Review

CpNifS-dependent iron-sulfur cluster biogenesis in chloroplasts

Hong Ye, Marinus Pilon and Elizabeth A. H. Pilon-Smits
Biology Department, Colorado State University, Fort Collins, CO 80523, USA

Summary

Iron-sulfur (Fe-S) clusters are important prosthetic groups in all organisms. The biosynthesis of Fe-S clusters has been studied extensively in bacteria and yeast. By contrast, much remains to be discovered about Fe-S cluster biogenesis in higher plants. Plant plastids are known to make their own Fe-S clusters. Plastid Fe-S proteins are involved in essential metabolic pathways, such as photosynthesis, nitrogen and sulfur assimilation, protein import, and chlorophyll transformation. This review aims to summarize the roles of Fe-S proteins in essential metabolic pathways and to give an overview of the latest findings on plastidic Fe-S assembly. The plastidic Fe-S biosynthetic machinery contains many homologues of bacterial mobilization of sulfur (SUF) proteins, but there are additional components and properties that may be plant-specific. These additional features could make the plastidic machinery more suitable for assembling Fe-S clusters in the presence of oxygen, and may enable it to be regulated in response to oxidative stress, iron status and light.

Introduction

The iron-sulfur (Fe-S) cluster is one of several ancient prosthetic groups, consisting of iron and sulfur atoms in different numbers. For a review of the architecture and types of Fe-S clusters, see Beinert et al. (1997). In chloroplasts, the common cluster types include 2Fe-2S, Rieske-type 2Fe-2S, 3Fe-4S, 4Fe-4S and siroheme-(4Fe-4S), in which the 4Fe-4S is covalently bound to a siroheme. The Fe-S cluster is usually bound to a polypeptide by covalent bonds between the iron atoms of the Fe-S cluster and the sulfur atoms of the polypeptide cysteines. However, in the Rieske-type 2Fe-2S protein, the iron atoms of the Fe-S cluster are coordinated to two histidines and two cysteines.

As an iron atom in the cluster can easily gain or lose an electron, thus switching between the redox states Fe$^{2+}$ and Fe$^{3+}$, the Fe-S cluster is capable of transferring electrons in electron transfer chains (for photosynthesis and respiration) and of catalyzing redox reactions. Additionally, Fe-S clusters act as catalytic centers, regulators of gene expression and sensors of iron and oxygen. As more and more essential proteins are found to be Fe-S proteins, the mechanism of Fe-S cluster biogenesis has become an active topic of research in recent years.

Fe-S cluster assembly systems in different organisms

The identification of the nitrogen fixation (NIF) gene cluster (Jacobson et al., 1989) and the characterization of NifS as a cysteine desulfurase in the nitrogen-fixing microbe, Azotobacter vinelandii (Zheng et al., 1993), marked the beginning of our
understanding of Fe-S cluster biosynthesis. To date, Fe-S biogenesis has been extensively studied in organisms from prokaryotes to eukaryotes, including bacteria (Johnson et al., 2005), yeast (Lill & Muhlenhoff, 2005), plants (Balk & Lobreaux, 2005) and humans (Rouault & Tong, 2005). Fe-S assembly systems can be generalized as follows. Sulfur is released from cysteine by a NiS-like protein. The sulfur combines with iron to form a pre-assembled cluster bound to a scaffold protein. Finally, the cluster is transferred to apoproteins. In bacteria, four machineries have been found to assemble Fe-S clusters, each of which is encoded by a gene cluster that includes a cysteine desulfurase (Mihara & Esaki, 2002; Johnson et al., 2005). Because the Fe-S assembly system in plastids has similarity to some of the components found in bacteria, a brief discussion of bacterial Fe-S assembly is warranted here.

The first bacterial Fe-S cluster biogenesis machinery to be identified was the NIF system, found in the nitrogen-fixing microbe A. vinelandii (Jacobson et al., 1989). The NIF system was named as such because it assembles Fe-S clusters for the maturation of nitrogenase. The nif gene cluster contains the genes icAICB-nifU-nifS-nifV-cysE1. Biochemical and genetic analysis has revealed the function of each gene product. NiS is a cysteine desulfurase, converting cysteine into alanine and sulfide, for assembly of sulfur into the Fe-S cluster (Zheng et al., 1993). NifU is a modular homodimeric scaffold protein where a transient 2Fe-2S cluster is assembled. The IscAIC may be an alternative scaffold to NifU for mediating nif-specific Fe-S cluster assembly. As key components of the NIF system, NiS and NifU are required for nitrogenase maturation, but cannot be used for the maturation of other Fe-S proteins, such as acinasa. The NifV protein in the system is a homocitrate synthase, probably providing homocitrate for the biosynthesis of the FeMo cofactor of nitrogenase. However, the relationship between the NifV and other NIF proteins in cluster assembly is unknown. The cysE1 protein in the NIF system is a serine acetyl transferase, which is important for cysteine synthesis. The probable role of the cysE-like gene product is to increase the cysteine pool needed for Fe-S cluster formation (Zheng et al., 1998).

The second identified bacterial machinery involved in Fe-S cluster biogenesis is the iron sulfur cluster (ISC) system, found in both A. vinelandii and Escherichia coli (Zheng et al., 1998). The ISC system is the housekeeping machinery that produces Fe-S clusters for the maturation of general Fe-S proteins. The isc gene cluster contains the genes iscRSUA-bscBA-fdx. IscS is a cysteine desulfurase (Zheng et al., 1998). IscU has sequence similarity to the N terminus of NifU and is a scaffold. IscS binds to IscU and directly transfers sulfur for the assembly of an Fe-S cluster on the scaffold. The heat shock cognate (hsc)A and hscB gene products are molecular chaperones. They bind to and form a complex with the IscU scaffold, playing a role in the assembly, stabilization, or transfer of Fe-S clusters formed on IscU. IscA has been proposed as an alternative scaffold, where a transient 2Fe-2S cluster is transferred to ferredoxin (Fd) or biotin synthase. However, more recent studies support a role of IscA as an iron-binding protein, which subsequently donates iron for the Fe-S cluster assembly on IscU (Ding et al., 2005). IscR is a repressor of the iscRSUA operon, which is activated by binding an 2Fe-2S cluster and represses the transcription of the isc operon. Fd, the fdx gene product, is a 2Fe-2S protein. Its role in the ISC machinery is unknown.

The third identified bacterial machinery involved in Fe-S cluster biogenesis is the mobilization of sulfur (SUF) system, found in Escherichia coli (Takahashi & Tokumoto, 2002) and Erwinia chrysanthemi. The SUF system is responsible for Fe-S cluster assembly under iron limitation or oxidative stress conditions (Nachin et al., 2001). The SUF gene cluster contains the genes sufABCDSE. SufS (also named CsdB) is a cysteine desulfurase (Mihara & Esaki, 2002). SufE is an activator of cysteine desulfurase, forming a complex with SufS and accepting sulfane sulfur from SufS (Outten et al., 2003). SufA is a scaffold (Ollagnier-de-Choudens et al., 2003). This contributes to the Fe-S cluster assembly under oxidative stress and ion limitation, but its exact role in the process is unknown.

More recently, a fourth bacterial Fe-S machinery, named CSD, was characterized in E. coli (Loiseau et al., 2005). This is a simple gene cluster composed of csdA-csdE (formally ygdK). CsdA is a cysteine desulfurase (Mihara & Esaki, 2002). CsdE is a SufE-like activator of cysteine desulfurase. The CSD machinery in E. coli constitutes a sulfur-generating system, which also contributes to Fe-S cluster biogenesis in vivo. The CSD system was proposed to supply Fe-S clusters for quinolinate synthetase NadA (Loiseau et al., 2005). However, the scaffold molecule for this system remains unknown, as at least one scaffold has been found to be required in all other Fe-S biosynthetic systems identified to date.

In summary, cysteine desulfurase activity (a NiS-like protein) is an essential part of all above-described systems. It can be considered to be the most important component of the Fe-S machinery. Moreover, the function of NiS-like proteins is not limited to Fe-S cluster assembly. They also supply sulfur for the synthesis of a variety of biologically active molecules, such as thiamine, biotin and molybdopterin (Mihara & Esaki, 2002). In addition, NiS-like proteins have selenocysteine lyase activity, providing the selenium for essential selenoproteins (Mihara & Esaki, 2002).

**Fe-S proteins in chloroplasts**

Chloroplasts host various Fe-S proteins, which are involved in photosynthesis, nitrogen and sulfur assimilation, and a variety of other plastidic processes (Fig. 1). As a result, plastids are a major subcellular sink of iron in plant cells. Fe-S proteins in plants have been reviewed by Balk & Lobreaux (2005). Fe-S clusters are pivotal to photosynthesis in the electron transport...
Electrons generated from H$_2$O molecules by light energy at photosystem II (PSII) are transferred to the cytochrome $b_6/f$ complex ($b_6f$), containing a Rieske-type protein (2Fe-2S). The electrons are subsequently delivered to photosystem I (PSI). PSI contains an interpolypeptide 4Fe-4S cluster, F(X), that bridges the PsaA and PsaB subunits, and two terminal 4Fe-4S clusters, F(A) and F(B), that are bound to the PsaC subunit. Through PSI, the electrons are passed to Fd (which is a 2Fe-2S protein). Finally, the electrons of Fd can be used for the Calvin cycle, or other reductive processes in chloroplasts. Ferredoxin-thioredoxin reductase (FTR), which is a 4Fe-4S protein, is able to accept the electrons from Fd and use them for light regulation of chloroplast enzymes.

The assimilation of sulfate into cysteine takes place mainly in chloroplasts (Pilon-Smits & Pilon, 2006). Sulfate is activated by reaction with ATP to form adenosine-5-phosphosulfate (APS). APS reductase, which is a 4Fe-4S protein, reduces APS to sulfite. Subsequently, sulfite reductase (Sir), containing a 4Fe-4S cluster, reduces sulfite to sulfide. Sulfide is incorporated into cysteine, which is the source of reduced sulfur in the cell.

In nitrogen assimilation, another central assimilatory pathway in chloroplasts, nitrite reductase (NiR), containing a 4Fe-4S cluster, reduces nitrite to ammonia (Lancaster et al., 1979). Ammonia is subsequently coupled to glutamate, producing glutamine. Glutamate synthase, also named GOGAT (glutamine:2-oxoglutarate amido transferase), a 3Fe-4S protein, transfers an NH$_2$ group from glutamine to $\alpha$-keto-glutarate, resulting in two glutamate molecules. Glutamate and glutamine are the source of reduced nitrogen in the cell. Amidophosphoribosyltransferase (ATase), also named glutamine phosphoribosylpyrophosphate (PRPP) amidotransferase, is a 4Fe-4S protein (Hung et al., 2004). It transfers an NH$_2$ group from glutamine to PRPP, resulting in 5-phosphoribosylamine, the first step in de novo biosynthesis of purine nucleotides.

Sirohydrochlorin ferrochelatase (SirB), which is a 2Fe-2S protein, catalyzes the last step of siroheme biosynthesis utilizing iron (Raux-Deery et al., 2005). Interestingly, in plants the
siroheme is only used by SiR and NiR, each of which contains a 4Fe-4S cluster. In fact, plant SiR and NiR represent a unique family of siroheme-(4Fe-4S) enzymes, in which a 4Fe-4S cluster and a siroheme are covalently bound, catalyzing a six-electron reduction.

Pheophorbide a oxygenase (PaO, or LLS1), a Rieske-type 2Fe-2S protein, is an enzyme involved in chlorophyll breakdown (Pruzinska et al., 2003). The PaO is located at the inner envelope membrane of chloroplasts. Chlorophyll a oxygenase (CAO), a Rieske-type 2Fe-2S protein, is involved in chlorophyll b formation from chlorophyll a (Tanaka et al., 1998). The CAO is probably located in the chloroplast stroma. Tic55, a Rieske-type 2Fe-2S protein, is a member of the chloroplastic inner envelope protein translocon (Caliebe et al., 1997). Choline monooxygenase (CMO) is a Rieske-type 2Fe-2S protein, catalyzing the first step of synthesis of the osmoprotectant glycine betaine (Rathinasabapathi et al., 1997). Finally, dihydroxy acid dehydratase (DAD), the third enzyme in the branched-chain amino acid biosynthetic pathway, also contains a 2Fe-2S cluster (Flint & Emptage, 1988).

The Fe-S cluster biogenesis machinery in chloroplasts

It was found, almost two decades ago, that in vitro-synthesized Fd acquires its 2Fe-2S cofactor after it is imported into chloroplasts (Li et al., 1990), suggesting that Fe-S clusters can be synthesized within this compartment independently of cytosol or other subcellular organelles, and thus that a separate Fe-S biogenesis machinery is present in chloroplasts. The chloroplast Fe-S biosynthetic machinery has been investigated using Arabidopsis thaliana as a model (Fig. 2).

CpNifS (At1g08490), also called AtNFS2, is a cysteine desulfurase (Leon et al., 2002; Pilon-Smits et al., 2002), converting cysteine into alanine and elemental sulfur, and providing sulfur for Fe-S assembly. Like all other NifS-like proteins, CpNifS also has selenocysteine lyase activity (Pilon-Smits et al., 2002). Its selenocysteine lyase activity is 300-fold higher than its cysteine desulfurase activity. CpNifS is most similar to SufS among all three NifS-like proteins in E. coli. So, CpNifS could also be termed CpSufS. As shown recently, CpNifS is...
essential for the Fe-S cluster formation activity of chloroplast stroma, and a fraction of it appears to be present in a complex of ~600 kDa (Ye et al., 2005). In Arabidopsis, a second cysteine desulfurase is probably present in mitochondria (Kushnir et al., 2001), where another ISC-like Fe-S biogenesis machinery is thought to be active (Leon et al., 2005). Additionally, a third cysteine desulfurase activity is found in the cytosol. It is assigned to an NifS-like domain of the ABA3 protein, unlikely to be relevant for Fe-S synthesis, but functioning in the sulfuration of molybdenum cofactor in aldehyde oxidase and xanthine dehydrogenase (Heidenreich et al., 2005). Therefore, at this point it appears that Fe-S proteins in cellular compartments other than the plastid and mitochondrion rely on one of these machineries for their Fe-S clusters. Indeed, the phenotype of the proteins in cellular compartments other than the plastid and with a glutaredoxin and thus involved in the oxidative stress machinery is thought to be active (Leon et al., 2005). Bacterial BolA proteins were hypothesized to be reductases interacting like domain (Xu & Møller, 2006; Ye et al., 2006). A human glutaredoxin, Grx5, was found to be required for Fe-S assembly in yeast and animals (Rodriguez-Manzaneque et al., 2002). These recent findings raise great interest in investigating the possible involvement of a Grx homologue in chloroplastic Fe-S biogenesis and its possible interaction with the BolA domain of CpSufE.

Nfu1–3 are scaffold proteins with sequence similarity to the C terminus of bacterial Nfu1 (Leon et al., 2003). Nfu1 (At4g01940) and Nfu2 (At5g49940) were able to restore the growth of a scaffold-mutated yeast, the Δnfs1Δnfs1 strain, suggesting a role of them as a scaffold. However, the complementation by Nfu3 (At4g25910) was not evident, probably as a result of its low expression in yeast (Leon et al., 2003). Recombinant Nfu2 contained a labile 2Fe-2S cluster, and could transfer it to apo-ferredoxin, resulting in holo-ferredoxin (Leon et al., 2003). Experiments with T-DNA insertion lines further revealed that Nfu2 is required for assembling 4Fe-4S clusters of PSI and 2Fe-2S clusters of Fd in chloroplasts (Touraine et al., 2004; Yabe et al., 2004). A subset, but not all, of chloroplast Fe-S proteins are affected in the Nfu2 mutant, suggesting that alternative scaffolds are functional in chloroplasts. Besides Nfu1–3, two more Nfu-like proteins (Nfu4 and Nfu5, which are probably mitochondrial) have been reported in plants (Leon et al., 2003).

CpSufA (At1g10500) is an additional scaffold protein (Abdel-Ghany et al., 2005a). CpSufA has sequence similarity to both bacterial IscA and SufA, but is more similar to IscA (Abdel-Ghany et al., 2005a; Yabe & Nakai, 2006). In vitro, by acquiring sulfur from CpNifS and ferrous iron from the media, CpSufA is able to assemble a 2Fe-2S cluster within its dimeric polypeptides, resulting in holo-CpSufA. The holo-CpSufA, stably holding the transient Fe-S cluster, can be isolated and is able to deliver the Fe-S cluster to apo-ferredoxin. CpSufA was reported to be mostly present in a ~600 kDa chloroplast stromal complex in Arabidopsis (Abdel-Ghany et al., 2005a), as were part of CpNifS (Ye et al., 2005) and CpSufE (Ye et al., 2006). Yabe & Nakai (2006), however, observed only a dimeric CpSufA complex upon in vitro import of CpslsA in pea chloroplasts. It was found recently (Yabe & Nakai, 2006) that CpslsA accumulation in plants depends on the presence of CpNfu2, suggesting that it functions downstream of this other scaffold. Homozygous knockout of CpslsA was not lethal (Yabe & Nakai, 2006). Taken together, the probable role of CpslsA in vivo is assembling Fe-S clusters for a subset of proteins, but its exact role remains to be determined. In plants, IscU scaffolds are found exclusively in mitochondria, indicating that the plastid Fe-S cluster biosynthetic machinery is IscU-independent (Leon et al., 2005).

Like their bacterial homologues, the chloroplast SufBCD proteins form a complex (Xu et al., 2005) displaying ABC/ATPase activity and probably involved in Fe-S cluster biogenesis. However, the exact role of the SufBCD complex in the process remains to be characterized. Arabidopsis SufB (At4g07700) can complement SufB deficiency in E. coli during oxidative stress, but unlike bacterial SufB, the AtSufB is a Fe-stimulated
ATPase (Xu et al., 2005). Arabidopsis SufC (At3g10670) is an ABC/ATPase (Xu & Möller, 2004), which can partially rescue growth defects in an E. coli SufC mutant during oxidative stress. SufD (Atlg32500) is a protein with homology to the bacterial SufD, the mutation of which results in impaired embryogenesis and abnormal growth of Arabidopsis (Hjorth et al., 2005).

High chlorophyll fluorescence 101 (HCF101) (At3g24430) is a P-loop ATPase (Lezhneva et al., 2004), which was originally identified in a photosynthetic mutant screen. It is required for the biogenesis of 4Fe-4S clusters for PSI and FTR in chloroplasts. However, a precise role of HCF101 in the process remains to be elucidated. Accumulation of photosystem one 1 (APO1) (At1g64810) is a member of a gene family only found in vascular plants, which was originally identified in a PSI mutant screen. It is involved in the assembly of 4Fe-4S cluster-containing complexes of chloroplasts (e.g., PSI and FTR) (Amann et al., 2004). However, a direct connection between APO1 and Fe-S assembly needs to be shown.

In summary, as chloroplasts contain proteins with sequence similarity to each of the bacterial SufABCDSE components, they apparently have conserved a complete Suf-type machinery for Fe-S biogenesis. It is reasonable that a Suf-like system, responsible for Fe-S biosynthesis under oxidative stress in bacteria, works well in the oxidative environment in chloroplasts. However, the entire chloroplast Fe-S machinery is apparently more complex, involving, in addition, three Nfu-like proteins, a HCF101 that has no homologue in bacterial Fe-S machineries, and probably an APO1 that is unique in vascular plants. There is a BolA domain that may interact with a glutaredoxin. The importance and complexity of the chloroplast Fe-S machinery is illustrated by the phenotypes caused by mutation of individual machinery components, for example embryonic lethality for CpSufE (Xu & Möller, 2006; Ye et al., 2006), dwarf and yellowish plants for Nfu2 (Yabe et al., 2004), abnormal plastid structure and impaired embryogenesis for SufC (Xu & Möller, 2004) or SufD (Hjorth et al., 2005), and seedling lethality and high chlorophyll fluorescence for HCF101 (Lezhneva et al., 2004).

Implications of the Fe-S machinery for the homeostasis of iron and sulfur in plastids

By supplying Fe-S clusters to a variety of proteins, the plastid Fe-S biogenesis machinery is essential for many vital processes, including photosynthesis, nitrogen assimilation and sulfur assimilation (Fig. 1). The efficiency of the Fe-S biosynthetic machinery directly affects those processes. Because of the importance of iron and sulfur for plant productivity and nutritional value, the impact of the plastid Fe-S machinery on the homeostasis of iron and sulfur is of particular interest.

Iron is an essential micronutrient of plants. Because iron is mainly present in soil as insoluble iron-oxide with low bioavailability, iron is one of the most limiting nutrients for plants. The uptake and transport of iron to leaf cells was reviewed by Curie & Briat (2003). In leaf cells, more than 90% of iron is located in chloroplasts. The molecular mechanism of iron transport into chloroplasts has not yet been identified, but the Fe$^{2+}$ transport across the chloroplast inner envelope membrane is measurable, and has been determined to be light-dependent. Fe$^{2+}$ transport across the inner envelope membrane is stimulated by an electrochemical proton gradient and is reduced by negating the potential gradient, suggesting that iron transport into chloroplasts is probably an Fe$^{2+}$/H$^+$ symport transport mechanism (Shingles et al., 2002). A recent study suggested that 40% of chloroplast iron is in the thylakoid (Abdel-Ghany et al., 2005b). In thylakoid membranes, iron is predominantly used for electron transfer. Approximately half of thylakoid iron is in PSI in the form of 4Fe-4S clusters, and the remaining half is present in cytochromes in the form of hemes and Rieske-type 2Fe-2S clusters. In the stroma, iron is used for assembling Fe-S clusters, hemes, Fe-SOD, etc. The excess of stromal iron is stored in ferritin. Because iron is one of the most limiting micronutrients to plants, plants might employ a sophisticated homeostasis mechanism to control cellular iron, particularly in plastids. At this point, the processes controlling plastid iron homeostasis are largely unknown. Conversely, the Fe-S biogenesis machinery may affect the available iron pool in a specific compartment. For instance, in yeast and mammalian cells, impaired mitochondrial Fe-S biogenesis results in iron overload in mitochondria (Rouault & Tong, 2005). Similar results were found for Arabidopsis mitochondria (Kushnir et al., 2001). Under particular physiological or pathological conditions, when the activity of the plastid Fe-S machinery is abnormally changed, it might affect iron homeostasis.

Sulfur is a macronutrient, widely present in a variety of biological molecules. Generally, it is not limiting for plants. Sulfur metabolism in plants has been recently reviewed by Pilon-Smits & Pilon (2006). The plastid is the predominant compartment for sulfur assimilation from sulfate to cysteine. In the process, out of four enzymes involved, two are Fe-S proteins: APS reductase and SiR (Fig. 1). Thus, sulfur assimilation is probably dependent on the plastid Fe-S machinery. In particular, the step catalyzed by SiR is expected to be dependent on the Fe-S machinery. The catalytic enzyme SiR employs a unique sioheme-(4Fe-4S) as its prosthetic group, requiring not only direct incorporation of a 4Fe-4S cluster, but also SIRB (which is a 2Fe-2S protein) for synthesizing the sioheme. Moreover, the six-electron reduction catalyzed by SiR needs Fd (a 2Fe-2S protein) for providing electrons. In the final step of sulfur assimilation, sulfide is incorporated into cysteine, which is the source of reduced sulfur in plastids. It is probable that the Fe-S machinery is important for sulfur assimilation. To supply reduced sulfur from cysteine, CpnNiFe-CpSufE activity is required, which provides sulfur not only for Fe-S biogenesis, but also probably for the synthesis of various sulfur-containing biomolecules.
Selenium is a group VIA metalloid without evident essenti-
ality for plants. It is metabolized by plants in the same path-
way as sulfur, because of their chemical similarity. Selenium metabolism in higher plants has been reviewed by Terry et al. (2000). Much of the selenium metabolism takes place in chloroplasts. Selenium is assimilated into seleno-cysteine and seleno-methionine, which can be nonspecifically incorpo-
rated into proteins by replacing sulfur amino acids. As cysteine residues are important for the structure and function of pro-
teins, the substitution of cysteine by seleno-cysteine results in the loss of protein function. Plants can employ the seleno-
cysteine lyase (SL) activity of NifS-like proteins to break seleno-cysteine into alanine and selenide, thereby avoiding selenium toxicity. In an initial approach, Arabidopsis was shown to acquire enhanced selenium tolerance and accumu-
lation when overexpressing a mouse SL (Pilon et al., 2003). Arabidopsis has its own SL, the CpNifS in chloroplasts. This is also the cysteine desulfurase component of the plastid Fe-S biogenesis machinery. Recently, transgenic Arabidopsis, over-
expressing the CpNifS in plastids, was obtained. These, too, displayed higher tolerance and accumulation of selenium (Van Hoeyyk et al., 2005). Therefore, altering the levels of this Fe-S machinery component clearly affects selenium metabolism. Understanding how the plastid Fe-S biogenesis machinery, particularly the CpNifS–CpSufE complex, is implicated in selenium homeostasis, will be applicable in selenium phytoremediation, using plants to clean up areas contaminated with selenium.

Investigations on how the Fe-S machinery is regulated by iron and sulfur levels, and how a modulated machinery affects iron and sulfur homeostasis, represents a promising research area. Transgenic plants, in which the expression levels of components of the plastidic Fe-S biosynthetic machinery are modulated from high overexpression to knockout, will be a useful tool in such studies.

Acknowledgements
Research in the authors’ laboratory is supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2003-35318-13758 to E.A.H.P.S. and M.P. We apologize for not being able to reference all the literature; this was because of space limitation.

References


