ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 1, 2011

DOI: 10.1089/ars.2010.3259

COMPREHENSIVE INVITED REVIEW

Iron–Sulfur Clusters: Biogenesis, Molecular Mechanisms, and Their Functional Significance

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Abstract

Iron–sulfur clusters [Fe-S] are small, ubiquitous inorganic cofactors representing one of the earliest catalysts during biomolecule evolution and are involved in fundamental biological reactions, including regulation of enzyme activity, mitochondrial respiration, ribosome biogenesis, cofactor biogenesis, gene expression regulation, and nucleotide metabolism. Although simple in structure, [Fe-S] biogenesis requires complex protein machineries and pathways for assembly. [Fe-S] are assembled from cysteine-derived sulfur and iron onto scaffold proteins followed by transfer to recipient apoproteins. Several predominant iron–sulfur biogenesis systems have been identified, including nitrogen fixation (NIF), sulfur utilization factor (SUF), iron–sulfur cluster (ISC), and cytosolic iron–sulfur protein assembly (CIA), and many protein components have been identified and characterized. In eukaryotes ISC is mainly localized to mitochondria, cytosolic iron–sulfur protein assembly to the cytosol, whereas plant sulfur utilization factor is localized mainly to plastids. Because of this spatial separation, evidence suggests cross-talk mediated by organelle export machineries and dual targeting mechanisms. Although research efforts in understanding iron–sulfur biogenesis has been centered on bacteria, yeast, and plants, recent efforts have implicated inappropriate [Fe-S] biogenesis to underlie many human diseases. In this review we detail our current understanding of [Fe-S] biogenesis across species boundaries highlighting evolutionary conservation and divergence and assembling our knowledge into a cellular context. *Antioxid. Redox Signal.* 15, 271–307.

Reviewing Editors: *Jason C. Crack, Huangen Ding, John H. Golbeck, Christopher H. Lillig, Anonymous, Tracey A. Rouault, and Anonymous*

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I. Introduction

A. History of iron–sulfur cluster research

It has been proposed that early life may have formed on the surface of iron sulfide minerals (316), indicating the important role of iron and sulfur in relation to our own existence. Iron and sulfur are two of the most crucial and versatile elements on our planet, and their importance in biology is unquestionable. The discovery of iron–sulfur proteins, which have iron–sulfur clusters ([Fe-S]) as cofactors, has further reinforced this notion.

[Fe-S], comprising iron and bridging sulfur elements at various molar ratios (27), are most often discussed in the context of the biological role for iron–sulfur proteins. The most common [Fe-S] are the rhombic [2Fe-2S] and the cubane [4Fe-4S] (Fig. 1), but there are also [3Fe-4S] in enzymes such as bacterial ferrodoxin I and more complex [8Fe-7S] belong to the P cluster of the MoFe nitrogenase (130). Cubane-type [4Fe-4S] can be assembled from two [2Fe-2S] units, whereas [3Fe-4S] and [8Fe-7S] can be assembled from [4Fe-4S] units *via* loss of one Fe and cluster fusion, respectively (130).

The ability to delocalize electron density over both Fe and S atoms makes [Fe-S] ideally suited for their primary role in mediating biological electron transport (103, 130, 303). Although the vast majority of electron transfer [Fe-S] are one-electron carriers, the double-cubane [8Fe-7S] that is found only in nitrogenases has the potential to act as a two-electron carrier (130, 223). Moreover, this cluster undergoes major structural changes on two-electron oxidation, involving Fe ligation by the amide N of one of the bridging cysteine residues and the O of a nearby serine, thereby providing a mechanism for coupling proton and electron transfer. These inorganic prosthetic groups participate in a variety of biochemical processes, including photosynthesis, respiration, nitrogen fixation (NIF), substrate binding and activation, redox catalysis, DNA replication and repair, regulation of gene expression, and tRNA modification (239).

Despite the importance of [Fe-S] within biology, research can only be traced back ~50 years. In 1960, Beinert and Sands discovered a new electron paramagnetic resonance (EPR) signal in beef heart mitochondria associated with a new nonhaem iron cofactor (28, 256). After this, similar EPR signals were also discovered in a diverse set of biological samples, including liver, plant tissue, and in both aerobic and anaerobic bacteria (275). In 1965, substantial quantities of iron and acid labile sulfide were found to be associated with succinate dehydrogenase isolated from pig heart (342) and in 1967 sulfur was shown, *via* isotropic substitution, to be an essential component of the nonhaem iron EPR signal (65). The interest surrounding these discoveries became evident as only a year later, in 1968, detailed investigations into these iron–sulfur proteins gained significant momentum (274). Since these early discoveries a wide spectrum of iron–sulfur proteins have been identified and characterized from a variety of organisms and this list is continuing to expand.

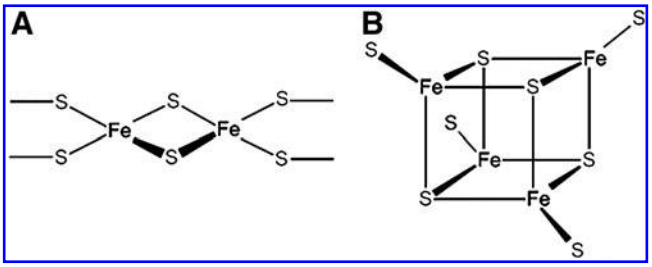

FIG. 1. The structures of the two most commonly found [Fe-S]: (A) rhombic and (B) cubane clusters. The ligand sulfur atoms of cysteine residues that coordinate the [Fe-S] in the polypeptide are also described. [Fe-S], iron–sulfur clusters.

In most iron–sulfur proteins, the iron elements are liganded by cysteine residues; however, histidine, serine, aspartic acid residues, or back-bone amides can also act as ligands (106, 119, 188, 281). Despite the large number of iron–sulfur proteins identified, there is no common consensus motif to predict whether a candidate protein can bind an [Fe-S]. However, some primary structural features are reoccurring (164, 188). These include the conserved positioning of cysteine residues such as the $CX_4CX_2CX_{30}C$ motif in plant and mammalian [2Fe-2S] ferredoxins (Fdx), the consensus motif $CX_2CX_2CX_{20–40}$ C for [4Fe-4S], which was originally defined in [4Fe-4S] Fdx but seems present in many other members of the [4Fe-4S] type. It appears that the spacing of the cysteine residues can also be reversed. Usually, a conserved proline (or sometimes glycine) flanks one of the cysteine residues (164).

In vitro chemical reconstruction of [Fe-S] was first demonstrated on apo-ferrodoxin by the addition of ferrous ions/ferric ions and sodium sulfide, and the reconstituted materials are identical to native Fdx with respect to enzymatic activity, acid-labile sulfide, and iron content in addition to ultraviolet and visible absorption spectra (179). However, as ferrous/ferric iron and sulfide sulfur are toxic *in vivo*, it was proposed that one or more coordinated biosynthetic pathways are required for [Fe-S] to be assembled in living cells. After the pioneering work of Dean's group on the function of the nitrogenases of *Azotobacter vinelandii*, several biosynthetic machineries have been discovered in bacteria and eukaryotic organisms, based on biochemical evidence and genetic analysis. These systems include the nitrogen fixation (NIF) system, the iron–sulfur cluster (ISC) system, and the sulfur utilization factor (SUF) system in bacteria, and the cytosolic iron–sulfur protein assembly (CIA) system in yeast (166, 289).

On the basis of sequence similarity, another pseudo-system for [Fe-S] biogenesis was identified in *Escherichia coli* consisting of only two genes, *csdA-csdE*, organized as an operon and named csdA-csdE system (25). CsdA shares high sequence identity with SufS and CsdE is a homolog of SufE. CsdE can enhance the cysteine desulfurase activity of CsdA by accepting persulfide sulfur on a conserved cysteine residue similar to SufS activation by SufE (170, 173). Indeed, CsdA-CsdE represents a cysteine desulfurase complex similar to SufS-SufE (SufSE). However, their function in [Fe-S] biogenesis has not been established. Within the context of evolution compartments or organelles became apparent adding a level of complexity to eukaryotic organisms. This cytosolic evolution presumably introduced the CIA [Fe-S] biogenesis system, which is supposed to be responsible for the maturation of iron–sulfur proteins in the cytosol and nucleus. Recent investigations have confirmed that this system is present in virtually all eukaryotes (162, 164–167). However, with the discoveries of several important factors of the ISC-like system in the cytosol of mammalian cells, it is evident that the *de novo* [Fe-S] biogenesis in the cytosol is not confined to a CIA-like system in eukaryotes (246, 247, 337).

It has been accepted that a minimal functional [Fe-S] biogenesis system must contain a scaffold protein(s), a sulfur donor, and an iron donor. The *in vivo* source of sulfur is cysteine from which sulfur is released by cysteine desulfurases such as NifS, IscS, and SufS. NifU and IscU have been confirmed to be scaffold proteins in the NIF and ISC systems, whereas SufB-SufC-SufD (SufBCD) complex has recently been suggested to be a scaffold complex for the SUF system in bacteria. When an assembled [Fe-S] is transferred to an apo-protein (protein lacking [Fe-S]), accessory proteins are required such as HscA and HscB components in the ISC system. However, a critical gap remained as to the nature of the iron donor for [Fe-S] biogenesis systems. Considering the labile nature of these clusters, storage components are important for maintaining a sustainable source of these cofactors.

Frataxin (bacterial homolog CyaY) has been suggested as a possible iron donor candidate for ISC-like systems, but more research is needed to confirm this and to identify which proteins provide iron for the NIF and SUF systems. Bacterial CyaY might also play a regulatory function in [Fe-S] biogenesis by modulating the enzymatic activity of the cysteine desulfurase (4). It would be reasonable to suggest that A-type proteins act as iron donors considering their conservation in all three systems, $IscA^{Nif}$ in NIF, IscA in ISC, and SufA in SUF. On the basis of evolutionary considerations, A-type proteins are divided to three subfamilies (313). There is evidence that IscA and SufA are iron-binding proteins that can provide iron for the [Fe-S] assembly in IscU in the presence of a thioredoxin reductase system (67–71, 175, 290, 332). However, experimental evidence supporting A-type proteins as alternative [Fe-S] scaffolds is steadily accumulating (142, 211, 212, 270, 341).

B. Detection of [Fe-S]

Although apo-proteins (without [Fe-S]) are generally colorless, holo iron–sulfur proteins (with [Fe-S]) often have different colors; for instance, some iron–sulfur proteins appear yellow at lower concentration and brown at higher concentrations. As insertion of [Fe-S] in apo-proteins will result in modification of their conformations, the clusters can be detected using spectroscopic methods. These methods include UV-visible spectroscopy, circular dichroism (CD) spectroscopy, EPR spectroscopy, Mössbauer spectroscopy, resonance Raman spectroscopy, extended X-ray fine-structure spectroscopy and electron nuclear double resonance spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy. Different [Fe-S] have different spectroscopic features. For example, a characteristic UV-visible absorption spectrum for oxidized [2Fe-2S] displays bands at 277, 325, 416, and 460 nm and a shoulder at around 560 nm (140, 228), whereas oxidized [3Fe-4S] consists of 281 and 408 nm peaks and a shoulder at 310 nm (123) and where [4Fe-4S] presents a broad peak at 420 nm (127, 153). Similarly, some [2Fe-2S] can be detected by EPR spectroscopy with absorptions at g-values of 2.02, 1.94 (140), whereas some [4Fe-4S] show g-values of 2.06, 1.93 (228) and [3Fe-4S] 2.03, 1.94 (123). On the basis of publications to date concerning characterization of iron–sulfur proteins, EPR spectroscopy, Mössbauer spectroscopy, and resonance Raman spectroscopy are most common. EPR spectroscopy is a well-accepted means to confirm the presence of [Fe-S] in proteins and assign them to specific cluster types and oxidation states. In general, combinations of different analysis approaches rather than a single method are necessary to determine the [Fe-S] type harbored by iron–sulfur proteins as evident from numerous reports. A yellowish sample protein, detected by UV-visible spectroscopy with 325, 416, and 460 nm absorption peaks, by EPR with g-values of 2.02 and 1.94, and by CD spectroscopy with a larger peak at 435 nm, could be confirmed to contain [2Fe-2S] (140).

C. Redox biochemistry of iron–sulfur proteins

[Fe-S], functioning primarily as electron transfer agents, are best known for their role in the oxidation–reduction (redox) reactions of mitochondrial and photosynthetic electron transport during which [Fe-S] interchange between different oxidation states.

Rhombic [2Fe-2S], coordinated by four cysteine ligands (in [2Fe-2S] Fdx) or by two cysteines and two histidines (in Rieske proteins), are able to exist in two different oxidation states, oxidized form $[2Fe\text{-}2S]^{2+}$ and reduced form $[2Fe\text{-}2S]^{+}$ ($[2Fe\text{-}2S]^{2+/+}$) (310). The oxidized form contains two Fe^{3+} ions, whereas the reduced form contain one Fe^{3+} and one Fe^{2+} ion. The redox potential of [2Fe-2S] Fdx is approximately −290 to −400 mV (pH 7.0), whereas that of the Rieske protein is approximately 290 mV (pH 7.0).

Cubane [4Fe-4S], coordinated by cysteine ligands, have three oxidation states $[4Fe\text{-}4S]^{+}$, $[4Fe\text{-}4S]^{2+}$, and $[4Fe\text{-}4S]^{3+}$ (310). The most investigated [4Fe-4S] proteins are electron-transfer [4Fe-4S] Fdxs. These can be further subdivided into low potential (bacterial-type) and HiPIP (high potential iron–sulfur proteins) Fdxs. In low potential Fdxs, clusters shuttle between $[4Fe\text{-}4S]^{+}$ and $[4Fe\text{-}4S]^{2+}$ ($[4Fe\text{-}4S]^{2+/+}$), whereas in HiPIP, clusters interchange between $[4Fe\text{-}4S]^{3+}$ and $[4Fe\text{-}4S]^{2+}$ ($[4Fe\text{-}4S]^{3+/2+}$). The Fdxs that contain $[4Fe\text{-}4S]^{2+/+}$ have a reduction potential ranging from −280 to −715 mM, whereas HiPIP Fdx have a reduction potential ranging from 90 to 450 mV in different native proteins (54).

II. [Fe-S] Biogenesis in Bacteria

The anoxic character of earth's early atmosphere (131) indicates an evolutionary timeline in terms of the different [Fe-S] biogenesis machineries. One can easily imagine that the NIF machinery might have been the primary system to have evolved followed by the ISC and SUF systems once production of O_2 occurred with the emergence of cyanobacteria (Fig. 2). After this the various [Fe-S] biogenesis systems then evolved in an orderly fashion in all other organisms (Fig. 3).

A. NIF system (A. vinelandii)

1. NifS and NifU. The NIF system was the first identified [Fe-S] biogenesis system that is used exclusively for the maturation of nitrogenase in *A. vinelandii* under nitrogen fixation conditions. Although there are a few exceptions where similar systems have been identified in organisms that do not fix nitrogen, such as *Helicobacter pylori* and *Entamoeba histolytica* (10, 213), the NIF system is generally regarded as a nitrogen-fixing-specific machinery for [Fe-S] assembly *in vivo*.

It is somewhat surprising that [Fe-S] biogenesis systems were not first identified from research focusing on the assembly of simple [2Fe-2S] or [4Fe-4S] but rather from attempt to understand the formation of nature's most complex [Fe-S], the FeMo-cofactor of the nitrogenize MoFe protein (93).

Nitrogenase is composed of two component proteins, the MoFe protein containing the substrate binding and reduction site, and the Fe protein serving as a specific source of electrons required for substrate reduction. The MoFe protein contains two types of [Fe-S], an [Mo-7Fe-9S] MoFe-cofactor and a P [8Fe-7S], whereas the Fe protein contains a single [4Fe-4S] cluster. Assembly of nitrogenase MoFe protein is assumed to be one of the most complex processes in the field of bioinorganic chemistry requiring at least the participation of *nifS*, *nifU*, *nifB*, *nifE*, *nifN*, *nifV*, *nifQ*, *nifZ*, *nifH*, *nifD*, and *nifK* gene products (118). Among all these *nif* gene products, only NifS and NifU, together with $IscA^{nif}$, are involved in [Fe-S] biosynthesis *in vivo*, which form the NIF system (130). This system provides [Fe-S] not only for Fe protein but also for the MoFe protein (129, 344).

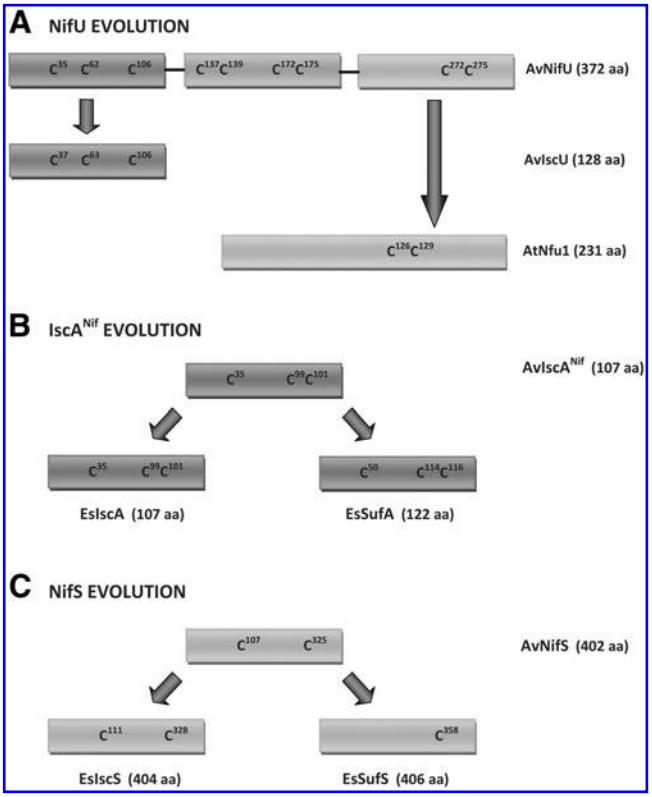

FIG. 2. Possible evolutionary route of NIF components. Because of the anoxic nature of earth's early atmosphere, the anaerobic NIF system is proposed to be the primary [Fe-S] biogenesis system. **(A)** AvNifU (NifU of *Azotobacter vinelandii*) contains three domains: N-terminal is similar to IscU such as AvIscU (IscU of *A. vinelandii*) and EsIscU (IscU of *Escherichia coli*); C-terminal to Nfu such as EsNfuA and AtNfu1 (Nfu1 of *Arabidopsis thaliana*); the middle part contains a permanent [Fe-S]. **(B)** $AvIscA^{nif}$ ($IscA^{nif}$ of *A. vinelandii*) is similar to IscA (such as *E. coli* EsIscA) and SufA (such as *E. coli* EsSufA) of bacteria and other organisms. **(C)** AvNifS (NifS of *A. vinelandii*) is similar to IscS (such as *A. vinelandii* AvIscS and *E. coli* EsIscS) and SufS (such as *E. coli* EsSufS) of bacteria and other organisms. The alternative possibility exists that ISC might be derived from NIF but SUF from ISC. The sizes of proteins are indicated in parentheses. aa, amino acid; At, *A. thaliana*; Av, *Azotobacter vinelandii*; Es, *Escherichia coli*; ISC, iron–sulfur cluster system; NIF, nitrogen fixation system; SUF, sulfur utilization factor system.

NifS has been confirmed to be a cysteine desulfurase and it is expressed only under nitrogen-fixing conditions. Purified NifS is a homodimer, always containing a pyridoxal phosphate (PLP) (64, 348). NifS catalyzes the release of sulfur from L-cysteine to yield L-alanine. In NifS there is a conserved Cys^{325} (Fig. 2), which is extremely active toward alkylating reagents, and a persulfide could be found at this site when NifS is incubated with equimolar amounts of L-cysteine (346, 347).

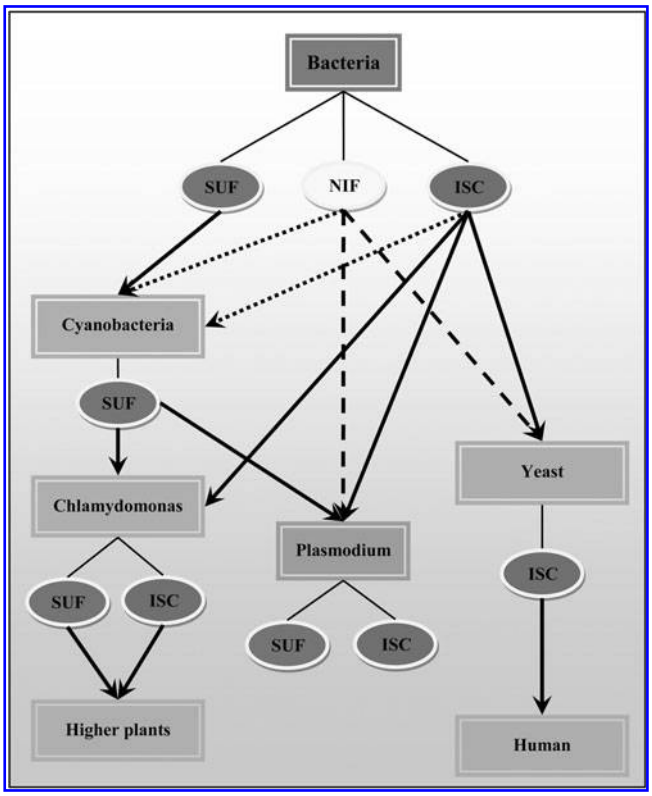

FIG. 3. Possible links of [Fe-S] biogenesis systems between different organisms ranging from prokaryotes to eukaryotes. Although the NIF system is generally specific for nitrogen fixing bacteria such as *A. vinelandii*, homologs of its components such as NifU (including IscU and Nfu domains), NifS, and IscA^{nif} are universally found in ISC and SUF systems in all organisms. Bacterial organisms contain NIF, ISC, and SUF systems. Cyanobacteria contain a complete SUF system but only a partial ISC system, both of which probably originate from bacteria. The SUF system of *Chlamydomonas* chloroplasts probably originates from cyanobacteria. As the ISC system of *Chlamydomonas* mitochondria is complete but not in cyanobacteria, it is reasonable to believe that the ISC system of *Chlamydomonas* is inherited from a bacterial ancestor. Some cyanobacterial species, specific for NIF, accommodate a functional NIF system (see text), which may come from bacterial NIF. The origins of the SUF and ISC systems in higher plants is undoubtedly from lower plants. Lower eukaryotes such as yeast have obtained a complete ISC system and Nfu components from bacteria, whereas higher eukaryotes, such as humans, have inherited ISC and Nfu components from lower eukaryotes. This schematic presents logical evolutionary links rather than real molecular connections. Continuous arrows indicate a highly probable inheritance and broken arrows indicate tentative inheritance. Although the evolutionary origin of *Plasmodium* is unclear, their SUF probably originated from cyanobacteria, whereas their ISC and Nfu genes probably arose from bacteria. Because of the lack of a common link between photosynthetic and nonphotosynthetic organisms, it is impractical to incorporate the CIA system in this schematic. CIA, cytosolic iron–sulfur protein assembly.

The mechanism for the function of NifS and other NifS-like cysteine desulfurases can be viewed as: (i) formation of a substrate L-cysteine-PLP (pyridoxal phosphate) ketimine adduct, (ii) formation of enzyme-bound persulfide through nucleophilic attack by the thiolate anion of the active-site cysteine on the sulfur of the substrate L-cysteine and alanine release, and (iii) subsequent transfer of substrate-derived sulfur of the cysteine persulfide residue to different biomolecules such as [Fe-S] scaffolds (25, 130). It is now known that NifS represents a broad class of cysteine desulfurases that use L-cysteine for general mobilization of sulfur for [Fe-S] formation. These proteins can be further classified into two groups based on their sequence composition: group I cysteine desulfurases contain a consensus sequence SSGSACTS and group II enzymes contain consensus sequence RXGHHCA (190). NifS belongs to group I.

The *cysE1* gene encodes an O-acetyl serine synthase, which catalyzes the rate-limiting step in cysteine biosynthesis. It is therefore proposed to augment the cysteine pool as a way to accommodate the activity of NifS (82, 345).

NifU is a scaffold protein for [Fe-S] biogenesis. Purified NifU is also a homodimer that contains one [2Fe-2S] (97). This cluster is thought to be a cofactor for the function of NifU itself (7, 74) so it is regarded as a permanent cluster. Sequence analysis revealed that NifU comprises three highly conserved primary amino acid sequences (Fig. 2): (i) the N-terminal region contains three highly conserved cysteine residues Cys^{35}, Cys^{62}, and Cys^{106}, (ii) the central domain contains four conserved cysteine residues Cys^{137}, Cys^{139}, Cys^{173}, and Cys^{175}, compatible with the [2Fe-2S] binding signature where permanent clusters reside, and (iii) the C-terminal region contains the two highly conserved cysteine residues Cys^{272} and Cys^{275} (32, 121). Either or both of the N-terminal and C-terminal domains could provide assembly sites for the formation of transient [Fe-S] destined for the maturation of nitrogenase (7, 74). The ability of the NifU N-terminal to assemble transient [Fe-S] was demonstrated *in vitro* on the purified N-terminal part of NifU, which was incubated with ferric ion, L-cysteine, and catalytic amounts of NifS (340). Approximately one transient [2Fe-2S] is assembled per homodimer, and the transient [2Fe-2S] species is labile and rapidly released on reduction. The ability of the NifU C-terminal domain to assemble transient [Fe-S] was suggested by the function of homologous proteins from other organisms that can accommodate formation of transient [2Fe-2S] and [4Fe-4S] (17, 157, 171, 172, 206, 298). Although the NIF system is generally found in nitrogen fixing organism, genes similar to the NifU C-terminus are ubiquitously found in other organisms (Fig. 3). The permanent [2Fe-2S] probably involve redox roles related to iron acquisition for cluster assembly (Fig. 2), release of the persulfide from NifS, release of the [Fe-S] precursor from NifU (93), or providing electron for S^0 reduced to S^{2-}.

NifU can interact with NifS forming a transient complex (340) that should be necessary for accepting the released elemental sulfur from NifS. However, NifU and NifS do not form a tight complex as was determined using two different approaches. First, NifU was not found to copurify with NifS when NifS was isolated from crude extracts prepared from nitrogen-fixing *A. vinelandii* cells. Second, specific immunoprecipitation of either NifU or NifS from *A. vinelandii* crude extracts did not result in the coprecipitation of the complementary protein. However, NifU and NifS can form a transient complex because an equimolar mixture of NifU and NifS results in the appearance of a new peak during size-exclusion column chromatography when compared with individually chromatographed samples of either NifU or NifS (340).

As *in vitro* chemical reconstruction of [Fe-S] can be demonstrated on any apo-iron–sulfur protein rather than scaffold proteins, such as apo-Fe protein, without any assistance of other proteins, it was vital to demonstrate that [Fe-S] formed on a proposed scaffold are transferred to a real target. A recombinant NifU was loaded with [Fe-S] by incubation with L-cysteine, ferric iron, and NifS which was then removed followed by mixing with separately prepared apo-Fe protein (74). The Fe-protein assay was immediately measured to verify the activation of Fe-protein. A high level of activation was achieved and maximum activity was obtained at an approximately equimolar concentration of NifU and apo-Fe protein (74). Similar *in vitro* activation could not be achieved by the coincubation of apo-Fe protein with L-cysteine, ferric iron, and NifS at concentrations equivalent to the [Fe-S]-loaded form of NifU (130). NifS was not required for cluster transfer but was necessary for cluster loading on NifU. This in effect means that activation of nitrogenase Fe protein is directed by NifU and NifS together (termed NifUS). Biosynthesis of MoFe-cofactor and P cluster in the MoFe protein of nitrogenase also involved NifUS (7, 74, 92, 94, 280, 340) and a detail procedure for the assembly of nitrogenase MoFe protein has been shown (118).

2. The function of $IscA^{Nif}$ and other A-type proteins. $IscA^{Nif}$, identified after IscA (Fig. 2), also has a function specifically related to the early stages of nitrogenase [Fe-S] assembly (130), but the biochemical functions and cellular roles of $IscA^{Nif}$ and other A-type (IscA and SufA) proteins remain unclear despite their presence in most studied systems (313), as discussed in the introduction. On the basis of current evidence, we propose that A-type proteins are most probably [Fe-S] storage proteins with which the requirement for [Fe-S] *in vivo* could be buffered to retain [Fe-S] homeostasis. Several pieces of evidence support this proposal. First, A-type proteins are iron–sulfur proteins that can accommodate [Fe-S]. As these proteins contain three highly conserved cysteine residues (C-X_{42-44}-D-X_{20}-C-G-C) in their C-terminal regions (345), this indicates a potential ability to hold [Fe-S] or Fe alone. Second, A-type proteins have been characterized to exist as a mixture of oligomeric forms in solution with dimeric and tetrameric forms predominating (142, 211, 212), and as a result it is reasonable that these proteins can bind [2Fe-2S], [4Fe-4S] or other forms of [Fe-S]. At least [2Fe-2S] and [4Fe-4S] have been discovered in *A. vinelandii* $IscA^{Nif}$ and *E. coli* IscA/SufA (108, 290). Third, [Fe-S] on A-type proteins can be transferred to apo-ferrodoxin but at a much lower efficiency than IscU (37). Fourth, *in vitro* studies showed that A-type protein can accept [Fe-S] from the scaffold protein IscU but cannot transfer [Fe-S] to IscU (212). Fifth, evidence shows that a deletion of A-type proteins does not cause phenotypic consequences under normal growth conditions (93). Further, studies involving the two yeast Isa1 and Isa2 proteins revealed that they are required for the function but not for the *de novo* synthesis of the [Fe-S] of biotin synthase in *Saccharomyces cerevisiae* (196). Although A-type proteins are proposed to be [Fe-S] storage proteins this does not contradict its ability to bind iron (71).

*B. ISC system (*A. vinelandii *and* E. coli*)*

A surprising result from the systematic analysis of *nif*-cluster genes of *A. vinelandii* is the ability of *nifS* and *nifU* deletion strains to produce low levels of active nitrogenase and to grow under nitrogen-fixing conditions at an extremely low rate, suggesting that some other housekeeping function related to generalized [Fe-S] biosynthesis could replace NifU and NifS functions in *A. vinelandii* (125, 126). Following a biochemical study focusing on a purified enzyme that had the same L-cysteine desulfurase activity as the NifS protein, designated IscS (87), the second [Fe-S] biogenesis system, ISC, was discovered. The *iscS* is contained within a gene cluster that includes a NifU homolog designated *iscU* and another gene, designated *iscA*, respectively (345). Apart from these three genes, the ISC system in *A. vinelandii* is made up of at least seven genes forming a gene cluster in the genome in the order *iscR-iscS-iscU-iscA-hscB-hscA-fdx-iscX,* among which *iscRSUA* forms an operon (Table 1). It has since been established that the ISC system universally exists in prokaryotic and eukaryotic realms (25, 93, 130, 165, 166). It represents the house keeping system for general [Fe-S] biogenesis in prokaryotes, including *E. coli* and *A. vinelandii* (25, 93).

IscS, like NifS, belongs to group I cysteine desulfurase, which contains the SSGSACTS signature. Purified *E. coli* IscS is a homodimer of 90 kDa and contains PLP (pyridoxal phosphate) as a cofactor (87). The crystal structure of IscS was solved to a resolution of 2.1 Å (63), which suggested that a significant conformational change must occur to allow the catalytic site Cys^{328} to participate in catalysis. IscS is essential for *A. vinelandii* as deletion of *iscS* is lethal (345), but deletion of *iscS* of *E. coli* causes severe growth defects (148, 204, 264, 288).

IscU has considerable primary sequence conservation with the N-terminal domain of NifU including the three conserved cysteine residues, suggesting that IscU is a scaffold component for the ISC system. Many *in vitro* experiments (18, 25, 130) have provided support and *in vivo* evidence further confirmed this to be the case in *A. vinelandii* (239). The *in vivo* experiments also revealed that apo-IscU and holo-IscU are most likely conformationally distinct and that [Fe-S] assembly is a dynamic process involving the association and dissociation of IscU and IscS (239). Conformation variation of IscU has attracted much attention. In *Haemophilus influenzae* IscU was identified as a monomer, dimers, and higher-order oligomers during purification from cell extracts of *E. coli* cells overproducing IscU. Fractions corresponding to monomeric IscU account for about 40% of the total IscU (236). *E. coli* IscU was also found as a monomer and as a covalently bound dimer involving the Cys^{63} residue (132); however, a separate line of analysis indicated that *E. coli* IscU was a folded compact monomeric molecule (5). A recent report showed that wild-type apo-IscU in solution exists in two distinct conformations: one largely disordered and one largely ordered except for the metal binding residues and the two states interconvert within milliseconds. The ordered conformation is stabilized by the addition of zinc, by the single-site IscU mutation D39A (135) and moreover an asymmetric trimeric architecture of [2Fe-2S] IscU was also reported in *Aquifex aeolicus* (276). These variations in IscU structure are in agreement with the NMR spectroscopy analysis of *Thermatoga maritima* IscU where IscU exhibits a tertiary structure that is fluxional among widely different conformational arrangements (31, 180, 181, 324).

As IscU needs to accept sulfur from IscS (Fig. 4), data reveal that there exists interaction between these two components as demonstrated by chemical, physical chemical, and biochemical methods (6, 132, 207, 208, 296, 305). *E. coli* IscS and IscU

Table 1. Conservation and Variation of Iron–Sulfur Cluster Biogenesis Systems

		Plasmodium		*Arabidopsis*			*Yeast*		*Human*	
	Bacteria	*Locus*	*Location*	*Name*	*Locus*	*Location*	*Name*	*Location*	*Name*	*Location*
ISC	IscR									
	IscU	PF14_0518	Mit	AtIscU1	At4g22220	Mit, Cyt	Isu1	Mit	ISCU	Mit, Cyt
				AtIscU2	At3g01020	Mit	Isu2	Mit		
				AtIscU3	At4g04080	Mit				
	IscS	MAL7P1	Mit	AtIscS	At5g65720	Mit	Nfs1	Mit, Cyt	ISCS	Mit, Cyt
	IscA	PFB0320c	Mit	AtIscA1	At2g16710	Mit	Isa1	Mit	ISCA1	Mit, Cyt
		PFC1005c	Mit[a]	AtIscA2	At2g36260	Mit	Isa2	Mit	ISCA1L	Mit
				AtIscA3	At5g03905	Mit			ISCA2	Mit
	HscA	PF11_0351	Mit	AtHscA1	At 4g37910	Mit, Cyt	Ssq1	Mit	HSPA9	Mit
				AtHscA2	At5g09590	Mit				
	HscB	PFI0985c	Mit	AtHscB	At5g06410	Mit, Cyt	Jac1	Mit	HSCB	Mit
	CyaY			AtFH	At4g03240	Mit	Yfh1	Mit	FXN	Mit, Cyt
	Fdx	PFL0705c	Mit	AtFdx	At 4g05450	Mit	Yah1	Mit	FDX1	Mit
					At4g21090	Mit				
	FNR	PF11_0407	Mit	AtFdr	At4g32360	Mit	Arh1	Mit	FDXR	Mit
	NfuA	PFI1835c	Mit	AtNfu4	At3g20970	Mit	Nfu1	Mit	NFU1	Mit, Cyt
				AtNfu5	At1g51390	Mit				
	Grx4	PFF0340c	Mit[a]	AtGrx4	At3g15660	Mit	Grx5	Mit	GLRX5	Mit
		MAL13P1	Mit	AtIsd11	At5g61220	Mit	Isd11	Mit	ISD11	Mit, Cyt
		PF11_0258	Mit	AtMge1a	At4g26780	Mit	Mge1	Mit	GrpE1	Mit
				AtMge1b	At5g55200	Mit			GrpE2	Mit
				AtIba57	At4g12130	Mit	Iba57	Mit	C1orf69	Mit
				AtInd1	At4g19540	Mit	Ind1	Mit	Ind1	Mit
				AtSufE1	At4g26500	Mit, Chl				
CIA		PF11_0296	Cyt[a]				Cfd1	Cyt	NUBP2	Cyt
		PFI0525w	Cyt	AtNbp35	At5g50960	Cyt	Nbp35	Cyt	NUBP1	Cyt
				AtNar1	At4g16440	Cyt	Nar1	Cyt	NARF	Cyt
				AtCia1a	At2g26060	Cyt	Cia1	Cyt	CIAO1	Cyt
				AtCia1b	At4g32990	Cyt				
		MAL8P1	Cyt	AtDre2	At5g18400	Cyt	Dre2	Cyt, Mit	CIAPIN1	Cyt, Nuc
SUF	SufA	PFE1135w	Api[a]	AtSufA	At1g10500	Chl				
	SufB	SufB	Api	AtSufB	At4g04770	Chl				
	SufC	PF14_0133	Api	AtSufC	At3g10670	Chl				
	SufD	PF11_0044	Api	AtSufD	At1g32500	Chl				
	SufS	PF07_0068	Api	AtSufS	At1g08490	Chl				
	SufE	PFB0270w	Api	AtSufE1	At4g26500	Chl, Mit				
				AtSufE2	At1g67810	Chl				
				AtSufE3	At5g50210	Chl				
	NfuA	PFI1050c	Api	AtNfu1	At4g01940	Chl				
				AtNfu2	At5g49940	Chl				
				AtNfu3	At4g25910	Chl				
				HCF101	At3g24430	Chl				
				APO1	At1g64810	Chl				
				GrxS12	At2g20270	Chl				
				GrxS14	At3g54900	Chl				
				GrxS16	At2g38270	Chl				
				GrxC5	At4g28730	Chl				

[a]Low confident prediction.

CIA, cytosolic iron–sulfur protein assembly; ISC, iron–sulfur cluster; SUF, sulfur utilization factor; Mit, mitochondria; Cyt, cytosol; Chl, chloroplast; Api, Apicoplast (Plastid); Nuc, Nucleus; Grx, glutaredoxin; Bacteria, *Escherichia coli*; Plasmodium, *Plasmodium falciparum*; Arabidopsis, *Arabidopsis thaliana*; Yeast, *Saccharomyces cerevisiae*; Human, *Homo sapiens*.

can form an $\alpha_2\beta_2$ heterotetrameric complex with a disulfide bond between Cys^{328} in IscS and Cys^{63} in IscU. A heterodisulfide complex between IscS Cys^{328} and IscU requires Cys^{37} on IscU of *A. vinelandii* (280), whereas in *E. coli* the heterodisulfide complex requires IscU Cys^{63} (132). All three conserved cysteine residues on *A. vinelandii* IscU can accept sulfur from IscS *in vitro* (280), but only Cys^{63} in *E. coli* was reported to be a single acceptor (132).

Time-course analysis *in vivo* of *A. vinelandii* [Fe-S] biogenesis on IscU demonstrated the serial production of apo-IscU, an IscU-IscS complex and an oxygen-labile [2Fe-2S] loaded form of IscU. However, no significant accumulation of an [4Fe-4S] cluster-loaded IscU species was observed (239). The reason for this might be that the [4Fe-4S] cluster-loaded IscU species is too labile for purification or very short-lived *in vivo*. The other possible reason is that the assembly of [4Fe-4S]

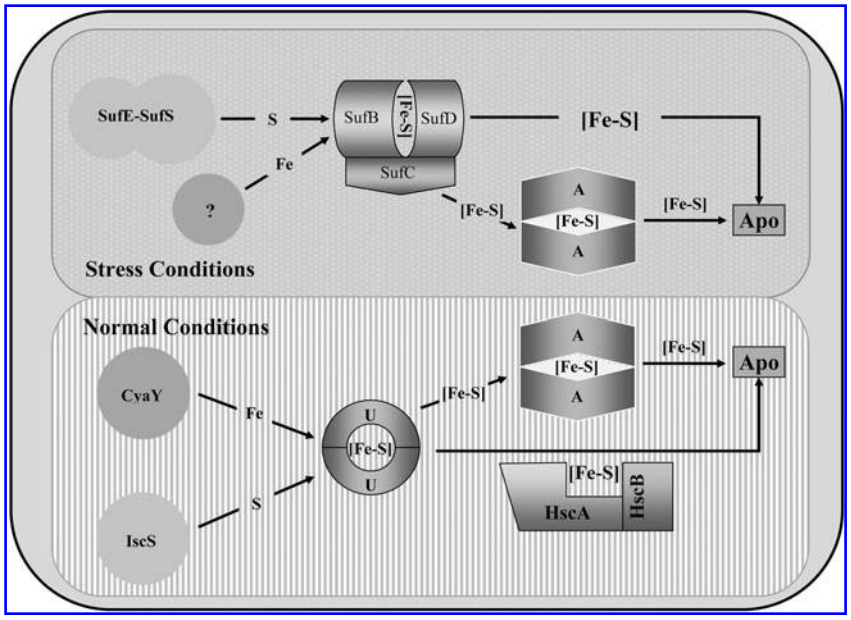

FIG. 4. [Fe-S] biogenesis in *E. coli*. The *upper panel* describes a working model of the SUF system under stress conditions. The SufBCD complex acts as a scaffold complex receiving sulfur from SufS-SufE and iron from a still unknown donor followed by assembly of [Fe-S] ultimately leading to cluster transfer to apo-protein (Apo) directly or just stored on an A-type protein (SufA). The *lower panel* shows a possible working model of the ISC system under normal growth conditions. The IscU dimer (U) acts as a scaffold complex. IscS provides sulfur and CyaY provides iron. Assembled [Fe-S] is transferred to apo-protein (Apo) with the help of the HscA-HscB complex or just stored on an A-type protein (IscA). The stored [Fe-S] on A-type proteins is then transferred to apo-proteins (Apo). SufBCD, SufB-SufC-SufD.

occurs at a slow rate as demonstrated *in vitro* where the process took several hours (53, 304). Reconstruction of *A. vinelandii* IscU revealed sequential formation of [Fe-S] on IscU: one [2Fe-2S] per dimer, two [2Fe-2S] per dimer, and one [4Fe-4S] per dimer (6, 53).

Once [Fe-S] are produced on IscU proteins two additional factors, HscA and HscB (also named Hsc66 and Hsc20) are needed to assist cluster transfer to apo-proteins (Fig. 4). HscA is an Hsp70 chaperone and HscB a J-protein cochaperone. The involvement of HscA and HscB in iron–sulfur protein maturation was first suggested based on the conserved linkage of *hscA* and *hscB* genes with *isc* genes in bacteria. ATPase assays of HscA, together with surface plasmon resonance and isothermal titration calorimetry studies, revealed the interactions between IscU and HscA or HscB (116, 278). It seems that HscA and HscB use IscU as a native substrate probably regulating [Fe-S] transfer from IscU to apo-proteins (278). Maturation of biotin synthase (BioB) confirmed that HscA can bind BioB forming the BioB-HscA complex, which further binds the [Fe-S] scaffold protein IscU in a noncompetitive manner generating a three protein complex, facilitating the transfer of [Fe-S] from IscU to BioB (241). HscB binds to and stabilizes the ordered state of IscU (135), which assists the delivery of IscU to HscA and stabilizes the chaperone and scaffold complex (311). Detailed genetic studies discovered a Leu-Pro-Pro-Val-Lys (LPPVK) motif in IscU, which is critical for HscA and IscU interaction (63, 115–117, 291) and NMR spectroscopy and genetic method also revealed that a conserved patch in HscB is the principal binding site for IscU (98, 135).

ATP also plays a critical role in the process of [Fe-S] transfer from IscU to apo-protein. CD spectra indicate that the rate of cluster transfer was stimulated more than 20-fold in the presence stoichiometric HscA and HscB and excess MgATP. No stimulation was observed in the absence of either HscB or MgATP. The results demonstrate that [2Fe-2S] cluster transfer from IscU is an ATP-dependent process (52). A structural change of holo-IscU occurs during HscA-ATP (T-state) transition to HscA-ADP (R-state) accompanying ATP hydrolysis (36). The HscA/HscB chaperone/cochaperone system is specific for the ISC system.

Electrons are needed for the reduction of element sulfur (S^0) released from cysteine to sulfide (S^{2-}). As Fdx are iron–sulfur proteins that mediate electron transfer in a range of metabolic reactions, Fdx is thought to provide electrons for the ISC system for [Fe-S] biogenesis in *E. coli* and *A. vinelandii* (25, 187). Fdx accepts electrons from Fdx reductase (FDXR) whose electrons are obtained from NADH or NADPH. On the basis of the discussion above, ISC-mediated [Fe-S] biogenesis in bacteria can be observed schematically as seen in Figure 4.

C. *SUF system (*E. coli *and* Synechocystis *sp. PCC 6803)*

In *A. vinelandii*, mutations altering several components of the ISC system are lethal, suggesting that the ISC system is essential for *Azotobacter*. In contrast, *E. coli* strains lacking the entire ISC system still remain viable indicating the existence of additional accessory functions. The SUF system was subsequently discovered in *E. coli* (289).

The SUF system in *E. coli* consists of six genes organized in the operon *sufABCDSE* (Table 1; Fig. 5). Before its role as a [Fe-S] biogenesis machinery was determined, *sufD* and *sufS* were discovered to be related and that together with the FhuF protein (containing [2Fe-2S] cluster) are able to utilize ferrioxamine B as an iron source. Further, it was discovered that their expression is regulated by the iron-dependent Fur repressor with increased expression under iron-deficient conditions (221). Genetic studies revealed that the *suf* operon is also activated by OxyR regulatory protein, which is an H_2O_2-sensing transcriptional regulator (349). Studies on the homologous *suf* operon in the plant pathogen, *Erwinia chrysanthemi*, also showed that *suf* gene expression can be induced under iron-deficient growth conditions (200). The function of the *suf* operon in [Fe-S] formation was clarified in *E. coli* by analysis of varied combinations of both ISC and SUF mutants (289). Inactivation of either the *isc* or *suf* operons in isolation does not lead to lethality in *E. coli*: both systems need to be

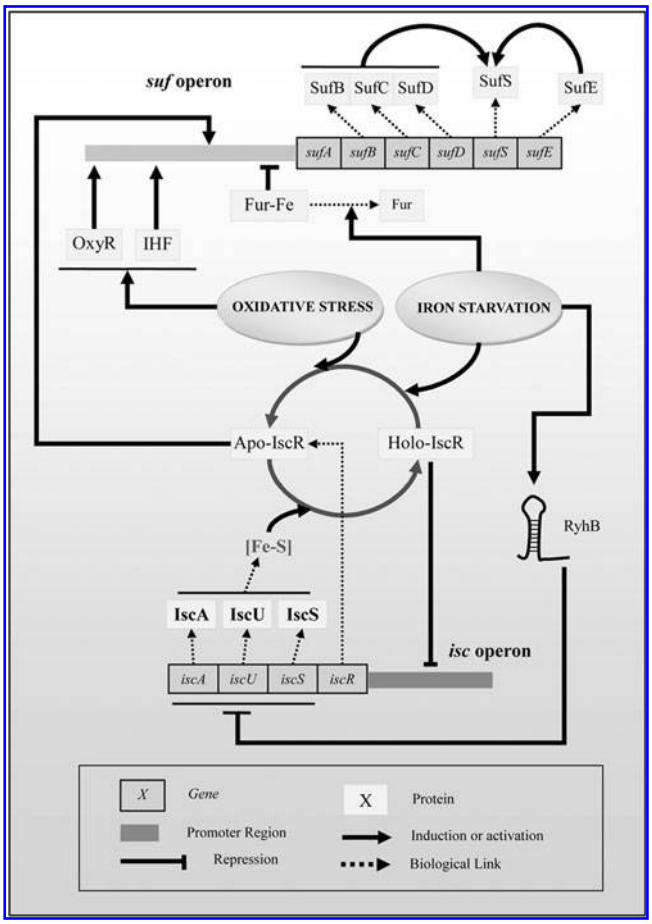

FIG. 5. The regulation of [Fe-S] biogenesis in *E. coli*. (i) Holo-IscR (activated IscR by [2Fe-2S]) depresses *isc* operon; (ii) small RNA RhyB is expressed under iron starvation and downregulates the *isc* operon; (iii) oxidative stress and iron starvation damages [Fe-S] of IscR leading to inactivation, which releases its depression on the *isc* operon, and apo-IscR activates the *suf* operon; (iv) oxidative stress activates the *suf* operon through OxyR and IHF; (v) iron starvation upregulates the *suf* operon through Fur inactivation by iron depletion, as holo-Fur depresses *suf* operon. (vi) In the SUF system itself, SufBCD or SufE activate SufS and upregulates the SUF pathway. The concerted performance of these actions determines which system (SUF or ISC) dominates [Fe-S] production.

inactivated to show a lethal phenotype. Because *suf* mutations did not cause severe defects in [Fe-S] enzyme activities, the SUF system was regarded as a minor contributor to [Fe-S] biogenesis. However, subsequent work established the SUF system as a vital [Fe-S] biogenesis machinery under stress conditions such as oxidative stress and iron starvation (18, 25, 88). On the basis of this it is also reasonable to assume that the SUF-like system in oxygen-producing plant chloroplasts forms the basis of the [Fe-S] biogenesis machinery (23, 229, 334), which was probably inherited from the cyanobacterial ancestor.

SufA exhibits significant identity with IscA and $IscA^{nif}$ (Fig. 2) and their function has been discussed above.

The scaffold issue of the SUF system in bacteria has recently been resolved. Fine systematic work has established the SufBCD complex as an [Fe-S] scaffold (49, 149, 216, 217) (Fig. 4). It was discovered earlier that SufBCD acts as a complex (174, 217). In addition to the SufBCD complex, SufBC and SufCD complexes were also discovered, which can exist in 2B-2C or 2C-2D forms (139, 226, 317). SufC contains a typical ATPase motif and was confirmed to have ATPase activity (139, 201, 238) and SufB can accelerate ATP cleavage on SufC (78, 238). Sequence analysis showed that SufB contains a putative [Fe-S] binding motif C-X2-C-X3-C and although it is not strictly conserved (18) it suggests that SufB may provide sites for [Fe-S]. It was discovered that SufE, which has received sulfur from SufS, in turn interacts with the SufB protein for sulfur transfer to SufB (149) and that this interaction only occurs if SufC is present. In these studies it as also confirmed that SufB can act as a site for [Fe-S] assembly (149). Using a combination of protein–protein interaction and *in vitro* [Fe-S] assembly assays, the relative roles of the SufBCD complex and the SufA protein during [Fe-S] biosynthesis were characterized. These studies revealed that SufA interacts with SufBCD to accept [Fe-S] formed *de novo* on the SufBCD complex (49) (Fig. 4). SufBCD is therefore a novel [Fe-S] scaffold system that assembles nascent clusters.

SufS is a group II cysteine desulfurase that is different from NifS or IscS, and its activity can be dramatically stimulated in the presence of SufE (174, 217). The reason for this is that SufE can interact with SufS (174) and receive sulfur from SufS (209). SufSE was therefore regarded as two-component cysteine desulfurase or cysteine desulfurase complex. The SufBCD complex was also found to interact with SufSE and to facilitate sulfur liberation from cysteine (217), and it is now known that the mobilized sulfur is used to assemble [Fe-S] on the SufBCD complex (49). A schematic model of the bacterial SUF system is outlined in Figure 4.

As chloroplasts have originated from endosymbiotic cyanobacteria, the SUF system in *Synechocystis* sp. PCC 6803 is also reviewed here. *Synechocystis* sp. PCC6803 contains all the SUF-like genes *sufA*, *sufB*, *sufC*, *sufD*, *sufS*, and *sufE* but only sufBCDS make up an operon *sufRBCSD* (273, 320). Although *Synechocystis* sp. PCC6803 contains an *IscA* (*slr1565*), two *IscS* like genes (*slr0387* and *sll0704*) and *HscA* (*sll0170*), no IscU and HscB homologs have been identified. Knockout experiments discovered that *SufB*, *SufC*, *SufD*, *SufS*, and *SufE* are essential genes, whereas no ISC components are vital for *Synechocystis* (20). On the basis of this it appears that the SUF proteins make up the dominating [Fe-S] biogenesis system in *Synechocystis* sp. PCC6803.

In some bacteria such as *T. maritima* the *suf* operon contains no *sufE* but *sufU*. SufU bears considerable primary sequence conservation, including three conserved cysteine residues (130) and SufU is structurally similar to IscU (242). SufU contains an 18–21 amino acids insertion located between the second and third conserved cysteines when compared with IscU and SufU lacks the signature motif LPPVK found in IscU that is required for HscA binding to IscU (130). Genome comparisons show that *sufU* is not genetically linked with *hscA* or *hscB* homologs, so SufU may function independently of the HscA/HscB chaperones. In fact, apo-SufU is an activator of the cysteine desulfurase SufS by enhancing its activity ~40-fold *in vitro* (9) similar to the SufE protein (174). Structural analysis showed that despite the lack of sequence homology the core of SufE is strongly similar to IscU (105). Combined, the data indicate that SufU functionally replaces SufE.

Although there is no NIF-like system in *E. coli* or *Synechocystis* sp. PCC6803, NfuA in *E. coli* (17) and ss12667 in *Synechocystis* sp. PCC6803 are homologous with the NifU C-terminal (named Nfu) from *A. vinelandii* (20). They might represent the remnant of Nfu (Fig. 3). Indeed, cyanobacterial Nfu can perform cluster transfer to apoferredoxin and act as a scaffolding protein for the insertion of [4Fe-4S] clusters into PsaC of photosystem I (PSI) (128, 206). Besides unicellular cyanobacterial species such as *Synechocystis*, there are also colonial cyanobacteria such as heterocyst-forming species *Nostoc punctiforme*, specialized for NIF, which contains full-length homologous NifU and NifS sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi), indicating a functional NIF system in these microorganisms.

D. Evolution of [Fe-S] biogenesis systems

It can be logically deduced that the anaerobic NIF was the primary [Fe-S] biogenesis system if the atmosphere of the early earth was indeed anoxic. There is, however, some difficulty in determining which system was the second system to emerge the following facts favor ISC rather than SUF. (i) Even though ISC can tolerate oxygen it is in fact an anaerobic system, whereas SUF is an aerobic system. (ii) On the basis of sequence characteristics and similarity, ISC has a closer relationship with NIF than SUF: IscS and NifS belong to group I cysteine desulfurases, but SufS is a group II cysteine desulfurase; IscU is homologous to N-terminal region of NifU, but SUF system does not contain NifU homolog (Fig. 2). However, if this second system indeed evolved from cyanobacteria, it must be oxygen tolerant, which would then favor the SUF system.

Eukaryotic systems are derived from bacterial counterparts (Fig. 3). As discussed above, [Fe-S] biogenesis in *Synechocystis* is dominated by the SUF system and it is expected that the endosymbiotic organelle from cyanobacteria, the chloroplast in plants and the plastid in *Plasmodium*, accommodate a functional SUF system, which has been inherited from cyanobacteria (Fig. 3). Similarly eukaryotic mitochondria, an anaerobic organelle when respiration occurs, has probably inherited a bacterial functional ISC system (Fig. 3). At present, it is difficult to locate the CIA system in this evolutionary context.

E. Communication between different systems

1. NIF and ISC in *A. vinelandii*. It is known that deletions of *nifU* and *nifS* does not deprive nitrogenase activity completely in *A. vinelandii* under nitrogen fixing conditions (125, 126) and that the ISC system can partially replace the NIF system in providing [Fe-S] (345). Genetic analysis from other organisms also provided evidence for crosstalk between the NIF-like system and the ISC or SUF systems (10, 213, 295). In these instances, these organisms are nonnitrogen-fixing, but they contain NifS- and NifU-like proteins that are required for the general maturation of iron–sulfur proteins. So, it is not surprising that the *nifUS*-like operon from *Helicobacter pylori* can functionally replace *isc* or *suf* operons in *E. coli* (295).

In *A. vinelandii*, the NIF and ISC systems are similar in that they include an enzyme for the mobilization of sulfur (NifS or IscS), an assembly scaffold (NifU or IscU) and an A-type protein (IscA and $IscA^{Nif}$). NifU specifically supplies [Fe-S] for maturation of nitrogenase, whereas IscU specifically provides [Fe-S] for nonnitrogenase enzymes such as aconitases. However, change on this kind of target specificity is expected under certain conditions. For example, if cells are cultured under low-oxygen-availability conditions, NifU of *A. vinelandii* expressed at normal levels can partially replace the function of IscU by providing [Fe-S] for aconitase (73). Further, if expression of NifU is elevated under normal conditions, NifU can replace IscU for the maturation of nonnitrogenase enzymes (73). The capacity of elevated NifU level to functionally replace IscU does not require NifS, indicating that the S^{2-} normally supplied to NifU by NifS can be replaced in some way by IscS. Similarly, elevated expression of ISC components, as a result of deletion of the negative regulatory *iscR* gene, can improve the capacity for nitrogen-fixing growth of strains deficient in either NifU or NifS (73). Together, these results indicate that bidirectional functional replacement can occur between NifU and IscU, notably when the expression level of the remaining scaffold is increased. Further, IscS can also partially replace the function of NifS but there is no experimental evidence that NifS can replace the function of IscS in *A. vinelandii* (73), indicating that they are two distinct systems even if there is crosstalk and overlapping function between them.

2. ISC and SUF in *E. coli*. The crosstalk observed between ISC and SUF contributed to the identification of the SUF system in *E. coli*. The YT1014 *E. coli* mutant, in which the entire *isc* operon has been deleted, grows very poorly and exhibits a marked decrease in the activity of iron–sulfur proteins (297); however, overexpression of the *suf* operon can restore normal growth and activity of iron–sulfur proteins (289). Disruption of the *suf* alone does not cause any major defects under normal growth conditions, but all of the *suf* genes, except *sufA*, are essential for viability in the mutant background of the *isc* operon. In effect this means that synthetic lethality will be observed when both the *isc* and *suf* operons are inactivated in *E. coli* (215, 289, 295). This suggests that bidirectional functional replacement of SUF and ISC is also possible. *E. coli* retains both the *suf* and *isc* operons, whereas other species possess only one of the two. This provides additional evidence for the redundant role of the SUF and ISC systems that operate in separate, parallel pathways.

F. Regulation of [Fe-S] biogenesis

1. Regulation of ISC and SUF in *E. coli*. As [Fe-S] are sensitive to oxygen and iron limitation, environmental stresses related with these two factors will certainly affect the actions of [Fe-S] biogenesis systems (Fig. 5). Many experiments have confirmed that SUF is comparatively more stress resistant than ISC (152, 215, 338) (Fig. 5) with the ISC system working as a house-keeping machinery.

In *E. coli*, *isc* operon *iscRSUA* expression is controlled by one of its products IscR (Fig. 5). IscR has two forms, holo- and apo-IscR. Holo-IscR contains an [2Fe-2S] cluster, which can bind to the promoter of the *isc* operon inhibiting its expression (265). When [Fe-S] is impaired or lost due to its sensitivity to oxygen, or iron depletion, IscR converts to the apo form and is then released from the *isc* promoter; this results in the activation of the *isc* operon. In addition to regulating *isc* operon activity, apo-IscR can act as an activator of the *suf* operon by directly binding to its promoter region under oxidative con-

ditions (102, 338). Apo-IscR contributes almost equally to OxyR to activate the *suf* operon in response to oxidants (338).

The *suf* operon (*sufABCDSE*) is also controlled by the transcription factors OxyR, IHF, Fur, and apo-IscR (152, 215) (Fig. 5), which was also confirmed in *E. chrysanthemi* (243). OxyR and IHF bind to different regions of the *suf* promoter and activate its expression under conditions of oxidative stress. The iron-rich form of Fur (Fur–Fe) normally binds to the *suf* promoter and represses its expression, whereas the iron-limiting form of Fur loses its binding ability; this results in the activation of the *suf* operon. SufE and SufBCD might also play a role in regulating [Fe-S] assembly, owing to their ability to promote SufS activity. IscR can regulate both the ISC and SUF systems (102, 338).

It is clear that IscR plays a central role in regulating the function of ISC and SUF, and also coordinating the consumption of iron and cysteine between these two systems (Fig. 5). Under normal growth conditions, IscR continuously oscillates between the holo and apo forms because of the sensitivity of [Fe-S] to oxygen and the feedback control of IscR on [Fe-S] synthesis. However, it is unlikely that SUF is fully activated by IscR since it would also require OxyR- and IHF-mediated activation and Fur derepression. It is, therefore, reasonable to assume that under normal conditions [Fe-S] is essentially generated by ISC. On the other hand, when bacteria are grown under oxidative-stress and iron-starvation conditions, which are detrimental to [Fe-S], IscR occurs mainly in its apo form (Fig. 5). Even though ISC is active, transcription factors apo-IscR, OxyR, and IHF activation and Fur derepression mediate SUF activation in concert. Further, SufE- and SufBCD-mediated enhancement of SUF activity establishes the central role of SUF for [Fe-S] biogenesis under these conditions (Fig. 5).

Recently, it was found that small RNAs also play an important role in regulating the ISC system (66). An iron-responsive sRNA, *RyhB*, is expressed under iron starvation some of which binds to the second cistron (*iscS*) of the polycistronic mRNA *iscRSUA* (Fig. 5). This promotes the cleavage of the downstream *iscSUA* transcript and downregulates the ISC system. This cleavage gives rise to the remaining 5′-section of the transcript encoding IscR. The stability of the *iscR* transcript depends on a 111-nucleotide long nontranslated RNA section located between *iscR* and *iscS*, which forms a strong repetitive extragenic palindromic secondary structure and may protect against ribonucleases degradation.

2. Regulation of SUF in *Synechocystis* sp. PCC 6803.

The *suf* operon *sufBCDS* in *Synechocystis* sp. PCC 6803 is composed of four genes and is highly conserved in the genomes of cyanobacteria (320). The open reading frame of SufR is located near the 5′ end of the *suf* operon but is transcribed in the opposite direction. SufR has two significant features: (i) a DNA-binding domain near the N terminus with high sequence similarity to transcription regulatory proteins and (ii) four highly conserved cysteine residues C-X_{12}-C-X_{13}-C-X_{14}-C near the C terminus representing a metal-binding site. In agreement with this, the expressed SufR harbored a [4Fe-4S]. Expression levels of the *sufBCDS* is elevated when cells are grown under conditions of oxidative and iron stress and are even higher in a null mutant where SufR was inactivated. SufR seems therefore to function as a transcriptional repressor of the *suf* regulon in cyanobacteria (320).

Consistent with its role as a transcriptional regulator, the intergenic region between *sufR* and the *sufBCDS* operon was found to contain two SufR binding sites with different affinities for SufR. The ability of SufR to regulate transcription depends on the presence or absence of the [Fe-S], which by definition must be sensitive to oxygen or otherwise labile. That DNA bending may be involved in SufR regulation is hinted at by the presence of two binding sites with inverted repeats, by the large distance (26 nucleotides) between these sites, and by the finding that SufR can form tetramers. Each of the two sites contains a perfect inverted repeat, CAAC-N6-GTTG, and are highly conserved in cyanobacteria. The high affinity binding site controls *sufBCDS* expression and the low affinity binding site controls sufR expression. The binding affinity depended on the presence and redox state of the [4Fe-4S]. It is possible that binding of SufR at the two sites leads to the bending of the DNA region in the vicinity of the *sufBCDS* and *sufR* promoters, which results in coordination of the regulation of the *sufBCDS* operon and *sufR* (269, 273, 320).

III. [Fe-S] Biogenesis in Yeast (*S. cerevisiae*)

The ISC system in *S. cerevisiae* mitochondria was among the first fully annotated eukaryotic systems responsible for [Fe-S] biogenesis and the CIA system was first reported in the *S. cerevisiae* cytosol followed by reports in *Arabidopsis*. *S. cerevisiae* is surely an ideal model for [Fe-S] biogenesis but because multiple comprehensive reviews have been published on [Fe-S] biogenesis in yeast we will only summarize our knowledge to date (162–167).

A. Mitochondrial system: ISC

The well-established ISC system in yeast includes 16 components (Table 1). Core components are inherited from bacteria and the evolutionary process has recruited more auxiliary proteins into this system. The basic mechanism of [Fe-S] biogenesis in yeast mitochondria involves two major steps. The first step involves the synthesis of a transiently bound [Fe-S] on the scaffold proteins Isu1 and Isu2. The second step involves the release of the [Fe-S] from scaffolds Isu1-Isu2 and its transfer and incorporation into recipient apoproteins.

The synthesis of a transiently bound cluster is supported by the so-called early components of the ISC machinery. These proteins include the cysteine desulfurase complex Nfs1-Isd11 (3, 321), which serves as the sulfur donor for scaffold proteins such as Isu1-Isu2. Nfs1 and Isd11 form a tight complex that is ~200 kDa in size (3, 321). It was thought that Nfs1 alone is sufficient to mobilize sulfur from cysteine because the recombinant protein releases sulfide *in vitro* under reducing conditions (194). However, it is accepted now that this reaction does not reflect the physiological situation *in vivo*, as no free sulfide is likely produced in this pathway. Recent research has documented that no [Fe-S] can be assembled *in vivo* on the scaffold Isu1-Isu2 without functional Isd11 (321). The protein is found in virtually all eukaryotes from yeast to human. However, no bacterial homologs of Isd11 have been identified to date, making it likely that Isd11 is a eukaryotic addition to the bacterium-derived ISC assembly machinery (167). The mode of action of Isd11 is similar to SufE and SufS, but no research has shown that Isd11 can promote the activity of Nfs1.

The iron-binding protein Yfh1 (frataxin) plays an essential role in yeast [Fe-S] biogenesis (75, 90, 99, 101, 154, 159, 198, 235, 268, 315, 319), where it acts as a putative iron donor. Yfh1 interacts with Isu1-Nfs1 *in vivo* in an iron-stimulated manner, and its depletion is associated with a specific defect in the *de novo* [Fe-S] assembly on Isu1 (101, 195). Similarly, *in vitro* studies have shown that Yfh1 is able to bind several atoms of iron with low affinity and stimulate [Fe-S] assembly on Isu1 (39, 60, 61, 339). Transient [Fe-S] assembly on Isu1-Isu2 also depends on the electron transfer chain consisting of the FDXR Arh1 and the [2Fe-2S] Fdx Yah1, which likely receives its electrons from NADH for reduction of the sulfane sulfur (S^o) to sulfide (S^{2-}) (13, 26, 197).

Research work in yeast provided the first *in vivo* evidence supporting U-type proteins (NifU, SufU, and IscU) as scaffolds for [Fe-S] assembly. By using radiolabeled ^{55}Fe in yeast cells, it was found that Fe was bound to Isu1p *in vivo*. This was further confirmed by Fe accumulation on Isu1p when downstream processes were affected. The binding of iron to Isu1p is strictly depended on the function of the sulfur donor Nfs1p. Therefore, it was concluded that iron is bound to Isu1p in the form of a nonchelatable [Fe-S] (195).

The release of the [Fe-S] from scaffolds Isu1-Isu2 and its transfer and incorporation into recipient apoproteins are facilitated by late components of the ISC assembly machinery. [Fe-S] release from Isu1-Isu2 is achieved by interaction of the Hsp70 chaperone Ssq1 (HscA in bacteria) with Isu1-Isu2. The DnaJ-like cochaperone Jac1 (HscB in bacteria) and the nucleotide exchange factor Mge1 assist the ATP-dependent function of Ssq1 (16, 76, 77, 137, 176, 285, 311, 315). Ssq1 interacts specifically with the highly conserved domain of Isu1 (LPPVK), which is thought to mobilize the [Fe-S] from Isu1, facilitating its transfer to apoproteins. Jac1 stimulates the ATPase function of Ssq1 and the nucleotide exchange factor Mge1 serves to exchange bound ADP for ATP to start a new cycle binding of Ssq1. Jac1 also binds to Isu1 and mutational studies suggest that the interaction might be beneficial for targeting Ssq1 to the Isu1 scaffold under conditions that have a high demand for iron–sulfur protein biogenesis. The role of Nfu1 is unknown.

A monothiol glutaredoxin 5 (Grx5) also plays an important function in [Fe-S] assembly (113). Grxs were first defined as glutathione (GSH)-dependent thiol-disulfide oxidoreductases (*redoxins*), which utilize the reducing power of GSH to maintain and regulate the cellular redox state and redox-dependent signaling pathways (168). Their functions include electron donor, reversible glutathionylation, iron homeostasis, and [Fe-S] assembly (168). Grxs are encoded by multigene families in those organisms possessing GSH and Grxs (249). Depending on the active site structure, these homologous proteins can be roughly classified into two groups: class I-dithiol Grxs containing the characteristic active site motif Cys-Pro-Tyr-Cys/Ser (CXXC/S) and class II-monothiol Grxs containing the Cys-Gly-Phe-Ser (CGFS) motif (168). Class II can be further divided into several subdivisions based on the active site sequence and conserved motifs involved in GSH binding (249). Among all these Grxs, only the monothiol class is involved in [Fe-S] biogenesis. It was first discovered in yeast that the mutants lacking mitochondrial monothiol Grx5 are highly sensitive to oxidative and osmotic stresses. Knock-out of Grx5 led to iron accumulation and inactivation of [Fe-S] -containing enzymes (244). The function of Grx5 in [Fe-S] biogenesis or repair was therefore suggested. It was later discovered that depletion of Grx5 from yeast cells resulted in an increase in the amount of iron loaded scaffold Isu1 implying that Grx5 is required in a step after [Fe-S] synthesis on Isu1 before the preassembled clusters are finally inserted into apo-proteins (195). The role of monothiol Grxs in [Fe-S] assembly is conserved throughout evolution (168); however, the exact biochemical function of monothiol Grxs in the synthesis of iron–sulfur proteins remains to be established.

B. Cytosolic system: CIA

The CIA system was first discovered in yeast and five components have been identified so far: Cfd1, Nbp35, Nar1, Cia1, and Dre2 (162–167). In yeast, the two P-loop NTPases Cfd1 and Nbp35 exhibit sequence similarity to each other (110, 111, 252) and they form a stable heterotetrameric complex and associate [4Fe-4S] at their C-termini (205), suggested to be scaffold proteins for cluster assembly. The scaffold activity *in vivo* depends on the function of the mitochondrial ISC components Nfs1 (homolog of IscS in *E. coli*) and Atm1, an ATP binding cassette (ABC) transporter. The *in vivo* cluster transfer reaction depends on the other two CIA components Nar1 and Cia1 (21, 22). Nar1 is conserved in virtually all eukaryotes and exhibits striking sequence similarity to bacterial iron-only hydrogenases. It contains two [Fe-S] of unknown type. Nar1 represents a crucial component of the CIA machinery and coimmunoprecipitation assays demonstrated a specific interaction between Cia1 and Nar1. In contrast to the mostly cytosolic Nar1, Cia1 is preferentially localized to the nucleus. Since WD40 proteins are known to act as protein interaction platforms, Cia1 may mediate the contact between different CIA proteins or between CIA and target iron–sulfur proteins to facilitate the transfer and incorporation of the [Fe-S] (23).

The recently discovered iron–sulfur protein Dre2 is also an essential conserved iron–sulfur protein implicated in extramitochondrial [Fe-S] assembly, similar to other components of the CIA pathway. It is partially localized to the mitochondrial intermembrane space and the cytosol. The real function of Dre2 is not known (343).

Even though there are CIA homologs in humans (Table 1), their function has not yet been confirmed. With the discoveries of functional cytosolic ISCS, ISCU, ISD11, NFU1, and Frataxin, it is obvious that at least an ISC-like system is responsible for the *de novo* [Fe-S] biogenesis in the mammalian cytosol (246, 247, 337).

C. Communication between ISC and CIA: mitochondrial export machinery

[Fe-S] assembly in the cytosol has been confirmed to be mitochondria dependent (166, 167). A still unknown compound (X) from the mitochondria is proposed to be exported to the cytosol by the export machinery. The central component of this export machinery is the ABC transporter Atm1 of the mitochondrial inner membrane (138). Depletion of Atm1 leads to a severe impairment of iron–sulfur protein maturation in the cytosol and nucleus, but mitochondrial iron–sulfur proteins are unaffected clearly indicating a relationship of Atm1 with the cytosolic and nuclear iron–sulfur proteins. ATPase hydrolysis by purified Atm1 reconstituted into proteoliposomes was specifically increased 3–5-fold by thiol-

containing compounds, in particular by micromolar concentrations of cysteine thiol groups in peptides. Because the ATPase of ABC transporters is typically stimulated by their physiological substrates, this may indicate that the substrate transported by Atm1 contains a sulfhydryl group in a peptidic environment (143).

The export reaction is further facilitated by Erv1 of the mitochondrial intermembrane space (147). The Erv1 protein contains a conserved C-terminal domain that exhibits sulfhydryl oxidase activity, that is, the oxygen-dependent formation of disulfide bonds in target proteins (151). The function of Erv1 needs flavin adenine dinucleotide. However, Erv1 has also been related to the mitochondrial import of intermembrane proteins (34), which lack classical mitochondrial targeting sequences. This reaction is catalyzed by two essential components, Mia40 and Erv1. Mia40 is a protein in the intermembrane space that directly binds newly imported proteins *via* disulfide bonds. Mia40 then becomes reduced and needs to be reoxidized to regain its activity. Oxidation of Mia40 is carried out by Erv1 by directly interacting with Mia40 and shuttling electrons from reduced Mia40 to oxidized cytochrome c (11, 81, 112, 277, 293). The oxidative activity of Erv1 strongly depends on the oxygen concentration in mitochondria. Thus, Erv1 appears to perform multiple functions, and combined Erv1 and Mia40 might be responsible for bidirectional transport across mitochondrial membrane.

A third component of the ISC export machinery is the tripeptide GSH (279). GSH is the most abundant low-molecular-weight nonprotein thiol in eukaryotic cells (222). It is involved in a variety of cellular functions: protection against oxidative damage, the maintenance of mitochondrial structure and membrane integrity, and cell differentiation and development (231). Due to its low redox potential and its high cellular concentration, GSH is a major redox buffer in thiol-based redox systems (308). The reduced sulfhydryl group in GSH, when oxidized, produces a disulfide, or oxidized GSH, and functions as a source of reducing equivalents to reduce cellular disulfide bonds, often in conjunction with Grxs (86). The real function of GSH in [Fe-S] biogenesis is unclear.

IV. [Fe-S] Biogenesis in Plants

Plant iron–sulfur proteins were among the first iron–sulfur proteins to be identified. Post-translational assembly of photosynthetic iron–sulfur proteins were analyzed even before [Fe-S] biosynthetic machineries were discovered (185). It is now clear that [Fe-S] biogenesis in plants is a complicated process, not only because [Fe-S] biogenesis is spatially separated in different cellular compartments and that the machineries contain more components than in bacteria, but the regulation and crosstalk also appear more complex.

According to the endosymbiotic theory, mitochondria arose from proteobacteria (in particular, Rickettsiales or close relatives) and chloroplasts from cyanobacteria, and these two organelles probably inherited [Fe-S] biogenesis systems from their ancestors (Fig. 3). Indeed, genetic and molecular experimental evidence has confirmed that both organelles contain their own [Fe-S] assembly machinery (23, 133, 229, 329, 336). Chloroplasts contain a complete SUF system, similar to bacterial SUF, whereas mitochondria accommodate a complete ISC-like system (Table 1). On the other hand, as [Fe-S] cofactors are also needed in the cytosol and nucleus, it is obvious that [Fe-S] are either assembled by a cytosolic system or provided by the systems residing in chloroplasts or mitochondria. With regard to the latter, transport systems across the double organelle membranes are required. Work on yeast and other model organisms has confirmed that both possibilities exist for eukaryotic organisms (162, 165–167). In this section, we will discuss [Fe-S] biogenesis systems in photosynthetic eukaryotes focusing on the chloroplast system (SUF-like), mitochondrial system (ISC-like), cytosolic system (CIA-like), and transporter systems. We will focus on four models: *Chlamydomonas reinhardtii*, *Arabidopsis thaliana*, *Oryza sativa,* and *Populus trichocarpa*, of which whole genomes have been sequenced.

A. SUF-like system in chloroplasts of A. thaliana

1. SufA.

In the *Arabidopsis* genome there are four *sufA/iscA*-like genes (23), among these the product of *At1g10500* (*AtSufA*) was confirmed to be localized to chloroplasts (1, 331) (Table 1), whereas the products of the other three genes are predicted to be mitochondrial. *AtSufA* mRNA was shown to be expressed in all tissues tested, with higher expression levels in green, photosynthetic tissues (1). Although the function of AtSufA is still unclear, the purified protein has [Fe-S]-binding capability and can significantly enhance AtIscS (IscS-like protein in *Arabidopsis*), mediating *in vitro* reconstitution of the [2Fe-2S] in apo-Fdx (1, 331). During this process, AtSufA was shown to acquire a transient [Fe-S], which was then transferred to apo-Fdx.

The *Chlamydomonas* genome contains two SufA/IscA-like proteins (104, 186): one predicted to be imported into chloroplasts, whereas the other appears to be in mitochondria (104). On the basis of sequence similarity and the conserved three cysteine residues, the NCBI database (www.ncbi.nlm.nih.gov), using the bacterial SufA/IscA sequence as an input query, identifies conserved *sufA/iscA*-like genes in *Oryza sativa* and *Populus trichocarpa*. *Oryza sativa* Japonica has six *sufA/iscA*-like genes in its genome (134): the products of two are predicted to be in chloroplasts, one in mitochondria, two in both mitochondria and chloroplasts, and one in both the cytosol and mitochondria (80). *Populus trichocarpa* also hosts six SufA/IscA-like proteins and according to the prediction (80), three are in chloroplasts, one in mitochondria, one to both chloroplasts and mitochondria, and one to chloroplasts or the cytosol.

2. SufBCD complex.

SufBCD form a functional complex as an [Fe-S] scaffold in bacteria (18, 49) and in plants these proteins have been retained in chloroplasts through endosymbiosis. *Arabidopsis* SufB (AtSufB or AtNAP1), SufC (AtSufC or AtNAP7), and SufD (AtSufD or AtNAP6) have been confirmed to be localized in chloroplasts (325, 327) (Table 1). All three proteins are members of the *Arabidopsis* ABC protein superfamily (255). AtSufB/AtNAP1 or AtSufD/AtNAP6 can interact with AtSufC/AtNAP7 as shown by yeast two hybrid (YTH) experiments and bimolecular fluorescence complementation (BiFC) analysis. AtSufB/AtNAP1 or AtSufC/AtNAP7 represent atypical or typical ATPases respectively and AtSufB-AtSufC-AtSufD are predicted to form an ATPase complex. As *atsufB* or *atsufC* can complement *E. coli sufB* or *sufC* knockout mutants this indicates that this ATPase

complex is involved in [Fe-S] biogenesis in *Arabidopsis* (325, 327). ATP and NADPH are essential for [Fe-S] formation as demonstrated on spinach Fdx (287). Although there is no direct evidence demonstrating that they are responsible for [Fe-S] metabolism, it is tempting to predict that *Arabidopsis* chloroplasts depend on the scaffold complex AtSufB-AtSufC-AtSufD to assemble [Fe-S] as knockout of *AtSufC* causes an embryo lethal phenotype (327) (Fig. 6). Sequence alignment of SufB-, SufC-, and SufD-like proteins from *E. coli*, higher plants, and from the malaria parasite *Plasmodium falciparum* demonstrates that SufB and SufC are much more conserved proteins than SufD, which only shows limited conservation. SufB-like proteins contain four conserved cysteine residues, but it is unclear if these residues take part in [Fe-S] assembly because the residual space between them is too large. Possible spatial folding could make these residues sufficiently close, however, to hold [Fe-S]. SufD-like proteins contain only one conserved cysteine residue and the function of this residue remains to be clarified (Fig. 7). The high conservative nature of SufB and SufC is compatible with the fact that in some organisms, such as *Methanococcus jannaschii*, which has a minimal SUF-like system, only contains SufB and SufC. SufD-like proteins seem to play an assisting role and indeed an *Arabidopsis atsufD* T-DNA insertion mutant demonstrates that AtSufD fulfils housekeeping functions during embryogenesis and in adult plants (114). In all plant genomes we have analyzed, *sufD*-like genes are universally conserved (Fig. 7). To our surprise, we did not find any *sufC*-like gene in the C4 crop Sorghum. Generally *sufB-*, *sufC-*, and *sufD*-like genes are only represented by a single copy in genomes. However, in *Arabidopsis* two copies (At4g04770 and At5g44316) of *sufB* is present although no cDNA has been successfully cloned for At5g44316, indicating this to be a pseudogene.

AtSufB/AtNAP1 is a conserved protein that is not exclusive to [Fe-S] biogenesis in *Arabidopsis*. A point mutant (C778T) of *AtSufB/AtNAP1*, *hmc1*, was found to accumulate 7-hydroxymethyl chlorophyll *a* (HMChl), an intermediate molecule of chlorophyll *b* to chlorophyll *a* conversion, and *AtSufB/AtNAP1* was suggested to be related to chlorophyll degradation (202). A *laf6* mutant of *AtSufB/AtNAP1*, which was inactivated by an insertion within the 5′UTR, presented a long hypocotyl phenotype and was suggested to be involved in communication between the nucleus and plastids (193). A homologous gene in *Nicotiana benthamiana*, NbNAP1, however, was proposed to affect chloroplast development (8). Although several lines of evidence suggest multiple roles for AtSufB/AtNAP1, they all involve chloroplasts and suggest that SufB is a versatile protein.

3. SufS-SufE. The *Arabidopsis* genome encodes two cysteine desulfurases: AtSufS/AtNFS2 in chloroplasts and AtIscS/AtNFS1 in mitochondria (144, 155, 230, 307, 334) (Table 1; Fig. 6). Green fluorescent protein (GFP)-tagged proteins, immunoblot analysis, and *in vitro* import proved their localization in plastids and mitochondria. Purified recombinant AtSufS/AtNFS2 can catalyze the desulfuration of cysteine, which is pyridoxal-5′-phosphate dependent, and confirmed its cysteine desulfurase activity. The essential role of AtSufS/AtNFS2 in *Arabidopsis* was studied using constitutive and inducible RNAi approaches (307). Plant lines in which AtSufS/AtNFS2 expression was significantly reduced displayed severely chlorotic cotyledons and died as seedlings. Induced silencing of AtSufS/AtNFS2 exhibited chlorosis, disorganized chloroplast structure, and stunted growth and eventually became necrotic and died before seed set. Photosynthetic electron transport and carbon dioxide assimilation were severely impaired in the silenced plant lines. The silencing of AtSufS/AtNFS2 decreased the abundance of all chloroplastic Fe-S proteins tested, representing all five [Fe-S] types. Mitochondrial iron–sulfur proteins and respiration were not affected, suggesting that mitochondrial and chloroplastic [Fe-S] assembly operate independently. These findings

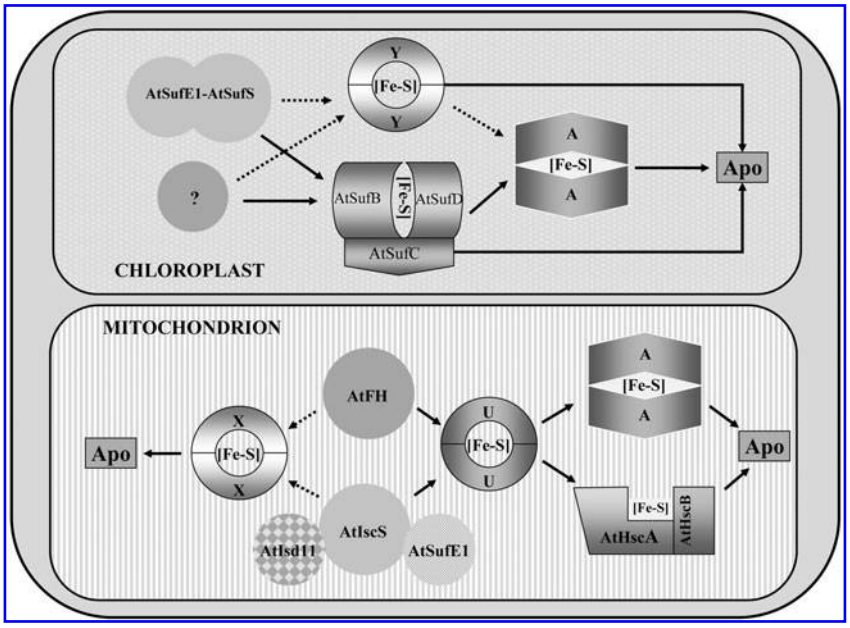

FIG. 6. [Fe-S] biogenesis in chloroplasts (AtSUF) and mitochondria (AtISC) in *Arabidopsis*. (i) In chloroplasts, AtSufB, AtSufC, and AtSufD can form a complex that acts as a scaffold for [Fe-S] biogenesis; In mitochondria, AtIscU1, 2, and 3 (U) act as scaffold proteins. (ii) AtSufS in chloroplast and AtIscS in mitochondria are sulfur donors. (iii) In chloroplasts, AtSufE1, AtSufE2, or AtSufE3 interacts with AtSufS and enhance its activity; AtSufE1 is also localized to mitochondria and enhances the activity of AtIscS, which is probably activated by AtIsd11. (iv) The iron donor for AtISC (mitochondria) is AtFH, a frataxin homolog, but for AtSUF an iron donor remains to be identified (?). (v) AtSufA and AtIscA work as storage proteins for [Fe-S] clusters. (vi) AtHscB-AtHscA are essential for [Fe-S] transfer from scaffolds to apo-proteins (Apo). (vii) Additional proteins X (such as Nfu, Glutaredoxin) in mitochondria or Y (such as HCF101, Glutaredoxin, Nfu, or APO1) in chloroplasts might also function as scaffold proteins. *Dashed arrows* indicate predictions. AtISC, *Arabidopsis* ISC system; AtSUF, *Arabidopsis* SUF system.

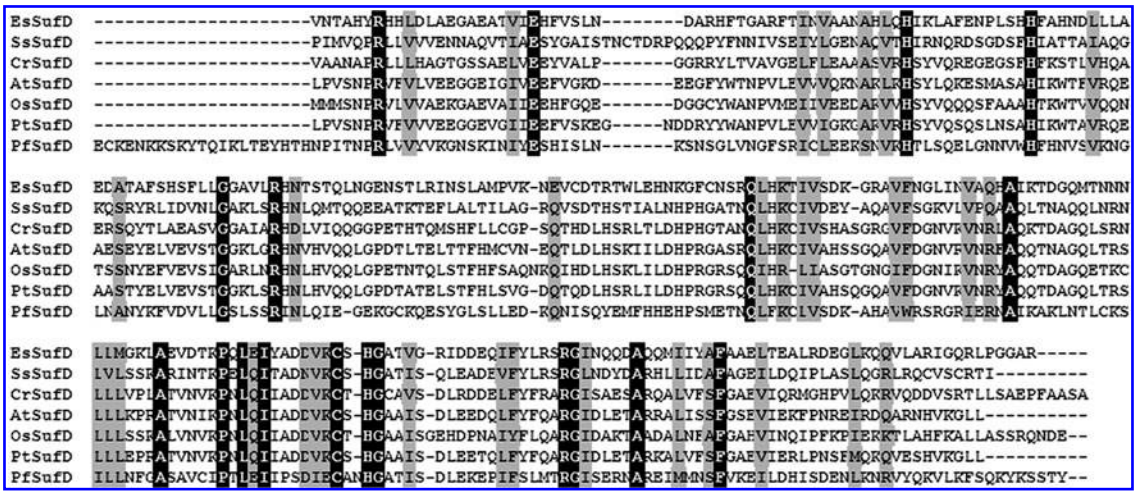

FIG. 7. Alignment of the most conserved regions of SufD-like proteins. Es, *E. coli*; Ss, *Synechocystis* sp. *PCC 6803*; Cr, *Chlamydomonas reinhardtii*; At, *A. thaliana*; Os, *Oryza sativa*; Pt, *Populus trichocarpa*; Pf, *Plasmodium falciparum*. Approximately two-thirds of the *Plasmodium* SufD C-terminal was omitted because this region is outside of the conserved SufD region. On the basis of sequence analysis, SufD-like proteins show the lowest homology among the three components of SufBCD complex. A cysteine residue is conserved in all these organisms, but its function is unclear.

indicate that AtSufS/AtNFS2 is necessary for the maturation of all plastidic Fe-S proteins.

As for SufS in bacteria, the specific activity of recombinant AtSufS/AtNFS2 is dramatically enhanced by the SufE-like proteins AtSufE1–3 (177, 328, 333). The amino acid sequence of AtSufE1 (also named AtSufE or CpSufE) contains an N-terminal SufE-like domain and a C-terminal BolA-like domain unique to higher plants (Fig. 8). AtSufE1 interacts with AtSufS, indicated by YTH, BiFC, gel filtration, native polyacrylamide gel electrophoresis, and affinity chromatography experiments (328, 333). As in bacteria, AtSufS and AtSufE1 form a two-component cysteine desulfurase complex. Detailed experimentation demonstrated initially that AtSufE1 localized to plastids; however, follow-up studies showed dual localization to both plastids and mitochondria (328, 333). Further evidence of dual localization has come from *in vivo* protein–protein interaction experiments in which AtSufE1 and the mitochondrial protein AtIscS/AtNfs1 show a clear interaction in mitochondria (328). AtSufE1 is essential for *Arabidopsis*, as a loss-of-function mutant is embryo-lethal. On the other hand, overexpression of AtSufE1 results in chlorosis and retarded plant development (328). This is compatible with the bacterial result that elevated expression of the *suf* operon in the *isc* deletion mutant YT1014 has an inhibitory effect on growth (289). It cannot be excluded that in bacteria SufE promotes IscS function as AtSufE1 does to AtIscS/AtNfs1.

AtSufE1 harbors of a SufE-like domain and a BolA-like domain, which is also the case in moss (*Physcomitrella patens*), rice (*Oryza sativa*), maize (*Zea mays*), and tree (*Populus trichocarpa*) but does not exist in bacteria (*E. coli*), cyanobacteria (*Synechocystis* sp. PCC6803), or *Chlamydomonas reinhardtii* (Fig. 8). BolA, a morphogene, is a general stress response gene in *E. coli* that induces a round morphology when overexpressed. Increased BolA levels can inhibit cell elongation mechanisms by affecting the filament architecture of MreB, whose polymerization is crucial for the bacterial cell cytoskeleton and essential for the maintenance of a cellular rod shape (96). The reason why the SufE domain and BolA domain have merged forming AtSufE1 is unclear. However, BolA-type proteins are frequently found adjacent to Grxs in many prokaryotic organisms. Grx is another important component for [Fe-S] assembly as will be discussed below and their co-occurrence is strong (62, 120). It is suggested that Grx-BolA interaction might constitute a general requirement for iron status control in organisms in which the two genes are present (248, 249).

In addition to AtSufE1, the *Arabidopsis* genome also encodes two other plastid localized proteins with SufE domains, AtSufE2 and AtSufE3 (177). Purified recombinant AtSufE2 could activate the cysteine desulfurase activity of AtSufS/AtNFS2 40-fold. AtSufE2 expression was flower-specific and high in pollen and was therefore hypothesized to be specifically functional in pollen [Fe-S] biosynthesis. AtSufE3, also a plastid targeted protein, is expressed at low levels in all major plant organs. The mature AtSufE3 contains two domains, one SufE-like and one with similarity to the bacterial quinolinate synthase, NadA (Fig. 9). Indeed AtSufE3 displayed both SufE activity (stimulating AtSufS cysteine desulfurase activity 70-fold) and quinolinate synthase activity. The full-length protein was shown to carry a highly oxygen-sensitive [4Fe-4S] at its NadA domain, which could be reconstituted by its own SufE domain in the presence of AtSufS/AtNFS2, cysteine, and ferrous iron. *Arabidopsis* AtSufE3 loss-of-function mutants are embryo lethal and it appears that AtSufE3 represents an NadA enzyme in *Arabidopsis* involved in a critical step during nicotinamide adenine dinucleotide biosynthesis (177).

It is evident that chloroplasts contain a complete set of SUF-like proteins. However, there are at least three other scaffold proteins for [Fe-S] biogenesis in *Arabidopsis*.

4. Other components. HCF101 (High Chlorophyll Fluorescence 101) may serve as a chloroplast scaffold protein that specifically assembles [4Fe-4S] and transfers them to the chloroplast membrane and soluble target proteins (158, 266).

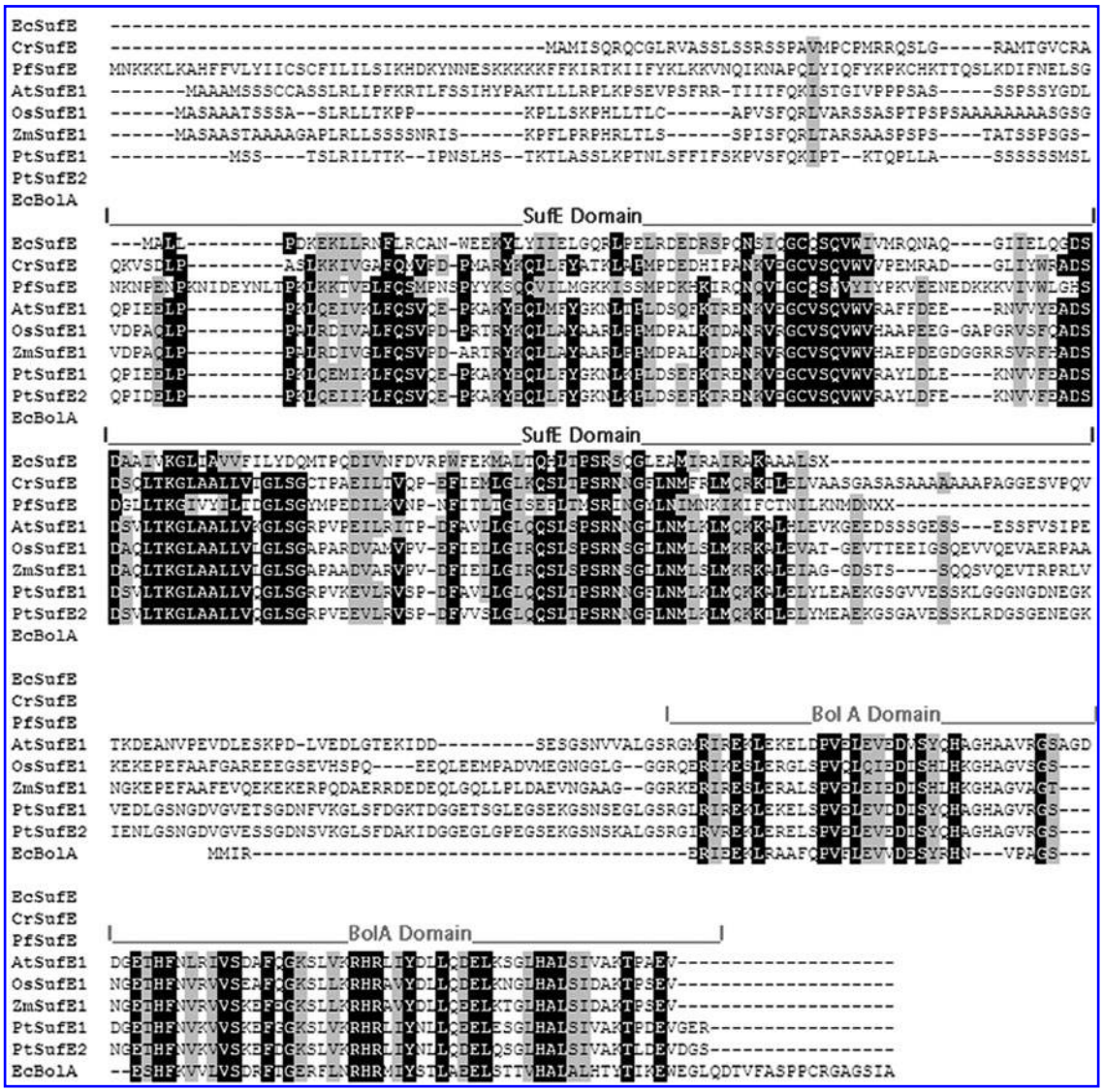

FIG. 8. Alignment of AtSufE1-like proteins, which contain SufE and BolA domains. Es, *E. coli*; Cr, *Chlamydomonas reinhardtii*; At, *A. thaliana*; Os, *Oryza sativa*; Zm, *Zea mays*; Pt, *Populus trichocarpa*; Pf, *P. falciparum*. Linkage of SufE and BolA is universally conserved in higher plants but does not exist in bacteria, *Chlamydomonas* and *Plasmodium*. The reason for this is unclear. A cysteine residue is conserved in all these organisms, indicating its importance. Populus contains two AtSufE1 like proteins, PtSufE1 and PtSufE2, possibly resulting from a duplication event during evolution. PtSufE2 does not have a transit peptide, indicating that it might be located in the cytosol.

HCF101 is plastid localized and belongs to an ancient and universally conserved family of P-loop ATPases previously designated as the MetG-related protein family. The homologous members were confirmed to play crucial roles in [Fe-S] biosynthesis in yeast (166). It was revealed that HCF101 binds a [4Fe-4S] suggested by Mössbauer and EPR spectroscopy and that the reconstituted cluster is transiently bound and can be transferred from HCF101 to a [4Fe-4S] apoprotein (266). ^{55}Fe incorporation studies of mitochondrially targeted HCF101 in *S. cerevisiae* confirmed the assembly of an [Fe-S] in HCF101 in an Nfs1-dependent manner. Site-directed mutagenesis identified three HCF101-specific cysteine residues required for assembly and stability of the cluster (266). PSI activity in the *hcf101* mutant is abolished because PSI core complexes (with [Fe-S]) fail to accumulate in *hcf101*, whereas levels of other thylakoid membrane proteins (without [Fe-S]) are unaffected (158). Mutant plants not only fail to accumulate mature PSI, which contains three [4Fe-4S] clusters, but are also characterized by reduced levels of the soluble [4Fe-4S] clusters-containing complex Fdx-thioredoxin reductase in the stroma. Levels of the [2Fe-2S] cluster-containing soluble and membrane proteins, Fdx and PetC, respectively, were unchanged in *hcf*101 plants. These data suggest a specific role of HCF101 in [4Fe-4S] biogenesis (158) (Fig. 6).

Recent results are also in favor of a role for Nfu proteins as scaffold protein for [Fe-S] biogenesis in plants (49, 157, 301, 330) (Fig. 6). These polypeptides share a conserved CXXC motif in their NFU domain homologous to the C-terminal of *A. vinelandii* NifU. The *A. thaliana* genome encodes *AtNFU1–5* genes, and AtNFU proteins (157, 330) are separated into two classes. AtNFU4 and AtNFU5 are part of the mitochondrial type, presenting a structural organization similar to *S. cere-*

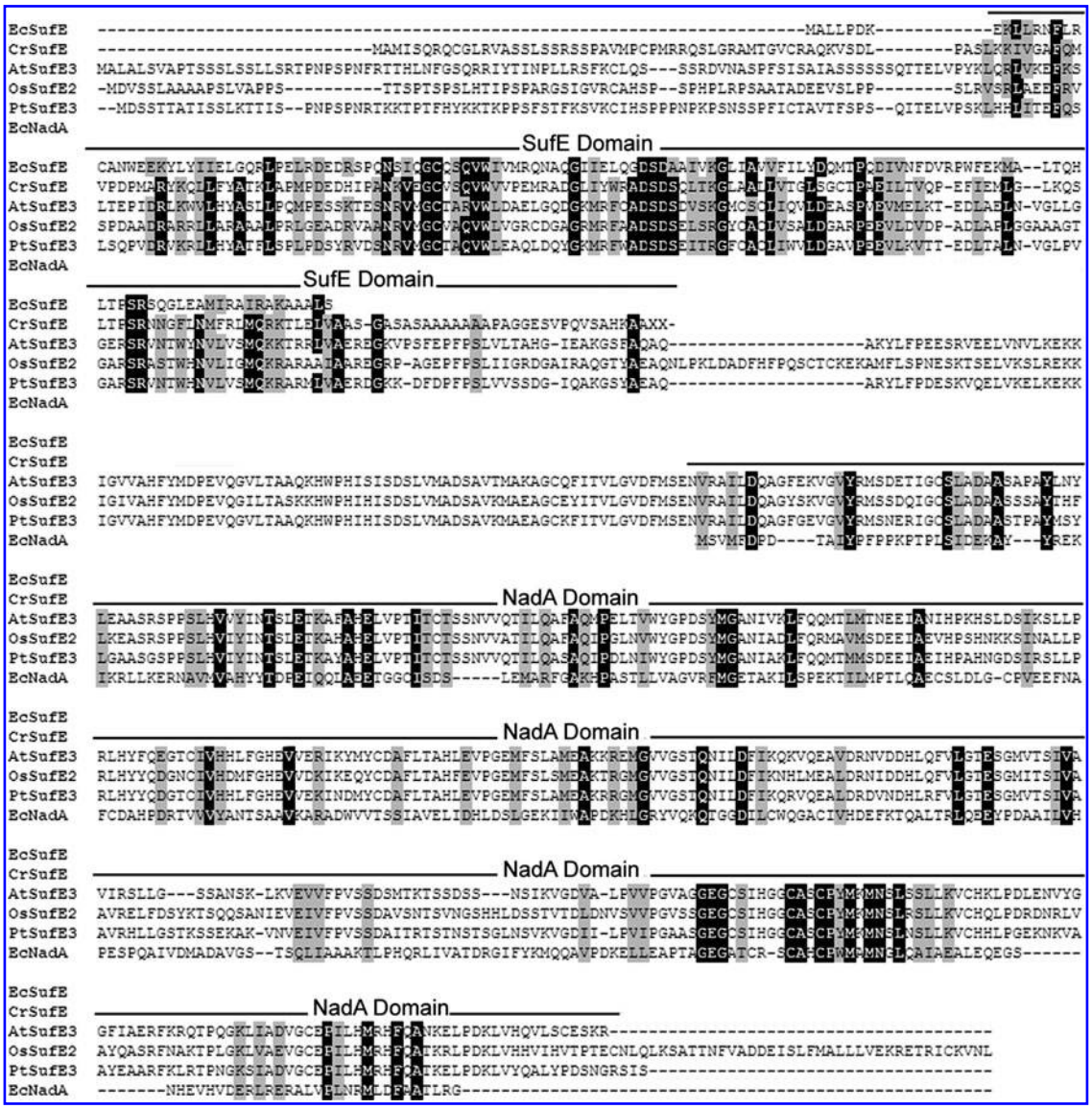

FIG. 9. Alignment of AtSufE3-like proteins, which contain SufE and NadA domains. Es, *E. coli*; Cr, *Chlamydomonas reinhardtii*; At, *A. thaliana*; Os, *Oryza sativa*; Pt, *Populus trichocarpa*. As AtSufE1, AtSufE3-like proteins are only conserved in higher plants and do not exist in bacteria, *Chlamydomonas* and *Plasmodium*. Two conserved cysteine residues are maintained in the NadA domain that might be responsible for holding the [4Fe-4S] (see text). As AtSufE3, homologs in Oryza and Populus appear to be located in plastids.

visiae Nfu1p. AtNFU1, 2, and 3 are unique to plants. These polypeptides are made of two NFU domains, the second having lost its CXXC motif. AtNFU1–3 proteins are more related to *Synechocystis* sp. *PCC6803* NFU-like proteins (206) and are localized to plastids. AtNFU1 and AtNFU2 are functional NFUs capable of restoring the growth of an *isu1/nfu1* yeast double mutant, when targeted to mitochondria (157). Mutant *Arabidopsis* lacking AtNFU1 exhibited a dwarf phenotype with faint pale-green leaves and drastically impaired PSI accumulation. Chloroplasts in the mutants also showed a decrease in both the amount of Fdx, a major electron carrier of the stroma that contains a [2Fe-2S] cluster, and in the *in vitro* activity of [Fe-S] insertion into apo-Fdx. When expressed in *E. coli*, AtNFU1 formed a homodimer carrying an [2Fe-2S] cluster, and this cluster could be transferred to apo-Fdx *in vitro* to form holo-Fdx (330). Two T-DNA insertion mutants of *AtNFU2* showed the same dwarf phenotype due to photosynthetic and metabolic limitations. Photosynthesis studies in these mutants revealed an altered PSI activity together with a decrease in PSI. Decrease of plastid [4Fe-4S] sulfite reductase activity correlates with the PSI decrease and supports an alteration of [4Fe-4S] biogenesis in *atnfu2* chloroplasts. The decrease of electron flow from the PSI is combined with a decrease in Fdx amounts in the *atnfu2* mutants (301). Further, AtNFU2 recombinant protein is capable of binding a labile [2Fe-2S] *in vitro* (157). These results are therefore in favor of a requirement of AtNFU2 protein for [4Fe-4S] and [2Fe-2S] Fdx assembly, conferring to this protein an important function for plant growth and photosynthesis.

As discussed for bacteria, the chaperone and cochaperone systems HscA (DnaK-like chaperone, Hsp70 protein) and HscB (DnaJ-like cochaperone) have important functions in iron–sulfur protein maturation within the ISC machinery. It is reasonable to question whether chloroplast Hsp70- and DnaJ-like proteins in chloroplasts are also involved in this process. This assumption might be supported by the finding that chloroplast stromal HSP70s are essential (286), which is a typical feature shared by all proteins with important roles in [Fe-S] biogenesis. However, analysis of the chloroplast DnaJ-like proteins CDJ3–5 of *Chlamydomonas*, which are recruited by chloroplast Hsp70 (Hsp70B), does not support this assumption even if CDJ3 and CDJ4 contain redox-active [Fe-S] (72).

Accumulation of photosystem ONE1 (*APO1*) contains two related motifs of ~100 amino acid residues that could potentially provide ligands for [4Fe-4S] (14). APO1 is essentially required for stable accumulation of other plastid-encoded and nuclear-encoded [4Fe-4S] complexes within the chloroplast, whereas [2Fe-2S] cluster-containing complexes appear to be unaffected. It is also required for photoautotrophic growth as levels of PSI core subunits are below the limit of detection in the *apo1* mutant. *In vivo* labeling experiments and analyses of polysome association suggest that translational elongation of the PSI transcripts *psaA* and *psaB* is specifically arrested in this mutant. Taken together, these findings suggest that APO1 is involved in the assembly of several [4Fe-4S] cluster-containing complexes in chloroplasts and interferes with translational events probably in association with plastid nucleotides (14).

Grxs, small oxidoreductases structurally related to thioredoxins and traditionally involved in redox reactions, are also required for [Fe-S] assembly (248, 249). Grx is encoded by multigene families and the largest number of *Grx* genes is found in plant *Populus trichocarpa* (62). They have two major proposed biochemical roles: the reduction of disulfide bonds and the binding of [Fe-S], both of which involve GSH. Their function related to [Fe-S] assembly has been firmly established in eukaryotic organisms (12, 85, 124, 192, 195, 244, 250, 312). *In vitro* kinetic studies of chloroplast monothiol Grxs, GrxS14 and GrxS16, monitored by CD spectroscopy indicate that [2Fe-2S] on Grxs are rapidly and quantitatively transferred to apo chloroplast Fdx, demonstrating that chloroplast monothiol Grxs have the potential to function as scaffold proteins for the assembly of [2Fe-2S] that can be transferred intact to physiologically relevant acceptor proteins (24).

5. AtSufE1- and AtSufE3-like proteins. Within the *Arabidopsis* SUF system (AtSUF), the SufE-like proteins show interesting features. First, SufE has three family members, AtSufE1, 2, and 3, whereas the other Suf proteins have only one homolog (Table 1). Second, AtSufE1 is functional in both mitochondria and chloroplasts, whereas the other Suf proteins are chloroplast specific. Third, AtSufE1 and AtSufE3 each contain two domains (Figs. 8 and 9) (177, 328, 333).

The AtSufE1 amino acid sequence contains three regions: (i) a transit peptide region specific for chloroplast and mitochondrial import, (ii) a SufE-like domain within the N-terminal region and, (iii) a BolA domain within the C-terminal region (328, 333) (Fig. 8). Separate SufE and BolA genes are ubiquitously conserved from bacteria to higher plants. However, plants are the only species that need both domains within one protein to be functional (Fig. 8). In *E. coli*, BolA is involved in the regulation of cell division in response to nutrition (95, 257–259); however, the molecular mechanism by which BolA functions is not yet resolved. Recently, a comparison of data from genomic sequences, YTH experiments, and three-dimensional structures, led to the hypothesis that the BolA protein may be a reductase interacting with a monothiol Grx (120). What the BolA domain does in AtSufE1 remains to be clarified.

AtSufE3 consists of both a SufE-like domain and a domain similar to the bacterial quinolinate synthase NadA (177) (Fig. 9). *E. coli* NadA is involved in the biosynthesis of nicotinamide adenine dinucleotide, a cofactor in numerous essential redox biological reactions and contains an [4Fe-4S] essential for activity (58, 182, 210, 251, 261, 262). It was suggested that the SufE domain of AtSufE3, *via* the conserved cysteine residue, is essential for [Fe-S] formation on the NadA domain of the same protein. The SufE domain specifically shuttles sulfur atoms from AtSufS/AtNfS2 to the NadA domain, for the assembly of the catalytically essential [4Fe-4S] cluster, through an intraprotein sulfur transfer from the conserved cysteine residue to the active site of the NadA domain (182). Similar to AtSufE1, AtSufE3 is only found in higher plant although NadA-like structures are ubiquitously conserved from bacteria to higher plants (Fig. 9).

B. ISC-like system in mitochondria of A. thaliana

During the time when the bacterial ISC genes were being identified, eukaryotic ISC homologs were also starting to be recognized in yeast. Along with a few additional auxiliary proteins, the ISC-like system constitutes the mitochondrial machinery for [Fe-S] biogenesis (165). Currently, the best studied ISC systems are from *E. coli* and *S. cerevisiae*. Discussion of the plant ISC-like system is based partly on experimental work and partly on genetic predictions. The ISC system in bacteria contains IscR, IscA, IscU, IscS, HscB, HscA, and Fdx. Three IscA/SufA like proteins are predicted to be localized in mitochondria in *Arabidopsis* (21). As discussed above, the function of these proteins is not clear, but we suggest a storage function for these A-type proteins based on their ability to hold [Fe-S] and transfer to apo-proteins, where they are not essential for growth.

Scaffold proteins represent one of the most essential parts for [Fe-S] assembly *in vivo* and IscU and Nfu-like proteins are scaffold proteins involved in [Fe-S] biogenesis playing a key role in yeast mitochondria and bacteria. The *A. thaliana Isu* gene family, *AtIscU1–3* (or AtIsu1–3), encodes polypeptides closely related to their bacterial and eukaryotic counterparts (156) (Table 1). AtIsu1–3 expression in an *S. cerevisiae isu1 nfu1* thermo-sensitive mutant led to growth restoration of this strain at 37°C, suggesting a scaffold role for AtIscU1–3. Using IscU-GFP fusions expressed in leaf protoplasts and immunodetection in organelle extracts, the *Arabidopsis* IscU proteins are shown to be located only in mitochondria, supporting the existence of an IscU-independent [Fe-S] assembly machinery in plant plastids (156). A basic local alignment search tool for protein search identified only two putative *IscU* genes in rice (302), and one of them *OsIscU1* (*OsIsu1*), when expressed in onion epidermal cells, localized to mitochondria. Northern blotting analysis showed that *OsIscU1* was downregulated in iron-deficient rice root. Further, *OsIs-*

cU1 promoter-GUS transgenes introduced into *A. thaliana* showed that *OsIscU1* expression was regulated in a stage- and tissue-specific manner (302).

AtIscS/AtNFS1 is a mitochondrial cysteine desulfurase (144, 227) that provides sulfur to AtIscU1, AtIscU2, or AtIscU3 for [Fe-S] assembly. As expected, assembly of [Fe-S] was observed *in vitro* under anaerobic conditions in the presence of AtIscU1, AtIscS, L-cysteine, and ferric ammonium citrate (91). The phenotypes of plants in which AtIscU1, 2, or 3, or AtIscS were downregulated by RNAi indicate that the AtIscS and the three AtIscU proteins are essential for normal plant growth and development (91). This is not surprising since the mitochondrial [Fe-S] assembly machinery is required for [Fe-S] in the respiratory complexes and cofactor biosynthesis, including biotin, lipoate, and Moco (144). In addition, a link between AtIscS and phytohormone biosynthesis was shown in AtIscS antisense lines through the strongly diminished activities of aldehyde oxidase (AO), which is a vital enzyme for ABA synthesis (91).

As discussed above, *Arabidopsis* AtSufE1 is also localized to mitochondria, where it interacts with AtIscS to enhance its cysteine desulfurase activity (328). An *atsufE1* mutant is embryonic lethal, which cannot be complemented by only plastid-targeted or only mitochondrial-targeted AtSufE1, demonstrating that organelle-specific AtSufE1 re-establishment is not sufficient to allow embryo progression, revealing that AtSufE1-mediated sulfur mobilization is crucial in both organelles. Because of this, AtSufE1 should be regarded as an important component of the mitochondrial ISC-like system of *Arabidopsis* (AtISC).

In bacteria, as the SUF and ISC systems function in the same compartment, it is difficult to determine by mutation which genes are essential for these systems separately. In contrast, yeast contains the mitochondrial-specific ISC-like system and many genes, including *Jac1* (HscB homolog), have been shown to be vital for growth (16, 176, 314). The alignment of HscB-like protein sequences of various organisms from bacteria to human highlighted interesting characteristics: (i) a "HPD" motif, special for J-domain proteins, is conserved in all HscB like proteins, (ii) leucine residues comprise 30% of all conserved amino acids, and (iii) apart from yeast Jac1, the transit peptide sequences of the other eukaryotic HscBs contain four conserved cysteine residues forming two CXXC domains (Fig. 10). AtHscB from *Arabidopsis* can rescue the *Jac1* yeast knockout mutant, suggesting a role for AtHscB in iron–sulfur protein biogenesis in plants (326). In contrast to

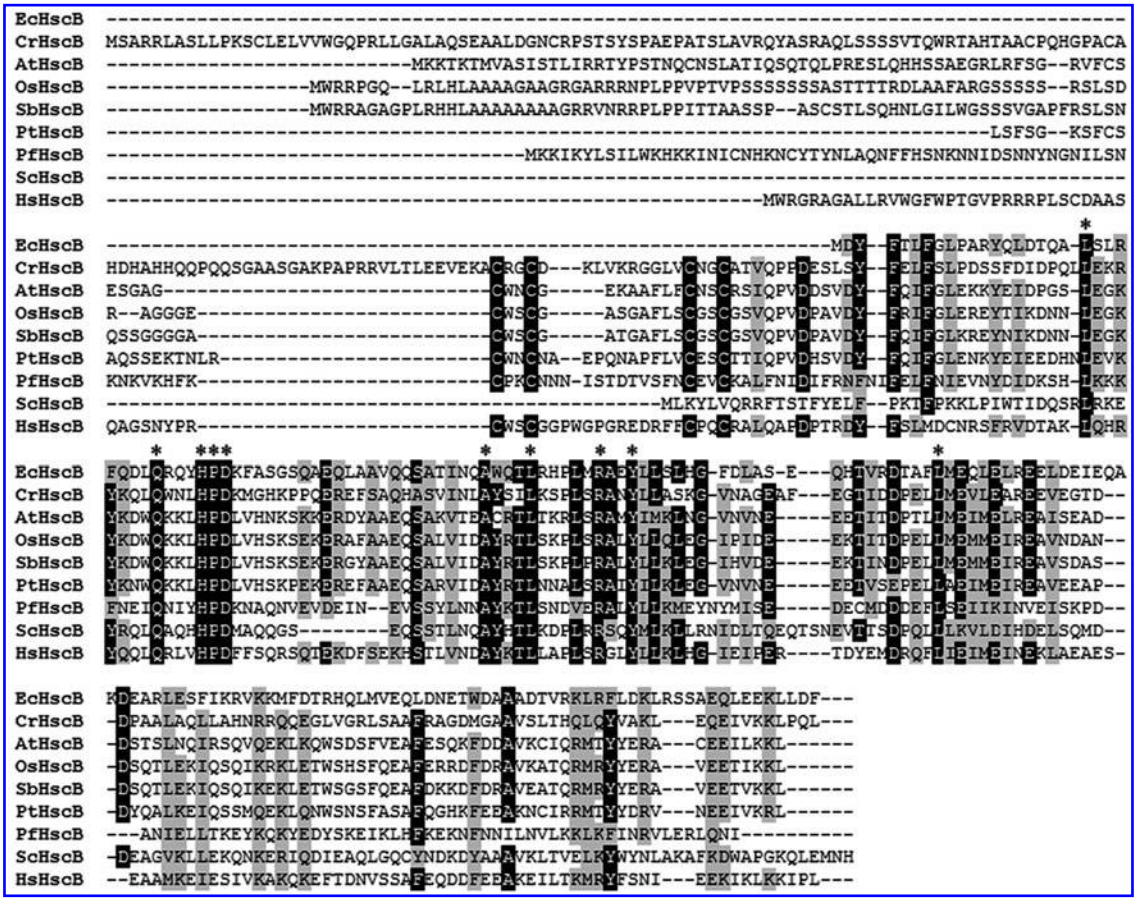

FIG. 10. Alignment of HscB-like proteins. Es, *E. coli*; Cr, *Chlamydomonas reinhardtii*; At, *A. thaliana*; Os, *Oryza sativa*; Zm, *Zea mays*; Sb, *Sorghum bicolor*; Pt, *Populus trichocarpa*; Pf, *P. falciparum*; Sc, *Saccharomyces cerevisiae*; Hb, *Homo sapiens*. The highly conserved residues are indicated by "*." The motif HPD is conserved in all these organisms from bacteria to humans, indicating its importance in activity. The transit peptide of the yeast HscB (Jac1) only contains 11 amino acid residues, which are sufficient to direct Jac1 to mitochondria. All transit peptides of the organisms shown, except yeast, contain four cysteine residues. The HscBs of yeast and *Plasmodium* are obviously different from that of other organisms.

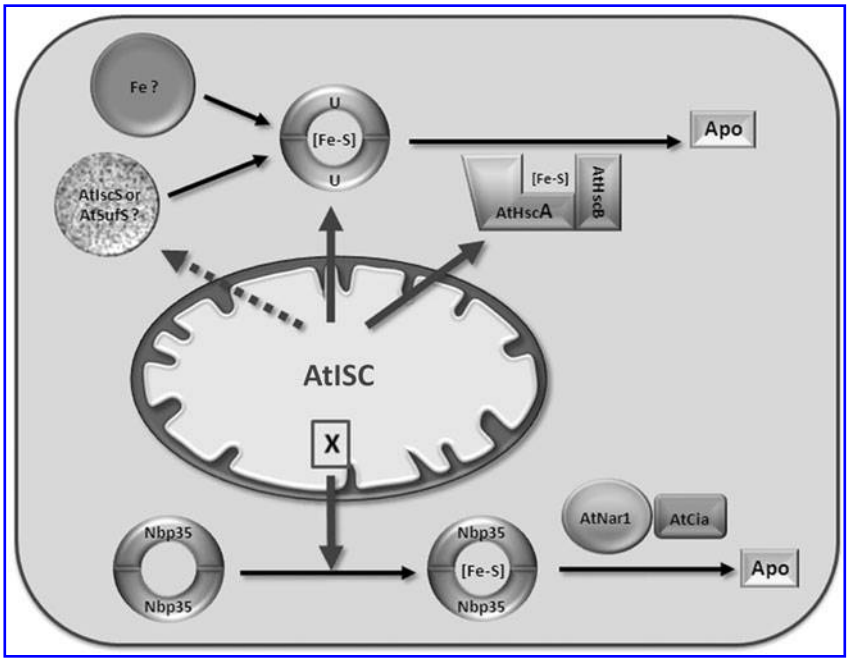

FIG. 11. [Fe-S] biogenesis in the cytosol of *Arabidopsis*. *Arabidopsis* contains most of the yeast CIA components except Cfd1 and AtNbp35, which has been confirmed to function as yeast Nbp35. AtNar1 and AtCia1 have not been characterized to date. However, experimental evidence has confirmed that AtHscB, AtHscA, and AtIscU1 are present in both the cytosol and mitochondria, which agrees with the result from human research, where ISCU, ISCS, ISD11, and NFU are also present as cytosolic variants (Fig. 13). The possible exported X factor from mitochondria, which was first proposed in yeast, has not been identified in any other organisms. This CIA part of this figure is mostly based on yeast research. Apo, Apo-protein; AtISC, *A. thaliana* ISC system containing at least AtIscA, AtIscU, AtIscS, AtHscB, AtHscA, and AtISD11 components.

mitochondrial Jac1, AtHscB localizes to both mitochondria and the cytosol (Fig. 11). AtHscB interacts with AtIscU1 revealed by YTH and BiFC, and through this interaction AtIscU1 is partially retained in the cytosol. Therefore, AtIscU1 is probably an important component of the cytosolic [Fe-S] assembly machinery (326). AtHscB is highly expressed in anthers and trichomes and an *AtHscB* T-DNA insertion mutant shows severely reduced seed set (<10 seeds can be obtained), a waxless phenotype and inappropriate trichome development as well as dramatically reduced activities of the [Fe-S] enzymes aconitase and succinate dehydrogenase (326).

Arabidopsis mitochondria, like yeast, also contain two Hsp70 chaperones, AtHscA1–2. AtHscA1 was demonstrated to functionally complement the yeast *Ssq1* knockout mutant (326). As expected, it is an ATPase and its ATPase activity can be enhanced by AtHscB and AtIscU1. AtHscA is also localized to both mitochondria and the cytosol (Fig. 11). These data suggest that AtHscB together with AtHscA and AtIscU1 play an important role in the biogenesis of iron–sulfur proteins in both mitochondria and the cytosol (326).

Members of the Grxs multigene family also exist in plant mitochondria. Yeast mitochondrial Grx5 plays a central role in the [Fe-S] assembly process through interaction with the A-type [Fe-S] scaffold proteins Isa1 and Isa2, and supports mitochondrial genome integrity (136). An *Arabidopsis* mitochondrial monothiol Grx, AtGrx4, was proposed to be involved with protecting cells against oxidative damage (56). However, its relationship with plant mitochondrial [Fe-S] biogenesis remains to be confirmed.

Frataxin has been proposed to be an iron chaperone providing iron for [Fe-S] assembly in yeast and humans (75, 90, 101, 146, 154, 160, 183, 191, 195, 247, 272). It is a highly conserved protein, and the frataxin homolog in *A. thaliana* (AtFH) is a single nuclear-encoded gene targeted to mitochondria sharing 65% similarity with animal frataxin (309). AtFH can complement an *S. cerevisiae* mutant (Δ*yfh*) lacking the Frataxin gene proving that AtFH is a functional protein. It is an essential protein in plants, required for full activity of mitochondrial iron–sulfur proteins and playing a protective role against oxidative damage and iron homeostasis (42, 43, 178, 184, 237, 309). Knockout of *AtFH* causes embryo lethality in *Arabidopsis* (309). However, an *atfh-1* knock-down T-DNA frataxin-deficient mutant contains increased total and organellar Fe levels. Frataxin deficiency leads also to nitric oxide (NO) accumulation in *atfh-1* roots (184), which might result from Fe surplus.

As in chloroplasts, the list of components to be discovered involved in [Fe-S] biogenesis in mitochondria will be steadily increasing. However, as there is only one cysteine desulfurase (IscS-like) in plant mitochondria providing element sulfur for scaffolds, one could envisage that the components in mitochondria form a more extended ISC system than that found in bacteria.

C. CIA like system in the cytosol of A. thaliana

The *Arabidopsis* genome encodes almost all the homologs genes of these components, but so far only the *Nbp35*-like proteins have been characterized. Genome analysis revealed that NBP35 is conserved in the green lineage but that CFD1 is absent (44) (Table 1, Fig. 11). Plant and algal NBP35 proteins lack the characteristic CXXC motif in the C terminus, thought to be required for [Fe-S] binding. However, chemical reconstitution and spectroscopy showed that *Arabidopsis* AtNBP35 bound a [4Fe-4S] in the C terminus as well as a stable [4Fe-4S] in the N terminus. Holo-AtNBP35 was able to transfer an [Fe-S] to an apoprotein *in vitro*. The *AtNBP35* gene is constitutively expressed *in planta*, and its disruption was associated with an arrest in embryo development. These results show that despite considerable divergence from the yeast Cfd1-Nbp35 [Fe-S] scaffold complex, AtNBP35 has retained similar [Fe-S] binding and transfer properties and performs an essential function (44). When expressed in yeast, it was revealed that AtNBP35 bound ^{55}Fe, dependent on the cysteine de-

sulfurase Nfs1 in mitochondria (44). This dependence is carried out through the function of the mitochondrial membrane transporter Atm1 of yeast. Although CFD1 is absent in the *Arabidopsis* genome, there are two Cia1-like proteins that are predicted to be cytosolic (Table 1).

In *Arabidopsis*, besides the CIA-like components, AtHscB, AtHscA, and AtIscU1 should also be considered. As discussed above, these three proteins can be detected in the cytosol and mitochondria, and their function related to [Fe-S] assembly has been confirmed (326). Because of this we include these three proteins within the *Arabidopsis* CIA system (AtCIA), which we have named the extended AtCIA (Fig. 11).

In human cells, the subcellular distribution of transiently expressed Nfs1 is restricted not only in mitochondria but also in the cytosol and nucleus, which was claimed to be achieved by alternative translation initiation from a single transcript (145). In yeast, attempts to detect the endogenous Nfs1 in the nucleus by immunoblotting or immunofluorescence failed most probably because Nfs1 is present at extremely low levels in the nucleus (199). Previous genetic studies (194, 203) in *S. cerevisiae* have suggested that this protein distributes between the mitochondria and the nucleus with biochemically undetectable amounts in the nucleus (termed "eclipsed distribution"). Direct evidence for Nfs1 nuclear localization (in addition to mitochondria) was recently obtained by using both α-complementation and subcellular fractionation (199). The mitochondrial and nuclear Nfs1 are derived from a single translation product. It was therefore suggested that the Nfs1 distribution mechanism involves at least partial entry of the Nfs1 precursor into mitochondria, and then retrieval of a minor subpopulation (probably by reverse translocation) into the cytosol and then the nucleus (199).

The re-entry of human and yeast Nfs1 into the cytosol is compatible with the theory of mitochondrial export. This hypothesis suggested that mitochondria possess specific mechanisms for export of proteins to other compartments (282). Many proteins that were originally characterized on the basis of nonmitochondrial functions have unexpectedly been shown to be identical to mitochondrial–matrix proteins. Most of these proteins are encoded by single nuclear genes and are initially targeted to the mitochondrial matrix.

D. Communication between different compartments

1. The importance of chloroplasts. By the very nature of [Fe-S], sulfur and iron sources are important factors affecting the functions of iron–sulfur proteins. The direct sulfur source has been determined to be cysteine from which cysteine desulfurases mobilize sulfur and provide this to the scaffold proteins. In plants, sulfur is taken up as sulfate and then incorporated into cysteine (240). Cysteine biosynthesis plays a central role in fixing inorganic sulfur from the environment and provides the only metabolic sulfide donor for the generation of methionine, GSH, phytochelatins, [Fe-S], vitamin cofactors, and multiple secondary metabolites (35). As this pathway is located in plastids (240, 254, 323), this compartment represents a cysteine storage facility in plant cell.

Iron absorbed by plant roots from soil is mainly (>90%) compartmentalized in plastids even though some is deposited in the apoplast and vacuoles (40, 41). Imported iron in plastids is mainly stored in ferritin, a globular protein complex consisting of 24 subunits with the ability to store up to 4500 iron atoms. *Arabidopsis* contains four ferritin genes, *AtFer1–4*, predicted with high confidence to be localized to plastids (41, 224, 225), consistent with the iron quantitative analysis (292). Therefore, plastids are both iron and cysteine storage sites in plant cells. Because of this, relocation of cysteine and iron will be vital for iron, cysteine, and [Fe-S] homeostasis. It is therefore necessary to know which factors determine the relocation of cysteine and iron from chloroplasts to other compartments.

2. AtSUF and AtISC. AtSufE1, which consists of a BolA domain and a SufE domain, is a dual localized protein functional in plastids and mitochondria and can regulate cysteine desulfurases in both organelles (328, 329). As an *AtSufE1*, targeted only to chloroplasts or mitochondria, cannot rescue the embryo-lethal phenotype of *atsufe1*, it is obviously essential for both organelles. The existence of AtSufE1 provides an important link for the communication between chloroplasts and mitochondria. This link might involve the relocation of cysteine and iron from chloroplasts to mitochondria. One would expect that the distribution of cysteine and iron between the spatially separated AtSUF and AtISC systems would be balanced according to the activity of their cysteine desulfurases.

3. AtISC and AtCIA. Mitochondrial chaperones provide some of the best studied examples pointing to roles for mitochondrial proteins in diverse cellular processes (282). It was proposed that specific export mechanisms exist by which certain proteins exit mitochondria, allowing these proteins to have additional functions at specific extra-mitochondrial sites. Gram-negative proteobacteria, from which mitochondria evolved, contain a number of different mechanisms for protein export. So, it is likely that mitochondria either retained or evolved export mechanisms for certain proteins (282). However, the information concerning these export mechanisms is lacking.

The AtHscA and AtHscB chaperone and cochaperone in *Arabidopsis* are dual localized proteins that are found in the cytosol and mitochondria (326). Since AtIscU1 interacts with AtHscB, this scaffold protein is probably exported from mitochondria together with AtHscB (Fig. 11).

Communication between AtISC and AtCIA is reinforced by the existence of homologs of a mitochondrial export machinery found in yeast (see yeast section, Fig. 11). *Arabidopsis* has three *Atm* genes, *AtAtm1*, *AtAtm2*, and *AtAtm3*. Among these, only *AtAtm3* has been characterized (30, 294). Analyses of selected metal enzymes of *Arabidopsis atm3* alleles showed that the activity of cytosolic aconitase ([Fe-S] dependent) was strongly decreased, whereas mitochondrial and plastid [Fe-S] enzymes were unaffected. Nitrate reductase activity (Moco, haem) was decreased by 50% in the strong *atm3* alleles, but catalase activity (haem) was similar to that of the wild type. Strikingly, in contrast to mutants in the yeast and mammalian orthologs, *Arabidopsis atm3* mutants did not display a dramatic iron homeostasis defect and did not accumulate iron in mitochondria. It was suggested that *Arabidopsis* AtAtm3 may transport (i) at least two distinct compounds or (ii) a single compound required for both [Fe-S] and Moco assembly machineries in the cytosol, but not iron (30).

The localization of AtHscB, AtHscA, and AtIscU in both the cytosol and mitochondria and the existence of a

mitochondrial export machinery provide strong links between these two compartments. This is important for both iron and [Fe-S] homeostasis and it will be very interesting to examine whether the existence of AtHscB, AtHscA, and AtIscU in the cytosol is connected to the mitochondrial export machinery.

V. [Fe-S] Biogenesis in Malaria Parasite (*P. falciparum*)

P. falciparum is a protozoan parasite, one of the species of *Plasmodium* that cause human malaria. It is transmitted by the female *Anopheles mosquito*. *P. falciparum* is the most lethal form as *P. falciparum*–derived malaria has the highest rates of complications and mortality. The complete sequence of the *P. falciparum* genome indicates the presence of 5432 genes spread across 14 chromosomes, a mitochondrial genome and a circular plastid (apicoplast) genome. However, >60% are hypothetical proteins (47, 100), which can explain why only few of the genes involved in [Fe-S] biogenesis have been functionally analyzed so far in contrast to the tremendous progresses made in bacteria, yeast, and other eukaryotic organisms. On the basis of the amino acid sequence of their predicted products, they can, however, be classified into SUF, ISC, and CIA systems accommodated in plastid (apicoplast), mitochondria, and the cytosol, respectively (Table 1).

A very interesting aspect of malaria parasites is that they contain plastids (apicoplast) that are usually thought to be within the plant kingdom. This organelle is no longer capable of photosynthesis, but is an essential organelle. The eubacterial ancestry of the organelle provides a wealth of opportunities for the development of therapeutic interventions. Morphological, biochemical, and bioinformatic studies of the apicoplast have further reinforced its plant-like characteristics and potential as a drug target (169). A predicted apicoplast proteome has been assembled and putative pathways for the biosyntheses of fatty acids, isoprenoids, and haem, and [Fe-S] have been mapped out in the apicoplast (234). Searches of the apicoplast proteome identified various [Fe-S] biosynthetic enzymes, including SufB encoded by the apicoplast genome and Nfu, SufA, SufC, SufD, and SufS in the nuclear genome of *P. falciparum* (79, 169, 234, 267) (Table 1). *PfSufD* is somewhat special compared to other orthologs as its gene product is approximately three times larger than other SufDs (Fig. 7). Using the *P. falciparum* database, we also found SufE-like homolog (Table 1; Figs. 8 and 9) which were claimed to be missing from malaria parasites. In *E. chrysanthemi*, the SUF system is essential for its infection of plants (200), and SufB, SufC, and SufE are vital components. It is expected that this system is also indispensable for *Plasmodium* (169).

The *Plasmodium* mitochondria contain both conserved and unusual features, including an active electron transport chain and many necessary enzymes for coenzyme Q and also [Fe-S] biosynthesis (306). Bioinformatic searches for [Fe-S] biosynthesis in the *P. falciparum* database has revealed homologs of the core components of the mitochondrial [Fe-S] assembly machinery (267). The components of ISC system in *P. falciparum* include two IscAs, IscS, IscU, HscB, HscA, Fdx, FNR, Isd11, and several auxiliary proteins (Table 1). The N-terminal leader sequence of IscS was cloned and was shown to target GFP to mitochondria, supporting that this process occurs in the *Plasmodium* mitochondria (260). However, the frataxin homolog is missing, which is thought to deliver iron to the IscU protein. Considering *Plasmodium* as a parasite whose growth and development depend on its host, the functional model of their metabolic pathways, such as ISC, might be different from what has been established on other organisms.

For the CIA system of *Plasmodium*, we only identified two Cfd1 (orNbp35)-like genes, namely, *PF11_0296* and *PFI0525w* (Table 1). Functional analysis is necessary to determine whether these proteins perform similar roles to their homologs in yeast or other eukaryotic counterparts. As the information of [Fe-S] biogenesis is so limited, the regulation of these processes and their communication is clearly lacking but represent exciting future challenges.

VI. [Fe-S] Biogenesis in Humans and Implications in Disease

Although most research on [Fe-S] biogenesis has focused on yeast, emerging evidence that defective [Fe-S] biogenesis causes a spectrum of disease states has sparked increased interest in understanding the process of [Fe-S] biogenesis in humans. Because [Fe-S] are crucial for a wide range of fundamental cellular activities, it is clear that disruption of biogenesis pathways affects several cellular processes that have implications for human disease (Fig. 12). The most studied [Fe-S] defect relates to the human disease Friedreich ataxia where Frataxin deficiency leads to diminished levels of iron–sulfur proteins in addition to iron overload in mitochondria and oxidative injury, which ultimately leads to defective mitochondrial functions (19, 220). In addition to research efforts on Friedreich ataxia, it is now evident that defective [Fe-S] biogenesis leads to sideroblastic anemia (45, 46) and myopathy (191, 214). More broadly, the mitochondrial status clearly plays an important role in supplying [Fe-S] to iron–sulfur proteins in mitochondria (33, 167). Further evidence underlining the importance of the functional status of mitochondria on [Fe-S] biogenesis linked to disease has been provided by recent studies on GSH-dependent oxidoreductases (GRX). Members of the monothiol GRX have been implicated in [Fe-S] biogenesis and findings have also implicated the mitochondrial dithiol Grx (GRX2) as being part of the mitochondrial [Fe-S] pathway in dopaminergic cells with a direct implication to Parkinson's disease (150).

A. [Fe-S] assembly pathways in humans

Because of the conserved nature of the [Fe-S] biogenesis proteins, it is not surprising that both the mitochondrial ISC and the putative cytosolic CIA systems are similar in yeast and in higher eukaryotes (Table 1). Frataxin has been widely studied in mammalian cells, including a mouse model, where frataxin depletion results in defects in mitochondrial iron–sulfur protein activities (183, 283). Indeed, frataxin has been shown to interact with components of the ISC machinery, including ISD11 and mortalin/GRP75 (272). ISD11 has been identified as a component of the ISCS/ISCU complex (3, 321), and these findings indicate that frataxin interacts with the ISCS/ISCU complex through ISD11. The ISC assembly pathway requires the concerted action of ISCS(Nfs1) and ISCU/(Isu1) and RNAi knock-down studies in cell cultures have shown that reduced levels of ISCS(Nfs1), and ISCU/(Isu1) results in inappropriate iron–sulfur protein activities in both mitochondria and cytosol (33, 89). In particular, the level of aconitase activity was reduced in both subcellular locations,

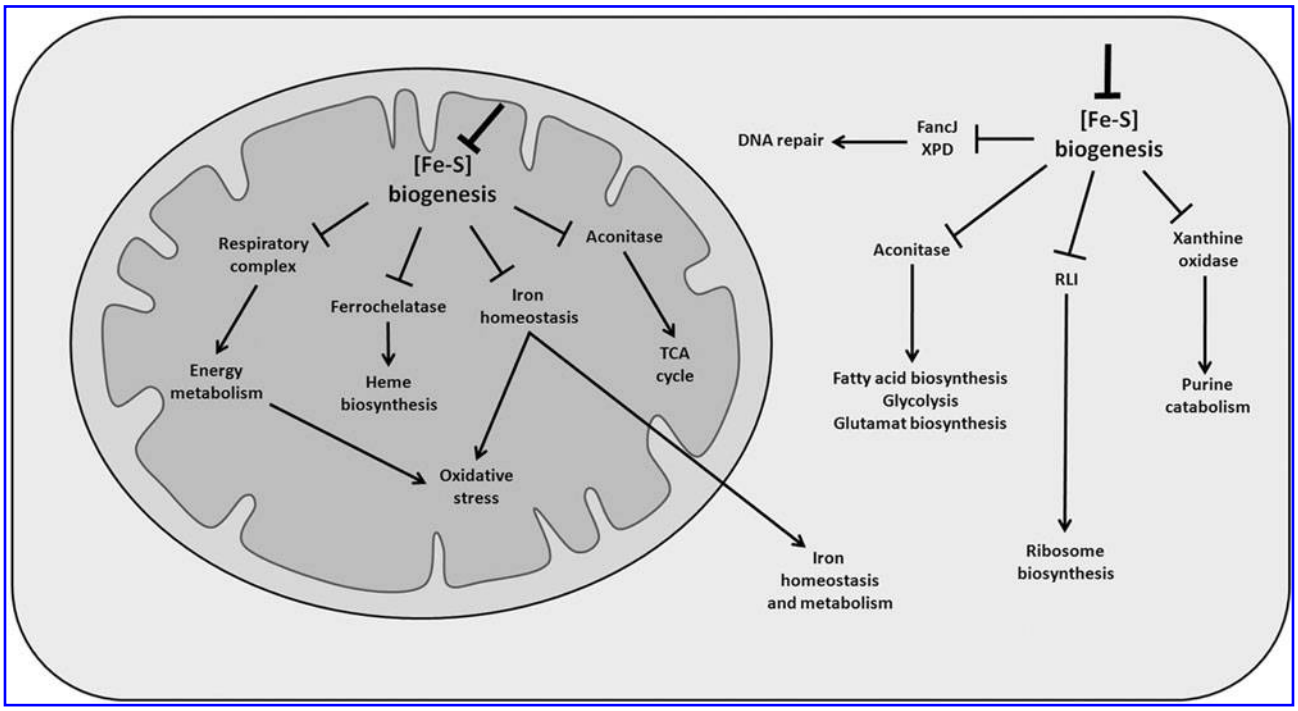

FIG. 12. Schematic diagram showing the effect of inappropriate [Fe-S] biogenesis in mitochondria and the cytosol of human cells. In mitochondria, defective [Fe-S] biogenesis results in altered energy metabolism, heme biosynthesis, oxidative stress, iron homeostasis, as well as effects on the TCA cycle. In the cytosol, defective [Fe-S] biogenesis results in altered fatty acid, glutamate, and ribosome synthesis as well as defective glycolysis and purine catabolism and DNA repair in the nucleus.

which is in agreement with the discovery of ISCS and ISCU localized in both the cytosol and mitochondria of human cells (246, 299).

The dual localization of ISCS and ISCU in the cytosol and mitochondria indicate that they might play important functions in cytosolic [Fe-S] biogenesis. Moreover, several additional factors correlated with mitochondrial [Fe-S] biogenesis, such as ISCA1, Nfu, GLRX5, and Frataxin (FXN), are also detected in the cytosol and variation of their activity clearly affect iron–sulfur proteins function in the cytosol (2, 59, 145, 298, 300). On the basis of this it is reasonable to assume that there exists an ISC-like system in the cytosol of mammalian cells, which can be named c-ISC (Fig. 13). The notion of a c-ISC system is also evident from work in yeast and *Arabidopsis*. For instance, the yeast cysteine desulfurase Nfs1 is a dual targeting enzyme functioning in both the cytosol and the mitochondrion; in the cytosol of *Arabidopsis* there are also components such as AtHscA, AtHscB, and AtIscU1, which belongs to mitochondrial ISC system AtISC (326) (Fig. 11).

Genetic conservation (Table 1) and experimental evidence suggest a CIA-like system also involved in the maturation of [Fe-S] proteins in both the nucleus and cytosol. The cytosolic

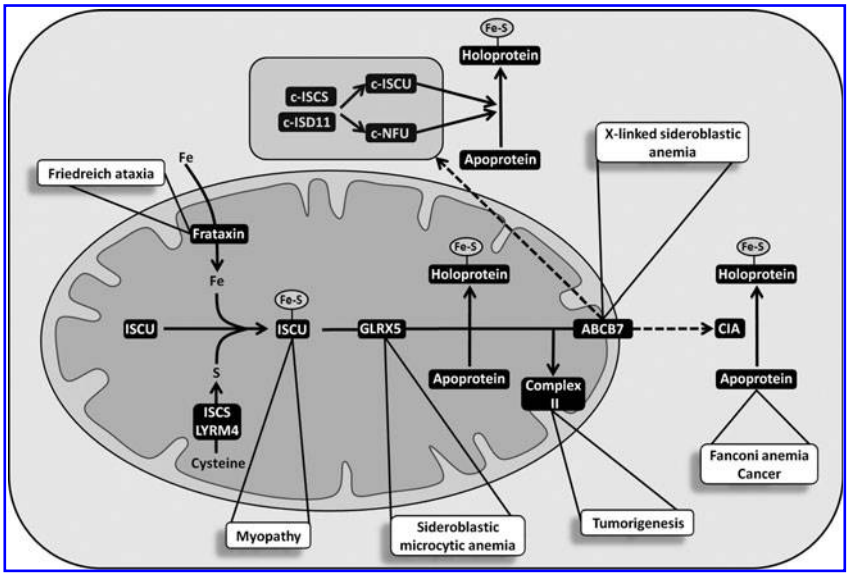

FIG. 13. Defective [Fe-S] biogenesis can result in various human disease states. Decreased levels of Frataxin lead to Friedreich ataxia, defective GLRX5 leads to sideroblastic microcytic anemia, defective ABCB7 leads to X-linked sideroblastic anemia, mutation in ISCU leads to myopathy, and defects in complex II can lead to tumorigenesis. In addition, the loss of [Fe-S] for insertion into apoproteins can lead to a multitude of diseases, including Faconi aneamia and various forms of cancers. ABCB7, homolog of yeast Atm1, is supposed to export an unknown factor either facilitating cytosolic [Fe-S] biogenesis or serving as a signal in the regulation of overall mitochondrial iron homeostasis, which will eventually affect [Fe-S] biogenesis in the cytosol and mitochondria.

P-loop NTPase NUBP1, the human homolog of the yeast Nbp35 protein, has been shown to be an iron–sulfur protein involved in maturation of iron–sulfur proteins as an integral part of the CIA system (284). It was shown that NUBP1 depletion in HeLa cells resulted in reduced cell growth and inappropriate maturation of the cytosolic iron–sulfur proteins glutamine phosphoribosyl pyrophosphate amidotransferase and the aconitase activity of IRP1. The effects observed were specific to the cytosol as mitochondrial aconitase and SDH activities remained unchanged in response to NUBP1 depletion (284). A second effect of NUBP1 depletion, due to impaired maturation of IRP1, relates to iron metabolism where cells show decreased cellular H-ferritin and elevated ferritin uptake. This is in contrast to yeast Nbp35, which does not play a role in iron metabolism and suggest a divergence in Nbp35/NUBP1 function during evolution. It has also been demonstrated that NUBP1 interacts with NUBP2, indicating the presence of a heterodimeric NTPase protein complex involved in cytosolic iron–sulfur protein maturation and iron regulation (284). In mouse models NUBP1 and NUBP2 interacts with the kinesin-like protein KIFC5A involved in bipolar spindle assembly and integrity, which suggests that NUBP1 and NUBP2 together with KIFC5A act in a common regulatory pathway controlling centromere duplication in mammalian cells (57).

In yeast, cytosolic [Fe-S] biogenesis is dependent on the ISC export machinery from mitochondria, which include members of the ABC class of proteins such as ATM1. Studies using RNAi technology have demonstrated that the human ATM1-like protein ABCB7, which involved in X-linked sideroblastic anemia with ataxia, has a similar function (48). ABCB7-deficient HeLa cells show a reduction in aconitase activity, particularly the IRP1 cytosolic form, which suggests that ABCB7 is involved in transfer of iron from mitochondria to the cytosol and also in the maturation of cytosolic iron–sulfur proteins. The vital role of ABCB7 was shown in mouse studies where depletion of ABCB7 resulted in embryo lethality (232). It is clear from the various studies on the ISC system that ISC-mediated assembly and export represent highly conserved processes.

B. Iron regulation by ISC and CIA

Iron is required in all mammalian cells but at high levels iron is toxic. Iron homeostasis is therefore highly regulated within cells and tissues and it is therefore not surprising that inappropriate iron balance represents one of the most prevalent human diseases. The regulation of iron homeostasis is mediated by iron regulatory proteins (IRP1 and IRP2) (246, 318) where IRP1 acts as a cytosolic aconitase when bound to a [4Fe-4S] cluster. In the absence of an [Fe-S], IRP1 interacts with IRE stem loops of iron-regulatory protein mRNAs. In contrast to IRP1, IRP2 does not contain an [Fe-S] but is regulated based on iron availability. The aconitase activity of IRP1 depends on [Fe-S] supply by the ISC machinery and because of this it is highly likely that iron–sulfur protein biogenesis influences iron homeostasis. Indeed, depletion of the human ISCU protein using RNAi results not only in decreased mitochondrial and cytosolic aconitase activities but also in a disruption in the intracellular iron homeostasis (300). ISCU itself in eukaryotes is regulated by microRNA-210 (miR-210) (50, 51, 55, 83, 84) as miR-210 can directly target ISCU-like RNAs and suppress their expression. ISCU levels are also suppressed by iron starvation, indicating that iron stress might induce the expression of miR-210, which is also induced by hypoxia (51).

Iron homeostasis is also regulated by the ISC export machinery. Mouse ABCB7 deficiency leads to iron accumulation in hepatocytes in the liver, which has been shown to be due to inappropriate [Fe-S] maturation on IRP1 (232). It has been postulated that ABCB7 deficiency leads to cells counteracting inappropriate [Fe-S] maturation by elevating iron levels and a similar situation also occurs in human cells: depletion of ABCB7 in HeLa cells results in iron depletion in the cytosol with an associated sixfold increase in iron accumulation in mitochondria (48). In addition to ABCB7, NUBP1 also affects iron homeostasis as NUBP1 depletion results in decreased H-ferritin and increased ferritin receptor levels (284).

In combination various studies indicate that the ISC and CIA machinery play a role in regulating iron homeostasis in mammalian cells and that inappropriate regulation has dramatic pathological consequences.

C. [Fe-S] biogenesis proteins and human disease states

A number of [Fe-S] biogenesis proteins have been shown, when mutated, to cause severe pathological disease states (Fig. 13). The most studied and direct effects are linked to Frataxin, GLRX5, and ISCU although other iron–sulfur proteins connected to human disease include those of the mitochondrial respiratory chain and DNA repair. Mutations in these genes do not completely inactivate the components, as this would most probably lead to lethality as observed in several model systems.

1. Friedreich ataxia and [Fe-S] assembly. Friedreich ataxia is a severe neurodegenerative disorder that results when frataxin levels are decreased by >70% (233). Upon frataxin deficiency spinocerebellar neurodegeneration, dysarthria, muscle weakness, cardiomyopathy, and tumor formation occur (218, 219). Friedrich ataxia normally develops during childhood where subjects generally pass away during early adulthood of cardiomyopathy. At the molecular level, subjects with Friedreich ataxia show inappropriate iron–sulfur protein activities such as deficiencies in complex I–III [Fe-S] enzymes in addition to accumulation of mitochondrial iron levels. Subjects with Friedreich ataxia have reduced aconitase and succinate dehydrogenase activities, and combined, this suggests that the biogenesis and/or stability of [Fe-S] are impaired (245, 271). It appears that frataxin's main function is to act as an iron binding chaperone during [Fe-S] assembly (29) where frataxin associates with ISCU and ferrochelatase enabling donation of iron to [Fe-S] (319). Frataxin deficiency also leads to iron accumulation in mitochondria in heart and brain tissue (189), and this accumulation may lead to mitochondrial damage by iron-catalyzed oxidation (15).

2. Sideroblastic microcytic anemia and Grx5. Sideroblastic anemia is characterized by red blood cell precursors having iron accumulation in mitochondria (38) and this is caused by mutation of GLRX5 (Table 1), which leads to defi-

ciency of human [Fe-S] assembly and cellular iron homeostasis (46, 247, 335, 337). The single mutation in GLRX5 exon 1 (penultimate nucleotide before the splice site) results in only 10% of wild-type GLRX5 mRNA expression. Subjects with sideroblastic anemia show low levels of aconitase and H-ferritin but with a concomitant increase in transferrin receptor where IRP1 becomes an active IRE binding protein that then blocks haem biosynthesis. In effect GLRX5 deficiency, resulting in inappropriate [Fe-S] biogenesis in mitochondria, would lead to [Fe-S]-deficient IRP1. Another possibility, based on iron homeostasis studies, may suggest that activation of the IRE-binding activity of IRP1 is due to GLRX5-induced mis-regulation of iron homeostasis resulting in cytosolic iron depletion and loss of cytosolic [Fe-S] assembly. Work on *shiraz* (*sir*) zebrafish mutants, caused by deficiency of *grx5*, uncovered a connection between haem biosynthesis and [Fe-S], indicating that hemoglobin production in the differentiating red cell is regulated through [Fe-S] cluster assembly (322). This result has been confirmed by human cell experiment that GLRX5 deficiency causes sideroblastic anemia by specifically impairing haem biosynthesis and depleting cytosolic iron in human erythroblasts (335).

3. Myopathy caused by ISCU mutation. Myopathy is a muscular disease and clinical analysis revealed that aconitase and succinate dehydrogenase activities are severely reduced and mitochondria are overloaded with iron in patient muscle samples (109). Unlike the yeast genome that contains two *ISCU*-like genes, *Isu1* and *IscU2*, humans only have one *ISCU* copy (Table 1). However, it encodes two ISU isoforms due to alternative splicing, named m-ISCU (mitochondrial form, principal form) and c-ISCU (cytosolic form) (299, 300), both of which are functional in terms of [Fe-S] biogenesis (161, 300). Complete inactivation of *ISCU* is thought to be lethal based on the result of yeast *isu1-isu2* double deletion mutant (263). However, a splice mutation caused by a homozygous point mutation G7044C at a potential splice site in *ISCU* was found among patients from northern Swedish descent, which is correlated with myopathy (191, 214). Another point mutation, G149A, was recently found in two patients with more severe myopathy who also bear the heterozygous G7044C mutation (141). Biochemical analysis revealed that ISCU expression was indeed dramatically diminished in the muscle samples of these patients (191, 214). Two pieces of evidence indicate that it is the ISCU deficiency that caused myopathy in these patients: (i) aconitase and succinate dehydrogenase are iron–sulfur enzymes; (ii) an siRNA-mediated silencing targeted ISCU caused the loss of mitochondrial and cytosolic aconitase activities (300).

4. Mitochondrial respiratory chain, DNA repair and tumorigenesis. Relatively recent data have suggested that iron–sulfur proteins are also involved in more common human diseases. The mitochondrial respiratory chain complex II enzyme succinate dehydrogenase functions as a tumor suppressor where impaired complex II activity results in succinate accumulation and inhibition of prolyl hydroxylase (107). This in turn results in loss of hydroxylation of hypoxia-inducible factor 1a, which translocates to the nucleus turning on genes responsible for tumor progression and cell survival. The lack of [Fe-S] formation in complex II of the mitochondrial respiratory chain may account for these observed effects.

Loss of p53 function occurs in many forms of cancers. In an approach to identify genes downstream of p53 that may mediate loss of apoptotic responses toward 5-fluorouracil, FDXR (Table 1) was identified (122). FDXR is a tumor suppressor localized to mitochondria and it has been suggested that FDXR contributes to p53-mediated apoptosis. However, a direct link to [Fe-S] biogenesis has not been established.

DNA helicases are essential components during DNA replication, recombination, repair, and transcription. The FancJ helicase protein involved in the Fanconi anemia repair pathway and XPD involved in nucleotide excision repair contain [Fe-S] (253). It has been demonstrated that the [Fe-S] in these two enzymes are essential for their activities and that loss of [Fe-S] in XPD and FancJ leads to loss of nucleotide repair and disease.

VII. Conclusions and Perspectives

It is clear from our current knowledge that [Fe-S] assembly and regulation represents a complex and fundamental biological process in all organisms studied. [Fe-S] biogenesis and the multitude of effects observed in response to malfunctioning [Fe-S] formation have clear implications beyond the laboratory bench.

In bacteria the predominant [Fe-S] biogenesis systems include ISC and SUF where regulatory aspects and cross-talk between the two systems are well established. Although the bacterial SUF system is operational in response to oxidative stress compared to the ISC system there are clear and direct links between the two machineries. For example, apo-IscR lacking an [Fe-S] can bind to the promoter region of the bacterial *suf* operon leading to activation in response to oxidative stress. Similarly, holo-IscR can bind to the promoter of the *isc* operon, which in turn represses *isc* operon expression. The fact that the ISC and SUF machineries are not separated into subcellular organelles may account for the more simple regulatory and communication aspect.

In yeast and higher eukaryotes the situation is more complex. In addition to harboring a cytosolic CIA [Fe-S] biogenesis machinery, dependent on at least the mitochondrial ISC machinery, the spatial sub-cellular distribution of the SUF, ISC, and CIA systems adds another level of complexity in terms of coordination of activities in response to endogenous and exogenous cues. In addition to fulfilling a role in [Fe-S] assembly, the mitochondrial ISC system is also responsible for ensuring appropriate ion homeostasis where correct cellular iron acquisition and mitochondrial iron accumulation are vital aspects. In plants the plastid-localized SUF system is of crucial importance where defective [Fe-S] formation leads to embryo lethality, but how the SUF machinery is interlinked with the mitochondrial ISC and cytosolic CIA machineries are still unclear.

Another interesting line of research relates to the parasite *Plasmodium* where several [Fe-S] biogenesis genes have been identified although many are still unknown. *Plasmodium* harbors both mitochondria and a simple plastid form, the apicoplast, and it will be interesting to learn how nonphotosynthetic plastid organelles contribute to the cellular need for [Fe-S].

It is becoming apparent that defective [Fe-S] biogenesis and iron homeostasis in humans has a direct effect on a variety of disease states beyond the classical neurodegenerative diseases and anemias. This is provoking renewed interest in the field, and although yeast has been used as a classical

model to unravel [Fe-S] biogenesis, emerging mouse models will undoubtedly shed light on both conserved and novel molecular mechanisms.

The field of [Fe-S] research still has exciting challenges ahead. First, there are a number of components that still need to be identified, and various research efforts are being undertaken to perform this task. Second, many of the molecular mechanisms underlying mutant and gain-of-function studies require detailed investigation to clarify to date the unclear mechanics of the system as whole. Related to this are the integration of the various machineries into a coherent cellular context, which is not a trivial task. Further, the effect on and the integrated nature of the [Fe-S] biogenesis pathways on other cellular mechanisms and pathways remain to be elucidated. Last but not least, the implications of [Fe-S] biogenesis on various known and newly discovered human disease states is important in efforts for better diagnosis, treatment, and patient care. The field of [Fe-S] research has clearly exciting times ahead.

Acknowledgments

This work was supported by The Norwegian Research Council and The Folke Hermansen Foundation for cancer research.

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Date of first submission to ARS Central, April 18, 2010; date of final revised submission, August 18, 2010; date of acceptance, September 2, 2010.

Abbreviations Used

ABC = ATP binding cassette
AtCIA = *Arabidopsis* CIA system
AtISC = *Arabidopsis* ISC system
AtSUF = *Arabidopsis* SUF system
BiFC = bimolecular fluorescence complementation
CD = circular dichroism
CIA = cytosolic iron–sulfur protein assembly
EPR = electron paramagnetic resonance
Fdx = ferredoxins
FDXR = Fdx reductase
[Fe-S] = iron–sulfur clusters
GFP = green fluorescent protein
GRX = GSH-dependent oxidoreductases
Grxs = glutaredoxins
GSH = glutathione
ISC = iron–sulfur cluster system
NIF = nitrogen fixation system
NMR = nuclear magnetic resonance
PLP = pyridoxal phosphate
PSI = photosystem I
SUF = sulfur utilization factor system
SufBCD = SufB-SufC-SufD
SufSE = SufS-SufE
YTH = yeast two hybrid

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