yes
SUFE2	AT1G67810	Yes	
SUFE3	AT5G50210		
SUFB/NAP1	AT4G04770	Yes (3 independent studies)	
SUFC/NAP7	AT3G10670	Yes (4 independent studies)	
SUFD/NAP6	AT1G32500	Yes (2 independent studies)	
NFU1	AT4G01940		
NFU2/CnfU	AT5G49940	Yes (2 independent studies)	
NFU3	AT4G25910	Yes	
SUFA/CpIscA	AT1G10500		
HCF101	AT3G24430	Yes (3 independent studies)	
GRXS14	AT3G54900	Yes (3 independent studies)	
GRXS16	AT2G38270	Yes (5 independent studies)	
Ferrochelatase I	AT5G26030	Yes	
Ferrochelatase II	AT2G30390	Yes (3 independent studies)	

A blank space denotes that the protein has not been found so far using proteomics; for *Arabidopsis*, the localization has generally been confirmed by other experimental techniques.

[a]Tandem mass spectrometry data, 2013 (except where a separate reference has been given).

[b]Data from Salvato et al. (100).

[c]ATM3 has also been identified in plastid membranes and the plasma membrane. For a discussion of these data, see Reference 13.

SUFE3 is fused to NADA, or quinolinate synthase, an enzyme in nicotinamide adenine dinucleotide (NAD) biosynthesis. Insertion mutants of *SUFE1* or *SUFE3* are embryo lethal, suggesting that there is no redundancy in function (81). Mutants in *SUFE2* have not been described.

The ISC and SUF systems in *E. coli* differ in their tolerance to oxidative stress (26). Comparison of crystal structures of the cysteine desulfurases showed that the active-site cysteine in SufS is deeply buried and therefore less accessible to oxygen or H_2O_2, whereas the active site of IscS is surface-exposed (24, 66). The close proximity of Fe-S cluster assembly to the oxygen-generating photosystem II may have exerted selective pressure on ancestral cyanobacteria toward the oxygen-tolerant SUF system, which was then inherited by plastids.

Fe and S Assembly on Scaffolds

The persulfide from NFS1/ISD11 or NFS2/SUFE is transferred to a scaffold protein. In this step, S^0 is reduced to S^{2-}, for which electron donors are required. In the ISC system, electrons are provided by mitochondrial ferredoxin and a ferredoxin reductase. Exactly how and when Fe is inserted has remained elusive despite years of investigation focused on the ISC system.

The proteins functioning as Fe-S scaffolds in the ISC and SUF systems are structurally very different (**Figure 2*b***, **Table 1**). The scaffold protein in the ISC system, called ISU in plant mitochondria, forms a homodimer. The *Arabidopsis* genome encodes three ISU proteins, but most plant species have only one or two *ISU* genes. The expression of *ISU1* is much higher in most tissues than that of *ISU2* and *ISU3* in *Arabidopsis*, and therefore ISU1 is likely the major scaffold protein

(32, 58). The bacterial IscU homolog has been proposed to undergo a cycle of conformations: Interaction with IscS induces IscU to change from a disorganized (unfolded) form to a structured form on which the cluster is assembled (69). An IscS-IscU complex with an Fe_2S_2 cluster has been trapped using a D39A mutant of IscU, and a crystal structure is available (68). The Fe_2S_2 cluster can be converted to an Fe_4S_4 cluster on the IscU scaffold, which requires another two electrons. Whether specific proteins are needed to mediate cluster interconversion is unknown.

The mature Fe-S cluster is released by the interaction of chaperones and ATP hydrolysis, and IscU returns to the disorganized form (69). The chaperone interaction cycle is well characterized in bacteria and yeast mitochondria (65, 97 and references therein), but very little is known in plants. An initial study on this topic showed protein interaction between the cochaperone HSCB and ISU1 (132).

The scaffold of the SUF system consists of a complex of three proteins: SUFB, SUFC, and SUFD, predominantly in a 1:2:1 (BC_2D) stoichiometry (90, 128). Available crystal structures of SufC suggest that the complex shares the overall conserved architecture of ABC transporters, but without being inserted into a membrane (51, 124). This architecture was predicted from amino acid sequence analyses and gave rise to the names NAP1, NAP7, and NAP6 (nonintrinsic ABC protein) for SUFB, SUFC, and SUFD, respectively, when the proteins were first characterized in *Arabidopsis* (131, 133). In *E. coli*, an Fe_4S_4 cluster can be assembled on SufB (56). SufB also has a conserved flavin adenine dinucleotide ($FADH_2$)–binding site (128). The flavin cofactor is thought to play a role in reducing S^0 to S^{2-}. More than 20 years ago, Takahashi et al. (109) showed that NADPH is required for cluster assembly in plastids, so the most likely scenario is that NADPH directly transfers electrons to FAD on SUFB. Both the SUFB and SUFC proteins in *Arabidopsis* have ATPase activity in vitro (131, 133). In analogy with the ISC system, ATP hydrolysis may induce conformational changes that release the Fe-S cluster.

In mitochondria, a protein called frataxin has been suggested to deliver Fe to ISU by direct interaction with the NFS1/ISD11/ISU1 complex. Frataxin is highly conserved in all eukaryotes and is also found in bacteria. The protein is named after the disease Friedreich's ataxia, which is caused by mutation of the *FRATAXIN* gene (93). However, alternative suggestions are that frataxin plays a direct role in Fe homeostasis, and as such indirectly affects Fe-S cluster assembly, or that it has a regulatory function in the assembly process (93). The frataxin homolog in *Arabidopsis* is essential for embryo development (16, 121). A viable mutant allele resulted in decreased activities of aconitase and succinate dehydrogenase, an approximately 60% increase in total Fe in cell extracts, and a 20% increase in Fe in the mitochondrial fraction (16, 70). However, the effect on Fe homeostasis in *Arabidopsis* is modest compared with the yeast frataxin mutant (65). In plastids, ferritin would be an obvious candidate to provide Fe to SUFB. Surprisingly, the triple mutant in the three major ferritins in *Arabidopsis* did not affect photosynthesis, which is highly dependent on Fe-S cluster assembly (96). It has therefore been proposed that the primary role of ferritin is as an Fe scavenger rather than an Fe donor.

Carrier Proteins and Cluster Insertion into Apoproteins

There are a number of additional genes that either are associated with *isc* and *suf* operons in bacteria, or have functionally been shown to be involved in Fe-S cluster assembly. These include genes encoding the NFU proteins, the ISCA and SUFA proteins (A-type carriers), the monothiol glutaredoxins, and the P-loop NTPases HCF101 and INDH (**Figure 2*b***, **Table 1**). Each type of protein can assemble an Fe-S cluster in vivo and in vitro, which is transferred to an apoprotein in vitro within minutes when the two proteins are mixed together (reviewed in 23, 97). By contrast, cluster transfer in the other direction, i.e., from the carrier to the scaffold,

does not occur. Carrier proteins have been suggested to provide specificity for a particular Fe-S target protein or group of targets. However, the functions of several carrier proteins are redundant, because knockout mutants in each of *Arabidopsis NFU2*, *SUFA*, and *GRXS14* and *GRSX16* (both encoding plastid-localized glutaredoxins) are viable (1, 23, 116, 135, 136). Therefore, the carrier proteins could form a flexible network to deliver Fe-S clusters, providing robustness when a plant is challenged by oxidative stress, and clusters need to be replaced rapidly (23).

HCF101 and INDH are P-loop NTPases with homology to NBP35, the proposed scaffold protein of the CIA pathway (19; also see below). In contrast to the genes encoding NFU proteins and A-type carriers, *HCF101* and *INDH* are essential. HCF101 is localized in the plastids and is required for assembly of photosystem I and ferredoxin-thioredoxin reductase (59, 101). INDH is localized in the mitochondria and is required for complex I assembly (18, 130). The glutaredoxins are thought to function as Fe-S cluster carriers, but there is no evidence yet for their role in Fe-S cluster assembly in planta (for a discussion of in vitro data, see 23).

Apoprotein: a protein stripped of any prosthetic group or metal ion normally associated with it (cf. holoprotein, defined above)

ATM: ABC transporter of the mitochondria

ASSEMBLY OF FE-S CLUSTERS IN THE CYTOSOL AND NUCLEUS

Based on studies in yeast, a set of seven proteins has been described that is required for assembly of cytosolic and nuclear, but not mitochondrial, Fe-S clusters. One of these proteins is a transporter in the mitochondrial membrane; the other six, of which five are conserved in the green lineage, are localized in the cytosol. The sources of Fe and S have not been established for cytosolic Fe-S proteins. Analysis of *Arabidopsis* mutants in each of its three cysteine desulfurases that could provide S^0—mitochondrial NFS1, plastid NFS2, and cytosolic ABA3—showed that the activity of the cytosolic Fe-S enzyme aconitase was only decreased in a viable *nfs1* mutant, and was not affected in *nfs2* and *aba3* mutants (14). In yeast, mitochondrial targeting of NFS1 is critical for Fe-S cluster assembly on cytosolic/nuclear Fe-S proteins (78). In human cells, which have a major mitochondrial and a minor cytosolic isoform of NFS1 (named ISCS), silencing of the mitochondrial isoform affected cytosolic Fe-S enzymes more than silencing of the cytosolic isoform (15, 31, 62). Therefore, it is possible that the mitochondrial cysteine desulfurase provides (per)sulfide to cytosolic Fe-S proteins.

Function of the ABC Transporter of the Mitochondria ATM3

In addition to NFS1, cytosolic Fe-S cluster assembly requires the activity of an ABC transporter of the mitochondria (ATM), which is a member of the ABCB superfamily. This was shown first for Atm1 in *Saccharomyces cerevisiae* (50) and later for ABCB7 in mammals (95) and ATM3 in *Arabidopsis* (13). Atm1 is located in the inner mitochondrial membrane, with the nucleotide-binding domains facing the matrix (57). The orientation and the fact that transporter mutants have a specific defect in cytosolic but not mitochondrial Fe-S enzymes suggest that ATMs export a molecule from the matrix to the intermembrane space. The molecule(s) may cross the outer mitochondrial membrane through pores. The precise chemical nature of the exported molecule is not known, but available data make speculation possible (see below). Yeast cells in which *ATM1* is deleted (Δ*atm1*) accumulate secondary phenotypes, such as loss of mitochondrial DNA (57). However, controlled downregulation of *ATM1* expression revealed two primary phenotypes in yeast: loss of activity of cytosolic Fe-S enzymes owing to lack of de novo Fe-S cluster assembly, as shown by ^{55}Fe labeling studies, and Fe accumulation in the mitochondria (50).

Arabidopsis has three *ATM* genes: *ATM1*, *ATM2*, and *ATM3*. The first two are not critical for plant growth, maturation of cytosolic Fe-S enzymes, or Moco biosynthesis (13). By contrast,

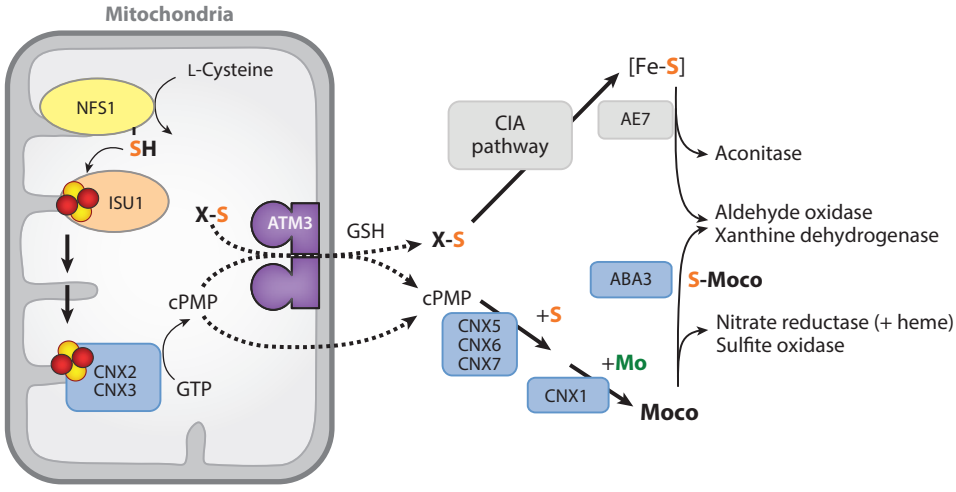

Figure 3

Intersections of Fe-S and molybdenum cofactor (Moco) assembly pathways. The first step of Moco biosynthesis is catalyzed by CNX2 and CNX3 in the mitochondria. CNX2 belongs to the radical *S*-adenosyl methionine enzymes, which depend on Fe_4S_4 as a cofactor. ATM3 is an ATP-binding cassette (ABC) transporter thought to export a molecule (X) containing persulfide (S, shown in *orange*), provided by NFS1. *atm3* mutants have decreased activities of both cytosolic Fe-S proteins (aconitase) and Moco enzymes. The enzymes aldehyde oxidase and xanthine dehydrogenase, which are similar in domain structure, bind two Fe_2S_2 clusters, flavin adenine dinucleotide (FAD), and Moco. Arrows with thick lines represent steps in cofactor assembly, arrows with dotted lines represent tentative steps in cofactor assembly, and thin arrows represent other reactions.

an insertion mutant of *ATM3* exhibited chlorosis and a dwarfed appearance (55). Analysis of Fe-S enzyme activities showed that the activity of cytosolic—but not mitochondrial—aconitase isozymes was strongly decreased in *atm3* alleles (13). Overexpression of *ATM3* can partially reverse the phenotypes of the Δ*atm1* yeast mutant (21, 55), showing that it is a functional ortholog of yeast *ATM1*. However, Fe accumulation in mitochondria was not observed in *atm3* mutant plants, in contrast to yeast cells depleted of Atm1 (13).

Interestingly, two mutant alleles of *ATM3* were isolated in genetic screens for sirtinol resistance designed to isolate mutants in auxin biosynthesis or signaling (13). The biochemical basis for this screen is that sirtinol mimics the effects of auxin after it is metabolized in several steps to 2-hydroxy-1-naphthoic acid (25). The last step is catalyzed by aldehyde oxidase, an enzyme with two Fe_2S_2 clusters, one FAD molecule, and a Moco cofactor (71) (see **Figure 3**). The aldehyde oxidase activity of all four isozymes was indeed decreased in *atm3* mutants (13, 114).

Additionally, activities of the Moco enzymes nitrate reductase and sulfite oxidase decreased by 50%, but only in stronger *atm3* alleles, correlating with an approximate 50% drop in Moco levels. By contrast, the Moco precursor cyclic pyranopterin monophosphate (cPMP) accumulated in mitochondria from the *atm3-1* mutant allele (114). These data indicate that ATM3 is also involved in Moco biosynthesis, downstream of cPMP biosynthesis. The findings also confirm that ATM3 is not required for maturation of mitochondrial Fe-S enzymes as synthesis of cPMP requires the Fe-S enzyme CNX2.

Cyclic pyranopterin monophosphate (cPMP): a precursor of Moco

The requirement of ATM3 for both Fe-S clusters and Moco connects the two biosynthetic pathways and provides new insights into the substrate of ATMs, an outstanding question for almost 20 years (50, 57). The current data suggest that cPMP might be a substrate for ATM3 (114). However, cPMP may also cross the membrane by passive diffusion, which is corroborated

by the fact that treatment of Moco deficiency in human patients can be achieved by intravenous cPMP injection (43). Moreover, cPMP is unlikely to be the sole substrate of ATM3, because Moco enzymes are not involved in Fe-S cluster assembly (71).

Fe accumulation in mitochondria from yeast *atm1* mutants has been taken as evidence that Atm1 exports a preassembled Fe-S cluster (49, 50). However, mitochondria of *atm3* mutants do not accumulate Fe (13), and studies in ABCB7 mouse models showed that the observed Fe accumulation in liver cells is not associated with mitochondrial Fe overload (95). Moreover, all yeast mutants in the ISC pathway accumulate Fe in the mitochondria, and it is therefore not an *atm1*-specific phenotype (65). Additionally, mitochondrial Fe overload is not observed when yeast cells are grown in anaerobic conditions (73). Rather, it has recently been shown that cellular and mitochondrial Fe accumulation occurs because the Fe sensor in yeast depends on an Fe-S cluster, and therefore lack of assembly of this cluster disrupts Fe homeostasis (60, 79) (see below).

The arguments outlined above suggest that ATMs transport a chemical form of S^0, which is required for both Fe-S and Moco assembly. ATMs may also transport cPMP (**Figure 3**). Another compound involved in ATM function is the tripeptide glutathione. In yeast, glutathione depletion in the Δ*gsh1* mutant resulted in strongly decreased activities of cytosolic Fe-S enzymes. Combination of Δ*gsh1* and downregulation of *ATM1* was synthetic lethal in yeast (103). In *Arabidopsis*, *atm3* mutants are sensitive to buthionine sulfoximine, an inhibitor of glutathione biosynthesis (T.A. Schaedler & J. Balk, unpublished results). Therefore, the S^0 may be transported as a glutathione-bound persulfide. In vitro and in vivo studies will hopefully identify the substrate of ATMs in the near future.

The CIA Pathway

The CIA pathway as described in yeast is essential for Fe-S cluster assembly on cytosolic and nuclear proteins and depends on an intact ISC machinery and a functional ATM. CIA proteins are highly conserved in eukaryotes and can be divided into three functional units based on protein interactions:

1. The diflavin reductase TAH18 and the Fe-S protein DRE2 form a short electron transfer chain (14, 86). In plants, yeast two-hybrid studies and pulldown assays showed that TAH18 (also called ATR3) interacts with *Arabidopsis* DRE2 (CIAPIN). TAH18 was able to transfer electrons from NAD(P)H to cytochrome *c* as a generic electron acceptor (14, 120). AtDRE2 can complement a yeast *dre2* mutant, but only when coexpressed with AtTAH18, providing further evidence that interaction between TAH18 and DRE2 is necessary for their function in the CIA pathway (14).
2. NBP35 is a nucleotide-binding protein thought to function as an Fe-S cluster scaffold because it can transfer a cluster to an acceptor protein in vitro (19). NBP35 occurs predominantly as a homodimer in vitro, and the fully reconstituted protein binds a stable Fe_4S_4 cluster at the N terminus of each protomer and a labile Fe_4S_4 cluster bridging the two protomers at the C terminus (19, 54). By contrast, in yeast and mammals, Nbp35 forms a heterotetrameric complex with Cfd1 (84, 85), a protein with homology to Nbp35 except that Cfd1 lacks the N-terminal Fe-S cluster–binding domain (40, 98). Cfd1 is not found in the green lineage, however (19, 54). The role of the nucleotide-binding domains of the Nbp35 and Cfd1 scaffold protein(s) is not understood, but it has been suggested that ATP hydrolysis is important for cluster loading on the Nbp35-Cfd1 complex in yeast (84).
3. A protein complex consisting of CIA1, CIA2, NAR1, and MET18/MMS19 acts downstream in the CIA pathway in yeast (64). Yeast two-hybrid and immunoprecipitation studies have

confirmed the formation of a protein complex of the four *Arabidopsis* homologs (CIA1, AE7/CIA2, NAR1, and MET18). A genetic interaction between a viable *ae7* allele, E51K, and the knockout *met18* mutant was also found (67). Cia1 from yeast has been crystallized and forms a seven-blade propeller structure typical for the WD40 family of proteins, which are important in mediating protein–protein interactions (7, 104). CIA2 is a 26-kDa protein with a DUF59 (domain of unknown function 59) protein domain that has a highly conserved, reactive cysteine residue (125). The DUF59 domain also occurs in the plastid HCF101 protein. NAR1 exhibits 40–50% amino acid sequence similarity to [FeFe] hydrogenases. It binds two Fe-S clusters but does not have hydrogenase activity (8, 20). MET18/MMS19 was found to interact with cytosolic and nuclear Fe-S apoproteins in human cell extracts (35, 105). In *Arabidopsis*, knockout mutant alleles of *MET18* have, surprisingly, no obvious phenotype, whereas knockout mutants in *CIA1*, *CIA2*, and *NAR1* are embryo lethal (67, 77, 83). These results indicate that the function of MET18 is redundant in plants, although no closely related paralogs are present in the genome. Currently, none of the molecular functions of the proteins of this unique complex are known, and their precise role in Fe-S protein biogenesis remains to be defined.

WD40: a tryptophan aspartate motif repeated every 40 amino acids

DUF59: domain of unknown function 59

ABA: abscisic acid

HOW DEFECTS IN FE-S CLUSTER ASSEMBLY AFFECT METABOLISM AND DEVELOPMENT

Given the essential functions of Fe-S proteins, it is perhaps no surprise that disruption of most individual genes involved in Fe-S cluster assembly results in embryo lethality. However, a small number of viable mutant alleles have been constructed by RNA interference (RNAi), identified in genetic screens, or selected from T-DNA mutants with an insertion in the promoter or untranslated region (summarized in **Table 3**). Also, knockout mutants of *ATM3* and *NFU2* are viable and have a clear phenotype. The biochemical data and phenotypes of mutants are providing valuable insights into the assembly proteins themselves, their downstream target proteins, and the importance of Fe-S cluster biosynthesis for plant metabolism and development. Currently, there are approximately 100 known Fe-S proteins in *Arabidopsis*, which underpin many basic processes required for photoautotrophic growth (9).

Hormone Biosynthesis and Growth

In an early review covering Fe-S proteins in plants, Imsande (46) pointed out that enzymes containing Fe-S clusters, such as aldehyde oxidases, might play key roles in hormone biosynthesis and plant development. Indeed, aldehyde oxidase isozymes were later shown to catalyze the last step in abscisic acid (ABA) biosynthesis (102) and auxin biosynthesis (25), and the activity of these enzymes is decreased in mutants affected in cytosolic Fe-S cluster assembly (13, 32, 114). In the *atm3-1* mutant allele, ABA levels are approximately 10% of those in wild-type seedlings. Although ABA levels in *atm3-1* mutant plants were induced by drought stress, they reached only 50% of wild-type levels (114). As mentioned above, mutant alleles of *atm3* were also found in screens for auxin mutants. The lack of auxin could induce the growth of axillary buds, resulting in a bushy appearance. This phenotype is seen not only in strong *atm3* mutants but also in plants with silenced expression of *NFS1* and *ISU1*, correlating with lower aldehyde oxidase activities (32). By contrast, altered plant architecture was not evident in mutants in frataxin (*fh-1*) or HSCB (16, 132).

Mutants in the mitochondrial Fe-S cluster assembly proteins frataxin, HSCB, and NFS1 have lower aconitase and succinate dehydrogenase activities (**Table 3**). *Arabidopsis* has three aconitase isoforms, which are localized in the mitochondria and cytosol. In green seedlings and young leaves, the mitochondrial isoforms represent the dominant activity (5, 13). Therefore, a decrease in total

Table 3 Comparison of phenotypes of viable mutant alleles of genes involved in Fe-S cluster assembly in *Arabidopsis*

Viable mutant allele[a]	Polymorphism	Fe-S proteins affected (% activity remaining)	Resulting phenotypes attributable to defective Fe-S enzyme	Other (unexplained) phenotypes	Reference(s)
Plastids					
grxS14 (*atgrxcp1*, *atgrxcp2*)	T-DNA in CDS (Salk_125903, Salk_056587)	Not investigated	Root growth is sensitive to H_2O_2		22
hcf101	Premature stop	PSI and FTR (Fe_4S_4) but not Fd or Rieske (Fe_2S_2)	Chlorosis, high chlorophyll fluorescence		59, 106
nfs2 (*cpNifS*)	Inducible RNAi	PSI, Fd, Rieske, NiR, SiR, and GOGAT	Chlorosis, impaired photosynthesis		119
nfu2	T-DNA in CDS (SALK_039254)	PSI, Fd, NiR, and SiR but not Rieske, GOGAT, or FNR	Chlorosis, impaired photosynthesis		116, 135
sufa (*CpIsca*)	T-DNA in CDS (SALK_114467)	None	N/A		1, 136
sufb	RNAi		Chlorosis, fewer chloroplasts	Decrease in mitochondrial membrane potential or number	3
sufb (*hmc1*)	Missense	Pheophorbide *a* oxygenase	Impaired chlorophyll degradation		82
sufb (*laf6*)	Ds element in promoter		Accumulation of protoporphyrin IX; no response to far-red light		76, 131
sufd	T-DNA −8 nt of AUG (SALK_055996)	Not investigated	Chlorosis, impaired growth, 30% embryo abortion		44
Mitochondria					
fh-1 (*frataxin*)	T-DNA in promoter (SALK_021263)	Aconitase (10%) and succinate dehydrogenase (70%)	Slow growth		16
grxS15 (*atgrx4-1*, *atgrx4-2*)	T-DNA in CDS	Not investigated	Root growth is sensitive to H_2O_2		
hscb	T-DNA in 3′ UTR (SALK_085159)	Aconitase (10%) and succinate dehydrogenase (10%)		Normal growth, fewer trichomes, waxless, lower seed set	132
indh	T-DNA in CDS (GK_956A05)	NADH:ubiquinone oxidoreductase (complex I, 0%)	Slow germination, delayed development	Pollen and ovule abortion	130

(*Continued*)

Table 3 (*Continued*)

Viable mutant allele[a]	Polymorphism	Fe-S proteins affected (% activity remaining)	Resulting phenotypes attributable to defective Fe-S enzyme	Other (unexplained) phenotypes	Reference(s)
isu1, *isu2*	Antisense RNA	Not investigated	Slow growth, more axillary stems		32
nfs1-2	T-DNA in 5′ UTR (SALK_083681)	Aconitase (30%)	Slow growth		14
nfs1	Antisense RNA	Aldehyde oxidases and likely all mitochondrial Fe-S proteins	Chlorosis, more axillary stems		32
Cytosol/nucleus					
ae7, *ae7-1*	Missense, E51K	Aconitase (cytosol 25% and mitochondria 40%), ROS1, and likely DNA repair enzymes but not aldehyde oxidases	Slow growth, dwarfed appearance, DNA damage, increased frequency of homologous recombination	Chlorosis	67
atm3-1[b]	Truncated fusion	Cytosolic aconitase (20%)[c] Aldehyde oxidases (0%)[c] Xanthine DH (20%)[c] NR and SO (Moco, 50%)[c] DNA repair enzymes	Dwarfed appearance Lower ABA levels, bushy architecture Enlarged nuclei, DNA damage, increased frequency of homologous recombination	Chlorosis, fewer chloroplasts, no palisade cells Serrated leaf edges (especially in *atm3-4 ae7-1* double mutant)	13, 55, 67
atm3-2	T-DNA in CDS (GK-714C03)				
atm3-3	Missense, R612K				
atm3-4	39-nt deletion in promoter				
met18-1 *met18-2*	T-DNA in CDS (SALK_121963, SALK_147068)	None			67
nar1-3/+	Premature stop	DEMETER?	Lower expression of FWA:GFP		83
nar1-4	T-DNA in 5′ UTR (SALK_151803)			Germination on 3 μM paraquat	83
nar1	RNAi		Dwarfed appearance Block in cell cycle	Same as wild type under low oxygen pressure	20, 77

Abbreviations: ABA, abscisic acid; CDS, coding sequence; Fd, ferredoxin; FNR, ferredoxin-NADP oxidoreductase; FTR, ferredoxin-thioredoxin reductase; GOGAT, glutamine:oxoglutarate aminotransferase; Moco, molybdenum cofactor; NiR, nitrite reductase; NR, nitrate reductase; nt, nucleotide; PSI, photosystem I; RNAi, RNA interference; SiR, sulfite reductase; SO, sulfite oxidase; UTR, untranslated region; xanthine DH, xanthine dehydrogenase.

[a]All mutant alleles are in the Columbia-0 background except for *ae7*, which is in the Landsberg *erecta* (L*er*) background.

[b]Knockout mutants of *ATM1* and *ATM2* have no obvious phenotype.

[c]In the knockout allele *atm3-2*.

aconitase as well as succinate dehydrogenase activities has a major impact on the tricarboxylic acid cycle and is expected to affect vegetative growth, as observed in *fh-1* and *nfs1* mutants. Surprisingly, plants with the *hscb* mutant allele were reported to have only 10% aconitase and 10% succinate dehydrogenase activity but still grew normally (132). The *hscb* plants displayed phenotypes that so far have not been observed in any other Fe-S cluster assembly mutants, such as fewer trichomes and a waxless epidermis. Further investigation of this allele as well as other *hscb* alleles will be necessary to establish the role of *HSCB* in Fe-S protein biogenesis.

DNA Metabolism

Plants with an *atm3-1*, *atm3-2*, or *ae*7 mutant allele have a dwarfed appearance, and the *atm3-4 ae7-1* double mutant is even smaller than either of its parent lines (67). RNAi silencing of *NAR1* in *Arabidopsis* also results in plants that are severely limited in growth (20, 77). The activities of mitochondrial Fe-S enzymes are normal in *atm3* mutants, and therefore the growth defect must be due to impairment of a biological process outside the mitochondria. Both *atm3-4* and *ae*7 mutants have larger cells, increased DNA breakage, induced expression of DNA repair genes, and a higher frequency of homologous recombination (67). Both *ae*7 and *nar1* mutants displayed an apparent block in cell cycle progression (67, 77). Interestingly, the induced expression of DNA repair enzymes was first observed in the *atm3-1* allele, along with larger, abnormally shaped nuclei (55). The enhanced phenotype of the *atm3-4 ae7-1* double mutant and the similar phenotypes of *atm3-4* and *ae7-1* single mutants present strong evidence that ATM3 and AE7 function in the same pathway and serve the same downstream target proteins involved in DNA maintenance. Several proteins required for DNA repair and/or replication contain Fe-S clusters, including Rad3/XPD (UVH6 in *Arabidopsis*); DNA helicases for nucleotide excision repair (so far uncharacterized in plants); endonucleases for base excision repair, such as NTH1 in *Arabidopsis*; PRI2, a DNA primase subunit for DNA synthesis and double-strand-break repair; and the eukaryotic DNA polymerases (for references, see 9, 67). In yeast, mutation of *MMS19* (*MET18*), encoding a protein that interacts with Cia2 (AE7 in *Arabidopsis*), decreased cluster assembly on DNA repair proteins such as Rad3 or the human homolog XPD (105). The activity and stability of Rad3/UVH6 have not been investigated in *Arabidopsis* Fe-S protein biogenesis mutants; however, the activities of at least two Fe-S-dependent DNA glycosylases, ROS1 and DEMETER, were decreased in *ae7-1* and *nar1-3*/+ mutants, respectively (67, 83). Both ROS1 and DEMETER excise 5-methyl cytosines to relieve DNA silencing of downstream genes (75). ROS1 regulates the expression of many genes, and its decreased activity in *ae7-1* was shown using a methylation-sensitive restriction enzyme followed by polymerase chain reaction (67). DEMETER activity is required for expression of the endosperm-specific *FWA* gene and other maternally imprinted genes. In heterozygous *nar1-3*/+ plants, 50% of the ovules exhibited decreased expression of FWA:GFP in the central cell, suggesting that DEMETER is not active in the female gametophytes that have inherited the *nar1-3* null allele (83). In summary, several recent studies suggest that assembly of Fe-S clusters, mediated by the mitochondrial ISC and cytosolic CIA pathways, is critical for DNA metabolism.

Photosynthesis and Other Plastid Functions

Mutants in plastid-localized Fe-S cluster assembly generally affect photosynthesis. In particular, the assembly of photosystem I, which has three Fe_4S_4 clusters, is affected in *nfs2*, *nfu2*, and *hcf101* mutants. Downregulation of *NFS2* led to lower levels and/or activity of all plastid Fe-S proteins tested, but there was no effect on mitochondrial respiration (119) or cytosolic Fe-S enzymes (14).

Knockouts of *NFS2*, *SUFB*, *SUFC*, *SUFD*, and *HCF101* die during embryo development and cannot be rescued with sucrose (59, 106, 119, 131, 133), unlike other photosynthetic mutants. This finding indicates that the proteins also provide Fe-S clusters to enzymes that are not directly involved in photosynthesis, for example, ISPG in isoprenoid biosynthesis, isopropyl malate isomerase in leucine biosynthesis, and phosphomethylpyrimidine synthase (THIC) in thiamine biosynthesis. The *NFS2* and *SUF* genes have been characterized mainly in reverse genetics studies, but viable *sufb* alleles have been found in two independent classical genetic screens. One mutant in *SUFB*, *long after far-red 6* (*laf6*), was identified in a screen for seedlings that did not respond to far-red light. The *laf6* mutant accumulated protoporphyrin IX, a precursor in chlorophyll biosynthesis involved in light signaling from the chloroplast to the nucleus (76). Another *sufb* mutant, called *hmc1*, was isolated because it accumulated 7-hydroxymethyl chlorophyll *a*, an intermediate in chlorophyll breakdown (82). Interestingly, chlorophyll *a* oxygenase and pheophorbide *a* oxygenase, two enzymes involved in chlorophyll biosynthesis and breakdown, respectively, have a Rieske-type Fe-S cluster, and other chlorophyll biosynthesis steps depend on ferredoxin. Therefore, these studies indicate that subtle defects in SUFB function affect growth and development through Fe-S-dependent steps in chlorophyll metabolism.

A puzzling phenotype of *atm3* and *ae*7 alleles is the observed chlorosis, even though the activity of chloroplast Fe-S enzymes such as photosystem I enzymes and nitrite reductase were not affected in *atm3* (13). In *atm3-1*, the palisade parenchyma is not developed (55). These cells have a high density of chloroplasts; therefore, the absence of palisade parenchyma appears to be the main reason for a 50% decrease in chloroplast numbers in the *atm3-1* mutant. The chloroplasts in *atm3-1* are slightly larger but contain the same amount of chlorophyll and Fe as the wild type (13). Interestingly, several of the phenotypes of strong *atm3* alleles and *ae*7 are strikingly similar to mutants in ribonucleotide reductase (RNR), a di-iron-dependent enzyme (36, 123), but a potential link between the CIA pathway and RNR remains to be investigated. Chlorotic spots were also observed in *NFS1*-silenced mutants (32). However, NFS1 is required for many essential processes, including respiration, biotin synthesis, and Moco synthesis, which all impact chloroplast development and photosynthesis.

REGULATION OF FE COFACTOR ASSEMBLY AND FE HOMEOSTASIS

The demand for Fe cofactors in the cell fluctuates depending on the expression levels of abundant Fe proteins, which vary in different tissues and developmental stages. Also, organisms adapt their use of Fe once Fe uptake cannot satisfy the Fe demands. In this situation, essential Fe proteins are prioritized and their levels maintained, whereas nonessential Fe proteins are downregulated when Fe becomes limiting. This is well documented in bacteria and the green alga *Chlamydomonas* (118). In higher plants, the levels of photosystem I (12 Fe) and complex I (22 Fe) are downregulated upon shifting seedlings from Fe-replete to Fe-deficient conditions (122), and the Fe is presumably recycled. Fe is also released upon damage to Fe-S clusters as a result of oxidative stress. Because of toxicity, Fe needs to be captured in a safe place. This role is performed by the multimeric ferritin protein, which can store Fe^{3+} in its cavity (96).

The expression patterns and global regulation of genes involved in Fe uptake from the soil and transport to the shoot are well characterized (53). By contrast, how Fe is distributed between cell compartments or between the Fe-S and heme biosynthesis pathways is not well studied (88). Even less is known about recycling of Fe in plant cells. Moreover, the Fe-sensing mechanism in plants also remains to be unraveled, and is likely to be entirely different from yeast and mammals (see sidebar Fe-Sensing Mechanisms in Yeast and Mammals).

To investigate global expression patterns of Fe cofactor assembly genes, we analyzed whole-genome microarray data from *Arabidopsis*, which are available in Genevestigator (45)

FE-SENSING MECHANISMS IN YEAST AND MAMMALS

The Fe levels inside a cell are tightly controlled, which requires precise coordination of the expression of genes involved in Fe uptake, distribution, and use—the so-called Fe regulon. Two elements are required for regulation: an Fe sensor and a response regulator. In bacteria, the sensor and regulator are often combined in one transcription factor, for example, the Fe-dependent transcriptional repressor Fur. In eukaryotes, different mechanisms seem to have evolved (reviewed in 65). In yeast, Fe homeostasis is disrupted both in mutants in the ISC pathway and in *atm1* mutants. Recent work has identified a cytosolic complex of glutaredoxin, the Fra1 and Fra2 proteins, and the transcription factor Aft1 as the Fe sensor (89). The complex binds a bridged Fe_2S_2 cluster using free glutathione as one of the ligands. When cellular Fe levels are replete, Aft1 is retained in the cytosol as part of the sensor complex. However, when cellular Fe levels are low or the biosynthesis of Fe-S clusters is disrupted, the labile Fe_2S_2 cluster falls apart, and Aft1 is then released and travels into the nucleus, where it activates the expression of genes involved in Fe uptake. If Fe-S cluster assembly is impaired but Fe levels are normal, then the cell takes up too much Fe, which accumulates in the vacuoles and mitochondria (65).

In mammals, a cytosolic aconitase functions as both a sensor and a translational regulator for Fe uptake and storage. When Fe levels are high, an Fe_4S_4 cluster is assembled on the protein that has aconitase activity. When Fe levels are low, the cluster is disassembled and the protein refolds to bind RNA hairpins known as Fe-responsive elements. The apo form of the protein, which is no longer a functional aconitase, is called iron regulatory protein 1 (IRP1). If the Fe-responsive element is situated at the 5′ end of an open reading frame, then IRP1 binding blocks translation, as is the case for the Fe storage protein ferritin. However, if the Fe-responsive element is located at the 3′ end, then the transcript is stabilized and translated, as is the case for the transferrin receptor. Therefore, under Fe-limiting conditions, no Fe is stored, and more Fe is taken up. When Fe-S cluster assembly is impaired, the cell becomes overloaded with Fe, similar to what occurs in yeast but based on a different mechanism.

Neither the yeast nor the mammalian Fe regulatory mechanism is conserved in plants, and the Fe sensor remains to be uncovered. All we know is that the Fe-sensing mechanism is unlikely to depend on mitochondrial Fe-S cluster assembly, because *Arabidopsis atm3* mutants do not have an obvious defect in Fe homeostasis (13).

(**Supplemental Figure 1**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). The genes/proteins involved in Fe-S cluster assembly were listed above (**Table 1**). We also included ferrochelatase I and II as the last step of heme biosynthesis. For Fe acquisition genes, we limited our investigation to genes that are known to be involved in Fe uptake in the organelles (88).

Supplemental Material

Expression patterns of genes involved in Fe cofactor assembly were clustered using the Hierarchical Clustering tool in Genevestigator. The maximum number of conditions (anatomy, perturbations) was selected to avoid bias. We found that the genes encoding plastid-localized proteins formed one cluster, and the mitochondrial and cytosolic assembly proteins formed another. In particular, the *SUFA*, *-B*, *-C*, *-D*, and *-E1* genes clustered together because of high expression in green tissues. The genes encoding proteins of the ISC and CIA pathways could not be separated based on expression patterns or by using a narrower data selection. The coclustering of ISC and CIA genes indicates a strong functional link between the two pathways, which is supported by experimental studies of *NFS1*, *ATM3*, and *AE7* (see above).

Three genes did not cluster with either the plastid or the mitochondria/cytosol group: *SUFE3-NADA*, *ISU2*, and *ISU3*. The different expression profile of *SUFE3-NADA* is in agreement with experimental data showing that the protein has a specific function in NAD biosynthesis, and it is therefore unlikely to have a generic role in Fe-S cluster assembly (81). The expression

of *ISU2* and *ISU3* is overall very low but highly induced in pollen, as borne out by both the microarray data and the experimental data (58). These data suggest that ISU2 and/or ISU3 may replace the function of ISU1 in pollen, but this remains to be investigated with mutant alleles of *ISU1*.

In general, we found that the expression of Fe-S assembly genes is not dramatically altered by environmental conditions. There was no diurnal pattern, as could be expected for the plastid assembly proteins. However, ISC and SUF genes were downregulated in response to pathogen infection (*Pseudomonas syringae*, FLG22 effector protein) or during hypoxia. Following pathogen infection, Fe-S clusters tend to be destroyed by oxidative stress. Downregulation of de novo Fe-S cluster assembly would limit the amount of Fe released from Fe-S clusters and the vicious cycle of increasing oxidative damage mediated by the Fenton reaction. This idea is supported by the isolation of mutants in Fe-S cluster assembly as suppressors of a *sod1* mutant in yeast (107). Under hypoxia, the lifetime of Fe-S clusters is longer, and there is likely to be a lower demand for Fe-S cluster biosynthesis. Interestingly, *nar1* mutants in plants, yeast, and nematodes display phenotypes at normal but not at low oxygen pressure (34, 77).

Only one gene was very responsive to a wide array of conditions: *SUFE2*. The SUFE2 protein has a single SUFE domain, unlike SUFE1-BOLA and SUFE3-NADA. Therefore, it is possible that SUFE2 serves as a regulator, controlling the flux of persulfide between BolA and NADA, the C-terminal domains of SUFE1 and SUFE3, respectively. This could be tested in vivo with the overexpressed proteins that are available (81).

Surprisingly, Fe deficiency barely affected the expression levels of *ISC* and *SUF* genes, except for *SUFB*, which is more than twofold decreased in most Fe-deficient plant samples. Downregulation of *SUFB* under Fe-deficient conditions was previously observed in *Arabidopsis* (131) and is also evident in rice (63). A decrease in SUFB levels is likely to adjust the overall rate of Fe-S cluster assembly in the plastids rapidly, because this is the primary scaffold protein. Indeed, mutations leading to a minor decrease in SUFB function affect Fe-S-dependent processes such as chlorophyll biosynthesis, as discussed above. In *E. coli*, the expression of the whole *isc* gene cluster is suppressed under low Fe, whereas *suf* gene expression is induced. However, in bacteria the ISC and SUF pathways operate in the same cell, whereas in plants these pathways are localized in different cell compartments and serve different essential Fe-S proteins. Therefore, differential regulation in response to Fe is unlikely in plant cells, and indeed was not found, although additional regulation may take place at the posttranscriptional level.

We also investigated the expression patterns of genes involved in Fe delivery to mitochondria and plastids. Transcript levels of plastid iron carrier 1 (PIC1) (28) and mitochondrial iron transporter 1 and 2 (MIT1 and MIT2, respectively) (10) were unaffected by Fe deficiency. As reported earlier, the expression of FRO3, a ferric chelate reductase localized to the mitochondria, is strongly induced under Fe deficiency, whereas the plastid-associated FRO7 is not Fe regulated (80). These data suggest that Fe delivery to the mitochondrial ISC pathway may be regulated at the level of transport of Fe into the organelle. By contrast, Fe delivery to heme and Fe-S cluster assembly in the plastids may be controlled downstream of Fe import into the organelle, for example, by SUFE2 or SUFB. Interestingly, sirohydrochlorin ferrochelatase in the plastids contains an Fe_2S_2 cluster, which may be involved in regulating the Fe insertion into siroheme (99).

Taken together, the studies described above suggest that Fe cofactor biosynthesis must be regulated in conjunction with Fe uptake in the cell because of the toxicity of free Fe. The available microarray data and a gene expression study in rice (63) offer a first glimpse of this regulation and identify potential key regulatory steps.

FUTURE ISSUES

In this review we have endeavored to summarize the current knowledge of, and provide insight into, the biosynthesis of Fe cofactors, with a focus on the recently discovered pathways of Fe-S cluster assembly. Despite steady progress in our understanding of Fe trafficking and assembly of Fe cofactors into proteins as catalytic sites, several open questions remain to be addressed. A short list is given here, which is by no means comprehensive.

1. How is Fe delivered to Fe-S scaffold proteins and ferrochelatases?
2. What is the Fe sensor in higher plants?
3. Do glutaredoxins and BOLA proteins play a role in Fe-S cluster assembly?
4. What is the substrate (or substrates) of the ABC transporter of the mitochondria?
5. What are the molecular functions of proteins of the CIA pathway?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Abdel-Ghany SE, Ye H, Garifullina G, Zhang L, Pilon-Smits EA, Pilon M. 2005. Iron-sulfur cluster biogenesis in chloroplasts. Involvement of the scaffold protein CpIscA. *Plant Physiol.* 138:161–72
2. Adam AC, Bornhövd C, Prokisch H, Neupert W, Hell K. 2006. The Nfs1 interacting protein Isd11 has an essential role in Fe/S cluster biogenesis in mitochondria. *EMBO J.* 25:174–83
3. Ahn CS, Lee JH, Pai H-S. 2005. Silencing of *NbNAP1* encoding a plastidic SufB-like protein affects chloroplast development in *Nicotiana benthamiana*. *Mol. Cells* 20:112–18
4. Arabidopsis Genome Init. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408:796–815
5. Arnaud N, Ravet K, Borlotti A, Touraine B, Boucherez J, et al. 2007. The iron-responsive element (IRE)/iron-regulatory protein 1 (IRP1)–cytosolic aconitase iron-regulatory switch does not operate in plants. *Biochem. J.* 405:523–31
6. Balk J, Lobréaux S. 2005. Biogenesis of iron-sulfur proteins in plants. *Trends Plant Sci.* 10:324–31
7. Balk J, Netz DJ, Tepper K, Pierik AJ, Lill R. 2005. The essential WD40 protein Cia1 is involved in a late step of cytosolic and nuclear iron-sulfur protein assembly. *Mol. Cell. Biol.* 25:10833–41
8. Balk J, Pierik AJ, Netz DJ, Muhlenhoff U, Lill R. 2004. The hydrogenase-like Nar1p is essential for maturation of cytosolic and nuclear iron-sulphur proteins. *EMBO J.* 23:2105–15
9. Balk J, Pilon M. 2011. Ancient and essential: the assembly of iron-sulfur clusters in plants. *Trends Plant Sci.* 16:218–26
10. Bashir K, Ishimaru Y, Shimo H, Nagasaka S, Fujimoto M, et al. 2011. The rice mitochondrial iron transporter is essential for plant growth. *Nat. Commun.* 2:322
11. Beinert H. 2000. Iron-sulfur proteins: ancient structures, still full of surprises. *J. Biol. Inorg. Chem.* 5:2–15
12. Beinert H, Holm RH, Münck E. 1997. Iron-sulfur clusters: nature's modular, multipurpose structures. *Science* 277:653–59

13. Bernard DG, Cheng Y, Zhao Y, Balk J. 2009. An allelic mutant series of *ATM3* reveals its key role in the biogenesis of cytosolic iron-sulfur proteins in Arabidopsis. *Plant Physiol.* 151:590–602
14. Bernard DG, Netz DJ, Lagny TJ, Pierik AJ, Balk J. 2013. Requirements of the cytosolic iron-sulfur cluster assembly pathway in Arabidopsis. *Philos. Trans. R. Soc. B* 368:20120259
15. Biederbick A, Stehling O, Rosser R, Niggemeyer B, Nakai Y, et al. 2006. Role of human mitochondrial Nfs1 in cytosolic iron-sulfur protein biogenesis and iron regulation. *Mol. Cell. Biol.* 26:5675–87
16. Busi MV, Maliandi MV, Valdez H, Clemente M, Zabaleta EJ, et al. 2006. Deficiency of *Arabidopsis thaliana* frataxin alters activity of mitochondrial Fe-S proteins and induces oxidative stress. *Plant J.* 48:873–82
17. Butterfield ER, Howe CJ, Nisbet RE. 2013. An analysis of dinoflogellate metabolism using EST data. *Protist* 164:218–36
18. Bych K, Kerscher S, Netz DJ, Pierik AJ, Zwicker K, et al. 2008. The iron-sulphur protein Ind1 is required for effective complex I assembly. *EMBO J.* 27:1736–46
19. Bych K, Netz DJ, Vigani G, Bill E, Lill R, et al. 2008. The essential cytosolic iron-sulfur protein Nbp35 acts without Cfd1 partner in the green lineage. *J. Biol. Chem.* 283:35797–804
20. Cavazza C, Martin L, Mondy S, Gaillard J, Ratet P, Fontecilla-Camps JC. 2008. The possible role of an [FeFe]-hydrogenase-like protein in the plant responses to changing atmospheric oxygen levels. *J. Inorg. Biochem.* 102:1359–65
21. Chen S, Sánchez-Fernández R, Lyver ER, Dancis A, Rea PA. 2007. Functional characterization of AtATM1, AtATM2, and AtATM3, a subfamily of *Arabidopsis* half-molecule ATP-binding cassette transporters implicated in iron homeostasis. *J. Biol. Chem.* 282:21561–71
22. Cheng N-H, Liu J-Z, Brock A, Nelson RS, Hirschi KD. 2006. AtGRXcp, an *Arabidopsis* chloroplastic glutaredoxin, is critical for protection against protein oxidative damage. *J. Biol. Chem.* 281:26280–88
23. Couturier J, Touraine B, Briat J-F, Gaymard F, Rouhier N. 2013. The iron-sulfur cluster assembly machineries in plants: current knowledge and open questions. *Front. Plant Sci.* 4:259
24. Cupp-Vickery JR, Urbina H, Vickery LE. 2003. Crystal structure of IscS, a cysteine desulfurase from *Escherichia coli*. *J. Mol. Biol.* 330:1049–59
25. Dai X, Hayashi K, Nozaki H, Cheng Y, Zhao Y. 2005. Genetic and chemical analyses of the action mechanisms of sirtinol in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 102:3129–34
26. Dai Y, Outten FW. 2012. The *E. coli* SufS-SufE sulfur transfer system is more resistant to oxidative stress than IscS-IscU. *FEBS Lett.* 586:4016–22
27. Davenport HE, Hill R, Whatley FR. 1952. A natural factor catalyzing reduction of methaemoglobin by isolated chloroplasts. *Proc. R. Soc. B* 139:346–58
28. Duy D, Wanner G, Meda AR, von Wirén N, Soll J, Philippar K. 2007. PIC1, an ancient permease in *Arabidopsis* chloroplasts, mediates iron transport. *Plant Cell* 19:986–1006
29. Fleischhacker AS, Kiley PJ. 2011. Iron-containing transcription factors and their roles as sensors. *Curr. Opin. Chem. Biol.* 15:335–41
30. Food Stand. Agency. 2002. *McCance and Widdowson's The Composition of Foods.* Cambridge, UK: R. Soc. Chem. 6th summ. ed.
31. Fosset C, Chauveau MJ, Guillon B, Canal F, Drapier JC, Bouton C. 2006. RNA silencing of mitochondrial m-Nfs1 reduces Fe-S enzyme activity both in mitochondria and cytosol of mammalian cells. *J. Biol. Chem.* 281:25398–406
32. Frazzon AP, Ramirez MV, Warek U, Balk J, Frazzon J, et al. 2007. Functional analysis of *Arabidopsis* genes involved in mitochondrial iron-sulfur cluster assembly. *Plant Mol. Biol.* 64:225–40
33. Freire P, Moreira RN, Arraiano CM. 2009. BolA inhibits cell elongation and regulates MreB expression levels. *J. Mol. Biol.* 385:1345–51
34. Fujii M, Adachi N, Shikatani K. Ayusawa D, 2009. [FeFe]-hydrogenase-like gene is involved in the regulation of sensitivity to oxygen in yeast and nematode. *Genes Cells* 14:457–68
35. Gari K, León Ortiz AM, Borel V, Flynn H, Skehel JM, Boulton SJ. 2012. MMS19 links cytoplasmic iron-sulfur cluster assembly to DNA metabolism. *Science* 337:243–45
36. Garton S, Knight H, Warren GJ, Knight MR, Thorlby GJ. 2007. *crinkled leaves 8*—a mutation in the large subunit of ribonucleotide reductase—leads to defects in leaf development and chloroplast division in *Arabidopsis thaliana*. *Plant J.* 50:118–27

37. Godman J, Balk J. 2008. Genome analysis of *Chlamydomonas reinhardtii* reveals the existence of multiple, compartmentalized iron-sulfur protein assembly machineries of different evolutionary origins. *Genetics* 179:59–68
38. Gutensohn M, Fan E, Frielingsdorf S, Hanner P, Hou B, et al. 2006. Toc, Tic, Tat et al.: structure and function of protein transport machineries in chloroplasts. *J. Plant Physiol.* 163:333–47
39. Hamza I, Dailey HA. 2012. One ring to rule them all: trafficking of heme and heme synthesis intermediates in the metazoans. *Biochim. Biophys. Acta* 1823:1617–32
40. Hausmann A, Netz DJ, Balk J, Pierik AJ, Muhlenhoff U, Lill R. 2005. The eukaryotic P loop NTPase Nbp35: an essential component of the cytosolic and nuclear iron-sulfur protein assembly machinery. *Proc. Natl. Acad. Sci. USA* 102:3266–71
41. Haussig JM, Matuschewski K, Kooij TW. 2013. Experimental genetics of *Plasmodium berghei* NFU in the apicoplast iron-sulfur cluster biogenesis pathway. *PLoS ONE* 8:e67269
42. Hayashi M, De Bellis L, Alpi A, Nishimura M. 1995. Cytosolic aconitase participates in the glyoxylate cycle in etiolated pumpkin cotyledons. *Plant Cell Physiol.* 36:669–80
43. Hitzert MM, Bos AF, Bergman KA, Veldman A, Schwarz G, et al. 2012. Favorable outcome in a newborn with molybdenum cofactor type A deficiency treated with cPMP. *Pediatrics* 130:e1005–10
44. Hjorth E, Hadfi K, Zauner S, Maier U-G. 2005. Unique genetic compartmentation of the SUF system in cryophytes and characterization of a *SufD* mutant in *Arabidopsis thaliana*. *FEBS Lett.* 579:1129–35
45. Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, et al. 2008. Genevestigator V3: a reference expression database for the meta-analysis of transcriptomes. *Adv. Bioinforma.* 2008:420747
46. Imsande J. 1999. Iron-sulfur clusters: formation, perturbation, and physiological functions. *Plant Physiol. Biochem.* 37:87–97
47. Ito J, Batth TS, Petzold CJ, Redding-Johanson AM, Mukhopadhyay A, et al. 2011. Analysis of the *Arabidopsis* cytosolic proteome highlights subcellular partitioning of central plant metabolism. *J. Proteome Res.* 10:1571–82
48. Kessler D, Papenbrock J. 2005. Iron-sulfur cluster biosynthesis in photosynthetic organisms. *Photosynth. Res.* 86:391–407
49. Kispal G, Csere P, Guiard B, Lill R. 1997. The ABC transporter Atm1p is required for mitochondrial iron homeostasis. *FEBS Lett.* 418:346–50
50. Kispal G, Csere P, Prohl C, Lill R. 1999. The mitochondrial proteins Atm1p and Nfs1p are essential for biogenesis of cytosolic Fe/S proteins. *EMBO J.* 18:3981–89
51. Kitaoka S, Wada K, Hasegawa Y, Minami Y, Fukuyama K, Takahashi Y. 2006. Crystal structure of *Escherichia coli* SufC, an ABC-type ATPase component of the SUF iron-sulfur cluster assembly machinery. *FEBS Lett.* 580:137–43
52. Klodmann J, Senkler M, Rode C, Braun HP. 2011. Defining the protein complex proteome of plant mitochondria. *Plant Physiol.* 157:587–98
53. Kobayashi T, Nishizawa NK. 2012. Iron uptake, translocation, and regulation in higher plants. *Annu. Rev. Plant Biol.* 63:131–52
54. Kohbushi H, Nakai Y, Kikuchi S, Yabe T, Hori H, Nakai M. 2009. *Arabidopsis* cytosolic Nbp35 homodimer can assemble both [2Fe-2S] and [4Fe-4S] clusters in two distinct domains. *Biochem. Biophys. Res. Commun.* 378:810–15
55. Kushnir S, Babiychuk E, Storozhenko S, Davey MW, Papenbrock J, et al. 2001. A mutation of the mitochondrial ABC transporter Sta1 leads to dwarfism and chlorosis in the Arabidopsis mutant *starik*. *Plant Cell* 13:89–100
56. Layer G, Gaddam SA, Ayala-Castro CN, Ollagnier-de Choudens S, Lascoux D, et al. 2007. SufE transfers sulfur from SufS to SufB for iron-sulfur cluster assembly. *J. Biol. Chem.* 282:13342–50
57. Leighton J, Schatz G. 1995. An ABC transporter in the mitochondrial inner membrane is required for normal growth of yeast. *EMBO J.* 14:188–95
58. Léon S, Touraine B, Briat J-F, Lobréaux S. 2005. Mitochondrial localization of *Arabidopsis thaliana* Isu Fe-S scaffold proteins. *FEBS Lett.* 579:1930–34
59. Lezhneva L, Amann K, Meurer J. 2004. The universally conserved HCF101 protein is involved in assembly of [4Fe-4S]-cluster-containing complexes in *Arabidopsis thaliana* chloroplasts. *Plant J.* 37:174–85

60. Li H, Mapolelo DT, Dingra NN, Naik SG, Lees NS, et al. 2009. The yeast iron regulatory proteins Grx3/4 and Fra2 form heterodimeric complexes containing a [2Fe-2S] cluster with cysteinyl and histidyl ligation. *Biochemistry* 48:9569–81
61. Li H-M, Theg SM, Bauerle CM, Keegstra K. 1990. Metal-ion-center assembly of ferredoxin and plastocyanin in isolated chloroplasts. *Proc. Natl. Acad. Sci. USA* 87:6748–52
62. Li K, Tong WH, Hughes RM, Rouault TA. 2006. Roles of the mammalian cytosolic cysteine desulfurase, ISCS, and scaffold protein, ISCU, in iron-sulfur cluster assembly. *J. Biol. Chem.* 281:12344–51
63. Liang X, Qin L, Liu P, Wang M, Ye H. 2014. Genes for iron-sulfur cluster assembly are targets of abiotic stress in rice, *Oryza sativa*. *Plant Cell Environ.* 37:780–94
64. Lill R. 2009. Function and biogenesis of iron-sulphur proteins. *Nature* 460:831–38
65. Lill R, Hoffmann B, Molik S, Pierik AJ, Rietzschel N, et al. 2013. The role of mitochondria in cellular iron-sulfur protein biogenesis and iron metabolism. *Biochim. Biophys. Acta* 1823:1491–508
66. Lima CD. 2002. Analysis of the *E. coli* NifS CsdB protein at 2.0 Å reveals the structural basis for perselenide and persulfide intermediate formation. *J. Mol. Biol.* 315:1199–208
67. Luo D, Bernard DG, Balk J, Hai H, Cui X. 2012. The DUF59 family gene *AE7* acts in the cytosolic iron-sulfur cluster assembly pathway to maintain nuclear genome integrity in *Arabidopsis*. *Plant Cell* 24:4135–48
68. Marinoni EN, de Oliveira JS, Nicolet Y, Raulfs EC, Amara P, et al. 2012. $(IscS\text{-}IscU)_2$ complex structures provide insights into Fe_2S_2 biogenesis and transfer. *Angew. Chem. Int. Ed.* 51:5439–42
69. Markley JL, Kim JH, Dai Z, Bothe JR, Cai K, et al. 2013. Metamorphic protein IscU alternates conformations in the course of its role as the scaffold protein for iron-sulfur cluster biosynthesis and delivery. *FEBS Lett.* 587:1172–79
70. Martin M, Colman MJR, Gómez-Casati DF, Lamattina L, Zabaleta EJ. 2009. Nitric oxide accumulation is required to protect against iron-mediated oxidative stress in frataxin-deficient Arabidopsis plants. *FEBS Lett.* 583:542–48
71. Mendel RR. 2013. The molybdenum cofactor. *J. Biol. Chem.* 288:13165–72
72. Merchant SS, Allen MD, Kropat J, Moseley JL, Long JC, et al. 2006. Between a rock and a hard place: trace element nutrition in Chlamydomonas. *Biochim. Biophys. Acta* 1763:578–94
73. Miao R, Kim H, Koppolu UM, Ellis EA, Scott RA, Lindahl PA. 2009. Biophysical characterization of the iron in mitochondria from Atm1p-depleted *Saccharomyces cerevisiae*. *Biochemistry* 48:9556–68
74. Mochizuki N, Tanaka R, Grimm B, Masuda T, Moulin M, et al. 2010. The cell biology of tetrapyrroles: a life and death struggle. *Trends Plant Sci.* 15:488–98
75. Mok YG, Uzawa R, Lee JH, Weiner GM, Eichman BF, et al. 2010. Domain structure of the DEMETER 5-methylcytosine DNA glycosylase. *Proc. Natl. Acad. Sci. USA* 107:19225–30
76. Møller SG, Kunkel T, Chua N-H. 2001. A plastidic ABC protein involved in intercompartmental communication of light signalling. *Genes Dev.* 15:90–103
77. Mondy S, Lenglet A, Cosson V, Pelletier S, Pateyron S, et al. 2014. GOLLUM [FeFe]-hydrogenase-like proteins are essential for plant development in normoxic conditions and moderate energy metabolism. *Plant Cell Environ.* 37:54–69
78. Mühlenhoff U, Balk J, Richhardt N, Kaiser JT, Sipos K, et al. 2004. Functional characterization of the eukaryotic cysteine desulfurase Nfs1p from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:36906–15
79. Mühlenhoff U, Molik S, Godoy JR, Uzarska MA, Richter N, et al. 2010. Cytosolic monothiol glutaredoxins function in intracellular iron sensing and trafficking via their bound iron-sulfur cluster. *Cell Metab.* 12:373–85
80. Mukherjee I, Campbell NH, Ash JS, Connolly EL. 2006. Expression profiling of the Arabidopsis ferric chelate reductase (*FRO*) gene family reveals differential regulation by iron and copper. *Planta* 223:1178–90
81. Murthy NUM, Ollagnier-de-Choudens S, Sanakis Y, Abdel-Ghany SE, Rousset C, et al. 2007. Characterization of *Arabidopsis thaliana* SufE2 and SufE3: functions in chloroplast iron-sulfur cluster assembly and NAD synthesis. *J. Biol. Chem.* 282:18254–64
82. Nagane T, Tanaka A, Tanaka R. 2010. Involvement of AtNAP1 in the regulation of chlorophyll degradation in *Arabidopsis thaliana*. *Planta* 231:939–49

83. Nakamura M, Buzas DM, Kato A, Fujita M, Kurata N, Kinoshita T. 2013. The role of *Arabidopsis thaliana* NAR1, a cytosolic iron-sulfur cluster assembly component, in gametophytic gene expression and oxidative stress responses in vegetative tissue. *New Phytol.* 199:925–35
84. Netz DJ, Pierik AJ, Stümpfig M, Bill E, Sharma AK, et al. 2012. A bridging [4Fe-4S] cluster and nucleotide binding are essential for function of the Cfd1-Nbp35 complex as a scaffold in iron-sulfur protein maturation. *J. Biol. Chem.* 287:12365–78
85. Netz DJ, Pierik AJ, Stümpfig M, Mühlenhoff U, Lill R. 2007. The Cfd1-Nbp35 complex acts as a scaffold for iron-sulfur protein assembly in the yeast cytosol. *Nat. Chem. Biol.* 3:278–86
86. Netz DJ, Stümpfig M, Dore C, Muhlenhoff U, Pierik AJ, Lill R. 2010. Tah18 transfers electrons to Dre2 in cytosolic iron-sulfur protein biogenesis. *Nat. Chem. Biol.* 6:758–65
87. Nitschke W, McGlynn SE, Milner-White EJ, Russell MJ. 2013. On the antiquity of metalloenzymes and their substrates in bioenergetics. *Biochim. Biophys. Acta* 1827:871–81
88. Nouet C, Motte P, Hanikenne M. 2011. Chloroplastic and mitochondrial metal homeostasis. *Trends Plant Sci.* 16:395–404
89. Outten CE, Albetel A-N. 2013. Iron sensing and regulation in *Saccharomyces cerevisiae*: ironing out the mechanistic details. *Curr. Opin. Microbiol.* 16:1–7
90. Outten FW, Wood MJ, Muñoz FM, Storz G. 2003. The SufE protein and the SufBCD complex enhance SufS cysteine desulfurase activity as part of a sulfur transfer pathway for Fe-S cluster assembly in *Escherichia coli*. *J. Biol. Chem.* 278:45713–19
91. Palmer T, Berks BC. 2012. The twin-arginine translocation (Tat) protein export pathway. *Nat. Rev. Microbiol.* 10:483–96
92. Pandey A, Golla R, Yoon H, Dancis A, Pain D. 2012. Persulfide formation on mitochondrial cysteine desulfurase: enzyme activation by a eukaryote-specific interaction protein and Fe-S cluster synthesis. *Biochem. J.* 448:171–87
93. Pastore A, Puccio H. 2013. Frataxin: a protein in search for a function. *J. Neurochem.* 126(Suppl. 1):43–52
94. Picciocchi A, Douce R, Alban C. 2003. The plant biotin synthase reaction. Identification and characterization of essential mitochondrial accessory protein components. *J. Biol. Chem.* 278:24966–75
95. Pondarre C, Antiochos BB, Campagna DR, Clarke SL, Greer EL, et al. 2006. The mitochondrial ATP-binding cassette transporter Abcb7 is essential in mice and participates in cytosolic iron-sulfur cluster biogenesis. *Hum. Mol. Genet.* 15:953–64
96. Ravet K, Touraine J, Boucherez J, Briat JF, Gaymard F, Cellier F. 2009. Ferritins control interaction between iron homeostasis and oxidative stress in Arabidopsis. *Plant J.* 57:400–12
97. Roche B, Aussel L, Ezraty B, Mandin P, Py B, Barras F. 2013. Iron/sulfur proteins biogenesis in prokaryotes: formation, regulation and diversity. *Biochim. Biophys. Acta* 1827:455–69
98. Roy A, Solodovnikova N, Nicholson T, Antholine W, Walden WE. 2003. A novel eukaryotic factor for cytosolic Fe-S cluster assembly. *EMBO J.* 22:4826–35
99. Saha K, Webb ME, Rigby SE, Leech HK, Warren MJ, Smith AG. 2012. Characterization of the evolutionarily conserved iron-sulfur cluster of sirohydrochlorin ferrochelatase from *Arabidopsis thaliana*. *Biochem. J.* 444:227–37
100. Salvato F, Havelund JF, Chen M, Rao RSP, Rogowska-Wresinska A, et al. 2014. The potato tuber mitochondrial proteome. *Plant Physiol.* 164:637–53
101. Schwenkert S, Netz DJ, Frazzon J, Pierik AJ, Bill E, et al. 2009. Chloroplast HCF101 is a scaffold protein for [4Fe-4S] cluster assembly. *Biochem. J.* 425:207–14
102. Seo M, Peeters AJ, Koiwai H, Oritani T, Marion-Poll A, et al. 2000. The *Arabidopsis* aldehyde oxidase 3 (*AAO3*) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. *Proc. Natl. Acad. Sci. USA* 97:12908–13
103. Sipos K, Lange H, Fekete Z, Ullmann P, Lill R, Kispal G. 2002. Maturation of cytosolic iron-sulfur proteins requires glutathione. *J. Biol. Chem.* 277:26944–49
104. Srinivasan V, Netz DJ, Webert H, Mascarenhas J, Pierik AJ, et al. 2007. Structure of the yeast WD40 domain protein Cia1, a component acting late in iron-sulfur protein biogenesis. *Structure* 15:1246–57

105. Stehling O, Vashisht AA, Mascarenhas J, Jonsson ZO, Sharma T, et al. 2012. MMS19 assembles iron-sulfur proteins required for DNA metabolism and genomic integrity. *Science* 337:195–99
106. Stöckel J, Oelmüller R. 2004. A novel protein for photosystem I biogenesis. *J. Biol. Chem.* 279:10243–51
107. Strain J, Lorenz CR, Bode J, Garland S, Smolen GA, et al. 1998. Suppressors of superoxide dismutase (*SOD1*) deficiency in *Saccharomyces cerevisiae*: identification of proteins predicted to mediate iron-sulfur cluster assembly. *J. Biol. Chem.* 273:31138–44
108. Tagawa K, Arnon DI. 1962. Ferredoxins as electron carriers in photosynthesis and in the biological production and consumption of hydrogen gas. *Nature* 195:537–43
109. Takahashi Y, Mitsui A, Fujita Y, Matsubara H. 1991. Roles of ATP and NADPH in formation of the Fe-S cluster of spinach ferredoxin. *Plant Physiol.* 95:104–10
110. Takahashi Y, Mitsui A, Matsubara H. 1991. Formation of the Fe-S cluster of ferredoxin in lysed spinach chloroplasts. *Plant Physiol.* 95:97–103
111. Takahashi Y, Tokumoto U. 2002. A third bacterial system for the assembly of iron-sulfur clusters with homologs in archaea and plastids. *J. Biol. Chem.* 277:28380–83
112. Tanaka R, Tanaka A. 2007. Tetrapyrrole biosynthesis in higher plants. *Annu. Rev. Plant Biol.* 58:321–46
113. Taylor NL, Heazlewood JL, Millar AH. 2011. The *Arabidopsis thaliana* 2-D gel mitochondrial proteome: refining the value of reference maps for assessing protein abundance, contaminants and post-translational modifications. *Proteomics* 11:1720–33
114. Teschner J, Lachmann N, Schulze J, Geisler M, Selbach K, et al. 2010. A novel role for *Arabidopsis* mitochondrial ABC transporter ATM3 in molybdenum cofactor biosynthesis. *Plant Cell* 22:468–80
115. Thornley JH, Gibson JF, Whatley FR, Hall DO. 1966. Comment on a recent model of the iron complex in spinach ferredoxin. *Biochem. Biophys. Res. Commun.* 24:877–79
116. Touraine B, Boutin J, Marion-Poll A, Briat JF, Peltier G, Lobréaux S. 2004. Nfu2: a scaffold protein required for [4Fe-4S] and ferredoxin iron-sulfur cluster assembly in *Arabidopsis* chloroplasts. *Plant J.* 40:101–11
117. Turowski VR, Busi MV, Gomez-Casati DF. 2012. Structural and functional studies of the mitochondrial cysteine desulfurase from *Arabidopsis thaliana*. *Mol. Plant* 5:1001–10
118. Urzica E, Pierik AJ, Muhlenhoff U, Lill R. 2009. Crucial role of conserved cysteine residues in the assembly of two iron-sulfur clusters on the CIA protein Nar1. *Biochemistry* 48:4946–58
119. Van Hoewyk D, Abdel-Ghany SE, Cohu C, Herbert S, Kugrens P, et al. 2007. The *Arabidopsis* cysteine desulfurase CpNifS is essential for maturation of iron-sulfur cluster proteins, photosynthesis, and chloroplast development. *Proc. Natl. Acad. Sci. USA* 104:5686–91
120. Varadarajan J, Guilleminot J, Saint-Jore-Dupas C, Piégu B, Chabouté ME, et al. 2010. *ATR3* encodes a diflavin reductase essential for Arabidopsis embryo development. *New Phytol.* 187:67–82
121. Vazzola V, Losa A, Soave C, Murgia I. 2007. Knockout of frataxin gene causes embryo lethality in *Arabidopsis*. *FEBS Lett.* 581:667–72
122. Vigani G, Maffi D, Zocchi G. 2009. Iron availability affects the function of mitochondria in cucumber roots. *New Phytol.* 182:127–36
123. Wang C, Liu Z. 2006. *Arabidopsis* ribonucleotide reductases are critical for cell cycle progression, DNA damage repair, and plant development. *Plant Cell* 18:350–65
124. Watanabe S, Kita A, Miki K. 2005. Crystal structure of atypical cytoplasmic ABC-ATPase SufC from *Thermus thermophilus* HB8. *J. Mol. Biol.* 353:1043–54
125. Weerapana E, Wang C, Simon GM, Richter F, Khare S, et al. 2010. Quantitative reactivity profiling predicts functional cysteines in proteomes. *Nature* 468:790–95
126. Wiedemann N, Urzica E, Guiard B, Müller H, Lohaus C, et al. 2006. Essential role of Isd11 in mitochondrial iron-sulfur cluster synthesis on Isu scaffold proteins. *EMBO J.* 25:184–95
127. Wollers S, Heidenreich T, Zarepour M, Zachmann D, Kraft C, et al. 2008. Binding of sulfurated molybdenum cofactor to the C-terminal domain of ABA3 from *Arabidopsis thaliana* provides insight into the mechanism of molybdenum cofactor sulfuration. *J. Biol. Chem.* 283:9642–50

128. Wollers S, Layer G, Garcia-Serres R, Signor L, Clemancey M, et al. 2010. Iron-sulfur (Fe-S) cluster assembly: the SufBCD complex is a new type of Fe-S scaffold with a flavin redox cofactor. *J. Biol. Chem.* 285:23331–41
129. Wu Y, Brosh RM Jr. 2012. DNA helicase and helicase-nuclease enzymes with a conserved iron-sulfur cluster. *Nucleic Acids Res.* 40:4247–60
130. Wydro MM, Sharma P, Foster JM, Bych K, Meyer EH, Balk J. 2013. The evolutionarily conserved iron-sulfur protein IND1 is required for complex I assembly and mitochondrial translation in *Arabidopsis*. *Plant Cell* 25:4014–27
131. Xu XM, Adams S, Chua N-H, Møller GM. 2005. AtNAP1 represents an atypical SufB protein in *Arabidopsis* plastids. *J. Biol. Chem.* 280:6648–54
132. Xu XM, Lin H, Latijnhouwers M, Møller SG. 2009. Dual localized AtHscB involved in iron sulfur protein biogenesis in Arabidopsis. *PLoS ONE* 4:e7662
133. Xu XM, Møller SG. 2004. AtNAP7 is a plastidic SufC-like ABC/ATPase essential for *Arabidopsis* embryogenesis. *Proc. Natl. Acad. Sci. USA* 101:9143–48
134. Xu XM, Møller SG. 2006. AtSufE is an essential activator of plastidic and mitochondrial desulfurases in *Arabidopsis*. *EMBO J.* 25:900–9
135. Yabe T, Morimoto K, Kikuchi S, Nishio K, Terashima I, Nakai M. 2004. The Arabidopsis chloroplastic NifU-like protein CnfU, which can act as an iron-sulfur cluster scaffold protein, is required for biogenesis of ferredoxin and photosystem I. *Plant Cell* 16:993–1007
136. Yabe T, Nakai M. 2006. *Arabidopsis* AtIscA-I is affected by deficiency of Fe-S cluster biosynthetic scaffold AtCnfU-V. *Biochem. Biophys. Res. Commun.* 340:1047–52
137. Ye H, Abdel-Ghany S, Anderson TD, Pilon-Smits EAH, Pilon M. 2006. CpSufE activates the cysteine desulfurase CpNifS for chloroplastic Fe-S cluster formation. *J. Biol. Chem.* 273:13264–72
138. Zhang B, Crack JC, Subramanian S, Green J, Thomson AJ, et al. 2012. Reversible cycling between cysteine persulfide-ligated [2Fe-2S] and cysteine-ligated [4Fe-4S] clusters in the FNR regulatory protein. *Proc. Natl. Acad. Sci. USA* 109:15734–39
139. Zheng L, Cash VL, Flint DH, Dean DR. 1998. Assembly of iron-sulfur clusters: identification of an *iscSUA-hscBA-fdx* gene cluster from *Azotobacter vinelandii*. *J. Biol. Chem.* 273:13264–72