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Iron–Sulfur Cluster Biogenesis Systems and their Crosstalk

Xiang Ming Xu and Simon G. Møller*[a]

The biogenesis of iron–sulfur clusters ([Fe–S]) plays a very important role in many essential functions of life. Several [Fe–S] biogenesis systems have been discovered, such as the NIF (nitrogen fixation), SUF (mobilisation of sulfur) and ISC (iron–sulfur cluster) systems in bacteria, and the ISC-like and CIA (cytosolic iron–sulfur protein assembly) systems in yeast. Experimental evidence has revealed that SUF and ISC in bacteria communicate with each other partly through IscR to coordinate the utilisation of iron and cysteine. The ISC-like system in yeast is localised to the mitochondria, while the ISC-dependent CIA system is localised to the cytosol; this suggests a possible role for the ISC mitochondrial export machinery in mediating crosstalk between the two systems. Based on genetic analysis, the model plant Arabidopsis thaliana *contains three [Fe–S] biogenesis systems similar to SUF, ISC and CIA named AtSUF, AtISC and AtCIA. Possible communication between these three systems has been proposed.*

1. Introduction

Iron–sulfur clusters ([Fe–S])—derived from two of the most versatile and abundant elements on our planet—are important cofactors of [Fe–S] proteins involved in numerous important biological processes.[1] Although simple in structure, [Fe–S] biosynthesis requires intricate interplay of a large number of proteins and can be divided into three basic steps: formation of elemental sulfur, sulfur and iron cluster assembly, and cluster insertion into apo proteins. Most research on [Fe–S] biogenesis has come from studies in bacteria and yeast,[2–7] and recent research has shed light on the seemingly intricate pathways of [Fe–S] biogenesis in plants.[8–10]

Since the first [Fe–S] biogenesis system was revealed by Dean's group nearly 20 years ago,[6] three complete bacterial [Fe–S] biogenesis systems have been discovered, termed NIF (**ni**trogen **f**ixation), ISC (**i**ron–**s**ulfur **c**luster), SUF (mobilization of **su**l**f**ur). In yeast and higher nonphotosynthetic organisms only the ISC-like system exists in the mitochondria. The CIA (**c**ytosolic **i**ron–sulfur protein **a**ssembly) system is regarded as an iron–sulfur protein assembly system, and recent evidence revealed that the two CIA proteins, Cfd1 and Nbp35, can form a scaffold complex for [Fe–S] assembly in the eukaryotic cytosol.[11] CIA is therefore a cytosolic iron–sulfur cluster biogenesis system in eukaryotic organisms. Based on amino acid sequence similarity, most components of CIA have counterparts in *Arabidopsis*. ISC is ubiquitously found in prokaryotes and in the mitochondria of both nonphotosynthetic and photosynthetic eukaryotes, whilst SUF is present in bacteria and plant plastids.

Although our knowledge about the mechanisms of [Fe–S] biogenesis in these systems is good, and the mode of action of several of the individual proteins is known, we still have little insight into the molecular and cellular relationship between individual elements and different systems. In this review we will provide insight into the crosstalk between different [Fe–S] biogenesis systems in bacteria, yeast and plants. As the NIF system generally only exists in nitrogen fixing bacteria, it will not be discussed here.

2. Bacteria: Crosstalk between ISC and SUF

In *Escherichia coli*, there are two [Fe–S] biosynthesis systems, ISC and SUF (Figure 1). The ISC system consists of IscR, IscA, IscS, IscU, HscA, HscB, Fdx and CyaY, and all genes except c*yaY* exist as a gene cluster in which *iscRSUA* forms an operon. IscR is a feedback repressor of this operon,[12] which will be discussed below. The group I cysteine desulfurase IscS mobilises sulfur from cysteine to the scaffold protein IscU, and both IscS and IscU represent key elements of ISC. Due to the excellent work of many research groups, there is now no controversy regarding their function.[2,4–6] CyaY, a frataxin-like protein, functions as an iron donor in the assembly of [Fe–S],[13,14] and the sulfur from IscS and iron from CyaY are used to assemble [Fe–S] on the IscU scaffold. Ferredoxin Fdx provides the necessary electron[15,16] (Figure 1). The specific functions of the chaperone/cochaperone HscA/HscB are still unknown, but they are speculated to mediate the transfer of synthesised [Fe–S] to IscA either indirectly, by acting as a carrier, or directly to [Fe–S] apo proteins (Figure 1). Although IscA might act as an [Fe–S] carrier, much research suggests that IscA is a scaffold protein.[17–19] However, there is no consensus regarding the number and the nature of [Fe–S] associated with IscA. Furthermore, this role has been challenged by IscA's possible role as an iron donor to IscU.[20,21] The ISC system appears to be a housekeeping system.

The SUF system harbours six protein members. SufA, which is a homologue of IscA, is thought to be a scaffold protein within the SUF system.[4] SufB, SufC and SufD are ABC (**A**TP-**b**inding **c**assette) superfamily members that form a protein complex.[22,23] SufC exhibits ATPase activity, which might provide energy for the [Fe–S] biogenesis process. Further, SufB

[a] *X. M. Xu, S. G. Møller*
Centre of Organelle Research, Faculty of Science and Technology
University of Stavanger, Stavanger 4036 (Norway)
Fax: (+47) 51-83-17-50
E-mail: simon.g.moller@uis.no

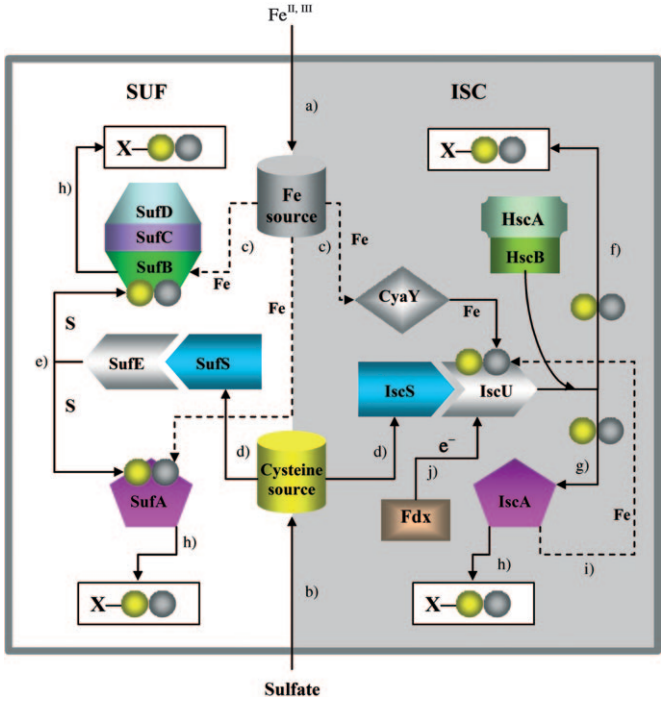

Figure 1. SUF-and ISC-mediated iron–sulfur cluster ([Fe–S]) biogenesis systems in bacterial cells. A white background indicates harsh/stress conditions and a grey background indicates normal conditions. a) Uptaken iron is stored within proteins (probably ferritin) that act as an iron source. b) Uptaken sulfate is assimilated to cysteine as a sulfur source. c) Stored iron might be redistributed directly or through other carriers to SufB, SufA, CyaY and possibly to IscA; the latter two deliver iron to IscU. Dotted lines indicate that these processes have not been confirmed. d) Cysteine is used by IscS or SufS to release sulfur, which can be accepted by IscU from IscS to assemble [Fe–S], or by SufE from SufS, and then e) transferred to SufB or SufA to assemble [Fe–S]. f) [Fe–S] synthesized on IscU is transferred to apo protein (X), likely with the help of the HscA–HscB chaperone–cochaperone complex, or g) to [Fe–S] carrier protein IscA, first for transient storage, and then h) to apo protein (X). i) IscA might also provide iron to IscU. j) Fdx can provide electrons to IscU for [Fe–S] synthesis, but an electron provider for the SUF system is not known.

has been discovered recently to bind a [4Fe–4S] cluster and to interact with SufE; this allows sulfur transfer from SufE to SufB,[24] and suggests a probable scaffold function for SufB. SufS, which is homologous to IscS, is a group II cysteine desulfurase the activity of which can be enhanced dramatically through interaction with SufE and SufBCD[23–25] (Figure 1). In contrast to ISC, SUF is specifically adapted to oxidative stress and iron starvation. For more detailed information about bacterial ISC and SUF, please see these excellent reviews [2,4–6].

Although ISC and SUF are two distinct [Fe–S] biogenesis systems, they have similar net functions, which dictates that they need to communicate with each other to provide appropriate levels of [Fe–S] under different growth conditions and to maintain iron and sulfur homeostasis.

2.1 Overlapping function of ISC and SUF

The SufA protein shares 40% sequence identity with IscA, and all analyses to date indicate that both proteins possess similar functional properties.[2,5,6] A series of in vitro studies have shown that SufA and IscA can act as interchangeable scaffold proteins; [Fe–S] biotin synthase (BioB) can be reconstituted by either holo-IscA or holo-SufA.[2] Equally, IscS is similar to SufS in that both proteins act as cysteine desulfurases during [Fe–S] biosynthesis. More surprisingly, although SufE is highly dissimilar in sequence to IscU, structural data have demonstrated that SufE is an IscU-like protein.[26] Therefore, it appears that SufS-mobilized sulfur from cysteine is transferred to SufE, whilst within the ISC system IscS releases sulfur followed by transfer to IscU. It will be interesting to investigate whether SufE can also functionally complement IscU.

At the primary sequence and structural level the SufBCD complex and the HscAB complex show no similarity. The only similarity between the two protein complexes is their ability to hydrolyse ATP, and thereby presumably provide energy for [Fe–S] biosynthesis. As far as we are aware, it is not known whether SufBCD can complement HscAB deficiency.

Although in silico analysis and genetic evidence for pathway coordination is helpful, experimental verification is vital. Several studies have now confirmed that ISC and SUF have overlapping functions in bacteria; lethality is only observed upon deletion of both the *isc* and *suf* operons, and furthermore, overexpression of the *suf* operon compensates for the absence of a functional ISC.[27,28] Despite these compensatory mechanisms, over-expression of the *suf* operon appears toxic, as elevated expression of the entire *suf* operon in the *isc* deletion mutant YT1014 has an inhibitory effect on growth.[28] In contrast, there is no experimental evidence to confirm whether excessive expression of the *isc* operon is harmful to bacteria. However, since excessive expression of the *isc* operon is tightly regulated by IscR autoregulation, it is highly likely that *isc* over-expression is detrimental to cells.

In conclusion, experimental evidence has confirmed that ISC and SUF are two distinct but overlapping systems, and suggests a level of communication between them.

2.2 Coordinating the use of iron and cysteine between ISC and SUF

In contrast to higher eukaryotes, bacteria lack organelles, and because of this SUF- and ISC-mediated [Fe–S] biosynthesis takes place in the same cellular compartment. As they have similar overall functions, coordination between SUF and ISC will undoubtedly be important for the regulated utilisation of iron and cysteine resources.

Sulfate taken up by bacteria is usually assimilated into cysteine for sulfur storage and is distributed for usage in, for example, [Fe–S] and GSH (glutathione) synthesis. Sulfur released from cysteine by cysteine desulfurases is accepted by SufE or IscU, and demonstrates that in bacteria the activity of cysteine desulfurases SufS and IscS determines sulfur flow to either SUF or ISC, respectively (Figure 1). Under normal conditions, ISC plays a housekeeping role for [Fe–S] synthesis and utilizes most of the sulfur, whilst SUF most probably only plays a minor role. However, when iron or sulfur metabolism is disrupted by iron starvation or oxidative stress, SUF plays a major role for [Fe–S] biosynthesis.[23]

Due to its toxicity, iron must also be assembled onto storage proteins. The most likely storage-protein candidate is the high iron affinity protein ferritin, which could then act as an iron source (Figure 1). Even though ferritin is the most probable iron source, no direct evidence has shown that ISC or SUF can acquire iron from ferritin. CyaY is an iron provider for ISC, but it is not clear whether CyaY can accept iron from ferritin. Furthermore, it is not known what provides iron for the SUF system. Combined, the lack of direct evidence for iron-provider proteins makes it challenging to analyze iron distribution between the two systems.

The regulation of ISC and SUF plays a key role in coordinating the distribution of iron and cysteine between the two systems (Figure 2). In ISC, regulation is controlled by IscR. IscR has two forms, holo- and apo-IscR. Holo-IscR contains a [2Fe–2S] cofactor, which can bind to the promoter of the *isc* operon (*iscRSUA*)[12] to inhibit its expression. When [Fe–S] is impaired or lost due to its sensitivity to oxygen, IscR converts to the apo form and is then released from the *isc* promoter; this results in the expression of *isc* operon.

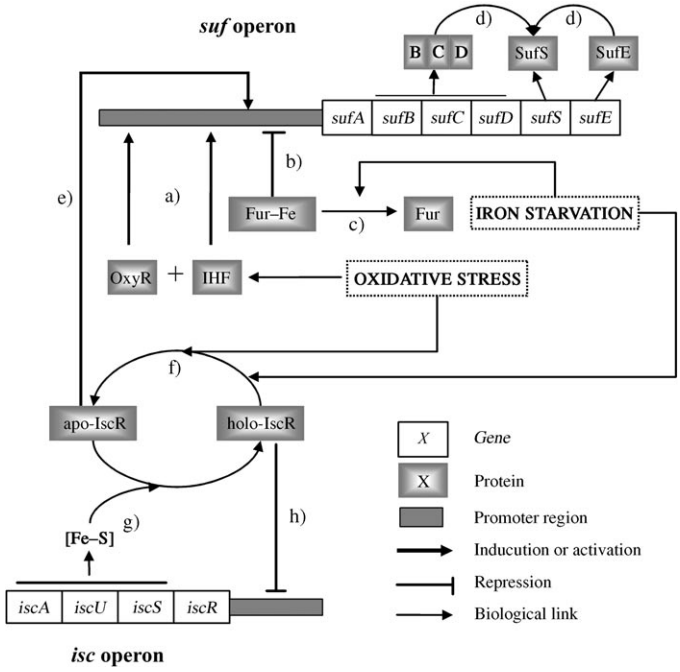

Figure 2. Regulation of the SUF and ISC pathways. a) Oxidative stress conditions induce *suf* operon (SufABCDSE) expression by causing OxyR to bind to the ORE-I site (−236 to −197 nucleotides upstream of the transcription start) and IHF to bind to ORE-II (−156 to −127).[31] b) The iron-rich form of Fur (Fur–Fe) binds at the promoter site (−32 to −3) and represses the transcription of the *suf* operon under normal conditions.[61] c) During iron starvation, Fur–Fe loses iron and Fur turns into its inactive form, which is unable to bind to the promoter; this results in derepression of the *suf* operon.[27,61] d) The SufE and SufBCD complex can enhance the cysteine desulfurase activity of SufS.[23,25] e) Apo-IscR (without [Fe–S]) can bind to the ORE-III site (−56 to −35) of the *suf* operon promoter and activate its expression to an almost equal level to OxyR in response to oxidative stress.[31] f) Stress conditions affect expression of the *isc* operon (iscRSUA—hscAB-Fdx is not part of the *isc* operon) through IscR,[27] which can be converted from holo-IscR to apo-IscR under these conditions. g) Expression of the *isc* operon facilitates [Fe–S] synthesis, which enables assembly of holo-IscR. h) Holo-IscR binds to the *isc* operon promoter and induces expression.[12] Under harsh conditions, [Fe–S] is impaired or lost; this leads to the formation of apo-IscR, which is unable to bind the promoter and the *isc* operon is inactivated.

The *suf* operon (*sufABCDSE*) is controlled by OxyR, IHF, Fur and apo-IscR[27,29] (Figure 2). OxyR and IHF bind to different regions of the *suf* promoter and activate its expression mainly under conditions of oxidative stress. The iron-rich form of Fur (Fur–Fe) normally binds to the *suf* promoter and represses its expression, whilst 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.

Interestingly, it has been demonstrated that IscR can regulate both the ISC and SUF systems.[30,31] 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 conditions.[30,31] Apo-IscR contributes almost equally to OxyR to activate the *suf* operon in response to oxidants.[31]

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 (Figure 2). 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 the ISC. On the other hand, when bacteria are grown under oxidative-stress and iron-starvation conditions, which are detrimental to [Fe–S], IscR mainly occurs in its apo form. Even though ISC is active, 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 (Figure 2).

3. Yeast: Crosstalk between CIA and ISC

Elegant work in the group of Roland Lill and others has established a well-defined mitochondria-localized [Fe–S] biogenesis ISC-like system and a cytosolic-localized CIA (**c**ytosolic **i**ron-sulfur protein **a**ssembly) [Fe–S] biogenesis system in yeast.[3,32,33] Yeast ISC not only provides [Fe–S] for mitochondrial proteins, but also has an important role for cytosolic [Fe–S] biogenesis, whilst CIA is essential for the maturation of iron–sulfur proteins in both the cytosol and nucleus. Because of this spatial [Fe–S] biosynthesis distribution, coordination in yeast is more complicated than in bacteria, and the fact that yeast contains many more ISC components than in bacteria strengthens this argument. For example, sulfur transfer to Isu1, which is the IscU homologue in yeast, requires Isd11 to form a complex with Nfs1,[34] whilst Mge1, which is a GrpE-like protein, is responsible for ADP/ATP exchange on Ssq1—the HscA homologue in yeast.[35] Further, Grx5, which is a monothiol glutaredoxin, is required after [Fe–S] assembly involved in transferring [Fe–S] to apo proteins.[36] Recent reviews[32,33] have presented excellent information regarding the function of all ISC ele-

ments and a good model for ISC- and CIA-mediated [Fe–S] biosynthesis.

Four CIA components have been identified to date and shown to be essential for [Fe–S] biosynthesis in the cytosol and nucleus.[32,33] Cfd1 and Nbp35 are P-loop NTPases and can form a stable protein complex, which can act as a cytosolic scaffold for [Fe–S] synthesis.[11] This raises the question: which components provide sulfur and iron for CIA?

It has become clear that CIA is a mitochondria-dependent system.[33] As cysteine desulfurase in yeast is found predominantly in the mitochondria, and the frataxin-like iron donor protein Yfh1 is also localized to mitochondria, [Fe–S] synthesis in the cytosol must dependent highly on mitochondrial functions. Nbp35 can further interact with the [Fe–S] protein Nar1 when [Fe–S] is transferred from Nbp35 to Nar1. Therefore, it appears that CIA is not a complete [Fe–S] biosynthesis system, and that mitochondrial export of a still unknown component(s) could be important in linking the yeast ISC and CIA systems together.[33]

In mitochondria, the export machinery associated with [Fe–S] biosynthesis consists of three components: Atm1, Erv1 and GSH (glutathione). Atm1 is an ABC transporter that is located in the inner membrane of mitochondria.[37] It exports a still unknown compound that is required for cytosolic and nuclear [Fe–S] protein maturation and iron-uptake regulation.[33] Erv1 is a sulfhydryl oxidase in the intermembrane space and is also required for protein import.[38] The other element of export machinery is GSH, but it is still unclear what role GSH plays in the export process, since GSH is the major redox buffer in yeast and is involved in detoxification processes and protection against oxidative stress.[33,39]

As cytosolic [Fe–S] protein maturation depends on ISC and the Atm1–Erv1–GSH export machinery, the activity of ISC and Atm1–Erv1–GSH most probably determines the ability of CIA in providing [Fe–S] for the cytosol and nucleus. The transport activity of Atm1 depends on its ATPase activity, which is stimulated by peptides that contain multiple cysteine residues. It has therefore been speculated that the physiological substrates of Atm1 include [Fe–S] stabilized by peptides, a sulfur compound needed for incorporation into cytosolic/nuclear [Fe–S], or a compound needed as a cofactor for the CIA system which contains free sulfhydryl groups.[33]

4. *Arabidopsis*: Possible Crosstalk between AtCIA, AtISC and AtSUF

The extensive and informative research on bacteria and yeast has encouraged an escalation in intensity within plant [Fe–S] biogenesis research. Based on genetic analysis, *Arabidopsis* harbours three [Fe–S] systems similar to ISC, SUF and yeast CIA (Figure 3). Most SUF-like components apart from AtSufE1, which shows dual localization in both plastids and mitochondria,[40,41] have been confirmed to be localized to plastids.[8,9] Together with the plastidic scaffold-like proteins AtNFU1, AtNFU2 and AtNFU3, these components constitute a functional AtSUF system (Figure 3).

The two important elements of the ISC-like system, AtIscS and AtIscU, are localized in mitochondria as are AtNFU4 and AtNFU5. Moreover, plants also contain homologues to IscA, HscA/HscB, Fdx (At4g21090 and At4g05450), CyaY (At4g03240), Isd11 (At5g61220) and Mge1 (At4g26780 and At5g55200), which are predicted to be localized in mitochon-

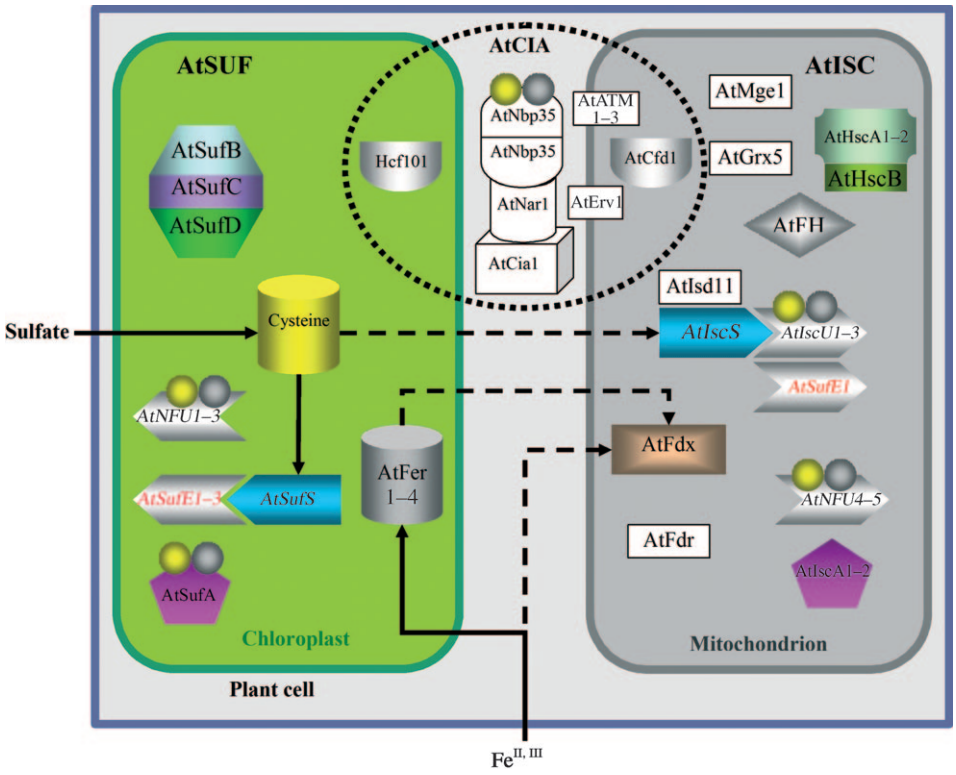

Figure 3. [Fe–S] biogenesis systems in *Arabidopsis*. Coloured objects indicate that these elements have homologues in both bacteria and yeast; the colourless objects only have homologues in yeast. The relationship between all of the proteins shown is not clear except: 1) in plastids, AtSufB, AtSufC and AtSufD can form an ATPase complex that might provide energy for [Fe–S] biogenesis in chloroplasts; 2) AtSufS of plastids and AtIscS of mitochondria are cysteine desulfurases; 3) in plastids, AtSufE1–3 can interact with AtSufS and accelerate its activity; 4) AtSufE1 is also localized in mitochondria and enhances the activity of AtIscS; 5) AtSufA and AtNFU1–2 act as scaffold proteins in plastids;[64–67] 6) AtIscU3–5 are scaffold proteins in mitochondria.[62,68] An extended description of AtSUF, AtISC and AtCIA is presented in the text. All functional and interaction data regarding AtISC-like AtHscA/AtHscB, AtFH, AtFdx, AtFdr, AtMge1, AtGrx5, AtIsd11 and nearly all AtCIA components are based on genetic analysis. To date, little has been reported on these elements. Although the assimilation pathway for sulfate and iron is to some extent clear, their delivery and redistribution inside plant cells is not clear (dashed lines).

Table 1. AtCIA (CIA-like and related mitochondria export elements in *Arabidopsis*).

Proteins in yeast	Homologues in *Arabidopsis*	Localization	Name
Nbp35, Cfd1	AT5G50960	cytosol[a]	AtNbp35
	AT4G19540	mitochondria[a]	AtCfd1
	AT3G24430	chloroplast[b]	HCF101
Nar1	AT4G16440	cytosol[a]	AtNar1
Cia1	AT2G26060	cytosol[a]	AtCia1a
	AT4G32990	cytosol[a]	AtCia1b
Atm1	AT4G28630	mitochondrial membrane[b]	AtATM1
	AT4G28620	mitochondrial membrane[b]	AtATM2
	AT5G58270	mitochondrial membrane[b]	AtATM3
Erv1	AT1G49880	mitochondria[b]	AtErv1
GSH (glutathione)	synthesized by GSH1 and GSH2		

[a] Predicted; [b] confirmed by experiment.

dria (Figure 3). By using the Grx5 sequence as an input to search for *Arabidopsis* homologues, many putatively corresponding loci can be identified, but to date it is unclear which locus is the real AtGrx5. All these together form a mitochondrial [Fe–S] biogenesis system named AtISC. Although no CIA-like components have been confirmed in *Arabidopsis*, sequence prediction analysis by using yeast CIA proteins as inputs has revealed a putative CIA-like system. Further in silico predictions suggest that Nbp35-, Nar1- and Cia1-like proteins might be cytosolic (Table 1, Figure 3). Cfd1 has two homologues in *Arabidopsis*; Hcf101 has been confirmed to be plastidic, whilst AtCfd1 is predicted to be mitochondrial. Together with mitochondrial export machinery homologues, they form the third system named AtCIA, and are predicted to serve the need for [Fe–S] by the cytosol and nucleus in *Arabidopsis* (Table 1, Figure 3).

Even though much progress has been made in deciphering the functions of the AtSUF and AtISC components, their relationship to each other and to AtCIA—a completely novel and putative system in plants—is far from clear. Since much research on *Arabidopsis* [Fe–S] biogenesis and crosstalk is based on progress from bacteria and yeast, an attempt is made (see below) to outline the *Arabidopsis* [Fe–S] biogenesis systems and their relationship, with reference to bacteria and yeast.

4.1 Relocation of cysteine and iron

As [Fe–S] biogenesis is central to iron and sulfur metabolism (Figure 4) its analysis will increase our understanding of iron and sulfur homeostasis in plants. Iron in plants cannot only be used for [Fe–S] synthesis, but also for heme assembly.[42] Cysteine is the primary product of the sulfur assimilation that occurs in plastids. Sulfur is not only used for [Fe–S] synthesis, but also to generate sulfur-rich proteins (SRPs, including thionins), GSH, glucosinolates and phytoalexins[43] (Figure 4).

Storage and buffering of iron at the subcellular level are crucial mechanisms that allow plants to cope with iron scarcity and toxicity. Although iron can be stored in the apoplast, organelles such as vacuoles and plastids, play a key role in the intracellular compartmentalization of iron. More than 90% of the iron in leaf cells is located in chloroplasts,[44] and in plastids the ferritins can store an important fraction of cellular iron.[42] Ferritin is a globular protein complex that consists of 24 protein subunits with the ability to store up to 4500 atoms of iron. It is the main intracellular iron storage protein in both prokaryotes and eukaryotes, and has the ability to keep iron in a soluble and nontoxic form. These proteins play various roles related to iron homeostasis during development or in response to environmental stresses. *Arabidopsis* contains four ferritin-encoding genes, (*AtFer1–4*) all predicted with high confidence to be localised to plastids;[45] this suggests that iron storage is mainly constrained to organelles.

Similar to bacteria, sulfur is taken up as sulfate in plants and is then incorporated into cysteine.[43] Sulfur assimilation occurs through the cysteine biosynthetic pathway, which is located in plastids; this indicates that plastids are the main source of cysteine in cells.[43,46]

As most cysteine and iron is be redistributed from plastids, communication between AtISC, AtSUF and AtCIA is essential for balancing the need for iron and cysteine for [Fe–S] biosynthesis in different organellar compartments. Iron and cysteine flux across the plastid membranes is therefore vital for cytosolic and mitochondrial [Fe–S] biosynthesis.

To date, few proteins involved in iron transport across plant organellar membranes have been characterized. However, recently the permease PIC1 (**p**ermease **i**n **c**hloroplasts **1**) was shown to function in iron transport across the inner envelope of chloroplasts and hence in cellular metal homeostasis,[47] but

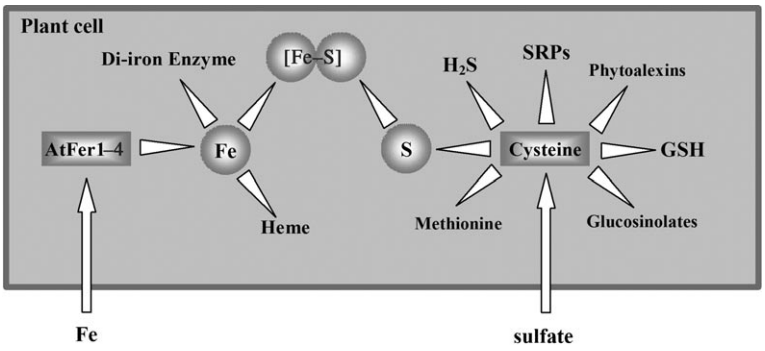

Figure 4. Iron and sulfur metabolism in plant cells. Sulfate taken up into the plant cell is incorporated into cysteine through the cysteine biosynthetic pathway, which is localized to plastids;[43,46] this highlights the importance of plastids in storing and redistributing cysteine in plant cells. The primary product of sulfur assimilation is incorporated into [Fe–S], SRPs (sulfur-rich proteins) and GSH and used to synthesize glucosinolates, phytoalexins, methionine.[43] Iron taken up by plant cells will most likely be stored within the ferritins AtFer1–4, which are all localized in plastids,[45] and iron is mainly used to generate [Fe–S] and heme.

it is not clear whether this protein is responsible for both iron uptake and efflux in chloroplasts.

Cysteine is a neutral amino acid the allocation of which is mediated by AAP permeases in *Arabidopsis*.[48] However, none of these has been confirmed to be cysteine-specific transporters. In yeast, cysteine transport is mediated by at least eight different permeases that are not specific for cysteine. Recently a new transporter, Yct1p, was found to have high-affinity towards cysteine, and transport experiments have revealed that Yct1p is the major contributor to cysteine transport.[49] Disappointingly, Yct1p does not have homologues in *Arabidopsis*. Whether there are cysteine-specific permeases in *Arabidopsis* remains an open question, but it is tempting to speculate that plant-specific permeases will be crucial for the redistribution of cysteine and other amino acids between different subcellular compartments in plant cells.

An interesting discovery was recently made by Heeg et al. who showed that mitochondria and not plastids play the most important role for cysteine synthesis in *Arabidopsis*. However, their data demonstrate that cysteine and also sulfide must be sufficiently exchangeable between the cytosol and organelles; this supports our notion outlined above.[50]

4.2 Communication between AtSUF, AtISC and AtCIA

In bacteria, genes that encode the ISC or SUF components form gene clusters or operons, and the regulation of these two systems is simpler than the regulation of eukaryotic cells. In *Arabidopsis*, genes that encode AtSUF or AtISC components are scattered throughout the genome and have partly unknown and complicated expression patterns. Three SufE-like proteins (AtSufE1–3) have been characterized and shown to accelerate the cysteine desulfurase activity of AtSufS.[40,41,51] AtSufE2 and AtSufE3 are plastidic proteins, whilst AtSufE1 is localized to both the plastid and mitochondria. Detailed experimentation has demonstrated that AtSufE1 localises initially to plastids followed by localisation to the mitochondria, which could explain slightly contradictory reports.[40,41] Further evidence of dual localisation has come from in vivo protein–protein interaction experiments in which AtSufE1 and the mitochondrial protein AtIscS (AtNfs1) show a clear interaction in mitochondria.[40] In addition, AtSufE1 can enhance the activity of AtIscS in mitochondria, and acts as a possible regulator of the AtISC system. AtSufE3 contains SufE- and NadA-like (bacterial quinolinate synthase) domains and not only stimulates cysteine desulfurase activity, but also acts as a quinolinate synthase.[51] AtSufE3, therefore, appears to be a quinolinate synthase in *Arabidopsis*.[51] The combined activities of AtSufE1–3 in plastids and AtSufE1 in mitochondria will most probably influence cysteine distribution between the AtSUF and AtISC pathways (Figure 3).

AtNAP1 (AtSufB) is the counterpart of SufB in bacteria that interestingly has acquired ATPase activity during evolution.[52] This is not entirely surprising considering that the oxidative environment in the chloroplast requires continuous [Fe–S] repair and/or synthesis. The activity of AtNAP1 is affected by iron levels; this indicates that it might also relate to iron homeostasis.[52] The *suf* operon in bacteria is regulated by Fur, which requires iron to repress this operon and also allows iron to be utilized for other processes, such as heme synthesis. AtNAP1 is the first AtSUF component found to be affected by iron levels. Interestingly, AtNAP1 can interact with AtSufE (X.M.X. and S.G.M., unpublished data), which is in agreement with recently published bacterial data,[24] but further work is needed to confirm that it can also assemble [Fe–S]. AtNAP7 (AtSufC) is another ATPase that can complement a bacterial SufC mutant under oxidative stress conditions.[53] Further, interactions between AtNAP1 and AtNAP7 and between AtNAP7 and the SufD-like protein AtNAP6[52,53] have been firmly established; this confirms the presence of a SufBCD complex in *Arabidopsis* plastids. Whether AtSufBCD can acquire iron and if this activity is regulated by AtSufEs remain to be elucidated, but are exciting prospects. In the mitochondria, iron acquisition and donation for [Fe–S] biosynthesis depend partly on the frataxin-like protein AtFH.[54,55] However, whether AtSufBCD and AtFH coordinate to balance iron distribution between AtISC and AtSUF is not clear.

An interesting aspect of the AtCIA system is that the yeast cytosolic Cfd1 homologues in *Arabidopsis* have been spatially separated during evolution: HCF101 is localized to chloroplasts and AtCfd1 to mitochondria (Figure 3). Based on our knowledge about yeast, it is reasonable to assume that AtNbp35 forms dimers in *Arabidopsis*, which might act in a similar fashion to the yeast Cfd1–Nbp35 complex, as a scaffold for [Fe–S] assembly. The HCF101 protein is plastid-localized and involved in [4Fe–4S] biosynthesis. The *hcf101* mutant is embryonic lethal and impaired with respect to photosystem I (PSI), which contains three [4Fe–4S], but has normal levels of [2Fe–2S]-containing proteins, such as ferredoxin and PetC.[56] AtCfd1 is predicted to be mitochondrial, but its function has not yet been characterized (Figure 3).

4.3 Crosstalk between AtISC and AtCIA

In the *Arabidopsis* genome, homologues of all the yeast ISC-related export machinery components exist, which are regarded as part of AtCIA (Table 1). Among them, the Atm1 homologues AtATM1–3 are confirmed mitochondrial proteins and AtATM1 and AtATM3 can complement the yeast *atm1* petite phenotype;[57] this indicates that they have transmembrane transport properties. The homologue of Erv1 in *Arabidopsis*, AtErv1, exhibits all of the characteristic features of the Erv1 protein family, which include a redox-active YPCXXC motif, noncovalently bound FAD and sulfhydryl oxidase activity.[58,59] Transient expression of an AtErv1–GFP fusion protein in plant protoplasts showed preferential localization to mitochondria. Moreover, in vitro assays with purified protein and artificial substrates have demonstrated a preference of AtErv1 for dithiols with a defined space between the thiol groups; this suggests a thioredoxin-like substrate[58] (Table 1). Glutathione (GSH) is the major redox buffer in yeast and is also involved in ISC-related export. In *Arabidopsis*, GSH is synthesized in two steps. The first step is controlled by γ-glutamate cysteine ligase (GSH1), which is exclusively located in plastids, and the second step by

glutathione synthetase (GSH2), which is located in both the cytosol and plastids.[60] Based on the above, *Arabidopsis* might have a mitochondrial export machinery that is involved in mediating cytosolic [Fe–S] assembly, similar to that of yeast (Figure 3). If plants have retained the function of the yeast export machinery, the *Arabidopsis* mitochondrial export complex should play a key role in AtISC and AtCIA crosstalk.

5. Conclusions

In bacteria, the SUF and ISC component genes are organized into operons or gene clusters and together with the lack of membrane-bound organelles, SUF and ISC communication and regulation are well understood (Figures 1, 2). Similarly in yeast, the mitochondrial ISC and the cytosolic CIA system have been well studied. However, although it is fairly clear that CIA-dependent [Fe–S] synthesis is dependent on ISC, their regulation at the gene and protein level remains largely unknown. Here, we have presented three [Fe–S] biogenesis systems AtSUF, AtISC and AtCIA from *Arabidopsis* and their possible crosstalk largely based on genetic analysis. Even though we have only just started to understand the communication between these seemingly interlinked [Fe–S] biogenesis systems, the evolutionary comparison of crosstalk between spatially separated fundamental biological processes paves the way for an exciting future within [Fe–S] biogenesis research.

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