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Key Players and Their Role During Mitochondrial Iron–Sulfur Cluster Biosynthesis

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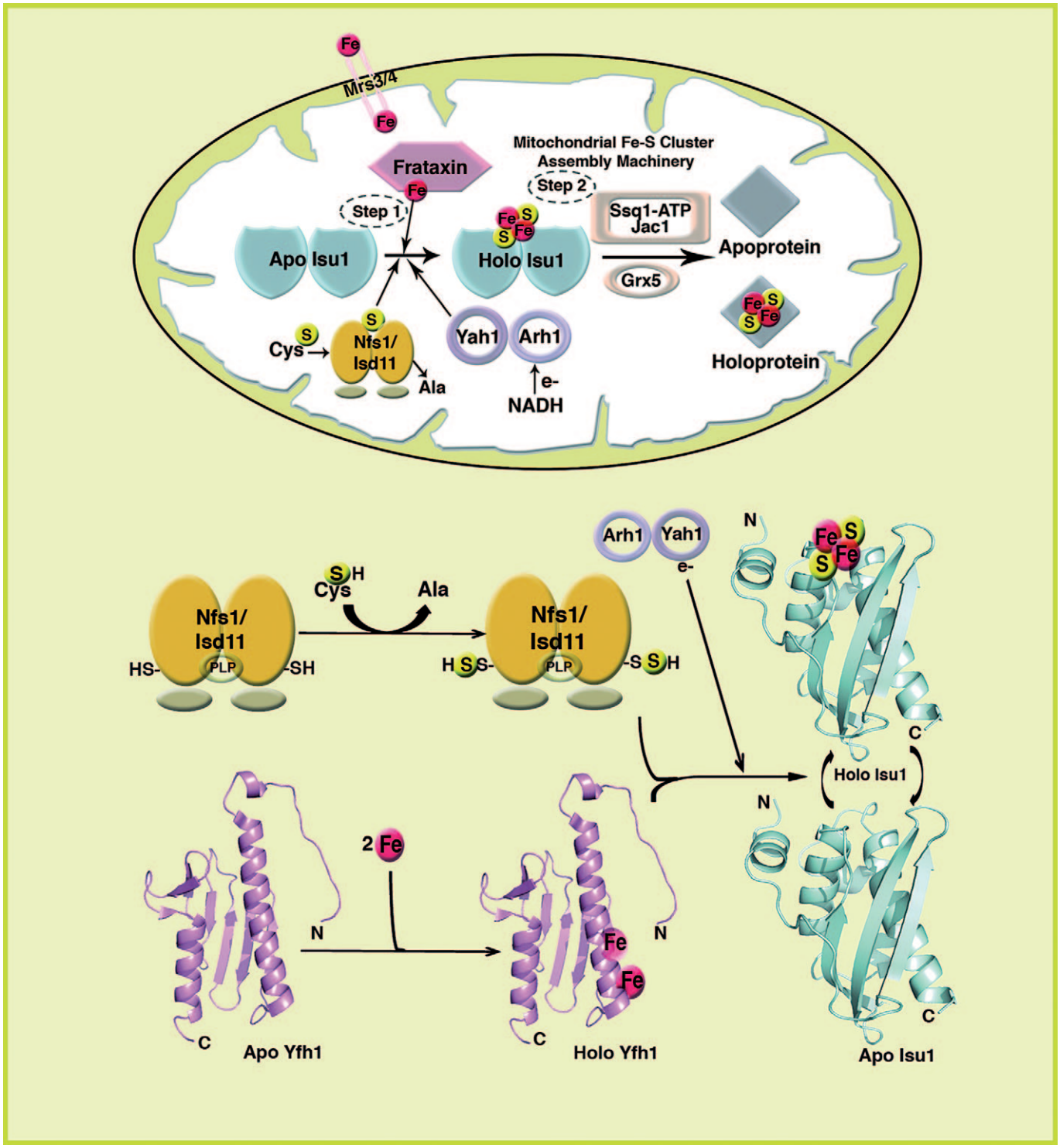

Abstract: Iron–sulfur clusters are multifaceted iron-containing cofactors coordinated and utilized by numerous proteins in nearly all biological systems. Fe–S-cluster-containing proteins help direct pathways essential for cell viability and participate in biological applications ranging from nucleotide biosynthesis and stability, protein translation, enzyme catalysis, and mitochondrial metabolism. Fe–S-containing proteins function by utilizing the unique electronic and chemical properties inherent in the Fe containing cofactor. Fe–S clusters are constructed of inorganic iron and sulfide arranged in a distinct caged structural makeup ranging from [Fe_2–S_2], [Fe_3–S_4], [Fe_4–S_4], up to [Fe_8–S_8] clusters. In eukaryotes, cluster activity is controlled in part at the assembly level and the major pathway for cluster production exists within the mitochondria. Recent insight into the pathway of mitochondrial cluster assembly has come from new in vivo and in vitro reports that provided direct insight into how all protein partners within the assembly pathway interact. However, we are only just beginning to understand the role of each protein within this complex pageant that is mitochondrial Fe–S cluster assembly. In this report we present results, using the yeast model for mitochondrial assembly, to describe the molecular details of how important proteins in the pathway coordinate for cluster assembly.

Keywords: biosynthesis · Fe–S clusters · frataxin · Isu scaffold · metalloproteins

Introduction

Iron–sulfur clusters are ancient cofactors utilized in nearly every life form.[1] These Fe–S clusters are constructed of inorganic iron and sulfide linked together in a branched fashion with each iron in direct contact with at least two sulfur atoms. The simplest and most common types of Fe–S clusters found within eukaryotes are the rhombic [Fe_2–S_2] and cubic [Fe_4–S_4] forms; however, examples of more complex forms up to the [Fe_8–S_8] can be found in bacteria and archea.[2] During the origin of ancient earth, spontaneous formation of Fe–S clusters took place easily under anaerobic conditions due to the high abundance of both iron and sulfur and their propensity to coordinate with each other. However, with a global shift to an aerobic environment, cells adapted to assemble these air-sensitive clusters by developing complex protein controlled mechanisms to complete cluster biosynthesis.[3,4] Once produced, Fe–S clusters are loaded onto proteins and these metalloproteins can be found in nearly every fundamental pathway in almost every cell. Some classic examples of where these important proteins function include within the mitochondrial complexes (I, II, and III) of the respiratory chain, as aconitase within citric acid cycle, and as ferredoxins used for electron-transfer and signaling.[5]

Biological pathways for assembling Fe–S clusters are accomplished using a highly conserved group of protein partners. While Fe–S clusters are simple in regards to their chemical structure and composition, their cellular synthesis must be kept tightly controlled given the high reactivity of iron and the instability of the cluster with regards to oxygen based chemistry. As a result, multifaceted protein-controlled machineries direct the production of the cluster. In bacteria, there are three different pathways for the production of Fe–S clusters: 1) the nitrogen fixation (Nif) pathway, used for the maturation of nitrogenase; 2) the general iron–sulfur cluster (ISC) pathway, which provides clusters for most cellular needs; and 3) the sulfur mobilization (SUF) pathway, highly active under oxidative stress conditions.[4,6,7] All three pathways involve a central scaffold protein, a sulfur donor, and an iron donor during biosynthesis of the clusters. Each of these pathways have been extensively studied for bacterial Fe–S cluster production predominately in the *Escherichia Coli* and *Azotobacter vinelandi* systems.[6,8,9]

In eukaryotes, the mitochondrial ISC assembly pathway is the central channel for the biological production of Fe–S clusters.[10] The mitochondrial Fe–S cluster assembly pathway utilizes several of the same players used within the different bacterial assembly systems.[11,12] General players utilized during the production stage include a scaffold protein that accomplishes assembly, a cysteine desulfurase that supplies sulfide to the scaffold protein, an iron chaperone that delivers metal to the scaffold protein, and a ferredoxin that provides reducing equivalents for cluster assembly.[13,14] For the most part, these proteins are highly conserved between eukaryotes and also within the different bacterial assembly production pathways.[5,15] Following assembly, a complex network of proteins participate in the transfer and delivery of functional clusters to ensure the newly produced Fe–S clusters are utilized properly. In this article, we will explore the structural and functional roles of key proteins within the mitochondrial Fe–S cluster assembly pathway, focusing on the yeast model system when giving specific examples.

Mitochondrial Fe–S Cluster Assembly Pathway

In yeast, mitochondrial Fe–S cluster assembly is a multistep and highly controlled protein driven process.[16,17] Since free iron and sulfur are toxic, it is important that assembly be

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tightly regulated within the cell. Given the high correlation between human and yeast systems, and the availability of well-established techniques for studying yeast genetics, a large amount of the details regarding eukaryotic mitochondrial Fe–S cluster assembly have come from studies performed using the yeast model system. Key molecular details for yeast cluster assembly pathway are given in Figure 1.

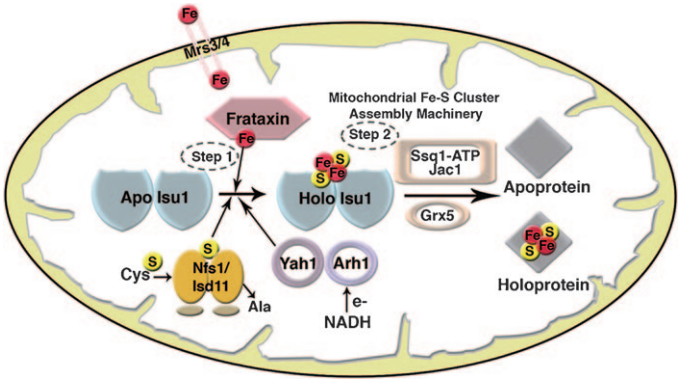

Figure 1. Model of yeast mitochondrial ISC assembly (step 1) and release (step 2) prior to the cluster being exported from the mitochondria.[13,18] Step 1: Mitochondria is shown as an oval, double membrane organelle. Iron is imported into the mitochondria through inner membrane proteins Mrs3/4. Assembly of the Fe–S cluster occurs on the Isu1 scaffold. Cysteine is acted upon by the enzyme cysteine desulfurase (Nfs1) and accessory protein (Isd11) for delivery of sulfur to Isu1. The metal has been proposed to be delivered by the potential iron chaperone protein frataxin (Yfh1). The reducing equivalents for the assembly reaction are provided by ferredoxin reductase (Arh1) and ferredoxin (Yah1). Step 2: Release of Fe–S clusters (red and yellow color circles) to apoproteins occurs by interaction between Isu1 and Hsp70 chaperone Ssq1 in the presence of Jac1, Mge1 and Grx5, followed by multiple additional protein interactions.

Overall, Fe–S cluster assembly involves two steps: step 1 is the transient synthesis of cluster production on a scaffold assembly protein and step 2 is the transfer of a formed cluster to target apo proteins.[13,18,19] Each stage is completed utilizing a coordinated set of proteins acting in a choreographed fashion.

For discussion, it is beneficial to break down each stage and look at the key players individually. In the first stage of Fe–S cluster assembly, iron is imported into the mitochondria. In yeast, iron is imported into the mitochondria by means of the inner membrane proteins Mrs3 and Mrs4, accomplished in a membrane potential, proton motive force driven fashion.[20] Iron delivery to the Fe–S cluster assembly protein (Isu1 and or Isu2 in yeast) takes place most likely in a protein-controlled manner, and this iron chaperone function has been proposed for the protein frataxin (Yfh1 in yeast).[21] The sulfur component of the Fe–S cluster originates from the amino acid cysteine through the activity of the cysteine desulfurase Nfs1, a pyridoxal phosphate containing enzyme. In eukaryotes Nfs1 must assemble with an essential small subunit of unknown function called Isd11.[22,23] A persulfide intermediate formed on the active site cysteine of Nfs1, perhaps requires an electron donor to convert the sulfane S^0 to sulfide S^{2-} during transfer to the Fe–S scaffold intermediate.[24] The ferredoxin reductase Arh1 and ferredoxin Yah1 have been suggested to be involved at this step, utilizing electrons from NADH[25]or NADPH.[26] The cluster produced on the scaffold protein (Isu1 or Isu2) is labile, suggesting that it may constitute an intermediate for transfer to apoprotein recipients during the second stage of the pathway.[5] Several proteins have been implicated in the transfer of functional clusters from the scaffold intermediate to their recipient apoproteins, although this assembly stage has not been biochemically characterized in detail. Four proteins are thought to participate in this transfer, including a chaperone protein of the Hsp70 ATPase family (Ssq1), a DnaJ-like cochaperone protein (Jac1), a nucleotide exchange factor protein (Mge1) and the monothiol glutaredoxin (Grx5).[3,5] Binding of Ssq1 to Isu1/2 (in the highly conserved LPPVK region) stimulates the ATPase activity of Ssq1 when Jac1 is also present, while Mge1 is required for exchanging bound ADP with ATP. The ATPase cycle somehow mediates release and transfer of the Fe–S cluster intermediates to apoproteins. Atm1, a mitochondrial inner membrane ABC transporter, is believed to export some component or signal for extramitochondrial Fe–S cluster assembly.[27–29] This component is essential for maturation of cytosolic Fe–S binding proteins. An intermembrane space sulfhydryl enzyme Erv1 plays an important role in forming disulfide bridges and facilitating export. Glutathione (GSH) assists in the transport process in a manner that is not clear.

In this concept article, we will focus only on the proteins in the mitochondrial assembly pathway (step 1). With regard to Fe–S cluster assembly, many of the players are known, but the molecular details of how they interact and in each case the role of an essential partner interaction remains unclear. In particular, the identity of the iron donor has been controversial. Frataxin is believed to perform this function, based on the protein's ability to bind iron, promote in vitro cluster assembly on Isu1 and an extensive amount of genetic evidence indicating Yfh1 interacts in vivo with Isu1 (and with Nfs1-Isd11), an interaction that is essential for cluster assembly.[21,30–33] However, recent reports suggest an alternative role for frataxin acting as a gatekeeper for the formation of clusters.[34,35] Regarding the scaffold protein, the mechanism for iron loading and details regarding the sequence of events for when/how protein partners interact with Isu1 remains unclear. Finally, the role of Isd11 is not well established yet. In this report, we will explore individually and in complex the molecular details of the interaction between assembly protein partners.

The Isu1 scaffold protein: This protein is a member of the U-type scaffold proteins and provides the primary site for [Fe_2–S_2] cluster assembly.[36] In yeast, two homologs exist (Isu1 and Isu2)[5,36,37] with a high degree of sequence homology compared to orthologs. Isu2 results from a recent gene duplication and is found only in some fungi such as *S. cerevi-*

siae; in the following description we will refer to the yeast scaffold as only Isu1. The protein sequence identity of Isu1 is very close to the human orthologue (72%), at 80% for the human to the *Drosophila melanogaster* (Dm) orthologue, but 28% for human to the bacterial orthologue from *Thermotoga maritima* (Tm). Isu orthologues are often but not strictly stable as protein dimers; the active state of yeast Isu1 is as a dimer.[38] Isu orthologues bind iron and sulfide for use during cluster assembly.[13,39] Their assembly active site is constructed of three conserved Cys residues[40] and a fourth residue of varying identity; either a His, Asp, Ser, or Lys have been proposed to complete the binding scheme.[4] In a highly related system, the assembly site in the *Aquifex aeolicus* IscU is constructed of Cys_3His coordination.[41] Isu1 contains the LPPVK motif required for interaction with Ssq1.[42] Finally, the general structures of Isu are highly flexible and this flexibility is most likely important for both cluster assembly and transfer.[43]

The iron binding ability of the Isu orthologs are well documented, although the molecular and mechanistic details of this interaction are unclear. Previous reports indicate *T. maritima* and *H. sapien* Isu bind a single ferrous iron atom at micromolar binding affinities (2.7 and 2 μM respectively).[44,45] Similar results have been obtained from our laboratory for the fly and yeast orthologues; however, both show slightly tighter binding affinities in the nanomolar range.[46] Spectroscopic evidence indicates each Isu orthologue initially binds iron in an oxygen/nitrogen-based ligand environment lacking any sulfur ligation. Based on this, we believe each Isu orthologue has an initial metal binding site distinct from the Cys_3X assembly site. Although the identity of this initial binding site is unclear, a comparison of the electrostatic potential surface of Isu1 indicates the protein's C-terminal helix has a charge distribution that would accommodate ferrous iron binding.[46] Upon addition of sulfide to yeast holo Isu orthologue, iron turns over during cluster formation leading to partial Fe–(S) coordination with a Fe···Fe vector of about 2.7 Å, consistent with our UV-Vis data suggesting 2Fe–2S cluster formation.[31]

In Isu1, the three conserved cysteine residues (Cys71, Cys98, and Cys159) at the assembly site are all essential for Fe–S cluster formation.[11] Site-directed mutagenesis studies, comparisons of iron-to-protein ratios, and correlations of cysteine content versus oligomeric state all confirm that one Fe–S cluster coordinates to each Isu monomer in the functional dimer.[47,48] Isu1 exists as a molten globular protein, confirmed by 1,8-anilinonaphthalenesulfonate (ANS) fluorescence measurements; however, this structure is dramatically altered following cluster production.[11,38,46] Using the crystal structure of IscU from *Aquifex aeolicus* with a $[Fe_2–S_2]$ cluster bound, one can develop a simulated model for Isu1 with the solvent-exposed Fe–S cluster-binding site containing the three conserved Cys residues and a semi conserved histidine as ligands.

The Nfs1 cysteine desulfurase: This protein provides sulfur required for Fe–S cluster biosynthesis.[49] Nfs1 is a pyridoxal 5′-phosphate (PLP)-dependent gamma-lyase enzyme with a high degree of homology with other ISC cysteine desulfurase orthologues (sequence identity of 64% for human, 67% for fly, 37% for Tm).[24] Nfs1 catalyzes the degradation of Cys as the substrate to the Ala product, liberating sulfur in the process which then forms an internal persulfide bond with a conserved internal Cys residue (Cys 421 in yeast and Cys 324 in *Thermotoga*) on Nfs1 (Figure 2). Studies of the

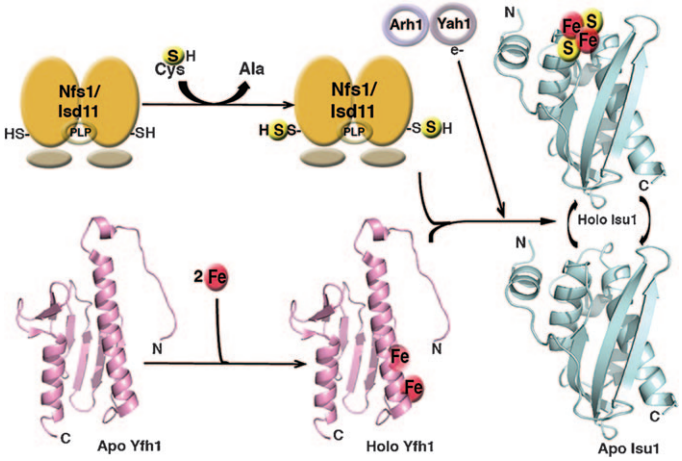

Figure 2. Molecular details of the assembly complex. Apo Yfh1 binds two ferrous iron atoms on the protein's α-helix 1/β-strand 1 region and delivers the metal to the Isu1 scaffold through a beta-sheet interface. The initial iron-binding site on Isu1 is still unknown. The simulated Isu1 structure shows the Fe–S-cluster-binding site towards N-terminal region, consisting of the 3 conserved cysteine residues. Nfs1 and Isd11, in presence of PLP cofactor, catalyses the conversion of Cys to Ala resulting in the formation of persulfide bound to Nfs1. Nfs1/Isd11 interacts with Isu1, in the presence of Yah1 and Arh1, which releases sulfur for the formation of Fe–S clusters.

orthologue NifS from *A. vinelandii* provide strong evidence that sulfane sulfur (i.e., S^0) in the persulfide bond is the activated form utilized for Fe–S cluster assembly.[50] Enzymatic production of the sulfide for Fe–S cluster assembly requires PLP, a derivative of vitamin B_6 that includes pyridoxamine 5′-phosphate and pyridoxine 5′-phosphate as cofactors. PLP is covalently bound to the ε-amino group of an internal conserved lysine residue (Lys 299 in yeast and Lys 203 in *Themotoga*), forming an internal aldimine.[51,52] There is shared formation of an external aldimine when the α-amino group of the substrate replaces the lysine. The activated form of sulfur within the persulfide moiety can then easily be transferred to Isu1 to promote Fe–S cluster assembly.

Structural details regarding the Nfs1 active site have come from studies from orthologues. Crystal structures of *E. coli* IscS and the *T. maritima* NifS orthologs have both been solved and both appeared as homodimers of a size about 45 kDa.[51,52] While the proteins share only a 40% sequence identity, their overall fold is similar to what is expected for members of the α-family of PLP enzymes. Both crystal structures indicate a two-domain protein dimer, with one domain harboring the pyridoxal phosphate binding site and the second smaller domain containing the active-site cys-

teine believed to transiently carry the sulfur released from cysteine as a persulfide adduct. In the *T. maritima* structure, the twelve residues including the persulfide forming cysteine (Cys324) are unfortunately located in a disordered region of the structure.[52] However, this region is well resolved in the *E. coli* structure and it shows this persulfide forming cysteine to be about 17 Å from the enzyme's active site.[51] The N-terminal β-strand in the bacterial protein, which is essential for activity, connects the two domains of the protein.

The Isd11 accessory protein: This protein is important for cysteine desulfurase activity.[13] Interestingly, the mature functional form of eukaryotic Nfs1 requires the presence of the accessory protein Isd11 for activity. However, no bacterial homolog of Isd11 has been found suggesting Isd11 is eukaryotic specific for Fe–S assembly. In vivo pulldown assays indicate yeast Nfs1, of a molecular size of ≈52 kDa, is associated with Isd11 (11 kDa in size) and together they form a complex of about ≈200 kDa in size.[22,23] Nfs1 by itself is prone to aggregation and degradation, however these traits are generally abolished when Isd11 is present.[53] Although the protein sequence of Isd11 is less conserved between eukaryotic orthologues, it is conserved from fungi to humans. In vivo depletion of Isd11 greatly reduces the levels of aconitase and Rieske containing proteins and absence significantly impairs the catalyzed formation of iron–sulfur clusters on Isu1.[22] The presence of Isd11 is required for in vivo activity of Nfs1. While these results suggest Isd11 may control the in vivo stability and function of Nfs1, additional results suggest Isd11 may also mediate the intermolecular interaction between Isu1, Nfs1, and frataxin.[33,53] Either way, Isd11 appears to play a functional role in mitochondrial Fe–S cluster assembly in eukaryotes.[54,55]

The Yfh1 frataxin protein: This protein is essential for cellular iron homeostasis and likely plays a direct role in Fe–S cluster assembly.[56,57] While the exact function of frataxin is unknown, it has been suggested to function in a variety of capacities including as an iron chaperone for both heme and Fe–S cluster pathways, as an iron storage protein in the mitochondria, within the aconitase repair pathway, in energy metabolism and recently as a gatekeeper in controlling NifS activity.[35] Frataxin deficiency in humans is the cause of the cardio-/neurodegenerative disorder Friedreich's ataxia (FRDA), which affects about 1 in 50000 live births.[58,59] Phenotypes of the disorder include mitochondrial iron overload, breakdown in Fe–S cluster and heme biosynthesis, and elevated reactive oxygen species levels likely due to the unregulated presence of high mitochondrial iron concentrations.[60,61] To begin to understand the function of frataxin, we will first explore the biophysical details of the molecule.

Structural and processing details indicate frataxin predominately functions as a soluble protein within the mitochondrial matrix.[62] In eukaryotes, frataxin is nuclear encoded but targeted to the mitochondria.[63–65] In vivo screens indicate mature processed Yfh1 (minus the targeting sequence) is found predominately in the matrix.[66] Solution and crystal structures have been reported for the yeast,[67] human,[68,69] and bacterial[70] frataxin orthologue monomers. Frataxin has a unique fold that combines two terminal α-helices, constructing one plane of the molecule, and five antiparallel β-strands, that construct the second plane, placing the protein in the α–β sandwich structural motif. A sixth and sometimes seventh β-strand are found connecting the two planes, giving a general shared frataxin topology of α1β1β2β3β4β5β6(β7)α2 that is structurally conserved between eukaryotes and prokaryotes.[56] In Yfh1 and in all other structurally characterized frataxin orthologues, numerous solvent exposed acidic residues line the α1 and β1 protein region, forming a contiguous anionic surface ideally suited for iron binding.

All frataxin orthologs bind iron, suggesting a direct role in cellular iron regulation and utilization. The iron binding activity of Yfh1 was first recorded for the frataxin orthologs.[71] When exposed to high iron concentrations at low salt in an environment uncontrolled for oxygen, Yfh1 assembles in a step-wise fashion to form 24-mer spherical aggregates that loosely resemble the iron storage protein ferritin.[72] While Yfh1 aggregation may be important under iron stressed conditions, the monomeric form of frataxin is the functional form during normal mitochondrial Fe–S cluster assembly.[33] Metal to protein stoichiometries, measured for monomeric bacterial[73], yeast[71], fly,[31] and human frataxin[30], show these proteins will tightly bind two, two, one, and up to six Fe^{II} atoms, respectively. Iron dissociation constants, measured for bacterial (CyaY), yeast (Yfh1), fly (Dfh), and human frataxin (HsFtx) give averaged K_D values of about 4, 3, 6 and 12–55 μM, respectively. NMR studies have implicated the conserved Asp, Glu, and His residues in the protein's α-helix 1 and β-strand 1 as being involved in iron binding (Figure 3).[67,71] Structural studies show frataxin

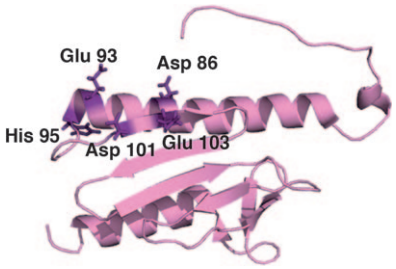

Figure 3. Proposed Yfh1 iron binding residues. Apo protein structure (PDB ID# 2A5) showing solvent exposed α-helix 1 (D86, E93, H95) and β-strand 1 (D101, E103) residue side chains involved in iron binding, as identified by NMR titrations[67,74,77]

binds Fe^{II} in a high-spin, symmetric, six-coordinate ligand environment constructed exclusively by oxygen and nitrogen based ligands, in good agreement with α-helix 1 and β-strand 1 Asp, His, and Glu residues identified as interacting with iron in the NMR titrations.[74–76]

Interaction between Assembly Protein Partners

Cofactor assembly is believed to proceed through formation of a macromolecular complex involving Nfs1, Isd11, Isu1, and Yfh1. Complex formation between protein partners ensures cluster assembly and delivery proceeds in a controlled manner. Regarding the Yfh1/Isu1 interaction, NMR chemical shift mapping studies implicate frataxin's β-sheet surface as the binding region onto Isu1.[46] Molecular details obtained from studying the Yfh1/Isu1 interaction indicate key residues on frataxin's β-sheet surface (N122, K123, Q124, W131) are important in forming the intermolecular interface with the scaffold protein.[78] In most cases, frataxin and Isu protein binding was iron dependent.[30] Although additional details of how all the yeast proteins interact are not yet clear, insight can be gained from looking at studies of orthologs. A recent crystallographic study of the bacterial system provides structural insight into how cysteine desulfurase (IscS) and the scaffold (IscU) interact.[79] The IscS/IscU complex structure suggests IscU interacts with the C-terminus of the cysteine desulfurase near the IscS active site cysteine (Cys328). The three conserved cysteines of the IscU active site project towards the IscS Cys328 containing loop. In vivo binding analysis places the bacterial frataxin binding interface on IscS in close proximity to the IscS/U binding surface.[79] NMR mapping studies of CyaY binding to IscS implicate the conserved residues in the frataxin helix-1/strand 1 ridge as interacting with the cysteine desulfurase.[35]

By simulating the structures of Isu1 and Nfs1, based on the structures of the bacterial orthologs, we have been able to propose a structural model for how the yeast proteins interact with frataxin.[46] In our model, Yfh1 could interact with both Isu1 (using frataxin's β-sheet surface) and Nfs1 (using frataxin's α1–β1 ridge) with minor closing of Nfs1 dimer exposed gap, placing both frataxin and the cysteine desulfurase in position to donate substrates to Isu1 for cluster assembly. However, the positioning, the stoichiometry, and the function of Isd11 are still poorly defined. As stated previously, in eukaryotes Nfs1 requires a small accessory subunit Isd11.[22] Isd11 has been proposed to stabilize Nfs1 through a direct interaction.[23] For the formation of the Fe–S cluster on Isu1/2, sulfur is provided by a direct interaction with the Nfs1/Isd11 complex.[32,40,80] A large number of the molecular details for this interaction have come from extensive studies of the bacterial cluster assembly pathways. The mechanism of sulfur transfer between cysteine desulfurase and the scaffold is unclear. Two hypothesis have been proposed: either transfer could occur through the formation of a heterodisulfide-bridged intermediate between Nfs1 and Isu1[81] or through a transpersulfuration reaction similar to that observed in the bacterial SUF system.[12] The sulfur transfer in the SUF system involves a conserved cysteine on the sulfur transfer protein (SufE), which binds to the sulfur released from the cysteine desulfurase (SufS) to form an intermediate persulfide bond before it is transferred to the corresponding scaffold protein. In this process SufE stimulates the activity of SufS.[82,83] Isd11 in yeast resembles SufE in some respects, but it is unlikely to function as a sulfur transfer protein because it completely lacks cysteine amino acids in its polypeptide backbone.

Although the exact role of frataxin in Fe–S cluster assembly is still not clear, this protein interacts with the scaffold protein, the cysteine desulfurase and with Isd11, either individually and within a multiprotein complex, suggesting it is a key player in regulating the pathway.[32,33] The binding interaction between holo Yfh1 and apo Isu1 has been shown to be submicromolar affinity (166 nM).[46] This binding affinity is tighter than iron binding affinity of frataxin suggesting the interaction is thermodynamically favorable for metal delivery if frataxin is the chaperone in the pathway. Further, co-purification studies from pulldown assays indicate Yfh1 forms a stable complex with Isu1/Nfs1.[32,33,84] Binding between Yfh1 and Isu1/Nfs1 was significantly higher when physiological concentrations of ferrous iron were maintained during complex isolation.[30,32] Mechanistic details of the frataxin/cysteine desulfurase interaction are, however, lacking. In the bacterial system, frataxin regulates the cysteine desulfurase in an iron dependent manner and evidence suggests, at high iron-to-protein ratios, frataxin perturbs the activity of the cysteine desulfurase and formation of the Fe–S cluster, suggesting frataxin acts as a negative regulator of the cysteine desulfurase.[35] In contrast, recent results with orthologues indicate frataxin positively regulates the K_m for cysteine desulfurase substrate binding, suggesting frataxin is acting as an allosteric activator for the enzyme.[85] The role of the frataxin/Isd11 interaction is also still unclear. Frataxin has been shown to interact with Isd11 using co-immunoprecipitation and mass spectrometry in mammalian cells.[84] However, this interaction of human frataxin and the human Isd11 orthologue (ISD11) decreases under clinical point mutations I154F and W155R, which can be restored through nickel supplementation.[84] Given the degree of binding overlap between each protein partners, it is reasonable to suggest in vivo cluster assembly occurs through the formation of a complex core constructed of all protein partners.

Summary and Outlook

Iron–sulfur containing proteins are found ubiquitously in the body and they participate in many fundamental biochemical tasks in nearly every living cell. The mitochondrial ISC pathway supplies clusters for proteins within the mitochondria, and also plays a role in cluster incorporation in the cytosol and throughout the cell.[3] Due to their widespread usage in many fundamental biochemical pathways, it is no wonder that humans, with a deficiency in the production of any of the protein partners responsible for cluster assembly, have serious disorders often incompatible with life.[5] Friedreich's ataxia, a deficiency in production of functional frataxin, is just one such disorder. Despite the importance of this pathway, we are just now beginning to understand the molecular events that lead to mitochondrial Fe–S cluster assembly. Future studies directed at understanding the path-

way at a molecular level will surely need to focus on the structural aspects of how the protein partners interact and the biophysical characterization of key molecular elements that drive these interactions. A glimpse of these structural details has recently been supplied in the bacterial system.[86] Once these details are provided, a mechanistic understanding of how these protein partners produce Fe–S clusters will surely follow.

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