Maturation of Iron-Sulfur Proteins in Eukaryotes: Mechanisms, Connected Processes, and Diseases

Roland Lill and Ulrich Mühlenhoff

Institut für Zytobiologie, Philipps Universität Marburg, Marburg D-35033, Germany; email: Lill@staff.uni-marburg.de

Annu. Rev. Biochem. 2008. 77:669–700

First published online as a Review in Advance on March 26, 2008

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

This article's doi: 10.1146/annurev.biochem.76.052705.162653

0066-4154/08/0707-0669$20.00

Key Words

CIA machinery, cysteine desulfurase, ferredoxin, Fe/S cluster, ISC assembly machinery

Abstract

Iron-sulfur (Fe/S) proteins are involved in a wide variety of cellular processes such as enzymatic reactions, respiration, cofactor biosynthesis, ribosome biogenesis, regulation of gene expression, and DNA-RNA metabolism. Assembly of Fe/S clusters, small inorganic cofactors, is assisted by complex proteinaceous machineries, which use cysteine as a source of sulfur, combine it with iron to synthesize an Fe/S cluster on scaffold proteins, and finally incorporate the cluster into recipient apoproteins. In eukaryotes, such as yeast and human cells, more than 20 components are known that facilitate the maturation of Fe/S proteins in mitochondria, cytosol, and nucleus. These biogenesis components also perform crucial roles in other cellular pathways, e.g., in the regulation of iron homeostasis or the modification of tRNA. Numerous diseases including several neurodegenerative and hematological disorders have been associated with defects in Fe/S protein biogenesis, underlining the central importance of this process for life.

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INTRODUCTION

Iron-sulfur (Fe/S) clusters are small inorganic cofactors, which are thought to be among the earliest catalysts in the evolution of biomolecules (1, 2). In the modern living world, Fe/S clusters are found in all kingdoms of life and are part of many proteins involved in numerous biochemical functions. The Fe/S clusters serve as catalysts in chemical reactions, electron carriers in redox reactions, regulatory sensors, and stabilizers of protein structure. Although Fe/S clusters can be assembled on purified proteins in vitro using chemical reconstitution protocols with ferrous iron and sulfide under anaerobic conditions, it is well established that biogenesis in a living cell is a catalyzed process involving the participation of a surprisingly large number of proteins. In bacteria, three systems assisting the assembly of Fe/S proteins have been identified (for excellent recent reviews on bacterial Fe/S protein biogenesis see References 3–8). In brief, the NIF (nitrogen fixation) system present in azototrophic bacteria is specifically required for generation of the metalloclusters in nitrogenase (9, 10). Bacterial housekeeping Fe/S proteins are matured with the help of the ISC (iron-sulfur cluster) assembly components encoded by the *isc* operon and/or the SUF (sulfur mobilization) system encoded by the *suf* operon (11–13). In some bacteria, including *Escherichia coli*, the ISC and SUF systems are present in parallel, whereas in other species only one of these machineries is encoded. The components of the *suf* operon are preferred for Fe/S protein maturation under oxidative stress conditions and during limited iron availability (14). However, it has been shown that, in principle, these systems are interchangeable, especially in an anaerobic environment (15). Two proteins, NifS and NifU, from *Entamoeba histolytica* with sequence similarity to components of the highest NIF system of azototrophic bacteria can replace the *E. coli* ISC and SUF machineries in the synthesis of housekeeping Fe/S proteins, defining

the minimal requirements for the making of Fe/S proteins in cells.

Two of the three biogenesis systems have been inherited by eukaryotic cells. The ISC system is found in mitochondria, and the more oxygen-stable SUF system is present in plastids (for comprehensive reviews on plant Fe/S protein biogenesis see References 16–18). In addition, two eukaryote-specific machineries have been identified, the mitochondrial ISC export apparatus and the cytosolic Fe/S protein assembly (CIA) machinery (19). Both systems are specifically involved in the maturation of cytosolic and nuclear Fe/S proteins, whereas the mitochondrial ISC assembly machinery is required for the generation of all cellular Fe/S proteins. In this review, we summarize our current knowledge of Fe/S protein maturation in (nonplant) eukaryotes. To avoid extensive overlap with review articles that have appeared in the past (3, 20–23), this work preferentially summarizes the most recent developments and integrates them into the emerging molecular model for this complex biosynthetic pathway. First, we provide a brief introduction into Fe/S clusters and a synopsis of the localization and functions of major eukaryotic Fe/S proteins. Next, we summarize knowledge derived from the yeast model system on Fe/S protein assembly and review and compare the pathways in higher eukaryotes. We then provide an overview on processes intimately connected to the biogenesis of cellular Fe/S proteins, such as iron homeostasis in yeast and mammals as well as the modification of ribonucleotides in cytosolic and mitochondrial tRNAs. Finally, we go over the currently known diseases linked to defects in either Fe/S protein biogenesis or Fe/S proteins themselves.

A PRIMER ON Fe/S CLUSTERS

The most common and simplest Fe/S clusters are of the rhombic [2Fe-2S] and cubic [4Fe-4S] types (24–27). In several Fe/S proteins, one Fe of a [4Fe-4S] cluster may be lost to generate the [3Fe-4S] cluster, which forms a distorted, elongated structure. Iron can alter between oxidation states +2 and +3 and is generally coordinated in a tetrahedral fashion with sulfide, which is always present in the oxidation state –2 (S^{2-}). In proteins, the iron ions are most typically bound to cysteine-sulfur groups, but coordination with nitrogen atoms of histidine, arginine, and serine residues or small molecules (CN^-, homocitrate) is also observed. Fe/S clusters have versatile electrochemical properties with reduction potentials ranging from +300 mV to –500 mV. On the basis of these simple cluster types, more complex Fe/S clusters can be generated, e.g., two [4Fe-4S] are joined. Often such complex cluster types contain additional metal ions, such as molybdenum, nickel, or vanadium, which are also coordinated to sulfides (28). Most prominent examples of such complex metalloclusters are the FeMo cofactor and the P-cluster of nitrogenase of nitrogen-fixing bacteria, the nickel-containing cluster of bacterial NiFe-hydrogenases, and the H-cluster of bacterial and algal iron-only hydrogenases (29). The binding motifs for Fe/S clusters in proteins are not highly conserved, yet some consensus motifs have been recognized, including the $CX_4CX_2CX_{\approx 30}C$ motif in mammalian and plant [2Fe-2S] ferredoxins and the $CX_2CX_2CX_{20-40}C$ motif, which was originally defined in [4Fe-4S] ferredoxins. Frequently, a proline (or less common a glycine) residue is located next to one of the cysteine residues. Many Fe/S clusters are fairly labile and can be destroyed under oxidative stress conditions.

WELL-KNOWN AND NEWLY IDENTIFIED EUKARYOTIC Fe/S PROTEINS

Eukaryotes contain Fe/S proteins in three cellular compartments: mitochondria, cytosol, and nucleus (**Figure 1**). The first known Fe/S proteins include mitochondrial complexes I, II, and III of the respiratory chain, aconitase of the citric acid cycle, and a [2Fe-2S] ferredoxin (termed adrenodoxin in higher eukaryotes and

ISC: iron-sulfur cluster

Nitrogenase: complex metalloenyzme of azototrophic bacteria, which converts molecular nitrogen into ammonia using the energy of ATP

CIA: cytosolic iron-sulfur protein assembly

Hydrogenase: enzymes that use metallocenters to generate or use molecular hydrogen, usually for redox reactions

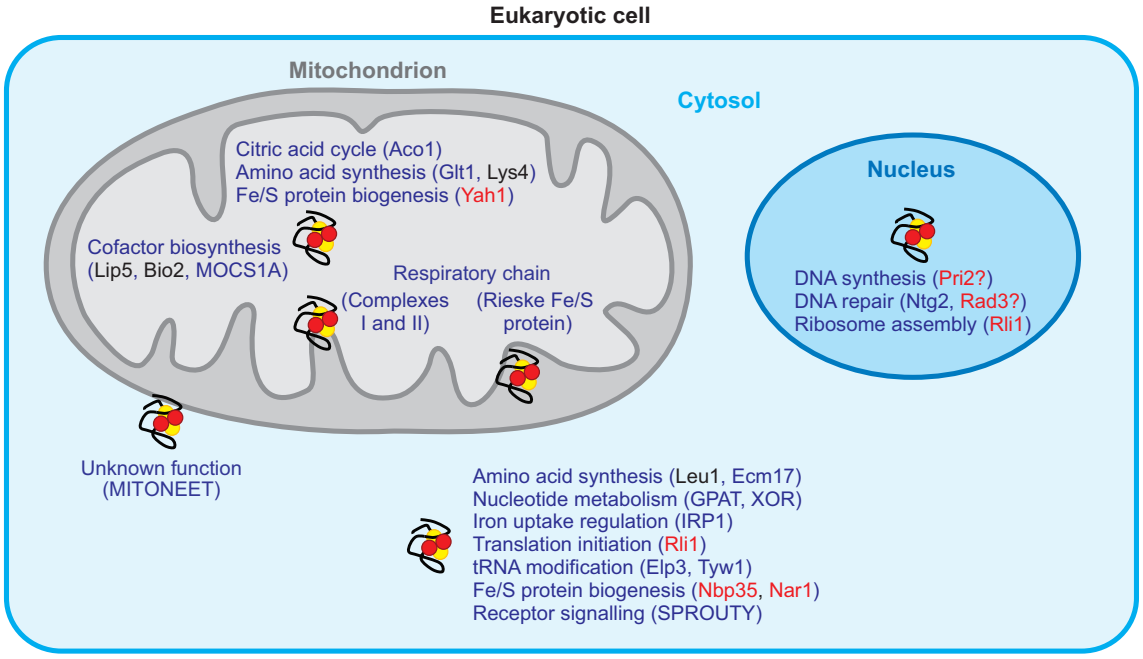


Figure 1

Functions and localization of Fe/S proteins in eukaryotes. Eukaryotic Fe/S proteins are localized in mitochondria, cytosol, and nucleus. Representative functions of selected Fe/S proteins (names in parenthesis) in their respective compartments are indicated. The names usually represent the abbreviations from the yeast *Saccharomyces cerevisiae*. Fe/S proteins essential for yeast viability are indicated in red. Fe/S proteins present in fungi, such as *S. cerevisiae* only, are presented in black. Some Fe/S proteins (depicted in capital letters) are present in higher eukaryotes only. Abbreviations: Aco1, aconitase; Bio2, biotin synthase; Ecm17, sulfite reductase β-subunit; Elp3, elongator protein 3; Glt1, glutamate synthase; GPAT, glutamine phosphoribosylpyrophosphate amidotransferase; IRP1, iron regulatory protein 1; Leu1, isopropylmalate isomerase; Lip5, lipoate synthase; Lys4, homoaconitase; mitoNEET, putative mitochondrial outer membrane Fe/S protein; MOCS1A, molybdenum cofactor synthesis protein 1A; Nar1 and Nbp35, members of the CIA machiery; Ntg2, DNA glycosylase 2; Pri2, primase 2; Rad3, DNA helicase; Rli1, RNase L inhibitor; SPROUTY, inhibitor of receptor tyrosine kinase signaling; Tyw1, tRNA wybutosine thio modification protein; XOR, xanthine oxidoreductase; Yah1, ferredoxin.

Yah1 in yeast), which also can be found in bacteria. Complex I of bacteria contains nine Fe/S clusters and that of eukaryotes eight Fe/S clusters, which are bound to domains exposed to the cytosol or mitochondrial matrix, respectively (30, 31). Complex II possesses three Fe/S clusters of the [2Fe-2S], [3Fe-4S], and [4Fe-4S] type (32). Complex III harbors the Rieske [2Fe-2S] cluster in which the Fe ions are coordinated by two cysteine and two histidine residues. Other mitochondrial Fe/S proteins include the two radical S-adenosyl-L-methionine (SAM) proteins biotin synthase (Bio2) and lipoate synthase (Lip5) of fungi. A recently identified matrix Fe/S protein is MOCS1A, involved in the synthesis of precursor Z required for molybdenum cofactor biosynthesis (33). Most of the mitochondrial Fe/S clusters face the matrix space. A notable exception is the Rieske Fe/S protein Rip1, which exposes its [2Fe-2S] cluster toward the intermembrane space (**Figure 1**). A recently identified Fe/S protein termed mitoNEET is associated with the outer face of the mitochondrial outer membrane, yet its physiological function and the role of the redox-active [2Fe-2S] cluster are still unclear (34).

SAM: S-adenosyl-L-methionine

In the cytosol, long-known Fe/S proteins include the fungal isopropylmalate isomerase, Leu1, the mammalian iron regulatory protein 1 (IRP1), sulfite reductase (Ecm17), and xanthine oxidoreductase (21). The list of known cytosolic Fe/S proteins is growing steadily (**Figure 1**). Two proteins of the CIA machinery, Nbp35 and Nar1, contain Fe/S clusters (35, 36). The ABC protein Rli1 (also termed ABCE1) is the first known essential yeast Fe/S protein outside mitochondria and performs a function in the export of ribosomal particles from the nucleus and in translation initiation (37–39). The majority of Rli1 is in the cytosol, yet minor amounts are located in the nucleus. Rli1 contains two Fe/S cluster binding motifs at its N terminus and holds two C-terminal ABC domains. The structure of an archaebacterial Rli1 homologue reveals two [4Fe-4S] clusters (39a). As described below, maturation of its Fe/S clusters essentially requires mitochondria, providing the first explanation of why these organelles, in particular, and Fe/S protein biogenesis, in general, are indispensable in yeast (40). Furthermore, novel Fe/S proteins include Elp3 and Tyw1, involved in tRNA modification (see below), and SPROUTY, a group of proteins acting as inhibitors of receptor tyrosine kinase signaling (41). The particular role of the Fe/S cluster in these proteins remains to be defined.

In the cell nucleus, most known Fe/S proteins are involved in DNA metabolism (**Figure 1**). Ntg2 and related proteins, such as the mammalian MutY homologue (MYH), function as DNA glycosylases in base excision during DNA repair (42). Yeast Rad3 and its mammalian homologues XPD and FancJ (see below) may contain a [4Fe-4S] cluster, similar to an archaebacterial counterpart (43). Recently, the primase Pri2 was shown to contain an Fe/S cluster, which is required for initiation of DNA replication via RNA primer synthesis (44). Both Rad3 and Pri2 are essential for yeast viability and represent two novel examples of the central importance of Fe/S protein biogenesis for life. It is expected that far more Fe/S cluster-containing proteins will be identified in the future.

IRP1: iron regulatory protein 1

ABC: ATP-binding cassette

XPD: xeroderma pigmentosum group D protein

A GENERAL OVERVIEW ON Fe/S PROTEIN BIOGENESIS

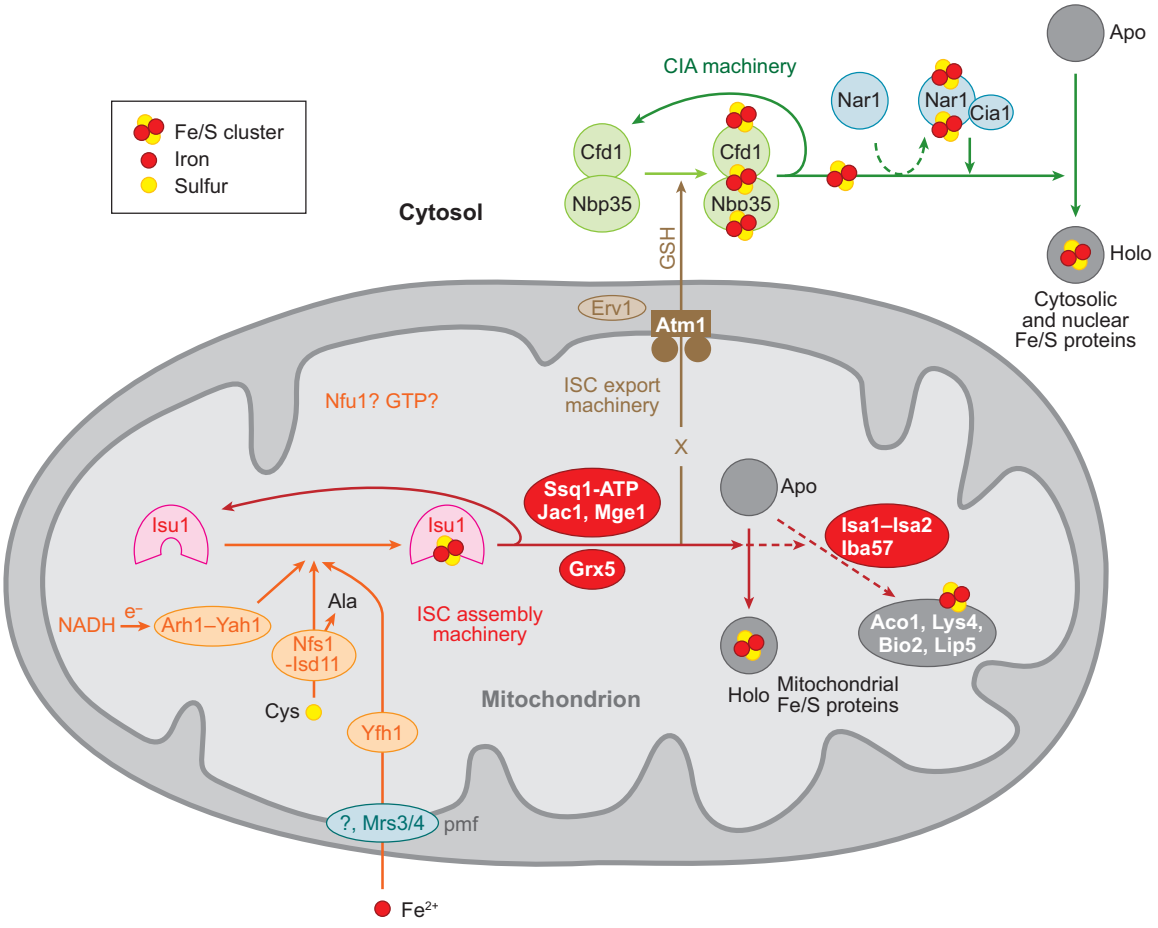
In this chapter, we provide a general overview on the basic steps underlying Fe/S protein maturation as worked out for the model organism yeast *Saccharomyces cerevisiae* in which most functional studies on eukaryotic biogenesis have been performed. Seminal findings on the components of the ISC assembly machinery were made by studying the bacterial representatives. Because these insights are largely transferable to the mitochondrial ISC assembly system, we briefly refer to some of these studies (details are summarized in References 3–8). Two highly sensitive methods are most often used to experimentally follow Fe/S protein biogenesis in vivo (45, 46). First, the activities of Fe/S cluster-dependent enzymes are monitored. Second, the incorporation of ^{55}Fe into Fe/S proteins is measured by immuno- or affinity isolation and scintillation counting of the radiolabeled proteins. The certain advantage of this method over the first approach is that it estimates de novo biosynthesis rather than steady-state levels. A whole spectrum of additional methods to follow in vitro Fe/S cluster association with proteins has been developed, including native gel electrophoresis, UV-visible spectroscopy, circular dichroism, electron paramagnetic resonance, and Mössbauer spectroscopy.

Mitochondrial Fe/S Protein Assembly: Step 1, Fe/S Cluster Assembly on Isu1

Mitochondria perform a central role in Fe/S protein maturation in eukaryotes because they are required for biogenesis of all cellular Fe/S proteins. They harbor the so-called ISC assembly machinery, which was inherited from bacteria in the endosymbiontic event leading to the engulfed precursor of present-day

mitochondria. To date, 15 yeast proteins are known to assist this complex biosynthetic process (**Figure 2**). Experimentally the reaction was subdivided into two major steps (47, 48). First, an Fe/S cluster is transiently assembled on the scaffold proteins Isu1 and Isu2 (orange arrows in **Figure 2**). (Because yeast Isu2 emanates from a recent gene duplication, is present only in some fungi such as *S. cerevisiae*, and is functionally redundant with Isu1, only Isu1 is mentioned in the following description.) In the second step, the cluster is released from Isu1, transferred to recipient apoproteins, and integrated into the Fe/S holoprotein by coordination with specific amino acid residues (red arrows in **Figure 2**). Both partial reactions need the assistance of specific ISC assembly components.

Isu1 is one of the best conserved proteins in evolution and is found in many bacteria (15) and virtually all eukaryotes (49). It contains three conserved cysteine residues that are critical for de novo Fe/S cluster assembly, a function that was established first for the N-terminal domain of bacterial NifU (50). These pioneering studies led to the concept of a scaffold function for NifU, the related bacterial IscU, and its mitochondrial counterpart Isu1 (51). IscU and Isu1 can assemble both [2Fe-2S] and [4Fe-4S] clusters (47, 52). The three-dimensional (3D) structure of a bacterial IscU dimer with a bound Zn ion instead of an Fe/S cluster has been solved (53). IscU possesses three conserved cysteine residues, which, in the folded structure, are in proximity and are exposed to solvent. In yeast, Fe/S



cluster assembly on Isu1 critically depends on the function of the cysteine desulfurase complex Nfs1-Isd11 (**Figure 2**) (47, 54, 55). It has long been thought that Nfs1 alone suffices to liberate sulfur from cysteine because the purified recombinant protein contains the enzymatic activity as a cysteine desulfurase and releases sulfide in vitro under reducing conditions (56). However, it is clear now that this reaction does not reflect the physiological situation in vivo, as in all likelihood no free sulfide is produced in this pathway. Thus, sulfide production in vitro may only be a convenient biochemical measure to monitor the activity of Nfs1 preparations. Recent experiments have documented that without functional Isd11 no Fe/S clusters can be formed in vivo on the scaffold Isu1 and consequently on target apoproteins (54).

Nfs1 is highly similar to its bacterial counterparts NifS, IscS, and SufS, which act as sulfur donors in the NIF, ISC, and SUF systems, respectively. These proteins belong to a subgroup of pyridoxal 5′-phosphate-dependent amino transferases. The 3D structures of several of these enzymes have been solved (see, for example, References 57 and 58). NifS converts cysteine to alanine by formation of a Schiff base of the amino function of the cysteine substrate with the pyridoxal 5′-phosphate cofactor (59). The conserved active-site cysteine residue in the C terminus of the NifS protein then performs a nucleophilic attack of the γ-sulfhydryl (SH) group of the cysteine intermediate causing the abstraction of γ-SH from the cysteine intermediate and the formation of a persulfide (-SSH) on the active-site cysteine. Eukaryotic Nfs1 is

Figure 2

A working model for the pathways of Fe/S protein biogenesis. Eukaryotic Fe/S protein biogenesis involves the crucial function of mitochondria. The organelles import iron in ferrous (Fe^{2+}) form from the cytosol in a membrane potential (pmf)-dependent fashion. Import is facilitated by the inner membrane carriers Mrs3 and Mrs4 and unknown proteins (?). Maturation of mitochondrial Fe/S holoproteins (Holo) involves two major steps. The synthesis of a transiently bound Fe/S cluster on the scaffold protein Isu1 (and Isu2 in yeast) is supported by the early components of the mitochondrial iron-sulfur cluster (ISC) assembly machinery (*orange arrows*). These proteins include the cysteine desulfurase complex Nfs1-Isd11, which serves as the sulfur donor for cluster synthesis, the iron-binding protein Yfh1 (frataxin) as the putative iron donor, and the electron transfer chain comprised of ferredoxin reductase Arh1 and ferredoxin Yah1, which receive their electrons (e^-) from NADH. The release of the Fe/S cluster from Isu1 and its transfer and incorporation into recipient apoproteins (Apo) are facilitated by late components of the ISC assembly machinery (*red arrows*). Fe/S cluster release from Isu1 is achieved by interaction of the Hsp70 chaperone Ssq1 with Isu1. The DnaJ-like cochaperone Jac1 and the nucleotide exchange factor Mge1 assist the ATP-dependent function of Ssq1. Furthermore, the monothiol glutaredoxin Grx5 is involved in this second major step. The ISC proteins Isa1, Isa2, and Iba57 are additionally and specifically required for the gaining activity of aconitase-type (Aco1, Lys4) and SAM-dependent (Bio2, Lip5) Fe/S enzymes (*dotted red arrows*). The role of Nfu1 is unknown, and the reaction in which GTP is required remains to be identified. Extramitochondrial Fe/S protein biogenesis requires the core ISC assembly machinery (but not the Isa and Iba57 proteins) and components of the mitochondrial ISC export machinery. The ABC transporter Atm1 of the inner membrane exports an unknown compound (X) to the cytosol for use in Fe/S protein assembly (*brown arrow*). Other members of this export machinery are the tripeptide glutathione (GSH) and the intermembrane space-located sulfhydryl oxidase Erv1, which introduces disulfide bridges into substrates. In the cytosol, the cytosolic Fe/S protein assembly (CIA) machinery catalyzes Fe/S protein maturation. The reaction can also be subdivided into two steps. First, Fe/S clusters are assembled on the P-loop NTPases Cfd1 and Nbp35, which form a heterotetrameric complex (*light green arrow*). Both mitochondrial ISC machineries are required for this reaction. The Fe/S clusters are bound to Cfd1-Nbp35 in a labile fashion and can be transferred to the Nar1 protein for its conversion to a functional holoprotein (*dotted green arrows*). Holo-Nar1 and the WD40 repeat protein Cia1 facilitate the dissociation of newly assembled Fe/S clusters from Cfd1-Nbp35 and their incorporation into cytosolic and nuclear apoproteins (*dark green arrow*). In addition to the indicated CIA protein interactions, Nbp35 interacts with Nar1.

Mitosome: mitochondria-derived, double-membrane-bounded organelle, which has lost many of the functions of a classical mitochondrion, yet has maintained ISC assembly components

believed to follow a similar mechanism. The persulfide group is subsequently transferred in an Isd11-dependent fashion from Nfs1 to Isu1.

Nfs1 and Isd11 form a tight complex, which has been estimated to be ~200 kDa in size (54, 55). The stoichiometry of Nfs1 and Isd11 is unknown so far. The amino acid sequence of Isd11 is only moderately conserved, yet the protein is found in virtually all eukaryotes from yeast to man. Even organisms lacking mitochondria and containing mitosomes instead (see below) contain a homologue of Isd11, indicating the importance of the role of Isd11 for Fe/S protein biogenesis (60, 60a). Conspicuously, no bacterial homologues of Isd11 have been identified to date, making it likely that Isd11 is a eukaryotic addition to the bacteria-derived ISC assembly machinery. It will be interesting to analyze the functional implications of Isd11 binding to Nfs1 and to learn how bacterial Nfs1 homologues may replace the apparently crucial function of this small protein.

Although the source of sulfur for Fe/S cluster formation is clear, the pathway of iron to Isu1 is less well defined. Iron can be imported into the mitochondrial matrix only in its reduced form (Fe^{2+}) (61). This step requires a membrane potential (**Figure 2**) and is facilitated by the integral inner membrane proteins Mrs3 and Mrs4, members of the mitochondrial carrier family (62–66). Yfh1 (also termed frataxin) binds iron in vitro and undergoes an iron-stimulated interaction with Isu1-Nfs1 (67–69). Therefore, the protein is thought to serve as the iron donor of the reaction. Mutations in the acidic ridge of Yfh1 impair both iron binding and the interaction with Isu1, showing the functional importance of this region (70). However, this region is not essential for formation of iron-induced aggregates, leading to the suggestion that frataxin functions as a monomer in vivo (71). Fe/S cluster assembly on Isu1 further depends on the electron transfer chain consisting of the ferredoxin reductase Arh1 and the [2Fe-2S] ferredoxin Yah1, which likely receives its electrons from NADH (**Figure 2**) (72–74). Even though it is plausible that electrons are needed for reduction of the sulfane sulfur (S°) to sulfide (S^{2-}), the requirement of this electron transfer chain in another step is not excluded. As is evident from this summary, the principles of Fe/S cluster formation on Isu1 have been established, yet the chemical mechanism is still unknown and awaits further in vitro studies with purified components.

Mitochondrial Fe/S Protein Assembly: Step 2, Fe/S Cluster Transfer from Isu1 to Apoproteins

The second major step of Fe/S protein biogenesis in mitochondria involves the release of the Isu1-bound Fe/S cluster, its transfer to apoproteins, and its assembly into apoproteins by coordination with specific amino acid ligands (**Figure 2**). To date, four ISC proteins are known to specifically assist this process, the mitochondrial monothiol glutaredoxin Grx5, the dedicated chaperone of the Hsp70 family termed Ssq1, the DnaJ-like cochaperone Jac1, and the nucleotide exchange factor Mge1 (8, 75–79). Ssq1 undergoes a specific protein interaction with a small highly conserved segment of Isu1 (LPPVK). This binding is thought to dislocate the Fe/S cluster from Isu1, thus facilitating its transfer to apoproteins. Jac1 is a cochaperone and stimulates the ATPase function of Ssq1. The nucleotide exchange factor Mge1 serves to exchange bound ADP for ATP to start a new cycle. Interestingly, the bacterial Mge1 homologue, GrpE, is not needed in Fe/S protein maturation owing to the labile character of Hsp66-bound ADP, which distinguishes the eukaryotic and prokaryotic ISC systems (8). Recent findings suggest that in addition to ATP there is a requirement for GTP in mitochondrial Fe/S protein maturation (79a). The step and the ISC component requiring GTP remain to be identified.

Jac1 also binds to Isu1, but this interaction is not essential in vivo (78). Mutational

studies suggest, however, that the interaction might be beneficial for targeting Ssq1 to the Isu1 scaffold under conditions that have a high demand for Fe/S protein biogenesis. A careful phylogenetic analysis has revealed that Ssq1 is present in some fungi only, and this raises the question, which component performs its function in other species (80)? It has been known for a long time that the *S. cerevisiae* mitochondrial Hsp70, termed Ssc1, can partially replace Ssq1 (79, 81). Further investigations suggested that in most organisms Ssc1 not only performs its well-studied functions in protein import and folding but also participates in Fe/S protein metabolism (80). Thus, Ssq1 may not be the direct orthologue of bacterial Hsc66 encoded by the *isc* operon, but Ssq1 may have arisen from a recent gene duplication in fungi and may have optimized its function for Fe/S protein maturation. The specific role of the chaperone system in Fe/S cluster transfer to apoproteins has been discovered mainly by in vivo studies in yeast (47, 48). These investigations received support from in vitro spectroscopic experiments with bacterial ISC proteins, showing the ATP-dependent stimulation of Fe/S cluster transfer from IscU to apoferredoxin by addition of both Hsp66 and Hsp20 (the bacterial Jac1 homologue) (82). This experimental system will allow further insights into the molecular events of this step. It may also be possible to unravel the precise molecular role of Grx5 participating in this partial reaction (47, 75).

A Specialized Function of the Isa Proteins in Fe/S Enzyme Activation

Although the ISC components discussed so far are required as general factors for biogenesis of all cellular Fe/S proteins, some of the ISC proteins play a more specialized role. A recent combination of genetic, cell biological, and biochemical approaches has shown that the function of Isa1 and Isa2 is critical for the enzymatic activity of mitochondrial aconitase-like proteins (Aco1 and Lys4) and the mitochondrial SAM-dependent proteins biotin synthase (Bio2) (83) and lipoic acid synthase (Lip5). These proteins are required in addition to and after the function of the ISC assembly proteins described above (see the dotted red arrows in **Figure 2**). Involvement of the Isa proteins in the functional activation of the above-mentioned four mitochondrial enzymes in yeast is suggested by the loss of mitochondrial DNA and auxotrophies for glutamate, lysine, biotin, and lipoic acid developed by Isa1- and/or Isa2-deficient cells (83–86; U. Mühlenhoff, unpublished). These observations are supported by biochemical experiments showing that these cells lack enzyme activities of these four mitochondrial proteins. Furthermore, cells depleted of the Isa proteins show no ^{55}Fe radiolabel incorporation into Aco1 and Lys4, directly demonstrating the need for the Isa proteins in Fe/S cluster assembly. Surprisingly, a different result was obtained for Bio2, which could assemble both its Fe/S clusters in the absence of the Isa proteins, yet the protein was devoid of its enzymatic function (83). This led to the working hypothesis that the Isa proteins may be involved in the regeneration of the [2Fe-2S] cluster, which, as discussed by Jarrett (87), might serve as a sulfur source for the conversion of desthiobiotin to biotin. Experimental verification of these ideas will require reconstitution of these processes in vitro with isolated components.

In bacteria, two roles for the homologous IscA proteins have been suggested: an alternative scaffold for de novo Fe/S cluster assembly or an iron donor for IscU (3, 88). Both of these functions can be excluded for the yeast Isa1-Isa2 proteins. They do not bind an Fe/S cluster but associate iron in vivo, yet this iron is not the source for Fe/S cluster assembly on Isu1 (U. Mühlenhoff, unpublished). These studies establish a novel function for Isa proteins in Fe/S protein assembly, representing the first example of ISC components exhibiting substrate specificity for the maturation of a subset of Fe/S proteins (**Figure 2**). Future biochemical studies will have to unravel their precise molecular function.

GSH: glutathione

Recently, another protein, designated Iba57 (also termed Caf17), was identified that physically interacts with both Isa1 and Isa2 (88a). Disruption of its gene elicits a phenotype highly similar to *ISA1* and/or *ISA2* deletion mutants (see above), suggesting that all three proteins are operating in the same pathway. As all three ISC proteins are conserved in eukaryotes, it seems likely that these proteins are specialized assembly factors for the maturation of aconitase-type Fe/S proteins and radical SAM proteins inside mitochondria.

The Export Function of Mitochondria in Cytosolic Fe/S Protein Maturation

All available evidence indicates that Fe/S protein maturation in both the cytosol and nucleus strictly depends on the function of the mitochondrial ISC assembly machinery. In principle, this could be explained by the presence of ISC components in the cytosol where they also might support Fe/S protein assembly. However, investigations in yeast cells have documented that Nfs1 or Isu1 when mistargeted to the cytosol by deleting the mitochondrial targeting sequences fails to support Fe/S protein assembly in both mitochondria and the cytosol (56, 89–91). Furthermore, cytosolic Isu1 is not able to assemble a transient Fe/S cluster, distinguishing it from its mitochondrial form. Hence, both yeast Nfs1 and Isu1 are needed inside mitochondria for function in cytosolic and nuclear Fe/S protein maturation. Small amounts of yeast Nfs1 have been genetically identified as present in the nucleus (90). Yeast cells without this extramitochondrial form of Nfs1 are not viable, but they maintain an active cellular Fe/S protein biogenesis in the presence of mitochondrial Nfs1. This means that the nuclear form of Nfs1 performs a yet to be identified essential function (also, see below).

Apparently, the function of the mitochondrial ISC assembly machinery is critical for the ability of the cell to generate extramitochondrial Fe/S proteins, but the molecular details of this dependence still have to be defined in more detail. According to a working hypothesis, the ISC assembly machinery produces a (still unknown) component (designated X in **Figure 2**), which is exported from the mitochondrial matrix to the cytosol where it performs an essential function in the maturation process. This transport step is accomplished by the so-called ISC export machinery (see the brown arrow in **Figure 2**). Its central component is the ABC transporter Atm1 of the mitochondrial inner membrane (89). Depletion of Atm1 leads to a severe impairment of Fe/S protein assembly in the cytosol and nucleus, but mitochondrial Fe/S proteins are unaffected. Purified Atm1 reconstituted into proteoliposomes possesses an ATPase activity that is stimulated by sulfhydryl groups, especially by cysteine residues of peptides (92). Because the ATPase of ABC transporters is typically stimulated by their physiological substrates, this may indicate that the substrate transported by Atm1 contains a sulfhydryl group in a peptidic environment.

The export reaction is further facilitated by the sulfhydryl oxidase Erv1 of the intermembrane space (**Figure 2**) (93). Erv1 catalyzes the formation of disulfide bridges (94), and recent investigations showed its involvement in the disulfide formation-driven import of proteins into this compartment by reoxidizing the import component Mia40 (95–97). Thus, Erv1 appears to perform multiple functions. The enzyme may deliver the electrons derived from the sulfhydryl oxidation process to either molecular oxygen or cytochrome *c* (98). A third component of the ISC export machinery is the tripeptide glutathione (99). Hitherto, the molecular role of Erv1 and GSH in Fe/S protein biogenesis was not understood. Depletion of each of the three ISC export components results in a highly similar phenotype, including normal biogenesis of mitochondrial Fe/S proteins, impairment of extramitochondrial Fe/S protein maturation, stimulation of the Aft1-dependent iron regulon (see below), and an iron accumulation inside mitochondria. Only the latter three

phenotypes are observed upon depletion of the major components of the ISC assembly machinery, allowing the distinction of the two ISC machineries.

An Emerging Mechanism for the Function of the CIA Machinery

Recent years have led to the identification of several components in the cytosol (and nucleus) with a function in the maturation of cytosolic and nuclear Fe/S proteins. All four proteins, Cfd1, Nbp35, Nar1, and Cia1, are essential for yeast cell viability, and together form the CIA machinery (35, 36, 100, 101) (**Figure 2**). The CIA components are highly conserved in eukaryotes. According to recent in vivo and in vitro studies, this process supported by the CIA proteins can be subdivided into two major partial reactions, similar to Fe/S protein assembly by the ISC assembly machinery (102). First, Fe/S clusters are transiently assembled on the soluble P-loop NTPases Cfd1 and Nbp35, which form a complex and serve as a scaffold (see the light green arrow in **Figure 2**). This step requires the mitochondrial ISC systems. Then, the Fe/S clusters are transferred to apoproteins, a reaction requiring the CIA proteins Nar1 and Cia1 (dark green arrows in **Figure 2**). Direct interactions between Nbp35-Nar1 and Nar1-Cia1 may be important for this step.

Both Cfd1 and Nbp35 contain four conserved C-terminal cysteine residues, but which ones may contribute to the assembly of the labile Fe/S clusters of the [4Fe-4S] type is unknown so far (102). Nbp35 possesses an additional Fe/S cluster at its N terminus, bound in a typical ferredoxin-like motif comprising four conserved cysteine residues (36). This segment is essential for function, yet its precise role is unclear. Likewise, both proteins contain a nucleotide-binding domain, but it is not known whether ATP or GTP is bound and what role the nucleotide may perform. Unlike the mitochondrial Isu1 scaffold, Cfd1-Nbp35 do not directly interact with a sulfur-donating protein, such as the extramitochondrial version of Nfs1, as genetic and biochemical studies did not establish a role for the protein in this process (see above). Rather, it has become clear that the mitochondrial version Nfs1, together with other mitochondrial ISC assembly and export components, is needed for extramitochondrial Fe/S cluster formation (102). Hence, Cfd1 and Nbp35 appear to receive their sulfur moiety from mitochondrial Nfs1, predicting that Atm1 may export a sulfur-containing compound.

The above model received further support from recent biochemical experiments in which the Fe/S cluster assembly process on the Cfd1-Nbp35 complex was reconstituted in vitro with isolated mitochondria under anaerobic conditions (R. Dutkiewicz, U. Mühlenhoff, & R. Lill, unpublished). Cluster formation on purified Cfd1-Nbp35 was followed by radiolabeling with either ^{35}S-cysteine or ^{55}Fe and required added iron or cysteine, respectively. The mitochondria needed to be energized with a membrane potential and matrix ATP, possibly reflecting the membrane potential-dependent import of iron and the ATP-dependent function of both Ssq1 and Atm1. Furthermore, efficient Fe/S cluster assembly on Cfd1-Nbp35 depended on a functional mitochondrial ISC assembly apparatus and the Atm1 and Erv1 components of the ISC export machinery. Interestingly, Fe/S cluster assembly did not occur on the apoform of Leu1 as a recipient protein, yet the Fe/S clusters could be transferred from Cfd1-Nbp35 to Leu1, demonstrating the critical importance of this complex as a scaffold for Fe/S protein formation in the cytosol.

Nar1 is structurally related to bacterial or algal iron-only hydrogenases (35). Similar to these proteins, Nar1 contains two Fe/S clusters of unknown type, which are coordinated by eight conserved cysteine residues (103; E. Urzica, unpublished). Assembly of these clusters on Nar1 in vivo requires the function of both Cfd1 and Nbp35 but not that of Cia1 (see the dotted green arrow in **Figure 2**) (101, 102). In contrast, neither Nar1 nor Cia1 is

RNAi: RNA interference method for specific degradation of endogenous mRNAs after binding of complementary RNA oligonucleotides

IRE: iron-responsive element

needed for Fe/S cluster incorporation into Cfd1-Nbp35, which is compatible with the idea that the former proteins are involved in a late step of biogenesis, e.g., in the transfer of the Fe/S cluster from the Cfd1-Nbp35 scaffold to target Fe/S apoproteins. In the in vitro Fe/S cluster transfer experiments mentioned above, Nar1 and Cia1 can be bypassed. A similar finding was made for the purified bacterial scaffold IscU, which can transfer Fe/S clusters to apoproteins independently of the chaperone system (5). The CIA proteins may dynamically interact with each other, and Cia1 might play an important role in these complex formations. As with other members of the large WD40 repeat protein family, Cia1 may serve as a protein docking site and bind Nar1 and possibly other CIA proteins. The recently solved 3D structure of Cia1 shows a seven-bladed β-propeller, which may, as shown for other WD40 β-propeller proteins, provide a binding platform (104).

Nuclear Fe/S proteins require the ISC and CIA machineries for assembly, but hitherto, it was unclear where maturation occurred (35, 36, 54, 101, 102). Either they are assembled in the cytosol and then imported as a holoprotein, or the assembly process takes place in the nucleus, requiring the import of the apoprotein into the nucleus. Because small amounts of the CIA proteins Cfd1, Nbp35, and Nar1 as well as the majority of Cia1 have been localized in the nucleus, both scenarios seem possible. Additionally or alternatively, the nuclear CIA components could be involved in repair of damaged Fe/S clusters in this compartment, but nothing is known to date about this potential process.

Fe/S PROTEIN ASSEMBLY IN HIGHER EUKARYOTES

The ISC and CIA components are highly conserved in eukaryotes from yeast to man (21). It was therefore expected that in higher eukaryotes these components fulfill similar roles as those identified in *S. cerevisiae*. Studies analyzing Fe/S protein biogenesis in higher eukaryotes have been published only recently and have generally confirmed this expectation. Functional investigations on Fe/S protein assembly in vertebrates have been greatly facilitated by the RNAi technology, which allows the specific depletion of a given component over time after targeted degradation of its mRNA. So far, analysis of the maturation of Fe/S proteins in mammalian cells has been restricted to the measurement of the steady-state levels of Fe/S cluster-dependent enzymes, such as mitochondrial aconitase as well as complexes II (succinate dehydrogenase) and III (cytochrome bc_1 complex) of the respiratory chain (**Figure 1**). For cytosolic Fe/S proteins, the aconitase activity of IRP1 has been widely used. Complementary to this assay, the increase in binding of the apoform of IRP1 to iron-responsive elements (IREs) of mRNAs can be used to indirectly monitor the Fe/S cluster status of this protein (see below). Recently, the tableau of cytosolic Fe/S protein targets has been extended to xanthine oxidoreductase (XOR) and glutamine phosphoribosylpyrophosphate amidotransferase (**Figure 1**) (105, 106). XOR has to be used with caution because it contains an additional molybdenum cofactor and FAD. Synthesis of the molybdenum cofactor itself is dependent on a mitochondrial Fe/S protein, MOCS1A (33). A decrease in XOR activity may therefore not necessarily reflect an impaired Fe/S protein biogenesis in the cytosol but may be due to multiple causes. Glutamine phosphoribosylpyrophosphate amidotransferase is a key enzyme of purine biosynthesis and contains a [4Fe-4S] cluster. Enzymatic activation requires the proteolytic cleavage of an N-terminal prepeptide, which strictly depends on the assembly of the Fe/S cluster. Hence, proteolytic processing can be used as a measure for Fe/S protein maturation and conveniently be followed by immunostaining (106). The ^{55}Fe radiolabeling assay developed for yeast (see above) has not been successfully employed so far for monitoring Fe/S protein maturation in higher eukaryotes. The major reason why this assay may be difficult to

establish in, e.g., human cell culture, is the detection limit for radiolabeled proteins. Unlike yeast cells, higher eukaryotes cannot be grown under iron depletion conditions without severe effects on growth and metabolism. As a consequence, the radioactivity incorporated into Fe/S proteins may be too low for detection. Currently, the unavailability of this assay for the analyses of Fe/S protein biogenesis in higher eukaryotes is a true shortcoming because the procedure measures de novo assembly rather than steady-state levels of Fe/S proteins. Furthermore, the lack of such a radiolabeling assay hampers the verification of the presence of an Fe/S cluster on newly identified (potential) Fe/S proteins under physiological conditions in vivo.

The first functional study of Fe/S protein biogenesis in higher eukaryotes was reported for human frataxin (related to yeast Yfh1) (107), a protein deficient in the neurodegenerative disease Friedreich's ataxia. Depletion of frataxin by RNAi technology resulted in a defect in both mitochondrial Fe/S enzymes and an increase in IRE binding of cytosolic IRP1. No effect on the biosynthesis of heme was observed under these conditions, indicating that frataxin has a primary function in Fe/S protein maturation in human cells. This study was widely confirmed in other biological organisms. In further RNAi and DNA array investigations, Fe/S protein defects were found as the primary consequence of frataxin depletion followed by oxidative stress and heme defects, suggesting that these consequences were secondary (108–110). The sequence of defects appeared to be similar in Friedreich's ataxia patients' cells (111). Recently, a mouse model has provided further evidence for a critical role of mitochondrial frataxin in cytosolic Fe/S protein maturation, as several Fe/S protein activities were affected in frataxin-deleted mouse tissues (106). Frataxin was found binding to other components of the ISC assembly machinery, such as the human Nfs1-Isd11 complex and the Ssq1 homologue Grp75, supporting its primary function in Fe/S protein biogenesis (112). This study suggests that the members of the mammalian ISC assembly machinery undergo interactions similar to those described for the yeast counterparts.

RNAi depletion studies were performed recently on human Nfs1 (huNfs1) and huIsu1, the two central players of the ISC assembly machinery (113–115). In both cases, their depletion in cell culture resulted in a strong impairment of mitochondrial and cytosolic Fe/S protein activities, which, in the case of huNfs1, could be restored by expression of the mouse homologue of Nfs1, indicating the specificity of the RNAi approach. Interestingly, depletion of huNfs1 resulted in a highly altered mitochondrial morphology (114). huNfs1-deficient mitochondria lacked cristae membranes and showed an onion-shaped membrane system with multiple concentric membranes. Although this morphology is clearly reminiscent of the fuzzy onion mutant of *Drosophila* encoding the Fzo1 protein involved in mitochondrial fission events, the molecular explanation of this potential role of huNfs1 in mitochondrial dynamics and morphology and the general specificity for a defect in Fe/S protein maturation remains to be established. RNAi was also used to knock down *Trypanosoma brucei* Nfs1 and Isu1, which are localized to the mitochondrion (116). Depletion results in diminished aconitase activities in both mitochondria and cytosol, mimicking the findings with yeast and human cells.

Recently, a zebrafish mutant designated *shiraz* was identified in which a member of the ISC assembly machinery, the mitochondrial monothiol glutaredoxin Grx5, was mutated (117). Even though biochemical studies on the Fe/S cluster metabolism are difficult to perform in this model organism, there is evidence for a role of zebrafish Grx5 in Fe/S protein biogenesis. First, zebrafish Grx5 can functionally replace its counterpart in yeast. Second, in the *shiraz* mutant, the function of IRP1 as an IRE-binding protein was activated, indicating impaired Fe/S cluster assembly on this protein. Phenotypically, the increased IRE binding of IRP1 leads to a translational arrest

of ALAS2, the erythroid δ-aminolaevulinate synthase, and consequently an impaired heme synthesis, explaining the hypochromic anemia observed in this mutant animal. Interestingly, in humans, a mutation in huGrx5 also increases IRE binding of IRP1, yet elicits sideroblastic microcytic anemia (118) (also, see below).

Several studies have provided evidence for a function of the ISC export machinery in cytosolic Fe/S protein biogenesis in higher eukaryotes. An RNAi study was performed on the human mitochondrial ABC transporter ABCB7, the functional orthologue of yeast Atm1 (119). Depletion of ABCB7 resulted in a specific increase in the IRE-binding capacity of IRP1, indicative of an impairment of Fe/S cluster assembly on IRP1. The activities of mitochondrial Fe/S proteins (aconitase and succinate dehydrogenase) were not significantly affected under these conditions. This finding closely mimics the situation found upon depletion of yeast Atm1 and reassures that these proteins perform the same function (120). Strong support for ABCB7 function in Fe/S protein biogenesis was obtained from a mouse knockout model. Universal ablation of the *ABCB7* gene in mouse is embryonically lethal in early stages of development, asserting the importance of this ABC transporter for life (105). The only viable conditional gene knockout of *ABCB7* was obtained for liver, which indicates that the encoded protein plays a less important role in this tissue or has a functional counterpart. Such a role may be expected for the ABC transporter ABCB6, which shows close homology to ABCB7, can functionally replace yeast Atm1, and is expressed in liver (121, 122). However, ABCB6 was recently located in the mitochondrial outer membrane and claimed to function as a transporter of porphyrin (122, 123). The gene knockout of *ABCB7* in liver had severe consequences on the cytosolic Fe/S proteins aconitase (IRP1) and xanthine oxidase with no detectable defects of mitochondrial Fe/S proteins (105). Hence, it was concluded that the function of ABCB7 is similar to that described for yeast Atm1. Together, these studies indicate a conservation of the ISC assembly and export machineries from yeast to man and suggest highly similar mechanisms of Fe/S protein maturation in virtually all eukaryotes.

Depletion of huNbp35, the human homologue of yeast Nbp35, by RNAi caused a massive reduction in growth rates showing the importance of this CIA component for cell viability (O. Stehling & R. Lill, unpublished). Moreover, cytosolic Fe/S protein activities were severely affected, whereas mitochondrial Fe/S proteins were fully functional. These findings indicate a functional similarity of this CIA component in yeast and human cells. Interestingly, mouse Nbp35 (also termed Nubp1) and the related mouse Cfd1 (also termed Nubp2) were found to interact with a kinesin involved in centrosome duplication (123a). Knockdown of mouse Nbp35 or Cfd1 elicited similar phenotypes as depletion of the kinesin. Either Nbp35 and Cfd1 perform a function in addition to Fe/S protein maturation or these data suggest the involvement of an Fe/S protein in this process.

In summary, these studies on mammalian ISC and CIA proteins provide a first glimpse of the pathways for Fe/S protein maturation in organisms other than yeast. In general, it became clear that the ISC and CIA components of higher eukaryotes studied so far perform similar, if not identical, roles as in yeast. This notion is also supported by several cases in which the mammalian proteins could functionally replace the yeast ISC or CIA counterparts, for instance Atm1, Yfh1, Grx5, and Cia1 (104, 117, 120, 124).

Several of the ISC assembly proteins have been detected in the cytosol and/or nucleus of human cells, including huNfs1, huIsu1, huNfu1, and frataxin (23). The amount of these proteins is usually low, and they are targeted via different mechanisms. huNfs1 was reported to use the second AUG start codon in its mRNA for targeting to the cytosol and nucleus. Translation from this second codon excludes mitochondrial-targeting

information and some N-terminal residues, which are highly conserved in huNfs1 homologues and were found essential for the function of yeast Nfs1 and bacterial IscS (56). In contrast, purified cytosolic huNfs1 was shown to be functional as a cysteine desulfurase in vitro and when combined with the cytosolic form of huIsu1 in converting IRP1 to its aconitase form (124a). huIsu1 and huNfu1 use alternative splicing mechanisms to create proteins with or without a mitochondrial presequence for targeting to mitochondria or cytosol, respectively (115, 125). The transcripts encoding the cytosolic versions are present in low abundance in expressed sequence tag databases and are usually derived from tumor cells (56). The potential function of these extramitochondrial ISC proteins is experimentally difficult to address as standard RNAi depletion approaches also deplete the mitochondrial version of these proteins. Therefore, only limited information has been derived so far as to the functional relevance of these ISC proteins. In the case of huNfs1, expression of a mouse Nfs1 homologue in the cytosol could not functionally rescue growth and the defects of cytosolic aconitase, indicating that this version cannot assist assembly of cytosolic Fe/S proteins, at least not without the presence of a functional mitochondrial huNfs1 (114). The presence of a unique nucleotide sequence for the cytosolic version of huIsu1 allowed the specific knockdown of this isoform, yet the depletion was without effect on the cytosolic aconitase activity of IRP1 (115). After treatment of the cells with H_2O_2 or an iron chelator, which destroys the Fe/S cluster on IRP1, the recovery of Fe/S cluster association on IRP1 was slightly delayed in the absence of cytosolic huIsu1. This suggests a role of cytosolic huIsu1 in the recovery of cytosolic Fe/S clusters, which are damaged by oxidative stress or iron deprivation, even though repair/regeneration apparently can take place in the absence of cytosolic huIsu1. It will be interesting to learn more about repair or regeneration of damaged Fe/S clusters in both yeast and higher eukaryotes.

The presence of ISC proteins in the cytosol of human cells raises several important questions. What is their specific role in the cytosol and nucleus, and why are these proteins present, in addition to the CIA machinery? It is well accepted that in yeast the ISC proteins are not functional in Fe/S cluster assembly outside mitochondria (22, 23). Therefore, knowing in which organisms functional ISC proteins were introduced in the cytosol during evolution and what evolutionary advantage this differential localization has provided to the higher eukaryote will be useful.

Several (pathogenic) organisms formerly called amitochondriates are known to lack classical mitochondria, yet they contain mitochondrial remnants, termed mitosomes or hydrogenosomes, instead (126, 127). These double-membrane-bounded organelles were derived in evolution from mitochondrial ancestors but have lost most of the functions of classical mitochondria, such as heme biosynthesis and respiration. Emerging data suggest that both mitosomes (in *Giardia* and two microsporidian organisms) (60a, 128) and hydrogenosomes (in *Trichomonas*) (129, 130) contain functional homologues of the mitochondrial ISC assembly components Nfs1, Isu1, Ssq1 and Yfh1. It appears from these studies that Fe/S protein assembly seems an indispensable function of this organelle. While this interpretation is consistent with the findings for the microsporidian *Encephalitozoon cuniculi* (60a), the issue might be more complex. *E. histolytica*, which contains mitosomes, is not known to encode any ISC components yet harbors NifS and NifU homologues in a so far unknown cellular location (21). Moreover, *Trachipleistophora hominis* Isu1 and frataxin are located in the cytosol requiring functional studies to clarify how these proteins might functionally interact with Nfs1-Isd11 and Ssq1 residing inside the mitosomes (60a).

Hydrogenosome: mitochondria-derived, double-membrane-bounded organelle, which metabolizes pyruvate to hydrogen, CO_2, and acetate for ATP generation

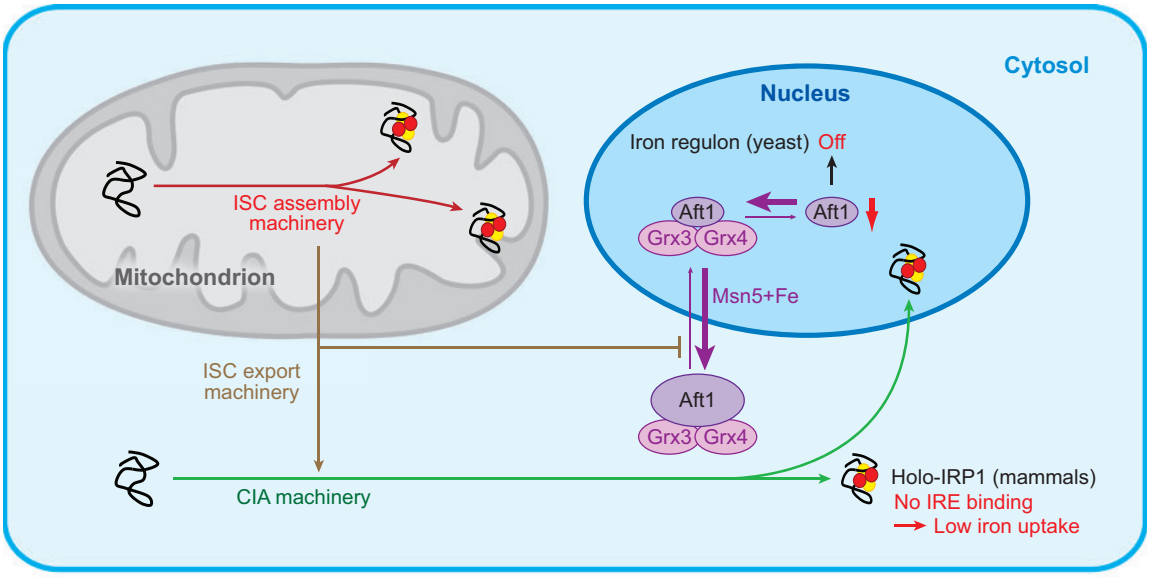
INVOLVEMENT OF THE ISC, BUT NOT THE CIA MACHINERIES IN YEAST CELLULAR IRON HOMEOSTASIS

The mitochondrial ISC assembly and export systems perform a critical influence on the uptake, intracellular distribution and utilization of iron in the yeast cell (131, 132). In fact, the importance of Yfh1 and Atm1 in iron homeostasis was realized well before the identification of their primary function in Fe/S cluster metabolism (133–135). Before we summarize the current knowledge on the role of Fe/S protein biogenesis in iron regulation in *S. cerevisiae*, we provide a brief summary of how this yeast regulates iron homeostasis (for more detailed reviews see References 132 and 136). The major regulatory factors controlling iron acquisition and intracellular iron distribution in response to different iron levels in the environment are the transcription factors Aft1 and Aft2 (137–139) (**Figure 3**). Because deletion of *AFT2* is associated with mild phenotypic effects, Aft1 may be the major iron-responsive transcription factor. Upon iron depletion, Aft1 translocates with help of the importin Pse1 from the cytosol into the nucleus, where it transcriptionally activates genes of the so-called iron regulon (140) (**Figure 3*b***). Encoded gene products include proteins involved in iron acquisition through the cell wall, plasma and endosomal membranes as well as in iron distribution within various cellular compartments. Another Aft1 target, the RNA-binding protein Cth2, degrades mRNAs of iron-containing enzymes, thus limiting the iron utilization under iron-deplete conditions (141). This post-transcriptional regulatory mechanism adds another level to balancing iron homeostasis. The targets of the Aft1 and Aft2 transcription factors overlap, but the majority of the regulated genes is specific for one of the two Aft proteins (139). Aft1 physically binds to the two functionally redundant cytosolic monothiol glutaredoxins, Grx3 and Grx4 (**Figure 3**) (142). These two proteins were recently shown to perform a role in iron regulation, as their deletion turned on genes of the iron regulon. In the absence of Grx3-Grx4, Aft1 activates transcription of the iron regulon, whereas overproduction of Grx4 attenuates Aft1 activity. Presumably, dissociation of Aft1 from Grx3-Grx4 precedes Aft1-DNA interaction, but it is unknown where dissociation might occur. Export of Aft1 from the nucleus under iron-replete conditions depends on a conformational change and an export signal in Aft1, which is recognized by the exportin Msn5 (142a) (**Figure 3*b***). Although the current data clearly show that

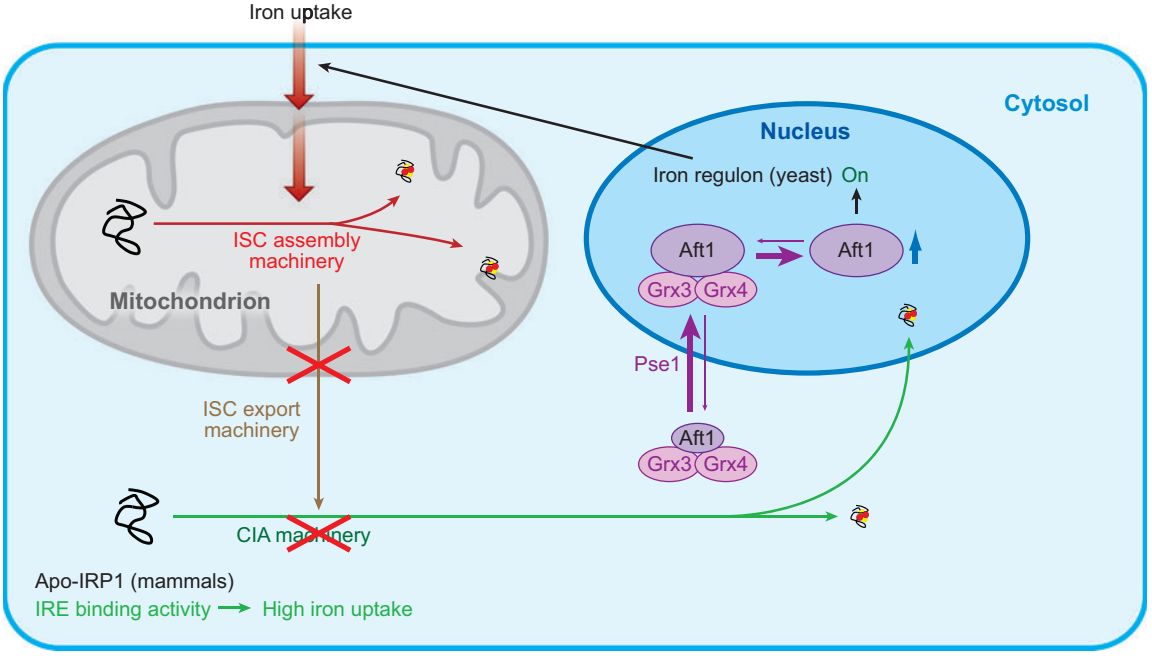
Figure 3

Comparison of the impact of Fe/S protein biogenesis on iron homeostasis in yeast and mammals. (*a*) Under iron-replete conditions, the iron-responsive transcription factor Aft1 (and possibly Aft2) translocates to the cytosol using the exportin Msn5. The yeast iron-sulfur cluster (ISC) assembly and export machineries provide a compound to the cytosol, which prevents translocation of Aft1 into the nucleus. Owing to the low amount of Aft1 in the nucleus, transcription of genes belonging to the yeast Aft1 iron regulon remains turned off. The monothiol glutaredoxins, Grx3 and Grx4, present in both cytosol and nucleus, bind to Aft1. In iron-replete mammalian cells, the ISC and cytosolic Fe/S protein assembly (CIA) machineries mature iron regulatory protein 1 (IRP1) to cytosolic aconitase (Holo-IRP1), which cannot bind to iron-responsive elements (IREs) of mRNAs, leading to, for example, high synthesis of ferritin and low amounts of transferrin receptor and, consequently, low iron uptake. (*b*) Upon defects in the yeast mitochondrial ISC machineries, Aft1 is no longer maintained in the cytosol but translocates with the importin Pse1 to the nucleus where it induces genes of the iron regulon. This leads to increased iron uptake, alterations in intracellular iron distribution, and an iron accumulation in mitochondria. Defects in any of the mammalian ISC or CIA machineries impair the maturation of IRP1. The apoform of IRP1 binds to IREs, leading to, for example, stimulation of the synthesis of transferrin receptor, blockage of ferritin synthesis, and, consequently, high uptake of iron, which may accumulate in mitochondria.

a Iron-replete cell



b Cell with Fe/S protein biogenesis defect



nuclear import-export trafficking is critical for iron-responsive Aft1 regulation of transcription, the molecular mode of how and where iron is sensed remains elusive.

Much information has been gained over the past few years about the mechanisms of the regulation of iron homeostasis by the mitochondrial ISC machineries. Several studies suggest that the mitochondrial ISC assembly machinery synthesizes a regulatory component that is exported to the cytosol by the ISC export component Atm1 to prevent the iron-responsive transcription factor Aft1 (and presumably Aft2) from entering the nucleus and turning on the iron regulon (**Figure 3*a***) (142–144). In the cytosol, the regulatory component seems to interact, directly or indirectly, with Aft1. Whether the regulatory compound is identical, similar, or different from the component X exported from mitochondria for cytosolic Fe/S protein assembly via the CIA machinery (**Figure 2**) is currently unknown. Solution of this problem will depend on the molecular identification of this (these) component(s).

Functional impairment of the ISC assembly and/or export machineries leads to a decrease of the regulatory component in the cytosol, translocation of Aft1 into the nucleus via the importin Pse1, and, consequently, the activation of the iron regulon (**Figure 3*b***) (142). Virtually all of the ISC assembly components, with the notable exception of the Isa1-Isa2 and Iba57 proteins (see above), have to be functional for appropriate Aft1 regulation. Under these conditions, a massive iron overload of mitochondria is observed (**Figure 3*b***) (89). Thus, iron deficiency in mitochondria, sensed by decreased mitochondrial Fe/S protein biogenesis activity and hence diminished amounts of the regulatory compound in the cytosol, may turn on cellular iron uptake and transfer into the mitochondria. The physiological meaning of this feedback response may be the tight coordination of the mitochondrial iron demands for use in Fe/S protein and heme biogenesis (see below) with cellular iron acquisition. In summary, activation of the Aft transcription factors is intimately linked to iron availability for Fe/S protein biogenesis in mitochondria.

As mitochondria utilize iron also for heme biosynthesis in the matrix, one might assume that a similar regulatory circuit exists for heme, which is synthesized in the mitochondrial matrix (145). However, a detailed genetic analysis showed that heme deficiency does not elicit iron trafficking into the yeast cell and does not lead to high levels of iron in mitochondria (146). In the absence of heme synthesis, transcription of the iron (and copper) regulon genes is decreased. Thus, heme deficiency in yeast leads to lower rather than higher iron uptake into the cell, distinguishing this situation from Fe/S protein biogenesis defects. Notably, heme deficiencies in mammalian erythroid cells lead to an iron overload situation, which indicates that yeast and the mammalian erythroid system differ markedly in their response to heme defects (147).

Various DNA microarray experiments showed a striking overlap in the global transcriptional responses to either iron starvation, Aft1-Aft2 activation, or impairment of the ISC assembly and export machineries (138, 139, 148–150). The depletion of the ISC proteins elicited numerous responses in addition to those contained within the Aft1-Aft2 iron regulon. These include the repression of genes involved in respiration and transcriptional remodeling of the citric acid cycle, heme metabolism, and biosynthetic pathways of ergosterol and biotin (150). The "retrograde response," normally elicited upon functional defects of mitochondria (151), was responsible for only few of the observed changes, suggesting a highly specific reason for these alterations. These data support the view that the mitochondrial ISC assembly and export machineries provide a key signal to the Aft-dependent iron regulon. However, in addition to the Aft-dependent alterations, deficiencies in the ISC machineries induce a more general remodeling of iron-dependent cellular processes. This includes the increased entry of iron into mitochondria, the

coordination of the iron distribution between Fe/S protein and heme biogenesis, and the balanced expression of respiratory components in response to the availability of Fe/S clusters and heme.

Before the identification of the CIA machinery, it was generally assumed that a cytosolic Fe/S protein could serve as the regulatory component or, alternatively, that the Aft1-Aft2 proteins, via two conserved cysteine residues, might directly bind an Fe/S cluster, which is assembled with the help of the mitochondrial ISC machineries (23, 131, 143). Surprisingly, depletion of any of the CIA components does not impose any significant effects on cellular iron metabolism nor does it dramatically alter the mitochondrial iron content, thus refuting the idea that canonical cytosolic Fe/S clusters are involved in the iron regulatory process in yeast (**Figure 3*b***) (35, 101, 144). These findings were further supported by DNA microarray data analyzing the responses to depletion of the CIA component Nbp35. Only weak transcriptional effects upon depletion of Nbp35 with no clear preference for a specific cellular process were observed (150). In conclusion, iron homeostasis in yeast is not regulated by a canonical cytosolic Fe/S protein that is matured by the CIA machinery.

THE ROLE OF THE ISC AND CIA MACHINERIES FOR IRON REGULATION IN MAMMALS

Iron homeostasis in mammals is largely regulated on the posttranscriptional level (23, 147, 152). Regulation is mediated by the iron regulatory proteins IRP1 and IRP2 (recently reviewed in References 153 and 154). IRP1 is an Fe/S protein and with a bound [4Fe-4S] cluster functions as a cytosolic aconitase. After loss of the Fe/S cluster, the apoform of IRP1 undergoes a substantial conformational change, which moves domains III and IV apart making space for the accommodation of the IRE stem-loop structure in mRNAs of several iron-regulated proteins (155). IRP2 does not associate with an Fe/S cluster yet is regulated by iron-dependent degradation. The form of iron that IRP2 senses is still unclear. The binding of IRP1 or IRP2 to an IRE has distinct effects depending on which side of the coding region the IRE is located. An IRE at the 5′-untranslated region of the mRNA blocks the passage of the scanning ribosome and thus inhibits translation initiation of proteins, such as ferritin, ALAS2, ferroportin, and mitochondrial aconitase. IRP binding to IREs at the 3′-untranslated tail results in mRNA stabilization and protection against RNase attack, hence increasing the translation efficiency of the encoded proteins. Examples of a 3′-located IRE include the transferrin receptor and the divalent metal ion transporter DMT1, both involved in iron import.

IRP: iron regulatory protein

As described above, the association of the Fe/S cluster with IRP1 critically depends on the function of several components of the mammalian ISC systems (**Figure 3*a***). Hence, it is expected that Fe/S protein biogenesis will influence iron regulation. This has been directly shown for the human Isu proteins in that the depletion of mitochondrial huIsu1 by RNAi resulted in increased iron levels inside the cell (115). This effect was observed when cells were treated with additional amounts of ferric iron, i.e., in the presence of high iron. Interestingly, in huIsu1-depleted cells, the levels of IRE-bound IRP2 also increased significantly, even though IRP2 does not carry an Fe/S cluster. This effect was explained by the influence of mitochondria on the cytosolic iron levels.

The ISC assembly machinery had a similar impact on the cellular iron status of the zebrafish mutant *shiraz*, which was defective in the mitochondrial glutaredoxin Grx5 (117). The defect in zebrafish Grx5 resulted in lower heme levels which were caused by low expression of ALAS2, the mitochondria-located erythroid-specific δ-aminolaevulinate synthase catalyzing the first committed step of heme biosynthesis. It was therefore reasoned that the IRP1 apoform was increased as a

XLSA/A: X-linked sideroblastic anemia and cerebellar ataxia

result of the zebrafish Grx5 defects, thus leading to decreased translation of ALAS2. This interpretation was substantiated because expression of ALAS2 lacking a functional 5′ IRE restored heme synthesis. Likewise, depletion of IRP1 (but not of IRP2) by the morpholino technique resulted in normal heme biosynthesis. These elegant studies suggest an intimate connection between Fe/S cluster biogenesis and heme biosynthesis in the erythroid system via the regulatory function of IRP1 on ALAS2 translation. It is therefore expected, although not directly shown, that zebrafish ISC component Grx5 has a direct impact on iron regulation.

A crucial function in the regulation of iron homeostasis has been assigned to a member of the mammalian ISC export machinery ABCB7 (see above). A conditional knockout of the murine *ABCB7* gene in liver caused a marked iron deposition in hepatocytes visualized by Prussian blue staining (105). Strikingly, iron was found in electron-dense rings surrounding a morphologically homogeneous center but not inside mitochondria as in patients with X-linked sideroblastic anemia and cerebellar ataxia (XLSA/A) in which *ABCB7* is mutated (156, 157). Although the identity of these rings remains to be clarified, the iron accumulation was attributed to the impaired maturation of the Fe/S cluster in IRP1 (**Figure 3*b***). The increased IRE-binding capacity of IRP1 indicates that ABCB7-deficient cells try to elevate iron uptake to counteract the impairment of cytosolic Fe/S protein maturation. This finding emphasizes the important role of mitochondria in mammalian iron regulation. Further support comes from RNAi studies in human cell culture. Silencing of *ABCB7* expression causes massive iron accumulation in mitochondria with an apparent concomitant iron depletion in the cytosol (119). Even though Fe/S protein biogenesis is normal in ABCB7-deficient mitochondria (see above), the increased levels of iron apparently cannot support the conversion of protoporphyrin IX to heme. The reason for this heme biosynthesis defect is unclear, yet the effect mimics the phenotype of the XLSA/A disorder.

Finally, depletion of the CIA component huNbp35 by RNAi caused an increase in the uptake of transferrin receptor as a result of the increased IRP1-IRE binding upon impaired IRP1 Fe/S cluster maturation (O. Stehling & R. Lill, unpublished). These findings indicate that the CIA machinery, via IRP1, has an effect on iron metabolism in higher eukaryotes clearly distinguishing it from *S. cerevisiae* (**Figure 3*b***).

Together, these studies indicate the important roles of the ISC assembly, ISC export, and CIA machineries for the regulation of iron metabolism in mammalian cells. This regulatory effect is mediated through increased IRE binding of IRP1 as a result of impaired Fe/S cluster assembly (**Figure 3*b***). Presumably, the situation is even more complex, as IRP2 binding to IREs is usually increased by defects in Fe/S cluster biogenesis (105, 115). The molecular events governing this effect have not yet been resolved. Because what IRP2 senses is largely unknown, predictions are relatively difficult to make. It is clear, however, that IRP2, directly or indirectly, responds to cytosolic iron levels and at higher iron concentrations becomes degraded by the proteasome. Hence, one simple, yet hypothetical, scenario may be that the iron accumulation in mitochondria, observed upon depletion of the ISC components, may lead to a concomitant iron depletion in the cytosol and hence a stabilization of IRP2.

THE ROLE OF THE ISC AND CIA MACHINERIES IN THIO MODIFICATION OF MITOCHONDRIAL AND CYTOSOLIC tRNA

It has long been recognized that bacterial cysteine desulfurase IscS performs a crucial role in tRNA thio modification by serving as a sulfur donor for this posttranslational maturation process (158, 159). The evident connection between the bacterial ISC assembly

pathway and tRNA thio modification is even more intimate as several tRNAs were shown to depend on Fe/S proteins (e.g., MiaB) for their modification (160, 161). Recent experiments in yeast and human cells indicate that a similar situation might prevail in eukaryotes. Thio modifications have been observed in both mitochondrial and cytosolic tRNAs with two uridine nucleosides in positions 34 (wobble base) and 35. The modification of the uridine in the wobble position seems to be present in virtually all organisms and is essential for yeast cell viability presumably because it increases the decoding efficiency of these tRNAs (162). A dependence of thio modification for both cytosolic and mitochondrial tRNAs on the function of yeast Nfs1 has been noted (56, 163). In early studies, no distinction between the role of mitochondrial and cytosolic-nuclear Nfs1 was made, and it was generally assumed that the extramitochondrial Nfs1 might be responsible for the thio modification of cytosolic tRNAs (**Figure 4**). However, a direct experimental test has refuted this idea (164). Instead, and quite surprisingly, thio modification depended on the mitochondrial version of Nfs1. In addition, other components of the ISC assembly machinery (Isu1 and Isu2) and members of the CIA machinery (Cfd1, Nbp35, and Cia1) are needed. These results indicate that thio modification of cytosolic tRNAs requires the cell's entire Fe/S protein assembly apparatus and thus may be an Fe/S protein-dependent pathway (**Figure 4**). This requirement might be explained, at least in part, by the function of the cytosolic [4Fe-4S] protein Elp3 (165), which, together with other proteins of the so-called elongator complex (termed Elp1, Elp2, Elp4, Elp5, and Elp6) and the tRNA methylase Trm9, is involved in the formation of the 5-methoxycarbonylmethyl group of the modified wobble nucleoside 5-methoxycarbonylmethyl-2-thiouridine in these tRNAs (**Figure 4**) (166–169). The thio modification is mediated by Tuc1, which is not known to contain an Fe/S cluster (162). The origin of sulfur in this reaction is presently unknown. It seems unlikely, although not impossible, that the CIA machinery is involved in this sulfur transfer reaction. Finally, generation of the wybutosine (Y) base of cytosolic $tRNA^{Phe}$ in *S. cerevisiae* requires the Fe/S protein Tyw1 and a number of other proteins (**Figure 4**) (170, 171).

Thio modification: posttranscriptional modification of uridine nucleosides of tRNA needed for efficient translation

As explained above thio modification of cytosolic tRNAs depends on functional Fe/S cluster biogenesis. In contrast, yeast mitochondrial tRNAs apparently do not require the function of an Fe/S protein, as Isu1 is dispensable for this process (**Figure 4**) (164). Mitochondrial Nfs1 (likely in complex with Isd11) serves as the sulfur donor for conversion of the 5-carboxymethylaminomethyluridine, synthesized by the proteins Mss1 and Mto1, to the 5-carboxymethylaminomethyl-2-thiouridine moiety (172). It should be noted that mammals, including man, synthesize the related group 5-taurinomethyl-2-thiouridine. The sulfur transfer step also involves the function of the mitochondria-specific 2-thiouridylase Mtu1 and possibly other unidentified proteins. This pathway seems to be conserved from yeast to man. Interestingly, the mutation A8344G in human mitochondrial $tRNA^{Lys}$, leading to the mitochondrial disease myoclonus epilepsy associated with ragged-red fibers, results in the lack of the 5-taurinomethyl-2-thiouridine modification, impressively showing the importance of this modification (173, 174). Collectively, these recent investigations have unraveled an intimate link of Fe/S protein biogenesis and function to the posttranscriptional modification of tRNA.

DISEASES RELATED TO Fe/S PROTEINS AND THEIR BIOGENESIS

A number of ISC and CIA components involved in Fe/S protein biogenesis have been linked to human disease (**Figure 5**). The first known examples were frataxin and ABCB7. Depletion of frataxin in human cells by more

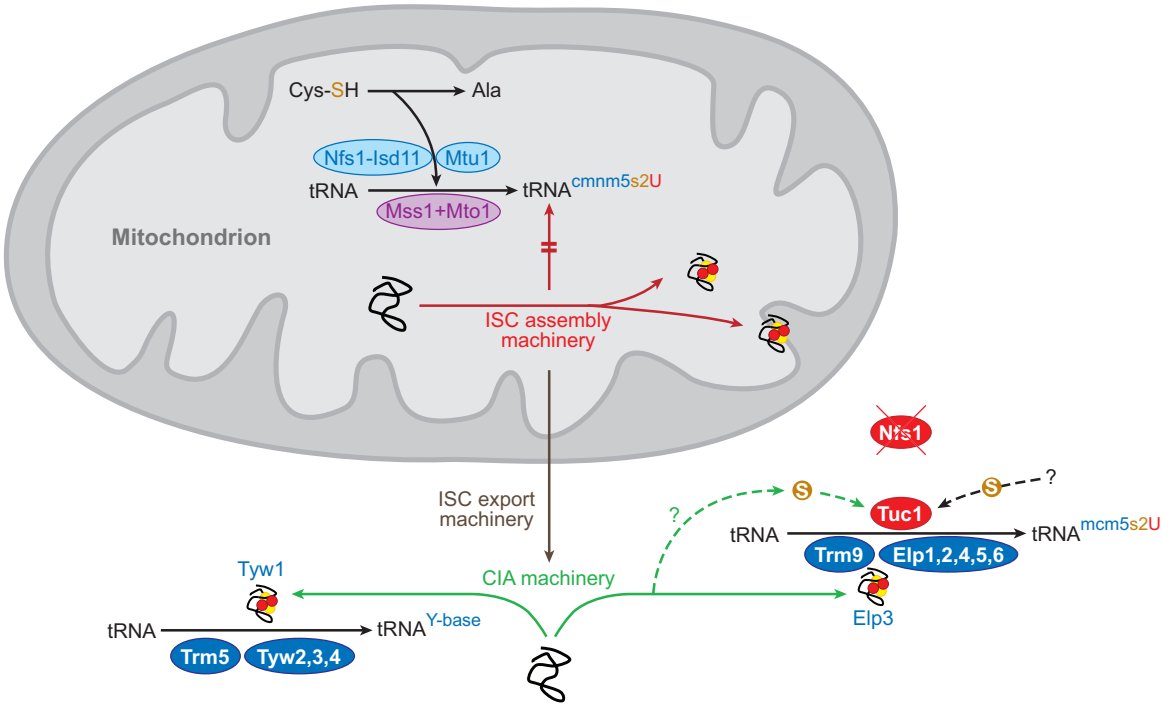


Figure 4

The role of the iron-sulfur cluster (ISC) and cytosolic Fe/S protein assembly (CIA) machineries and of Fe/S proteins in the modification of mitochondrial and cytosolic tRNAs. Some mitochondrial and cytosolic tRNAs need to be chemically modified after transcription and processing to become functional in translation. In mitochondria, alkylation of the uridine residue of tRNAs with a 5-carboxymethylaminomethyl group (cmnm5) is achieved by Mss1 and Mto1. Subsequently, thio modification to 5-carboxymethylaminomethyl 2-thiouridine (cmnm5s2U) requires the function of the Nfs1-Isd11 desulfurase complex as a sulfur donor and additional proteins involved in sulfur transfer (e.g., Mtu1). These reactions occur independently of other ISC assembly components, i.e., independently of Fe/S proteins. In the cytosol, wybutosine base (Y-base) attachment (*left side*) and thio modification (*right side*) of various tRNAs involve specific Fe/S proteins, which explains why both the ISC and CIA systems are required for these modification processes. Alkylation of cytosolic tRNAs with a 5-methoxycarbonylmethyl group is facilitated by the Fe/S protein Elp3, five indicated members of the elongator complex (Elp) and the tRNA methylase Trm9. Thiouridine modification to yield 5-methoxycarbonylmethyl-2-thiouridine (mcm5s2U) requires the Tuc1 protein and other proteins awaiting identification. Notably, the source of sulfur for cytosolic thio modification is unknown. The extramitochondrial version of Nfs1 does not support thio modification (164). Synthesis of the Y-base of yeast $tRNA^{Phe}$ requires the Fe/S protein Tyw1 and other indicated components.

than 70% causes the neurodegenerative disorder Friedreich's ataxia, the most common autosomal recessive ataxia (175). Patients show signs of mitochondrial Fe/S protein defects and accumulate iron in heart and neuronal tissues (176). Mouse models have been developed and show that ablation of the *FRDA* gene is embryonically lethal, whereas targeted deletion in muscle, neuronal, or liver tissues reproduces many of the pathophysiological and biochemical phenotypes observed in patients, i.e., cardiac hypertrophy, large sensory neuron dysfunction, deficiencies in Fe/S enzymes (complexes I–III of the respiratory chain and aconitase), time-dependent mitochondrial iron accumulation, decreased life

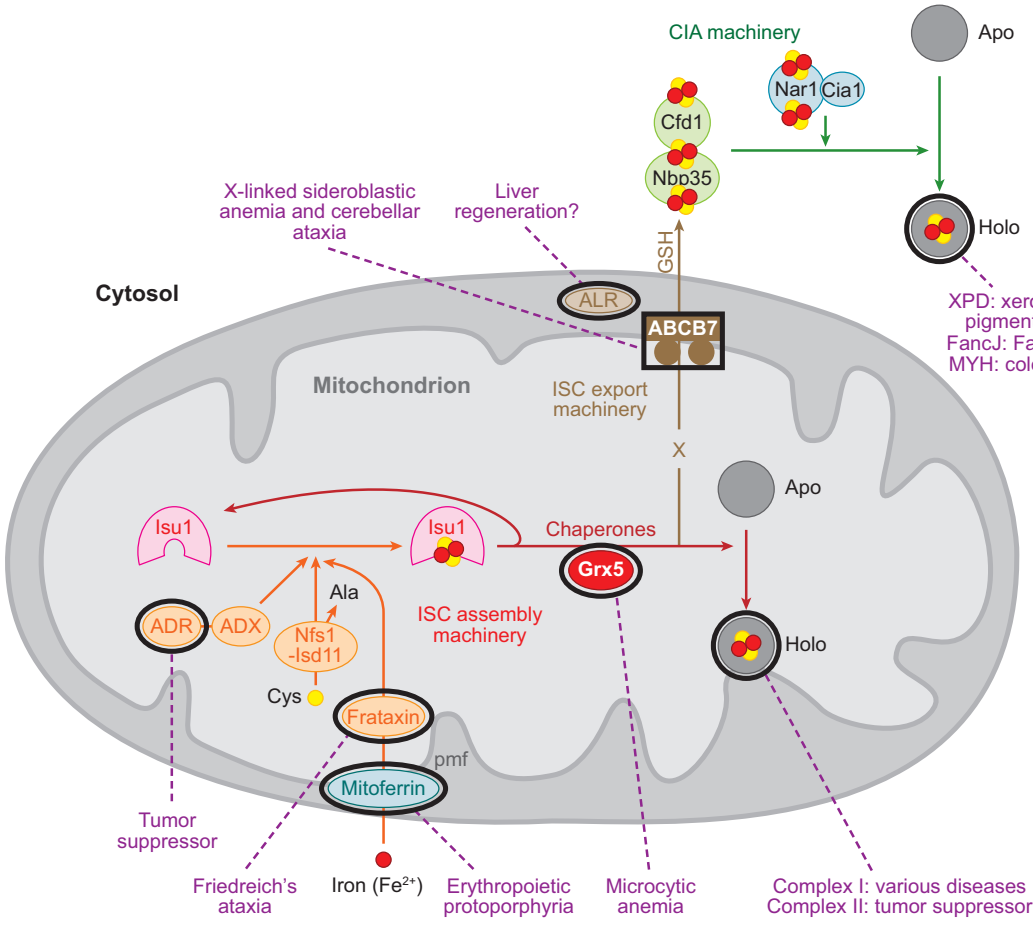


Figure 5

Diseases associated with Fe/S proteins or components of the iron-sulfur cluster (ISC) and cytosolic Fe/S protein assembly (CIA) machineries. Numerous components of cellular Fe/S protein biogenesis and a few Fe/S proteins are linked to the indicated human diseases. These proteins are circled by black lines. Abbreviations: ABCB7, mitochondrial ABC transporter B7; ADR, adrenodoxin reductase; ADX, adrenodoxin; Ala, alanine; ALR, augmenter of liver regeneration homologue; Apo, apoprotein; Cys, cysteine; Holo, holoprotein; XPD, xeroderma pigmentosum protein D; FancJ, Fanconi anemia protein J; MYH, MutY homologue.

span, and tumor growth (177–179). Mutations in the human *ABCB7* gene cause XLSA/A (156, 157). In this disease, mitochondria accumulate iron and form so-called ring sideroblasts (i.e., iron-loaded ring-shaped tubules, which are concentrated around the nucleus). The disease is rare, and only few patient families have been described (180). There exist striking similarities in the phenotypes developed by *ATM1/ABCB7* gene inactivation in yeast, mouse, and man. These include Fe/S protein defects in the cytosol (but not in mitochondria), heme defects (in yeast these are seen only upon strong depletion of Atm1), and mitochondrial iron accumulation (89, 105, 119, 135, 181). This verifies that yeast serves as an excellent model system to study the biochemical effects of *ABCB7* mutations.

Recently, several other cases have been added to these two classical Fe/S diseases (**Figure 5**). A functional defect in the human

glutaredoxin Grx5 was found in a patient who had microcytic anemia with an iron overload (118). Consistent with a primary defect in Fe/S protein biogenesis and hence increased IRE-binding activity of IRP1, the patient's cells showed low levels of aconitase and H-ferritin but an increased transferrin receptor. This phenotype reproduces features found in the zebrafish mutant *shiraz*, described above (117). These findings and the data derived for yeast Grx5 suggest a highly conserved function of this ISC assembly component (75). A splice variant of the human erythroid isoform of mitoferrin (the yeast Mrs3/4 homologue) leads to a nonfunctional protein and causes a variant form of erythropoietic protoporphyria (182). The classic case of this disease is due to a functional defect in ferrochelatase catalyzing the last step of heme biosynthesis. Although a biochemical analysis of this form of erythropoietic protoporphyria is still pending, this disease is expected to be characterized by primary Fe/S protein and heme biosynthesis defects.

Other ISC components have been linked to disease, yet the functional connection to Fe/S protein defects is less evident in these cases. The adrenodoxin reductase, ADR (the functional homologue of yeast Arh1), has been described as a tumor suppressor (183). Disruption of the *ADR* gene is lethal, and its expression is regulated by the p53 tumor suppressor. It was thus hypothesized that ADR contributes to p53-mediated apoptosis through the generation of oxidative stress in mitochondria. The Erv1 homologue ALR (augmenter of liver regeneration) was claimed to enhance liver regeneration after hepatectomy as an extracellular growth factor (184). However, because ALR expression is not restricted to liver cells, the primary function of ALR may be inside the cell, namely in Fe/S protein biogenesis and protein import (93, 95).

Recent findings indicate that two classes of Fe/S proteins are connected to human disease. The first class contains components of the mitochondrial respiratory chain (**Figure 5**). Succinate dehydrogenase (complex II) functions as a tumor suppressor (185). Impaired function of complex II leads to accumulation of succinate, which inhibits prolyl hydroxylase in the cytosol. In turn, hypoxia-inducible factor 1α is not hydroxylated and translocates to the nucleus inducing genes favoring tumor progression and decreased apoptosis. It is conceivable that decreased Fe/S cluster assembly in complex II might contribute to these effects. Complex I (NADH-ubiquinone oxidoreductase) defects are associated with a number of mitochondrial diseases, including mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; Leigh syndrome; and Leber's hereditary optic atrophy (for review see Reference 186). However, neither the assembly nor the function of the eight Fe/S clusters in complex I is related to the cause of the disease in these cases. The second class of disease-relevant Fe/S proteins encompasses components involved in DNA repair. The ATP-dependent DNA helicase XPD and related proteins, such as FancJ, show striking similarity to an archaebacterial helicases bearing an Fe/S cluster and thus are predicted to be Fe/S proteins (43). XPD shares a similar Fe/S cluster-binding domain. All these proteins, including the yeast homologue Rad3, are involved in nucleotide excision repair and are essential for life. Recently, the iron binding domain of Rad3 was shown to be essential for its function as a helicase (186a). Inherited mutations in *XPD* are found in cluster-coordinating residues and are associated with diseases such as xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy (187). *FancJ* mutations are found in Fanconi anemia patients. Finally, the MutY homologue MYH (related to yeast Ntg2) functions as a DNA glycosylase in base excision repair and has been associated with colon cancer (42, 188).

The relevance of both Fe/S proteins and their biogenesis factors for human disease impressively shows the general importance of this biosynthetic pathway. Because the process is essential for life and may represent one

of the elementary metabolic reactions, there may be little flexibility for alterations. This notion is supported by the high conservation of the process from yeast to man. Fe/S protein biogenesis has now been studied for almost a decade. The next period of research will encounter the discovery of new components, the clarification of the still ill-defined molecular mechanisms, the identification of links to other cellular processes, and possibly the identification of newly associated diseases. Thus, years of exciting research are lying ahead.

SUMMARY POINTS

1. Iron-sulfur (Fe/S) protein assembly in eukaryotes is an essential process for life and is facilitated by three complex proteinaceous machineries, which are conserved from yeast to man. Plants and algae contain an additional assembly machinery in chloroplasts.
2. The mitochondrial ISC assembly machinery is required for biogenesis of all cellular Fe/S proteins, including those in cytosol and nucleus. The mitochondrial ISC export apparatus and the cytosolic CIA system are specific for maturation of extramitochondrial Fe/S proteins.
3. Fe/S protein assembly can be divided into two major steps: (*a*) transient de novo Fe/S cluster assembly on a scaffold protein requiring sulfur and iron donors and (*b*) Fe/S cluster dislocation from the scaffold, followed by transfer and insertion into recipient apoproteins.
4. Defects in the mitochondrial ISC machineries result in an impaired iron homeostasis with increased cellular iron acquisition and iron accumulation in mitochondria.
5. Fe/S protein biogenesis is tightly linked to numerous other basic cellular processes, such as heme biosynthesis, respiration, DNA synthesis and repair, thio modification of tRNA, ribosome assembly, and translation initiation.
6. Defects in Fe/S protein biogenesis components or Fe/S proteins themselves are the cause of a number of diseases, including several neurodegenerative and hematological diseases, and tumor formation.

FUTURE ISSUES

1. The molecular mechanisms of Fe/S cluster synthesis on Isu1 need to be determined. For this biochemical task, knowing the 3D structures of ISC proteins will be helpful. Furthermore, the targets of the electron transfer chain, consisting of NADH, Arh1, and Yah1, need to be identified.
2. The molecular function of the ISC chaperones and the glutaredoxin Grx5 in Fe/S cluster dislocation from Isu1 and its transfer to apoproteins need to be resolved.
3. The specific molecular role of the two Isa proteins and Iba57 in the activation of aconitase-like and biotin synthase-like proteins needs to be clarified. These studies will unravel why only these latter, but not other, Fe/S proteins depend on these three biogenesis factors.

4. The molecular identities of the substrates of Atm1 for cytosolic-nuclear Fe/S protein maturation and iron regulation have to be defined. This knowledge will have a broad impact on the understanding of the role of Erv1 and GSH in these processes.
5. The molecular mechanisms of how the Fe/S clusters are assembled on Cfd1 and Nbp35 need to be established, and potential new components participating in this reaction have to be identified.
6. The roles of the CIA proteins Nar1 and Cia1 in Fe/S cluster release from the Cfd1-Nbp35 complex and Fe/S cluster transfer to apoproteins need to be defined. Additional components of the CIA machinery are likely to be identified, and their functions need to be characterized.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We dedicate this article to the commemoration of Prof. Helmut Beinert (Madison), the doyen of research on Fe/S proteins. We thank Dr. Y. Nakai for discussion, Dr. A. Sheftel for critical reading, and the members of our group for their excellent work. We acknowledge generous support from Deutsche Forschungsgemeinschaft (SFB 593 and TR1, Gottfried-Wilhelm Leibniz program, and GRK 1216), the German-Israeli foundation GIF, the European Commission, Rhön Klinikum AG, Alexander-von-Humboldt Stiftung, and Fonds der chemischen Industrie.

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