

The Cell's Cookbook For Iron–Sulfur Clusters: Recipes For Fool's Gold?

Janneke Balk and Roland Lill*^[a]

1. Introduction

Iron–sulfur (Fe–S) proteins are present in all forms of life and perform essential roles in, for example, respiration, photosynthesis, and nitrogen fixation. They contain prosthetic groups of complexed iron and “acid-labile” sulfide. These Fe–S clusters were identified about 40 years ago;^[1] however, it was not until the late 1990s that biosynthetic routes for *in vivo* assembly started to emerge.^[2] The last few years have seen great strides in our knowledge of the proteins involved in Fe–S cluster assembly, the core biochemical mechanism, and the specific features of the biosynthesis pathway in eukaryotes. This review will focus on what has been learned so far from Fe–S protein biogenesis in baker's yeast, a model organism for higher eukaryotes. Certain aspects of this topic have been discussed in more detail in recent reviews: comparison of Fe–S cluster assembly in bacteria and eukaryotes,^[3] the role of chaperones in Fe–S cluster biogenesis,^[4] and a pathological perspective.^[5]

The most common Fe–S clusters found in nature are the rhombic [2Fe–2S] and the cubane [4Fe–4S] clusters (Figure 1).

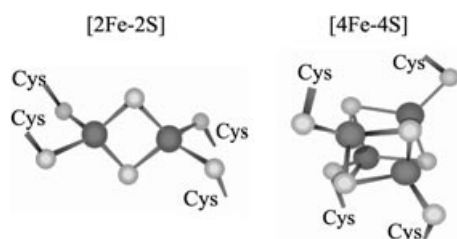


Figure 1. Simple Fe–S clusters. The rhombic [2Fe–2S] and cubane [4Fe–4S] clusters are most commonly found as protein cofactors. Fe^{2+/3+} is indicated in dark grey, S²⁻ in light grey. The Fe^{2+/3+} ions are most frequently liganded by the sulfur atoms of cysteine residues.

The [4Fe–4S] cluster can be seen as two modules of [2Fe–2S] and interconversion can take place, both by chemical induction and in biology.^[6] More complex clusters are also found.^[7,8] For example, Fe-only hydrogenases contain a so-called H cluster in their reactive center that is composed of two subclusters, a cubane [4Fe–4S] cluster and a binuclear [2Fe] subcluster, bridged by a cysteine sulfur atom. The FeMo protein of nitrogenase contains two types of complex metal clusters, the P cluster and the FeMo cofactor. The P cluster can be described in its reduced form as two regular [4Fe–4S] clusters that share a corner sulfur atom, an arrangement resulting in an [8Fe–7S] cluster. The FeMo cofactor ([Mo–7Fe–9S]) has a similar modular structure as the P cluster in that it consists of two bridged,

partial cubanes. One iron center is replaced by molybdenum and the partial cubanes are linked by three sulfur atoms and a light (N, C, or O) atom. In most Fe–S proteins, the iron atoms of the cluster are liganded by four cysteinyl residues, but there are exceptions. For instance, in the Rieske protein, the [2Fe–2S] cluster is liganded by two cysteinyl and two histidyl residues. Furthermore, CO and CN[−] may serve as ligands to the iron atoms of the hydrogenase active site. The number, nature, and spacing of the amino acid ligands, together with the protein environment, determine the chemical properties of the Fe–S cofactor.

The versatility of Fe–S clusters results in a surprisingly wide range of functions of Fe–S proteins. (For a detailed review, see ref. [9].) First, Fe–S proteins are abundant as electron-transfer proteins in ferredoxins, the respiratory chain, and photosynthetic complexes. For example, complex I of the bacterial or mitochondrial respiratory chain is a large, multisubunit assemblage containing 8–9 Fe–S clusters. Further down the respiratory chain, Fe–S clusters are found in subunit 2 of succinate dehydrogenase and the aforementioned Rieske protein of cytochrome c reductase. Second, Fe–S clusters can also serve as the catalytic sites of enzymes. In aconitase, one of the iron atoms of the [4Fe–4S] cluster has no protein ligand and functions as a Lewis acid to abstract a hydroxyl group and proton from adjacent carbons of the substrate, citrate. More recently, it has been observed that [4Fe–4S] clusters can serve as primary electron donors in initiating reactions that require a free radical, for example, in the anaerobe (class III) ribonucleotide reductase and in biotin synthase. Interestingly, Huber and Wächtershäuser have proposed that (nickel-)iron–sulfur surfaces may have provided life's earliest catalysts and, as such, Fe–S clusters may have played an important role in the origin of life.^[10] A third function of Fe–S proteins is in the sensing of oxygen, superoxide anions, iron, or even whole Fe–S clusters linked to the regulation of transcription or translation.^[11] Classical examples are FNR, the oxygen-sensing transcriptional regulator that governs the switch from anaerobic to aerobic growth in *Escherichia coli* and other bacteria, and mammalian IRP-1, a homologue of aconitase that regulates iron uptake and storage in the cytosol by a cunning translational mecha-

[a] Dr. J. Balk, Prof. R. Lill
Institut für Zytobiologie und Zytopathologie
Philipps-Universität Marburg
Robert-Koch Strasse 6, 35033 Marburg (Germany)
Fax: (+49) 6421-286-6414
E-mail: lill@mail.uni-marburg.de

nism. Fourth, Fe–S clusters can have a structural function. In a sense, this is the case in FNR, where oxygen converts the [4Fe–4S]-bridged dimer into [2Fe–2S]-containing inactive monomers. A strictly structural function has been assigned to the [4Fe–4S] cluster in the *E. coli* endonuclease III and its evolutionary pervasive homologues, in which the cluster is necessary to stabilize the DNA binding site.

2. Mitochondria, the Essential Cooking Pot

How do apoproteins, after synthesis by ribosomes, acquire their Fe–S cofactor(s)? For a long time, it was thought that the simple Fe–S clusters assemble spontaneously, because they could be reconstituted on apoproteins *in vitro*, by using iron salt, inorganic sulfide, and a sulfhydryl reagent under anaerobic conditions. However, because of the toxicity of Fe^{2+/3+} and S²⁻, the protocols applied are unlikely to resemble the process that takes place in a living cell. Instead, specific enzymes are required at various stages of Fe–S protein biogenesis, including assembly, insertion, and repair of the Fe–S clusters. In eukaryotic cells, further components and complexity of the biosynthetic route are necessary to ensure the presence of Fe–S cofactors in different cell compartments (Figure 2). Most of

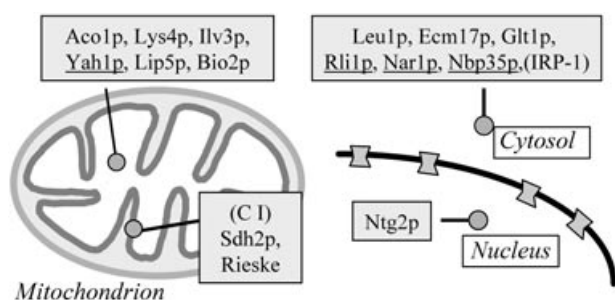


Figure 2. Subcellular localization of Fe–S proteins. In eukaryotic cells, Fe–S proteins have been localized to the mitochondria, cytosol, and nucleus. Proteins that are essential for viability in baker's yeast are underlined. Respiratory complex I (CI) is not present in *S. cerevisiae*; the cytosolic aconitase homologue IRP-1 is found in mammals only. See the *Saccharomyces genome database* (<http://www.yeastgenome.org>) for details on the yeast proteins.

what we know about Fe–S cluster biosynthesis in eukaryotes has been gleaned from baker's yeast (*Saccharomyces cerevisiae*). In this organism, the mitochondria are the primary site of Fe–S cluster biosynthesis^[12,56] and are also required for the maturation of extramitochondrial Fe–S proteins, including a number of essential proteins.^[13,14] This explains why mitochondria are essential to the yeast, even though this organism can do without respiration, the best-known function of mitochondria that depends on Fe–S proteins. In fact, it was recently found upon closer investigation that even so-called "amitochondriates" have some sort of mitochondria, sometimes small and hardly recognizable, in the form of so-called hydrogenosomes or mitosomes.^[15] For example, the parasitic protist *Giardia* was found to possess mitosomes harboring two key enzymes of Fe–S cluster biosynthesis. A cell fraction enriched in those organelles facilitated the reconstitution of Fe–S clusters

on apoferritin, a result strongly suggesting a primary role in Fe–S cluster biosynthesis for these remnant mitochondria.^[16] Why there seems to be a need to compartmentalize Fe–S cluster biosynthesis remains to be resolved.

Some 12 mitochondrial matrix proteins are known to be involved in Fe–S cluster assembly (Figure 3). Most of them have originally been identified based on homology to genes in the

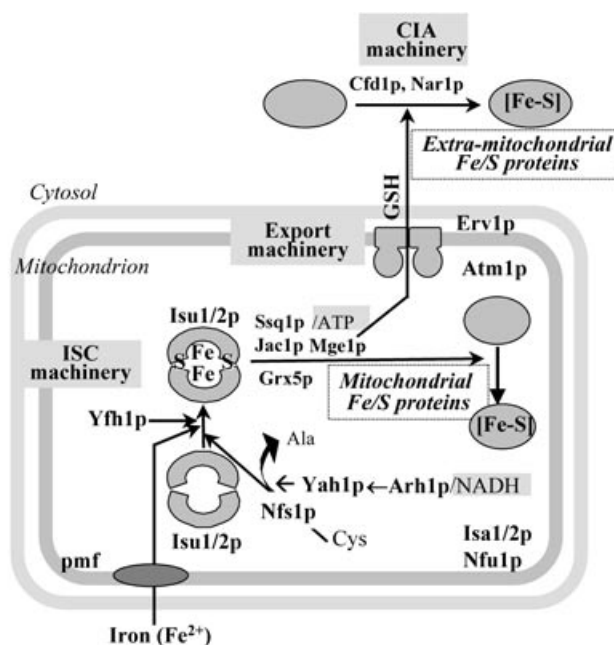


Figure 3. A model for Fe–S protein biogenesis in yeast. An Fe–S cluster is assembled and inserted into mitochondrial apoproteins mediated by the ISC assembly machinery. For detailed information about these proteins and the mechanism, see the text. The ABC transporter Atm1p and the sulfhydryl oxidase Erv1p are associated with the mitochondria but are specifically required for the maturation of extramitochondrial Fe–S proteins. The P-loop ATPase Cfd1p and the Fe-only hydrogenase-like Nar1p are the first cytosolic proteins known to be involved in extramitochondrial Fe–S protein biogenesis. pmf = proton motive force.

bacterial *isc* (for iron–sulfur cluster) gene cluster.^[2,13,17,18] In addition, a small number of yeast proteins have been found for which the role in Fe–S protein maturation is restricted to the maturation of extramitochondrial Fe–S proteins. Over the past few years, progress has been made predominantly in two areas. One is the interaction and molecular function of the so-called ISC proteins in the mitochondrial matrix. Another is the identification of the first proteins involved in the maturation of Fe–S clusters on cytosolic and nuclear Fe–S proteins.

Step 1. The basic ingredients: Nfs1p, Isu1p/Isu2p, and Yfh1p

There appears to be a basic molecular mechanism for Fe–S cluster assembly involving a sulfur donor, an iron donor, and a construction frame (scaffold) for forging the metal cluster together.^[19] In yeast, the elemental sulfur is delivered by the cysteine desulfurase Nfs1p and the iron appears to be donated by Yfh1p (yeast frataxin homologue) onto the scaffold proteins

Isu1p/Isu2p. In support of this, the complex of Nfs1p and the Isu proteins has been shown to physically interact with Yfh1p in the presence of iron.^[20,57]

Nfs1p is a pyridoxal phosphate (PLP) dependent enzyme that catalyzes the desulfuration of L-cysteine to yield L-alanine and elemental sulfur. The enzyme belongs to a widely distributed class of cysteine desulfurases involved in biological sulfur mobilization for the synthesis of Fe–S clusters, thiamine, and thiolated tRNAs.^[21] Among the desulfurases, Nfs1p is most homologous to NifS, required for metallocluster assembly on nitrogenase, and IscS, the desulfurase of the “housekeeping”

Fe–S cluster biosynthesis pathway in bacteria.^[2,22] Complementation studies have shown that IscS is a functional orthologue of yeast Nfs1p.^[13] Several desulfurases have been crystallized, including IscS from *Thermotoga maritima*^[23] and *E. coli*.^[24] They are homodimers, and each ≈ 45 kDa subunit consists of a small and a large domain. The active site is located at the surface of the large domain and consists of a cysteine residue situated on a loop that could be brought into close proximity of the PLP cofactor by relatively large conformational changes. Based on detailed studies on the reaction mechanisms of the bacterial NifS and IscS it has been proposed that, first, the substrate cysteine residue binds to the PLP cofactor to form a Schiff base. The sulfur atom is then removed through nucleophilic attack by the active-site cysteine residue, thereby resulting in the formation of an enzyme-bound persulfide. The flexible C-terminal loop on which the reactive cysteine is located may then transfer the elemental sulfur to a cysteine residue on the scaffold protein. In yeast, Nfs1p is predominantly localized in the mitochondrial matrix; however, tiny amounts are needed in the nucleus to provide elemental sulfur for the production of thionucleotides of tRNAs.^[25]

Yeast possesses two homologues (Isu1p and Isu2p) of the bacterial IscU, a protein that is highly conserved through evolution. Mutation or deletion of one of the *ISU* genes has a weak or no effect on Fe–S cluster assembly, whereas deletion or down-regulation of both genes is lethal.^[17,26,58] Isu1p is more abundant than Isu2p, and the expression of the *ISU* genes is differentially regulated during different growth phases. The Isu proteins are located in the mitochondrial matrix. The ≈ 16 kDa proteins form a dimer and have three highly conserved cysteine residues which are essential for their function, and, in analogy with the bacterial homologues, are the site of cluster assembly.^[27] An NMR spectroscopy structure of part of IscU (*Haemophilus influenzae*) showed that the three cysteines are positioned closely together at a protruding end of the protein molecule.^[28] The transfer of cysteine-derived sulfur atoms from IscS to IscU has been demonstrated experimentally, although the precise molecular mechanism of Fe–S cluster assembly is not known. For example, there are conflicting reports on whether the sulfur or iron atom is delivered first to the IscU protein.^[19]

The role of yeast frataxin as the iron donor to the nascent Fe–S cluster has been suggested recently.^[20,29] The protein is well-studied because reduced levels of frataxin in humans are the cause of Friedreich ataxia, a neurodegenerative disease

which is associated with defects in iron homeostasis.^[30] After years of speculative proposals about its cellular role to explain the defect in iron homeostasis (reviewed in ref. [5]), the protein–protein interaction with Isu1p can be viewed as strong evidence for a direct function of frataxin in Fe–S cluster biosynthesis. Furthermore, it was shown that frataxin is required for iron delivery to the Isu1p scaffold protein.^[20,27] As a consequence, the biogenesis of downstream Fe–S proteins is affected as well.^[31,32] The ≈ 18 kDa, monomeric protein probably functions as an iron chaperone.^[29] An iron-storage function of frataxin has been suggested based on in vitro experiments; however, the in vivo relevance of this finding has not been documented.^[33]

Step 2. Stir in electrons, adenosine triphosphate (ATP), and chaperones..

The biosynthesis of Fe–S clusters appears to involve at least one step that requires reduction and therefore the need for NADH.^[12] The electrons are transferred by an electron-transport chain encompassing the adrenodoxin reductase homologue Arh1p and the [2Fe–2S] cluster containing ferredoxin Yah1p, the yeast homologue of human adrenodoxin. Depletion of Yah1p or Arh1p leads to a strong defect in Fe–S cluster assembly^[34,35] including cluster assembly on Isu1p.^[27] Exactly which step requires electrons is not known, other than that it must be early in the pathway. It is tempting to speculate that the elemental sulfur released by Nfs1p is reduced to sulfide on the scaffold protein Isu1p, but additional reduction step(s) later in Fe–S cluster assembly cannot be excluded.

Yeast mitochondria possess a low-abundance chaperone system that appears to be specifically dedicated to Fe–S cluster assembly, as is the case in the bacterial ISC machinery.^[4] In yeast, the system consists of the Hsp70 family member Ssq1p and its cochaperone Jac1p. In vitro studies with either the *E. coli* proteins or the yeast homologues have demonstrated that the scaffold protein (IscU/Isu1p) is a substrate of the chaperones. The cochaperone facilitates the interaction of IscU/Isu1p with the Hsp70 protein, thereby stimulating its ATPase activity.^[36,37] Despite the striking similarities, significant biochemical differences exist between the yeast and *E. coli* systems, particularly in the requirement for the nucleotide release factor Mge1p in yeast. This could be linked to the fact that Ssq1p and its bacterial counterpart do not appear to share the same phylogenetic origin. The precise function of the Ssq1p/Jac1p/Mge1p system with relation to Fe–S cluster assembly by the core ISC machinery is not known. The chaperones may be important for either establishment or dissociation of the interactions between the Isu proteins, Nfs1p and Yfh1p, or, alternatively, for facilitating the transfer of the nascent Fe–S cluster to the acceptor apoprotein. By using ⁵⁵Fe-labeling studies in vivo, it has been determined that depletion of either Ssq1p or Jac1p leads to accumulation of an Fe–S cluster on Isu1p, a result suggesting that the chaperones act in a step after cluster assembly on Isu1p.^[27]

A number of other mitochondrial proteins have been shown to affect Fe–S cluster assembly, but their role or even step in

the assembly pathway has so far remained elusive. *Isa1p*, *Isa2p*, and *Nfu1p* all have homologues in the bacterial *nif* and/or *isc* gene clusters. The bacterial proteins are able to assemble a labile cluster on the conserved cysteine residues.^[38–41] Therefore, it is thought that they may function as alternative scaffolds. However, neither *Isa1p*, *Isa2p*, nor *Nfu1p* are essential for viability in yeast, and *Nfu1p* is not a substrate for the *Ssq1p*/*Jac1p* chaperone system either, despite a genetic interaction with *SSQ1* resulting in synthetic lethality.^[17,37] An involvement in Fe–S cluster assembly has also been observed for the glutaredoxin *Grx5p*,^[42] this appears to be in a step after cluster assembly on *Isu1p*.^[27]

Step 3. Serving suggestions to other cell compartments

As mentioned above, a number of Fe–S proteins are localized outside the mitochondria (Figure 2), whereas the functional location of the ISC proteins appears to be in the mitochondria (except for a fraction of *Nfs1p*, see above). *NFS1*, *YFH1*, *ISU1*, *ISU2*, *YAH1*, *ARH1*, *ISA1*, and *ISA2* have all been shown to be required for ⁵⁵Fe incorporation and/or activity of cytosolic Fe–S proteins, most commonly *Leu1p* (see below). In addition, two mitochondrial proteins have been identified that are specifically required for the maturation of cytosolic Fe–S proteins. These are the mitochondrial ABC (ATP binding cassette) transporter *Atm1p* and the sulfhydryl oxidase *Erv1p*. $\Delta atm1$ cells present, among other phenotypes, a leucine auxotrophy that is due to the loss of the Fe–S cofactor from the second enzyme in the leucine biosynthetic pathway, isopropylmalate isomerase (*Leu1p*).^[13] Close homologues of *ATM1* are found in virtually all eukaryotes as well as in a few bacteria such as *Rhodobacter* and *Rickettsia prowazekii*, an obligatory intracellular bacterium and a close relative of present-day mitochondria. Mutations in the human homologue *ABC7* cause a condition known as X-linked sideroblastic anaemia and cerebellar ataxia, an iron-storage disease.^[43,44] In yeast, inactivation of *ATM1* and other *ISC* genes leads to iron accumulation with increases up to 30-fold.^[56] It has recently been shown that the transcriptional regulation of iron homeostasis responds to mitochondrial Fe–S metabolism rather than to iron.^[59] Further studies into the molecular basis of this observation might eventually explain why defects in Fe–S protein maturation lead to constitutive up-regulation of the iron regulon. Human *ABC7* can revert the phenotypic defects of the yeast $\Delta atm1$ mutant including mitochondrial iron accumulation, a fact showing that it is a true orthologue of *ATM1* and that yeast may be a suitable model organism to study the functional defects underlying this disease.

The ABC domains of *Atm1p* face the matrix space; this suggests that it might function as an exporter. The substrate of *Atm1p* has not been identified yet. High-copy expression of *Mdl1p*, another mitochondrial ABC transporter with 49% sequence similarity to *Atm1p*, suppressed the $\Delta atm1$ phenotype.^[45] *Mdl1p* has been shown to function in the export of peptides from the mitochondrial matrix.^[46] A partly overlapping function of *Mdl1p* and *Atm1p* may explain why deletion

of *ATM1* is not lethal, whereas most matrix ISC proteins and *Erv1p* are essential for viability.

In addition to *Atm1p*, the sulfhydryl oxidase *Erv1p* is needed for Fe–S cluster assembly on the cytosolic Fe–S proteins *Leu1p* and *Rli1p*.^[14] This essential protein has been localized to the intermembrane space of mitochondria. The flavin- and oxygen-dependent enzyme functions as a dimer and introduces disulfide bridges into target proteins,^[47] but so far the physiological targets have remained elusive.

Furthermore, the tripeptide glutathione (GSH) has been shown to be specifically involved in the maturation of cytosolic Fe–S proteins in yeast, but it is not known in which cellular compartment.^[48] In a $\Delta gsh1$ mutant affected in the first step of GSH biosynthesis, addition of the reducing agent dithiothreitol complemented the general growth defect associated with the absence of the cellular antioxidant GSH. However, this treatment did not restore cytosolic Fe–S protein biogenesis.

Step 4. The second course: Cytosolic iron–sulfur protein assembly (CIA) machinery

Recently, the first cytosolic protein involved in extramitochondrial Fe–S protein assembly has been isolated in yeast.^[49] By using a sophisticated genetic screen, a mutant called *cfp1* was identified that lacked cytosolic aconitase activity of transgenic IRP-1 as well as *Leu1p* activity, despite an increase in the respective protein levels. Whole cell spectroscopy studies confirmed the absence of Fe–S clusters on IRP-1. Fe–S cluster assembly on mitochondrial Fe–S proteins was not affected as judged from the enzyme activities of mitochondrial aconitase and succinate dehydrogenase. *CFD1* encodes a putative P-loop ATPase, homologous to bacterial *ApbC* that has been shown to be required for Fe–S cluster dependent metabolism in *Salmonella enterica*.^[50] Another homologue, the Fe protein of nitrogenase (encoded by *nifH*) is involved in biosynthesis and insertion of the complex FeMo cofactor of nitrogenase,^[51] a fact suggesting an important role for P-loop ATPases in Fe–S cluster assembly.

A second important player of the emerging cytosolic iron–sulfur protein assembly (CIA) machinery is *Nar1p*, a protein with striking homology to bacterial Fe-only hydrogenases. *Nar1p* is predominantly localized in the cytosol and is required for Fe–S cluster assembly on *Leu1p* and *Rli1p*.^[60] Like hydrogenases, *Nar1p* is itself an Fe–S protein and possesses two interacting clusters. The biosynthesis of those clusters is dependent on the mitochondrial ISC machinery and *Atm1p*. This finding supports the crucial role of mitochondria in cellular Fe–S protein maturation. Both *CFD1* and *NAR1* are essential and it will be interesting to investigate the molecular relationship between the two gene products because they colocalize. *Nar1p* is also needed for Fe–S cluster assembly on the protein *Ntg2p*, which is exclusively located in the nucleus.^[60] At present it is not known whether the cluster of *Ntg2p* is inserted in the cytosol or after nuclear import. The *Ntg2p* apoprotein is rather unstable and therefore may depend on cluster assembly before intracellular trafficking. In fact, virtually nothing is known

about Fe–S cluster insertion into cytosolic apoproteins, for example, whether this occurs co- or posttranslationally.

3. The Finishing Touches

In addition to Cfd1p and Nar1p, more components are expected to be found that are involved in the cytosolic branch of Fe–S cluster assembly. This would be in line with the large number of mitochondrial factors already identified. A good candidate is the P-loop ATPase Nbp35p, which is a close homologue of Cfd1p but carries an additional N-terminal ferredoxin motif. Despite the discovery of the first two cytosolic components, the question remains of exactly what compound is exported by Atm1p for the maturation of cytosolic Fe–S proteins. One obvious scenario is that an Fe–S cluster is exported (Figure 4). This idea is based on the fact that all mitochondrial

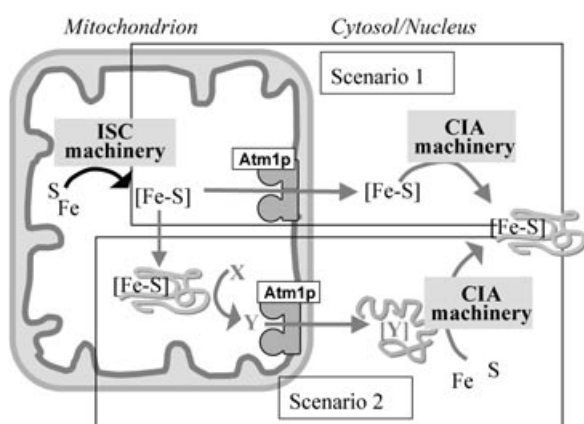


Figure 4. Two scenarios to explain the role of mitochondria in the maturation of extramitochondrial Fe–S proteins. In scenario 1, Fe–S clusters are assembled in the mitochondria and exported by the ABC transporter Atm1p for the biogenesis of cytosolic and nuclear Fe–S proteins. The cytosolic iron–sulfur protein assembly (CIA) machinery may function in cluster stabilization, insertion, or repair of Fe–S clusters outside the mitochondria. In scenario 2, the CIA machinery assembles Fe–S clusters de novo into cytosolic and nuclear apoproteins, but requires an unknown cofactor that is produced in the mitochondria involving a catalytic step by an Fe–S protein. More complex scenarios are not excluded.

ISC components known to date are needed for Fe–S protein maturation in the cytosol. Given the fragility of Fe–S clusters, they certainly must be stabilized. Strong candidates as stabilizing/chelating agents are peptides and/or the tripeptide glutathione (GSH). The latter compound is a substrate of certain ABC transporters that mediate the transport of GSH–metal conjugates into vacuoles as a strategy to detoxify heavy metals.^[52] In the case of Fe–S cluster export by Atm1p, the cytosolic machinery (Cfd1p, Nar1p) may function in cluster insertion into apoproteins or may be necessary for cluster completion and repair.

In a second scenario (Figure 4), the cytosolic machinery can assemble Fe–S clusters de novo but requires an as yet unidentified cofactor that is synthesised by an Fe–S protein located in mitochondria and that is exported by Atm1p. In vitro reconstitution of the transport reaction will be necessary to gain in-

sight into the precise mechanism underlying the mitochondria–cytosol relationship with respect to Fe–S cluster assembly.

In human cells, it has been proposed that separate ISC complexes for Fe–S cluster assembly exist in the mitochondria, cytosol, and nucleus.^[53–55] The human homologues of Nfs1p, Icu1p, and Nfu1p have all been shown to exist as isoforms that, due to alternative translation initiation or splicing, are localized to different cellular compartments. To date, it has not been shown whether the cytosolic and nuclear isoforms are functional in vivo. In fact, the existence of functional ISC complexes outside the mitochondria in mammalian cells seems unlikely, because 1) the shorter, cytosolic isoform of Icu1p lacks a conserved tyrosine residue at its N terminus, thus compromising its function,^[57] 2) even in organisms where ATP production takes place exclusively in the cytosol (such as *Giardia*), Fe–S cluster assembly is compartmentalized in mitochondrial remnants; 3) mammalian adrenodoxin and adrenodoxin reductase appear to be located exclusively in mitochondria, thereby raising the question of what would replace those essential proteins in the cytosol for Fe–S cluster assembly; 4) a highly conserved CIA machinery is being identified that differs from the mitochondrial ISC machinery. The existence of duplicates of the ISC machinery in the cytosol and nucleus would demand an explanation of why additional components are needed to assemble Fe–S proteins.

4. Summary and Outlook

The past years have brought major advances in our understanding of the molecular events leading to Fe–S protein biogenesis in eukaryotes. Some 15 mitochondrial and 2 cytosolic proteins have been shown to be involved in Fe–S protein assembly. Whereas the so-called ISC machinery in the mitochondrial matrix is required for the maturation of all cellular Fe–S proteins investigated to date, a couple of mitochondrial proteins are needed specifically for the maturation of extramitochondrial Fe–S proteins, including the ABC transporter Atm1p located in the mitochondrial inner membrane. In addition, the first cytosolic proteins involved in Fe–S protein maturation have been identified recently, the P-loop ATPase Cfd1p and the hydrogenase-like Nar1p. Despite the considerable progress made, many exciting aspects of biogenesis remain to be addressed in the future. These include the further elucidation of the molecular mechanism of the ISC complex and its dedicated chaperone system, the analysis of the substrate and export reaction of Atm1p, the unravelling of the cytosolic pathway, and an investigation of the role of mitochondria in iron homeostasis. Clearly, we are only beginning to learn the secrets of nature's recipes for Fe–S clusters. Furthermore, the high conservation of the Fe–S protein biogenesis apparatus in eukaryotes makes yeast an excellent model organism to study Fe–S cluster assembly in organisms as diverse as protists, plants, and humans. Studies in yeast have thereby opened up the way to understanding the molecular basis of human diseases associated with Fe–S defects, such as Friedrich's ataxia.

Keywords: biosynthesis · cofactors · iron–sulfur clusters · metalloproteins · mitochondria

- [1] H. Beinert, W. Lee, *Biochem. Biophys. Res. Comm.* **1961**, *5*, 40–45.
- [2] L. Zheng, V. L. Cash, D. H. Flint, D. R. Dean, *J. Biol. Chem.* **1998**, *273*, 13264–13272.
- [3] U. Mühlhoff, R. Lill, *Biochim. Biophys. Acta* **2000**, *1459*, 370–382.
- [4] E. A. Craig, J. Marszalek, *Cell. Mol. Life Sci.* **2002**, *59*, 1658–1665.
- [5] J. Gerber, R. Lill, *Mitochondrion* **2002**, *2*, 71–86.
- [6] H. Beinert, R. H. Holm, E. Münck, *Science* **1997**, *277*, 653–659.
- [7] C. L. Drennan, J. W. Peters, *Curr. Opin. Struct. Biol.* **2003**, *13*, 220–226.
- [8] D. C. Rees, *Ann. Rev. Biochem.* **2002**, *71*, 221–246.
- [9] H. Beinert, *J. Biol. Inorg. Chem.* **2000**, *5*, 2–15.
- [10] C. Huber, G. Wächtershäuser, *Science* **1998**, *281*, 670–672.
- [11] P. J. Kiley, H. Beinert, *Curr. Opin. Microbiol.* **2003**, *6*, 181–185.
- [12] U. Mühlhoff, N. Richhardt, J. Gerber, R. Lill, *J. Biol. Chem.* **2002**, *277*, 29810–29816.
- [13] G. Kispal, P. Csere, C. Prohl, R. Lill, *EMBO J.* **1999**, *18*, 3981–3989.
- [14] H. Lange, T. Lisowsky, J. Gerber, U. Mühlhoff, G. Kispal, R. Lill, *EMBO Rep.* **2001**, *2*, 715–720.
- [15] T. M. Embley, M. van der Gezen, D. S. Horner, P. L. Dyal, S. Bell, P. G. Foster, *IUBMB Life* **2003**, *55*, 387–395.
- [16] J. Tovar, G. León-Avila, L. B. Sanchéz, R. Sutak, J. Tachezy, M. van der Gezen, M. Hernández, M. Müller, J. M. Lucocq, *Nature* **2003**, *426*, 172–176.
- [17] B. Schilke, C. Voisine, H. Beinert, E. Craig, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10206–10211.
- [18] J. Strain, C. R. Lorenz, J. Bode, S. Garland, G. A. Smolen, D. T. Ta, L. E. Vickery, V. C. Culotta, *J. Biol. Chem.* **1998**, *273*, 31138–31144.
- [19] J. Frazzon, D. R. Dean, *Curr. Opin. Chem. Biol.* **2003**, *7*, 166–173.
- [20] J. Gerber, U. Mühlhoff, R. Lill, *EMBO Rep.* **2003**, *4*, 906–911.
- [21] H. Mihara, N. Esaki, *Appl. Microbiol. Biotechnol.* **2002**, *60*, 12–23.
- [22] L. Zheng, R. H. White, V. L. Cash, R. F. Jack, D. R. Dean, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 2754–2758.
- [23] J. T. Kaiser, T. Clausen, G. P. Bourenkow, H. D. Bartunik, S. Steinbacher, R. Huber, *J. Mol. Biol.* **2000**, *297*, 451–464.
- [24] J. R. Cupp-Vickery, H. Urbina, L. E. Vickery, *J. Mol. Biol.* **2003**, *330*, 1049–1059.
- [25] Y. Nakai, N. Umeda, T. Suzuki, M. Nakai, H. Hayashi, K. Watanabe, H. Kagamiyama, *J. Biol. Chem.* **2004**.
- [26] S. A. Garland, K. Hoff, L. E. Vickery, V. C. Culotta, *J. Mol. Biol.* **1999**, *294*, 897–907.
- [27] U. Mühlhoff, J. Gerber, N. Richhardt, R. Lill, *EMBO J.* **2003**, *22*, 4815–4825.
- [28] T. A. Ramelot, J. R. Cort, G. T. Montelione, M. A. Kennedy, **2003**, Molecular Modeling Database accession no. 25392.
- [29] T. Yoon, J. A. Cowan, *J. Am. Chem. Soc.* **2003**, *125*, 6078–6084.
- [30] H. Puccio, M. Koenig, *Curr. Opin. Genet. Dev.* **2002**, *12*, 272–277.
- [31] U. Mühlhoff, N. Richhardt, M. Ristow, G. Kispal, R. Lill, *Hum. Mol. Genet.* **2002**, *11*, 2025–2036.
- [32] O. S. Chen, S. Hemenway, J. Kaplan, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12321–12326.
- [33] S. Park, O. Gakh, H. A. O'Neill, A. Mangravita, H. Nichol, G. C. Ferreira, G. Isaya, *J. Biol. Chem.* **2003**, *278*, 31340–31351.
- [34] H. Lange, A. Kaut, G. Kispal, R. Lill, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1050–1055.
- [35] J. Li, S. Saxena, D. Pain, A. Dancis, *J. Biol. Chem.* **2001**, *276*, 1503–1509.
- [36] K. G. Hoff, J. J. Silberg, L. E. Vickery, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 7790–7795.
- [37] R. Dutkiewicz, B. Schilke, H. Kniesner, W. Walter, E. A. Craig, J. Marszalek, *J. Biol. Chem.* **2003**, *278*, 29719–29727.
- [38] C. Krebs, J. N. Agar, A. D. Smith, J. Frazzon, D. R. Dean, B. H. Huynh, M. K. Johnson, *Biochemistry* **2001**, *40*, 14069–14080.
- [39] M. Wollenberg, C. Berndt, E. Bill, J. D. Schwenn, A. Seidler, *Eur. J. Biochem.* **2003**, *270*, 1662–1671.
- [40] S. P. Wu, J. A. Cowan, *Biochemistry* **2003**, *42*, 5784–5791.
- [41] S. Ollagnier-de Choudens, T. Mattioli, Y. Takahashi, M. Fontecave, *J. Biol. Chem.* **2001**, *276*, 22604–22607.
- [42] M. T. Rodríguez-Manzanares, J. Tamarit, G. Bellí, J. Ros, E. Herrero, *Mol. Biol. Cell* **2002**, *13*, 1109–1121.
- [43] R. Allikmets, W. H. Raskind, A. Hutchinson, N. D. Schueck, M. Dean, D. M. Koeller, *Hum. Mol. Genet.* **1999**, *8*, 743–749.
- [44] S. Bekri, G. Kispal, H. Lange, E. Fitzsimons, J. Tolmie, R. Lill, D. F. Bishop, *Blood* **2000**, *96*, 3256–3264.
- [45] M. Chloupková, L. S. LeBard, D. M. Koeller, *J. Mol. Biol.* **2003**, *331*, 155–165.
- [46] L. Young, K. Leonhard, T. Tatsuta, J. Trowsdale, T. Langer, *Science* **2001**, *291*, 2135–2138.
- [47] J. Lee, G. Hofhaus, T. Lisowsky, *FEBS Lett.* **2000**, *477*, 62–66.
- [48] K. Sipos, H. Lange, Z. Fekete, P. Ullmann, R. Lill, G. Kispal, *J. Biol. Chem.* **2002**, *277*, 26944–26949.
- [49] A. Roy, N. Solodovnikova, T. Nicholson, W. Antholine, W. E. Walden, *EMBO J.* **2003**, *22*, 4826–4835.
- [50] E. Skovran, D. M. Downs, *J. Bacteriol.* **2003**, *185*, 98–106.
- [51] A. C. Robinson, D. R. Dean, B. K. Burgess, *J. Biol. Chem.* **1987**, *262*, 14327–14332.
- [52] T. Ishikawa, Z.-S. Li, Y.-P. Lu, P. A. Rea, *Biosci. Rep.* **1997**, *17*, 189–207.
- [53] T. Land, T. A. Rouault, *Mol. Cell* **1998**, *2*, 807–815.
- [54] W. H. Tong, T. A. Rouault, *EMBO J.* **2000**, *19*, 5692–5700.
- [55] W. H. Tong, G. N. Jameson, B. H. Huynh, T. A. Rouault, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9762–9767.
- [56] R. Lill, G. Kispal, *Trends Biochem. Sci.* **2000**, *25*, 352–356.
- [57] A. Ramazzotti, V. Vanmansart, F. Foury, *FEBS Lett.* **2004**, *557*, 215–220.
- [58] J. Gerber, K. Neumann, C. Prohl, U. Mühlhoff, R. Lill, *Mol. Cell Biol.* **2004**, *24*, 4848–4857.
- [59] O. S. Chen, R. J. Crisp, M. Valachovic, M. Bard, D. R. Winge, J. Kaplan, *J. Biol. Chem.* **2004**, *28*, in press.
- [60] J. Balk, A. J. Pierik, D. Aguilar Netz, U. Mühlhoff, R. Lill, *EMBO J.* **2004**, *23*, 2105–2115.

Received: March 1, 2004