Iron–sulfur-protein biogenesis in eukaryotes

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Iron–sulfur (Fe–S) clusters (ISCs) are versatile, ancient co-factors of proteins that are involved in electron transport, enzyme catalysis and regulation of gene expression. The synthesis of ISCs and their insertion into apoproteins involves the function of complex cellular machineries. In eukaryotes, the mitochondrial ISC-assembly machinery is involved in the maturation of all cellular iron–sulfur proteins. A mitochondrial export machinery and a recently discovered cytosolic assembly system specifically participate in the maturation of cytosolic and nuclear iron–sulfur proteins. Of the ~20 assembly components, more than ten are encoded by essential genes, which indicates that the process is indispensable for life. Mutations in two of the assembly components lead to neurological diseases. The essential character of Fe–S-protein biogenesis in eukaryotes and its importance for human disease identifies this evolutionary ancient process as one of the most important biosynthetic pathways of life.

Introduction

Iron–sulfur (Fe–S) clusters (ISCs) were discovered by Helmut Beinert and others in the early 1960s [1–3]. Until the late 1990s, the complexity of the in vivo assembly process of the ISC from its constituents, Fe$^{2+}$/S$^{2-}$, and its incorporation into an apoprotein was not anticipated. It is now known that bacteria possess three distinct biosynthetic systems to generate Fe–S proteins (for a review, see Ref. [4]). The first discovered system, termed nitrogen-fixation (NIF) machinery, is dedicated to the assembly of the complex Fe–S protein nitrogenase, which is responsible for the conversion of N$_2$ to NH$_3$ in nitrogen-fixing bacteria [5,6] (Figure 1a). The second system, the ISC-assembly machinery, is required for generation of the majority of cellular Fe–S proteins and, thus, might perform a general ‘house-keeping’ biosynthetic function [7]. The ISC-assembly machinery consists of approximately ten proteins. Finally, the SUF (sulfur-mobilization) machinery was discovered as an independent assemblage that might be used predominantly under iron-limiting or oxidative-stress conditions [8–11]. Although many bacteria contain both the ISC and SUF machineries, others harbour just one of these systems (Figure 1a). Strikingly, the NIF system of the eukaryotic parasite Entamoeba histolytica can replace both the ISC and SUF systems in Escherichia coli under anaerobic conditions, which shows that, in principle, these systems are interchangeable [12].

In eukaryotes, Fe–S proteins are present in the mitochondria, the cytosol and the nucleus (Table 1).

![Figure 1](https://www.sciencedirect.com)
Consequently, in all of these compartments, components have been identified that participate in Fe–S-protein maturation. Several mitochondrial proteins share high sequence similarity to the bacterial ISC-assembly proteins [13] (Figure 1b). These proteins are conserved in the majority of eukaryotes (Table 2). It is most likely that the mitochondrial ISC-assembly machinery was inherited from the endosymbiotic bacterial ancestor of these organelles. By contrast, the SUF machinery has so far been found only in photosynthetic eukaryotes, in which the SUF genes have been inherited from cyanobacteria, the evolutionary ancestor of plastids. Only a subset of the suf genes is present in Archaea, the believed host of endosymbiosis. Hence, it seems unlikely that the eukaryotic SUF proteins were inherited from Archaea and relocated to plastids after the endosymbiotic event, although this scenario cannot be excluded entirely. Nevertheless, the evolutionary outcome seems biologically meaningful because the oxygen-sensitive ISC system has been located to mitochondria, which provide a reducing environment, and the oxidative stress-resistant SUF machinery is present in oxygen-evolving plastids.

The mitochondrial ISC-assembly machinery is not only required for biosynthesis of mitochondrial Fe–S proteins, but also for the biosynthesis of cytosolic and nuclear Fe–S proteins [15,16]. Furthermore, maturation of extra-mitochondrial Fe–S proteins depends on mitochondrial proteins, designated ‘ISC-export machinery’, and proteins – the majority of which are cytosolic but are also found in the nucleus – termed ‘cytosolic Fe–S-protein assembly (CIA) machinery’ (Figure 1b). Unlike the ISC-assembly machinery, the ISC-export and CIA machineries are responsible exclusively for the generation of cytosolic and nuclear Fe–S proteins. The components of these latter two machineries are highly conserved in eukaryotes (Table 2; see later). Here, we summarize the discoveries of the past five years regarding the biogenesis of Fe–S proteins in (non-photosynthetic) eukaryotes. We introduce the three known machineries regarding the biogenesis of Fe–S proteins in (non-photosynthetic) eukaryotes. We introduce the three known machineries for the generation of cytosolic and nuclear Fe–S proteins.

### Table 1. Inventory of known Fe–S proteins in (non-photosynthetic) eukaryotes: localization and function

<table>
<thead>
<tr>
<th>Fe–S protein</th>
<th>Yeast gene</th>
<th>Cluster type</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mitochondrial Fe–S proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aconitase</td>
<td>Aco1</td>
<td>4F</td>
<td>Citric acid cycle</td>
</tr>
<tr>
<td>Homoaconitase</td>
<td>Lys4</td>
<td>4F</td>
<td>Biosynthesis of lysine</td>
</tr>
<tr>
<td>Dihydroxy-acid dehydratase</td>
<td>Ilv3</td>
<td>4F</td>
<td>Biosynthesis of branched-chain amino acids</td>
</tr>
<tr>
<td>Lipate synthase</td>
<td>Lip5</td>
<td>2F?, 4F</td>
<td>Biosynthesis of lipoic acid</td>
</tr>
<tr>
<td>Biotin synthase</td>
<td>Bio2</td>
<td>2F, 4F</td>
<td>Biosynthesis of biotin</td>
</tr>
<tr>
<td>Ferredoxin</td>
<td>Yah1</td>
<td>2F</td>
<td>Maturation of Fe–S proteins, biosynthesis of haeme A, steroid biosynthesis in mammals (adenodoxin)</td>
</tr>
<tr>
<td>Ferrochelatase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex I</td>
<td></td>
<td>8-9 clusters of 2F, 3F, 4F</td>
<td>Electron transport chain (NADH ubiquinone oxidoreductase)</td>
</tr>
<tr>
<td>Complex II</td>
<td>Sdh2</td>
<td>2F, 3F, 4F</td>
<td>Electron transport chain (succinate dehydrogenase)</td>
</tr>
<tr>
<td>Complex III</td>
<td>Rip1</td>
<td>2F</td>
<td>Electron transport chain (ubiquinone cytochrome c oxidoreductase)</td>
</tr>
<tr>
<td><strong>Cytosolic Fe–S proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isopropylmalate isomerase</td>
<td>Leu1</td>
<td>4F</td>
<td>Biosynthesis of leucine</td>
</tr>
<tr>
<td>Iron regulatory protein 1</td>
<td></td>
<td>4F</td>
<td>Post-transcriptional control of iron uptake, storage and use in mammals (‘cytosolic aconitase’)</td>
</tr>
<tr>
<td>Sulfite reductase</td>
<td>Ecm17</td>
<td>4F</td>
<td>Biosynthesis of methionine, contains siroheme</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>Glt1</td>
<td>4F</td>
<td>Biosynthesis of glutamate</td>
</tr>
<tr>
<td>ABC protein Ri1</td>
<td></td>
<td>4F, complex type</td>
<td>Biogenesis of ribosomes, rRNA processing, translation initiation</td>
</tr>
<tr>
<td>P-loop NTPase Nbp35</td>
<td></td>
<td>4F</td>
<td>Maturation of cytosolic and nuclear Fe–S proteins</td>
</tr>
<tr>
<td>Hydrogenase-like Nar1</td>
<td>Nar1</td>
<td>4F</td>
<td>Maturation of cytosolic and nuclear Fe–S proteins</td>
</tr>
<tr>
<td>MOCS1A</td>
<td>Nar1</td>
<td>3F?, 4F, complex type</td>
<td>Biosynthesis of Moco (molybdenum co-factor)</td>
</tr>
<tr>
<td>Dihydro-pyrimidine dehydrogenase</td>
<td></td>
<td>4xF</td>
<td>Degradation of pyrimidine nucleotides</td>
</tr>
<tr>
<td>CMP-N-acetyl-uricam acid</td>
<td></td>
<td>2F</td>
<td>Biosynthesis of N-glycoyl uricam acid, Rieske-like protein</td>
</tr>
<tr>
<td>Hydroxylase</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Xanthine dehydrogenase</td>
<td></td>
<td>2x 2F</td>
<td>Degradation of xanthine to urate, contains FAD and molybdopterin</td>
</tr>
<tr>
<td><strong>Nuclear Fe–S proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA glycosylase</td>
<td>Ntg2</td>
<td>4F</td>
<td>DNA repair (endonuclease III-like glycosylase 2)</td>
</tr>
</tbody>
</table>

*Fe–S proteins are involved in (i) metabolic reactions and enzyme catalysis, (ii) electron transfer, and (iii) regulatory processes. Most Fe–S proteins contain either a [2Fe–2S] cluster (2F) or a [4Fe–4S] cluster (4F). In some cases, multiple ISCs are present, for example, in respiratory complexes I and II, which also contain [3Fe–4S] clusters (3F). The structure of some ISCs, for example, those of Nar1 and Rli1 has not been resolved yet. A '?' is used where the type of cluster is unknown or uncertain.

Fe–S-protein assembly in mitochondria – a complex process

**The basic concept: scaffold plus sulfur donor**

The discovery of the first components of mitochondrial Fe–S-protein biogenesis almost coincided with the identification of the bacterial ISC machinery and showed the remarkable similarity of these proteins [15,17,18,19]. The fact that the bacterial ISC components are encoded by an operon [7] was exploited to postulate that the eukaryotic equivalents might be mitochondrial proteins and have a role in Fe–S-protein assembly (Figure 2); this prediction
has now been verified for all these proteins. Not surprisingly, our knowledge of the assembly of mitochondrial Fe–S proteins largely benefited from seminal findings made for the ISC proteins from various bacterial sources, mainly *Azotobacter vinelandii* and *E. coli* [4]. It seems that the processes in bacteria and eukaryotic mitochondria (studied mainly in the model organism *Saccharomyces cerevisiae*) are quite similar. Many bacterial proteins can complement the functional defects of the yeast ISC mutants, and the biochemical mechanisms underlying biogenesis are akin. The general concept of ISC biogenesis is the *de novo* synthesis of an ISC on a so-called scaffold protein, with sulfur being delivered by a sulfur donor [20]. Both synthesis of the transiently scaffold-bound ISC and its final transfer to apoproteins requires the help of additional ISC proteins.

The sulfur donor in both the mitochondrial ISC-assembly machinery (Nfs1) and in all three bacterial systems (IscS, NifS, and SuF5) is a pyridoxal phosphate-dependent cysteine desulfurase [15,16,21–23]. The sulfur atom of free cysteine is first transferred to a conserved cysteine residue of these enzymes to create a persulfide as a reaction intermediate [24,25]. Sulfur is then transferred directly to the scaffold proteins, namely IscU in bacteria and Isu1 (and Isu2 in yeast) in eukaryotes [18,26–29]. Usually, the scaffold and sulfur donor form heterotetrameric complexes, which might facilitate the sulfur transfer. The formation of an ISC on IscU/Isu1 has been observed both *in vitro* and *in vivo* [20,30]. IscU and Isu1 are small proteins of ~14 kDa, and are among the most conserved proteins in evolution. A 3D structure of bacterial apo-IscU derived by NMR indicates that the three conserved cysteine residues involved in ISC synthesis are surface-exposed [31,32]. Pioneering biochemical studies indicated that either a [2Fe–2S] or a [4Fe–4S] cluster can be assembled on an IscU dimer, suggesting that cysteine residues from both subunits participate in ISC assembly [33]. However, the chemical details of ISC synthesis are not yet clear. According to an attractive model the sulfur is first transferred from IscS or Nfs1 to a conserved cysteine residue on IscU/Isu1 [27,28]; the scaffold proteins then bind reduced iron, resulting in ISC synthesis.

### Early components of biogenesis

Several additional proteins are required for Fe–S protein maturation in mitochondria (Figure 2). They can be divided into two classes, depending on whether or not they are required for ISC synthesis on Isu1 *in vivo* [30]. The first class includes Yfh1, Yah1 and Arh1. It has become clear recently that Yfh1, the yeast homologue of human frataxin, performs its primary function in Fe–S-protein assembly. A deficiency of frataxin is associated with impaired Fe–S-protein biogenesis in yeast, conditional knockout mice and human cell culture [34–38]. Other (secondary) consequences of frataxin depletion are mitochondrial iron accumulation, haeme deficiency and oxidative stress [39,40]. Yfh1 interacts in an iron-dependent manner with Isu1 *in vivo* and its depletion is associated with a defect in the synthesis of the transient ISC on Isu1 [41]. *In vitro* studies indicate that Yfh1/frataxin can bind iron and might deliver bound iron to Isu1 for assembly of the ISC [42]. Thus, frataxin can be regarded as an iron donor for ISC formation on Isu1.
Because Yfh1 can be deleted in yeast with comparatively mild effects, and because some organisms lack frataxin homologues, this reaction can be bypassed and is not as central to biogenesis as reactions catalysed by Isu and Nfs1. In humans, however, depletion of frataxin leads to the neurodegenerative disease Friedreich's ataxia, which is associated with defects in Fe–S proteins [43].

An important pair of proteins required for Fe–S-protein maturation is the ferredoxin reductase Arh1 and the ferredoxin Yah1, which itself is a [2Fe–2S] protein (Figure 2 and Table 1). Depletion of these proteins in yeast drastically affects the maturation of Fe–S proteins [44,45]. In particular, the de novo assembly of the transient ISC on Isu1 is impaired in the absence of Yah1, suggesting that the proteins are required early in biosynthesis [30]. One reasonable possibility is that the proteins function as an electron-transfer chain to provide electrons for reduction of sulfur (S⁰) formed by the cysteine desulferase reaction to sulfide (S²⁻) present in ISC, but experimental evidence for this idea is still lacking. Electrons are provided by NADH, as shown by in vitro reconstitution of biosynthesis employing detergent extracts of isolated mitochondria [46]. Interestingly, the electron-transfer chain performs additional tasks. In yeast, Yah1/Arh1 – together with Cox10 and Cox15, are responsible for the synthesis of haeme A – a constituent of cytochrome oxidase [47]. In mammalian cells, the counterparts of Yah1/Arh1, adrenodoxin and adrenodoxin reductase, are involved in the conversion of cholesterol to, for example, aldosterone and cortisol in the adrenal gland.

**Late components of biogenesis**

In contrast to Nfs1, Yfh1 and Yah1/Arh1, other mitochondrial ISC components (i.e. the chaperone proteins Ssq1 and Jac1, and the glutaredoxin Grx5) are not required for ISC synthesis on Isu1 in vivo, and therefore might function in later steps of biogenesis [30,48,49] (Figure 2). One might speculate that they participate in the dissociation of the ISC from Isu1 and/or in the transfer of the ISC to apoproteins. Interaction of the chaperone proteins with Fe–S apoproteins is not excluded, but a direct association has not been observed so far. The mitochondrial chaperones Ssq1 and Jac1, like their bacterial counterparts Hsc66 and Hsc20, respectively, bind to Isu1/IscU as a substrate [48,50,51]. Jac1/Hsc20 function to target Ssq1/Hsc66 to the highly conserved binding motif, Leu-Pro-Pro-Val-Lys, on Isu1/IscU [52,53]. Cycles of ATP

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**Figure 2.** A current working model for the mechanism of Fe–S-protein biogenesis in eukaryotes. The biogenesis of Fe–S proteins first requires the membrane-potential-dependent import (pmf) of reduced iron into mitochondria by the carrier proteins Mrs3 or Mrs4 and other unknown transporters. The metal then binds to Isu1/Isu2 (green), which serves as a scaffold for synthesis of the ISC. Metal delivery to Isu1/Isu2 is assisted by frataxin (Yfh1; yellow), which directly binds to the Isu proteins. ISC synthesis on Isu1/Isu2 further requires the cysteine desulferase Nfs1 (orange), which mediates the release of sulfur from cysteine and the electron-transfer chain consisting of NADH, ferredoxin reductase (Arh1) and ferredoxin (Yah1). The chemical mechanism of ISC synthesis is still unknown. The chaperone system, which consists of Ssq1–ATP, Jac1 and Mge1 (representing DnaK-, DnaJ- and GrpE-like chaperones, respectively), and the glutaredoxin Grx5 are required after ISC assembly on the Isu proteins, for example, in ISC transfer to the apoproteins [30]. The role of further proteins (Isa1/Isa2 and Nfu1) in biogenesis is well established, but their function is still unclear. The maturation of extra-mitochondrial Fe–S proteins, for instance the essential ABC protein Rli1, requires the cooperation of the mitochondrial ISC assembly and export machineries and the cytosolic CIA machinery. The ABC transporter Atm1 of the inner membrane exports an unknown compound derived from the ISC-assembly machinery to the cytosol. Other components of the export system are the sulfhydryl oxidase Erv1 of the intermembrane space and the tripeptide glutathione (GSH). Maturation of cytosolic and nuclear apoproteins is facilitated by recently identified proteins (Nar1, Cfd1 and Nbp35) of the CIA machinery. The mechanism of the maturation process in the cytosol has not been resolved yet. '*' denotes components encoded by essential genes in yeast.
binding and hydrolysis lead to dissociation of Ssq1/Hsc66 from Isu1/IscU. At present, it is not understood in molecular terms what the functional contribution of the chaperones to Fe–S-protein maturation might be. Conspicuously, chaperone binding occurs to both the apo- and holo-forms of Isu1/IscU. Although the mitochondrial and bacterial chaperones share most properties, they differ in the requirement for the ADP–ATP exchange factor Mge1 in yeast or GrpE in bacteria [53]. The higher affinity of ADP for Ssq1 might demand participation of Mge1 in nucleotide turnover, whereas Hsc66 functions without further assistance from GrpE.

Further auxiliary proteins
The involvement of Isa1 and Isa2 in Fe–S-protein biogenesis is well documented, but their precise function is not yet clear (Figure 2). Depletion of the proteins is associated with defects in the activity and de novo biogenesis of various mitochondrial Fe–S proteins [54–56]. Double deletion of both genes does not exacerbate these defects, which might indicate that the proteins perform an overlapping function. Like the Isa proteins, the Isa proteins and their bacterial homologue IscA contain three conserved cysteine residues that are crucial for their function. Numerous in vitro studies suggest different molecular roles for this class of proteins. The predominant idea is that they serve as scaffolds for the assembly of ISCAs, similarly to Isu1/IscU proteins [57,58]. The 3D structure of bacterial IscA suggests that the nascent ISC might be formed on cysteine residues between two dimers of IscA [59,60]. As an alternative to the scaffolding theory, E. coli IscA might bind mononuclear iron, which, under iron-limiting conditions, can be specifically delivered to IscU for subsequent ISC assembly [61]. To determine which of the proposed functions might be most relevant for mitochondrial Isa1/Isa2 proteins requires further study in vivo.

A final component of the ISC-assembly machinery is Nfs1 [18]. The various forms of this protein in different species exhibit sequence similarity in a 60 amino acid residue segment to the C-terminal domain of bacterial NiU [20]. Deletion of yeast Nfu1 is without severe phenotypes, but the synthetic lethality of Nfu1 and several other ISC genes (e.g. ISU1 and SSQ1) indicates an auxiliary function of Nfu1 in mitochondria. By contrast, several Nfu1-like proteins are present in plant mitochondria, chloroplasts and cyanobacteria, in which they have central roles in Fe–S-protein assembly [62–64].

Other consequences of defects in ISC synthesis
Defects in the mitochondrial ISC system are associated with a drastic accumulation of iron in mitochondria [13] and with decreased haeme biosynthesis and cytochrome content. Although the connection between Fe–S protein biogenesis and iron homeostasis is not yet understood, progress has been made in defining the cause of the haeme-synthesis defect in ISC mutants [39]. Ferrochelatase, which catalyses the last step of haeme biosynthesis, is reversibly inhibited when the biosynthesis of mitochondrial Fe–S proteins is impaired. The challenge will now be to unravel the nature of the inhibition.

Genetic experiments in yeast have assigned a central role to Nfs1 in the nucleus, even though the protein was detectable in mitochondria only [65]. Recent studies show that nuclear Nfs1 does not play a part in Fe–S-protein assembly, not even in biogenesis of nuclear Fe–S proteins [16]. Instead, the protein performs its crucial nuclear role as a sulfur donor in the thiolation of tRNA, thus resembling the function of the bacterial protein IscS, which is involved in ISC, thiamine and NAD+ formation in addition to tRNA thiolation [66]. Both Nfs1 and Isu1/Isu2 depend on mitochondrial localization for participation in cytosolic Fe–S-protein maturation [67]. The reason for this strict compartmentalization is unknown, but the low oxygen content inside the matrix might be favourable for the (oxygen-sensitive) synthesis of ISCAs.

**Biogenesis in human cells**
In human cells, low levels of some ISC components have been detected in the cytosolic and nuclear compartment, namely Nfs1, Isu1 and Nfu1 [68]. It was suggested that these proteins might be involved in the generation of Fe–S proteins in the cytosol; however, presently, no functional data are available to examine this hypothesis. The cytosolic forms of human Nfs1 and Isu1 were suggested to be generated by alternative usage of an internal start codon or alternative splicing, respectively, from those genes that encode the predominant mitochondrial form of the proteins. However, the proposed targeting mechanisms generate proteins that lack essential, conserved residues at their N termini that are important for function [16,67]. Based on these observations, it seems unlikely that these truncated ISC proteins perform a functional role in the mammalian cytosol or nucleus. Nevertheless, a role of a functional (longer) isoform of human Nfs1 in nuclear tRNA thiolation, like in yeast, seems highly probable, but direct biogenesis studies in mammalian cells are necessary to examine these functional aspects. A recent advance in this vein has been the development of an experimental system that enables the study of Fe–S-protein assembly in human cells. Depletion of mitochondrial frataxin by RNAi technology causes defects in mitochondrial Fe–S proteins and in the maturation of cytosolic iron regulatory protein 1 (IRP1) [38]. This might be taken as a first indication that the mitochondria play a decisive part in the biogenesis of cytosolic Fe–S proteins in higher eukaryotes.

**A function of mitochondria in cytosolic and nuclear Fe–S-protein biogenesis**
One of the earliest observations in the elucidation of Fe–S-protein biogenesis was that mitochondria are not only required for maturation of their own Fe–S proteins, but that they also participate in the formation of Fe–S proteins outside the organelle [15]. For the majority of mitochondrial ISC-assembly proteins, it has now been shown that their function is needed for maturation of cytosolic and nuclear Fe–S proteins. The first known protein with a specific function in cytosolic Fe–S-protein biogenesis was the ATP-binding cassette (ABC) transporter Atm1 of the mitochondrial inner membrane [15] (Figure 2). Deletion of ATMT is associated with a strong
growth defect and a leucine auxotrophy, an observation that lead to the determination of a specific role of Atm1 in the maturation pathway of Leu1, a cytosolic Fe–S protein (isopropylmalate isomerase; Table 1). In the meantime, the common requirement of Atm1p for biogenesis of cytosolic and nuclear Fe–S proteins has been documented. The function of Atm1 seems to be widely conserved because both human and plant homologues can complement a yeast ATM1-deletion mutant [69,70]. Mutations in human ABCB7 are the cause of X-linked sideroblastic anaemia and cerebellar ataxia (XLSA/A), an iron-storage disease [71].

The molecular nature of the substrate transported by Atm1 is unknown, but the available information suggests that the mitochondrial ISC-assembly machinery produces a component that is exported via Atm1p to be used in cytosolic and nuclear Fe–S-protein formation. It has been proposed that mitochondria might produce an ISC or a precursor thereof, and, after appropriate stabilization, this moiety would be exported for incorporation into cytosolic or nuclear apoproteins [13]. Because Atm1 can be functionally replaced by over-production of the peptide ABC transporter Md11, such stabilization might be achieved by binding of a peptide. Although this model is attractive, so far it lacks experimental proof. Moreover, other (more indirect) scenarios for the identity of the expected Atm1 substrate are conceivable, for instance, a mitochondrial Fe–S protein produced by the ISC-assembly machinery could generate a compound that is necessary in the cytosol for extra-mitochondrial Fe–S-protein biogenesis [72]. This idea would fully explain why the entire ISC-assembly machinery is necessary for generating functional Fe–S proteins in the cytosol and nucleus.

The sulfhydryl oxidase Erv1 of the intermembrane space is specifically required for biogenesis of extra-mitochondrial Fe–S proteins [73]. Deletion of ERV1 results in loss of cell viability, indicating that this export pathway is a fundamental reaction of eukaryotic cells (see later). Erv1 can be functionally complemented and the biogenesis of extra-mitochondrial Fe–S protein is restored by targeting the human homologue ALR (augmenter of liver regeneration) to the intermembrane space. Together with the functional similarity of human, plant and yeast Atm1 homologues, this result indicates that the export pathway is conserved in lower and higher eukaryotes.

A third component of the ISC-export machinery is the tripeptide glutathione [74]. Depletion of glutathione results in a severe defect in cytosolic, but not mitochondrial Fe–S proteins. This defect cannot be restored by the addition of dithiotreitol, which can rescue the phenotypic consequences associated with the role of glutathione in maintaining the redox potential in yeast. The precise function of glutathione remains to be determined, but it is tempting to speculate that the compound closely cooperates with Erv1 and Atm1. Another phenotypical reason why glutathione could be considered a member of the ISC-export machinery is the drastic iron accumulation in mitochondria upon depletion of glutathione [74]. Similar effects are observed upon inactivation of almost all members of the ISC-assembly and -export machineries, suggesting a close connection of Fe–S-protein assembly and iron homeostasis, which is regulated by the yeast transcription factors Aft1 and Aft2 [13,75].

An emerging cytosolic machinery for Fe–S-protein assembly

The presence of Fe–S proteins in the cytosol and nucleus made the existence of biogenesis components in these compartments likely. However, it was not until 2003 that a function of a first cytosolic component in this process was documented [76]. Using a sophisticated genetic screen intended to study the maturation of the mammalian Fe–S protein IRP1 in yeast, the soluble P-loop NTPase Cfd1 was identified and shown to be required for cytosolic, but not for mitochondrial Fe–S-protein maturation. Recently, a related protein termed Nbp35 was shown to perform a similar function (A. Hausmann et al., unpublished). These two proteins are present in almost all eukaryotes, and they contain three conserved cysteine residues, which is typical for this subclass (designated Mrp/Nbp35) of the large family of P-loop NTPases (Figure 3). Two of these residues were shown to be essential for the function of Cfd1 [71]. Nbp35 contains a characteristic N-terminal extension of ~50 residues with four essential cysteine residues that bind an ISC. A similar protein, with only two of the three conserved cysteine residues, was identified in the bacterium Salmonella where it is required in the maintenance of Fe–S-protein activity [77]. Hence, members of the Mrp/Nbp35 subgroup of P-loop NTPases seem to perform a crucial function in Fe–S-protein biogenesis.

The third protein of the CIA machinery is another Fe–S protein, termed Nar1 [78]. Nar1 shares striking sequence similarity with bacterial iron-only hydrogenases, but has no known function in hydrogen production or consumption. At its N terminus, Nar1 contains a typical ferredoxin motif that probably coordinates a [4Fe–4S] cluster, whereas another four cysteine residues corresponding to the so-called H (hydrogenase) cluster of functional hydrogenases are scattered throughout the middle and C-terminal regions of the protein (Figure 3). Electron paramagnetic resonance studies indicate that these residues also hold an ISC that is electronically coupled to the N-terminal ISC, but the precise structure could not yet be unravelled. At present, we know nothing about the molecular mechanism of how the CIA components might facilitate the assembly of cytosolic and nuclear Fe–S proteins. Progress towards this challenging problem might come from an in vitro reconstitution of the biogenesis process using either isolated components or cell extracts.

Fe–S-protein biogenesis in eukaryotes that lack mitochondria

Several organisms from diverse branches of the eukaryotic phylogenetic tree are known to lack classical mitochondria. These include Microsporidia (e.g. Encephalitozoon cuniculi), Apicomplexa (e.g. Cryptosporidium parvum), Diplomonads (e.g. Giardia intestinalis) and Trichomonads (e.g. Trichomonas vaginalis) [79]. In light of the importance of mitochondria for the biosynthesis of cellular Fe–S proteins (see earlier), the question arises of how and where these proteins are matured in such organisms. Genomic analyses have shown, for several of these organisms, that they
Fe–S-protein biogenesis is essential. Why?

Deletion of the numerous yeast genes implicated in Fe–S-protein biogenesis is either lethal (Figure 2) or associated with strong growth defects, even on glucose-containing rich media (fermentative conditions). Which Fe–S proteins make this biosynthetic pathway essential? In mitochondria, only one Fe–S protein is known to be indispensable for viability – the ferredoxin Yah1. However, this protein is involved in Fe–S–protein biogenesis, that is, it is required for its own maturation [44]. This cannot explain the essentiality of the process. Likewise, in the cytosol, the essential Fe–S proteins Nbp35 and Nar1 are components of Fe–S–protein biogenesis [78]. The only known yeast Fe–S protein that is a true target of Fe–S–protein biogenesis is Rli1, one of the most conserved proteins in evolution. The protein carries N-terminal ISCs and two ABC domains (Table 1), and it has a crucial role in ribosome biogenesis and function [83,84]. In particular, it might be involved in rRNA processing. Thus, the intimate link between two evolutionary ancient processes, Fe–S–protein assembly and ribosome biogenesis, is the first known explanation for the essential character of Fe–S–protein biogenesis and, in turn, mitochondria in yeast.

Future perspectives

Tremendous progress has been made recently in understanding the fundamentals of Fe–S–protein biogenesis, yet we are only at the beginning of unravelling this crucial process. Future research efforts will have to concentrate on further analysing the (bio)chemical mechanism of ISC assembly in the ISC system using purified components. Moreover, we need to understand the contribution of mitochondria to cytosolic and nuclear Fe–S–protein maturation in molecular detail. It is likely that more CIA components will be identified, a prerequisite for defining the pathway of Fe–S–protein assembly in these compartments. Even though the overall process seems to be highly conserved, comparative investigations in yeast and mammals will have to elucidate the differences and similarities between higher and lower eukaryotes. Finally, we need to understand the connection between Fe–S–protein formation and (intra)cellular iron homeostasis. The latter aspect can be regarded as a prerequisite for the elucidation of the molecular basis of diseases associated with Fe–S–protein defects.

Note added in proof

During revision of this article another excellent review, mainly focused on bacterial Fe–S protein biogenesis, was published:

www.sciencedirect.com
References
15 Kispal, G. et al. (1999) The mitochondrial proteins Atm1p and Nfs1p are required for biosynthesis of cytosolic Fe/S proteins. EMBO J. 18, 3981–3989
41 Gerber, J. et al. (2003) An interaction between frataxin and Isu1Nfs1 which is crucial for FeS cluster synthesis on Isu1. EMBO Rep. 4, 906–911
Gro Harlem Brundtland, director-general for the WHO, said that this initiative was ‘perhaps the biggest step ever taken towards reducing the health information gap between rich and poor countries’.

See http://www.healthinternetwork.net for more information.