Iron-sulfur clusters belong to the most ancient co-factors of proteins involved in electron transfer, catalysis and regulatory processes (Beinert et al., 1997). The simplest Fe/S clusters are of the [2Fe-2S] and [4Fe-4S] types which contain either ferrous (Fe²⁺) or ferric (Fe³⁺) iron and sulfide (S²⁻) and which are usually integrated into proteins via coordination of the iron ions by cysteine or histidine residues (Fig. 1A). While (bio)chemists have worked out reconstitution procedures to assemble Fe/S clusters into apoproteins in vitro, cell biological and genetic studies over the past decade have provided ample evidence that the maturation of Fe/S proteins in living cells is a catalyzed rather than spontaneous process. Despite the chemical simplicity of Fe/S clusters their biosynthesis is rather complex requiring numerous components. Pioneering studies in bacteria have identified three different biosynthesis machineries; the NIF system for specific maturation of nitrogenase in azototrophic bacteria, the ISC assembly and the SUF systems for generation of house-keeping Fe/S proteins under normal and oxidative-stress conditions, respectively (Fontecave et al., 2005; Johnson et al., 2005). The latter two machineries were inherited by eukaryotes which contain components homologous to those of the bacterial ISC assembly system inside mitochondria (see below; (Lill and Kispal, 2000)) and SUF components in plastids (Balk and Lobreaux, 2005).

Fe/S proteins in eukaryotes have been localized in mitochondria, cytosol and nucleus where they are involved in rather diverse functions such as the TCA cycle, amino acid biosynthesis, bacterial and mitochondrial respiration, co-factor biosynthesis, ribosome assembly, regulation of protein translation, DNA replication and DNA repair (Fig. 1B). The yeast Saccharomyces cerevisiae has served as an excellent model organism to unravel the complex biosynthesis pathways, but recent investigations in human cell culture and transgenic mice have demonstrated that the process is highly conserved in eukaryotes from yeast to man. Since almost a decade my group is dedicated to the identification of the components and mechanisms underlying Fe/S protein biogenesis in eukaryotes using yeast and human cell culture as our major experimental systems (Lill et al., 2006; Lill and Mühlenhoff, 2006). This overview briefly summarizes the principles of how eukaryotic cells generate their Fe/S proteins in the different compartments. It has been noted early on in the studies of eukaryotic Fe/S protein biogenesis that mitochondria perform a central role as they are required for biogenesis of all cellular Fe/S proteins (Kispal et al., 1999; Schilke et al., 1999). As noted above they harbor the so-called ‘ISC assembly machinery’ which was inherited from bacteria during evolution. To date 15 proteins are known to assist this complex biosynthetic process which can be sub-divided experimentally into two major steps (Fig. 2; (Mühlenhoff et al., 2003)). First, an Fe/S cluster is assembled de novo on the scaffold protein Isu1 which serves as a transient assembly and binding platform. Then, the Fe/S cluster is transferred from Isu1 to recipient apoproteins for incorporation into the Fe/S holoprotein by coordination with specific amino acid residues. Both partial reactions need the assistance of specific ISC assembly components. Only the most important factors will be addressed here. Fe/S cluster assembly on Isu1 critically depends on the function of the cysteine desulfurase complex comprised of Nfs1 and Isd11 (Fig. 2; (Adam et al., 2006; Wiedemann et al., 2006)). Even though Nfs1 contains the enzymatic activity as a cysteine desulfurase and releases sulfur from cysteine to form alanine and a Nfs1-bound persulfide, the Nfs1-Isd11 complex is the functional entity for sulfur transfer from Nfs1 to Isu1 in vivo. This reaction is facilitated by direct interaction of Nfs1 and Isu1. Upon binding of iron to Isu1 the Fe/S cluster is formed by a still unknown biochemical mechanism. Yfh1 (also termed frataxin; Table 1) functions as an iron donor by undergoing an iron-stimulated interaction with Isu1-Nfs1. Iron is imported into the mitochondrial matrix...
Fe/S clusters are assembled on the P-loop NTPase complex Cfd1-Nbp35 (light green arrow). The Fe/S clusters are substrates. In the cytosol, the CIA machinery catalyzes Fe/S protein maturation in two major steps. First, an Fe/S compound (X) to the cytosol for use in Fe/S protein assembly (blue arrow), and is assisted by the tripeptide mitochondrial ISC export machinery. The ABC transporter Atm1 of the inner membrane exports an unknown mitochondrial Fe/S protein biogenesis requires, in addition to the ISC assembly machinery, components of the ISC assembly machinery (red arrows) including the ATP-dependent Hsp70 chaperone Ssq1, the DnaJ-like co-chaperone Jac1 and the nucleotide exchange factor Mge1 (Schilke et al., 2006; Vickery and Cupp-Vickery, 2007).

The second major step of biogenesis involves the release of the Isu1-bound Fe/S cluster, its transfer to apoproteins and its assembly into the apoprotein by coordination with the specific amino acid ligands. This step is specifically assisted by a dedicated chaperone system comprised of the Hsp70 family member Ssq1, the DnaJ-like co-chaperone Jac1 and the nucleotide exchange factor Mge1 (Schilke et al., 2006; Vickery and Cupp-Vickery, 2007).

According to a working hypothesis, the ISC assembly machinery produces a (still unknown) component X which is exported from the mitochondrial matrix to the cytosol where it performs an essential function in the maturation process (Fig. 2). The export reaction is accomplished by the ABC transporter Atm1 of the mitochondrial inner membrane. Further, the sulfhydryl oxidase Erv1 of the intermembrane space and glutathione (GSH) are required. Together, these three components have been designated 'ISC export machinery', because depletion of these proteins results in a highly similar phenotype including normal biogenesis of mitochondrial Fe/S proteins and an impairment of extramitochondrial Fe/S protein maturation. Maturation of the cytosolic and nuclear Fe/S proteins is catalyzed by the cytosolic iron-sulfur protein assembly (CIA) system comprised of five known proteins (Fig. 2). According to recent in vivo and in vitro studies, this process can be sub-divided into two major partial reactions (Netz et al., 2007). First, an Fe/S cluster is transiently assembled on the P-loop NTPases Cfd1 and Nbp35 which serve as a scaffold. This step essentially requires mitochondria. Then, the Fe/S cluster is transferred to apoproteins by the CIA proteins Nar1, Cia1 and Cia2. Unlike the mitochondrial Isu1 scaffold, Cfd1 and Nbp35 do not directly interact with a sulfur-donating protein such as the extra-mitochondrial version of Nfs1, as genetic and biochemical studies did not establish a role of this protein in this process (Biederbick et al., 2006; Mühlenhoff et al., 2004; Nakai et al., 2001). Rather, it has been shown that the mitochondrial version Nfs1 and other mitochondrial ISC assembly components are needed for extra-mitochondrial Fe/S cluster formation. Cfd1 and Nbp35 are involved in the activation of the CIA protein Nar1 by assembly of two Fe/S clusters on this iron-only hydrogenase-like protein (Fig. 2). Thus, Nar1 is both a target and a component of the cellular Fe/S protein biogenesis machinery creating an interesting "chicken and egg" situation for its maturation process (Balk et al., 2004). Nar1 holoprotein assists Fe/S cluster transfer to target apoproteins by interacting with Cia1, a WD40 repeat protein which serves as a docking platform for binding Nar1 and Cia2 (Sriniwasan et al., 2007). To date, it is still unclear where maturation of the nuclear Fe/S proteins occurs. Either they are assembled in the cytosol and are then transferred to the nucleus in the holoform, or the assembly process takes place in the nucleus requiring the import of the apoprotein. Since small amounts of the CIA proteins Cfd1, Nbp35, Nar1 and Cia2 and the majority of Cia1 have been found in the nucleus, both scenarios seem possible. Most of the more than 20 ISC and CIA components are essential for viability of yeast and human cells (Biederbick et al., 2006; Lill and Mühlenhoff, 2005). In fact, Fe/S protein biogenesis is the only known function of mitochondria that is indispensable for viability of yeast cells. In comparison, yeast mutants in

Figure 2: A model for Fe/S protein biogenesis in eukaryotes. Eukaryotic Fe/S protein biogenesis involves the crucial function of mitochondria. The organelles import iron in ferrous (Fe²⁺, red circle) form from the cytosol in a membrane potential-dependent fashion. Import is facilitated by the inner membrane carriers Mr3 and Mr4. Maturation of mitochondrial Fe/S holoproteins (Holo) involves two major steps. The synthesis of a transiently bound Fe/S cluster (red and yellow circles) on the scaffold protein Isu1 (and Isu2 in yeast) is supported by the early components of the ISC assembly machinery (orange arrows). These proteins include the cysteine desulfurase complex Nfs1-Yah1 which serves as the sulfur (yellow circle) donor for cluster synthesis, the iron binding protein Yah1 (frataxin) as the iron donor, and the ferredoxin Yah1 which provides electrons (e⁻) for sulfur reduction. The release of the Fe/S cluster from Isu1, and its transfer and incorporation into recipient apoproteins (Apo) is facilitated by late components of the ISC assembly machinery (red arrows) including the ATP-dependent Hsp70 chaperone Ssq1, the DnaJ-like co-chaperone Jac1, the nucleotide exchange factor Mge1, and the monothiol glutaredoxin Grx5. Extra-mitochondrial Fe/S protein biogenesis requires, in addition to the ISC assembly machinery, 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 (blue arrow), and is assisted by the tripeptide glutathione (GSH) and intermembrane space sulfhydryl oxidase Erv1 which introduces disulfide bridges into substrates. In the cytosol, the CIA machinery catalyzes Fe/S protein maturation in two major steps. First, Fe/S clusters are assembled on the P-loop NTPase complex Cfd1-Nbp35 (light green arrow). The Fe/S clusters are bound to Cfd1-Nbp35 in a labile fashion, and by assistance of Nar1, the WD40 repeat protein Cia1 and Cia2 can be transferred to cytosolic and nuclear apoproteins (dark green arrows).
oxidative phosphorylation result in a respiratory growth defect, but they can grow on glucose-containing media. Interestingly, the essential character of mitochondria is explained by their role in the maturation of extra-mitochondrial Fe/S proteins. The first known example of an essential cytosolic-nuclear Fe/S protein is Rli1, a component involved in ribosome assembly and export from the nucleus (Kispal et al., 2005; Yarunin et al., 2005). Maturation of its Fe/S clusters strictly depends on the two mitochondrial ISC and CIA machineries providing a tight link between mitochondrial function and cytosolic protein translation. Recently, two other essential (nuclear) Fe/S proteins were identified with a function in nucleotide excision repair (Rad3) and RNA primer synthesis for DNA replication (Pri2) (Klinge et al., 2007; Rudolf et al., 2006). It seems likely that their maturation requires mitochondria possibly linking these organelles to another two fundamental processes of life. The central importance of Fe/S protein biogenesis for life is impressively documented by numerous diseases associated with defects in Fe/S protein biogenesis components or Fe/S proteins itself (Table 1). Defects in frataxin, the putative iron donor for Isu1 cluster formation on Isu1 cause the neurodegenerative disease Friedreich’s ataxia. Defects in the glutaredoxin Grx5 and in mitoferrin (the mitochondrial iron importer) are associated with diseases exhibiting mainly hematological phenotypes, microcytic anemia and erythropoietic protoporphyria, respectively (Table 1; (Shaw et al., 2006; Wingert et al., 2005)). This can readily be explained by the fact that Fe/S protein biogenesis is crucial for cellular iron uptake regulation which, in mammals, is mediated by the iron-regulatory proteins (IRP) 1 and 2 (Hentze et al., 2004). IRP1 is a cytosolic Fe/S protein requiring mitochondrial ISC and CIA components for its maturation to an aconitase. In iron-deplete situation (or upon impairment of the ISC or CIA machineries) its Fe/S cluster dissociates and IRP1 instead binds to a stem-loop structure in mRNAs encoding proteins in iron uptake, distribution, utilization or storage (Walden et al., 2006). One such example is the erythroid ALAS2 (5-aminolaevulinate synthase), catalyzing the committed step of heme biosynthesis in mitochondria. Translation of this protein in the cytosol is largely decreased when iron is scarce or Fe/S protein biogenesis is hampered intimately integrating the efficiency of heme and Fe/S protein biosynthesis, the two major iron-consuming processes in the cell. Some Fe/S proteins appear to be connected to human disease (Table 1). Succinate dehydrogenase (complex II of the respiratory chain) has been described as a tumor suppressor, as has the ISC assembly protein adrenodoxin reductase, ADR. The putative human Fe/S proteins XPD and FancJ are causative of Xeroderma pigmentosum and Fanconi anemia. Despite the impressive progress in research on Fe/S protein biogenesis during the past eight years, exciting years of further exploration are ahead of us, reaching from basic cell biologi-

<table>
<thead>
<tr>
<th>Human protein</th>
<th>Yeast homolog</th>
<th>Function</th>
<th>Associated disease</th>
</tr>
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<tbody>
<tr>
<td>Mitoferrin</td>
<td>Mrs3, Mrs4</td>
<td>Mitochondrial carrier, putative iron transporter</td>
<td>Erythropoietic protoporphyria</td>
</tr>
<tr>
<td>Frataxin</td>
<td>Yfh1</td>
<td>Iron donor for Isu1 scaffold</td>
<td>Friedreich’s ataxia</td>
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<tr>
<td>Grx5</td>
<td>Grx5</td>
<td>Monothiol glutaredoxin, transfer of Fe/S clusters from Isu1 to apoproteins</td>
<td>Microcytic anemia</td>
</tr>
<tr>
<td>ADR</td>
<td>Arh1</td>
<td>Adrenodoxin reductase, electron transfer from NAD(P)H to adrenodoxin, also involved in steroid hormone biosynthesis</td>
<td>Tumor suppressor</td>
</tr>
<tr>
<td>ABCB7</td>
<td>Atm1</td>
<td>ABC transporter, ISC export component</td>
<td>X-linked sideroblastic anemia and cerebellar ataxia (XLSA/A)</td>
</tr>
<tr>
<td>ALR</td>
<td>Erv1</td>
<td>Sulphydryl oxidase, disulfide bridge formation, also involved in protein import</td>
<td>Augmenter of liver regeneration (?)</td>
</tr>
</tbody>
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Table 1: Diseases related to Fe/S proteins and their biogenesis
cell and biochemical research to molecular medicine. The pathways of biosynthesis will be described in molecular detail and eventually be analyzed based on 3D structures of participating proteins, new components will be identified, the precise role of mitochondria in cytosolic and nuclear Fe/S protein biogenesis will be unraveled, and possibly new diseases will be associated with this fundamental process of life.

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