CHARACTERIZATION OF THE ROLES OF A-TYPE PROTEINS IN IRON-SULFUR CLUSTER BIOGENESIS

by

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(Under the Direction of Prof. Michael K. Johnson)

ABSTRACT

The A-type proteins are a class of [Fe-S]-cluster biogenesis proteins which are found in all domains of life. However, the specific function(s) of these A-type proteins within the mitochondrial and bacterial Fe/S-protein biogenesis are still unknown. The ability of *Azotobacter vinelandii*(*Av*) IscA and *Arabidopsis thaliana*(*At*) SufA1 to bind Fe and [Fe-S]-cluster has been investigated to assess the potential role(s) of A-type proteins in [Fe-S]-cluster biogenesis. Both A-type proteins have been shown to bind one Fe(III) per homodimer with an intermediate-spin (S = 3/2) Fe(III) center that is most likely 5-coordinate with two or three cysteinate ligands. Further, *At*SufA1 is shown to bind one $[2Fe\text{-}2S]^{2+}$-cluster with complete cysteinyl ligation. In addition, *in vitro* UV-visible CD studies of rapid cluster transfer from *At*GrxS14 to *At*SufA1 yielding a rate constant of 50000 $M^{-1}min^{-1}$ and >95% completion within 3min indicate that these two classes of proteins work together in cellular [Fe-S]-cluster trafficking.

INDEX WORDS: Iron-sulfur cluster assembly, A-type protein, Monothiol glutaredoxins, Cluster transfer.

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DEDICATION

To my dearest parents

Mr. T.B Randeniya and Mrs. H. M. Randeniya

and my loving husband

Dr. Aruna Ramanayaka

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Iron-Sulfur Proteins: Background

Iron-sulfur (Fe-S) clusters are functionally versatile and ancient prosthetic groups that are found in almost all forms of life (*1-3*). These clusters are found in a variety of proteins (such as ferredoxins, NADH dehydrogenase, hydrogenases, succinate, and nitrogenase) and play a major role in metabolic processes (including respiration, photosynthesis, biosynthesis of amino acids, vitamins, coenzymes and other cofactors) as well as in fundamental metabolism of chemical elements (specifically carbon, oxygen, hydrogen, sulfur, nitrogen, and iron) (*2;4-6*). Their functions include electron transport in the respiratory and photosynthetic chains and a wide range of enzyme complexes, binding and activating substrates in a range of enzymes, regulation of genes expression, and DNA repair (*5*). Despite the fact that these clusters play a crucial role in many biological processes, the detailed mechanism of Fe-S cluster biosynthesis and the maturation of Fe-S proteins still has many unresolved questions.

Structure and Properties of Iron-Sulfur Clusters

Iron-sulfur clusters are biological cofactors containing non-heme iron and inorganic sulfide, with the simplest types having $[Fe_2(\mu_2\text{-}S)_2]$, $[Fe_3(\mu_3\text{-}S)(\mu_2\text{-}S)_3]$, and $[Fe_4(\mu_3\text{-}S)_4]$ cores that are attached to the protein via cysteinyl-S ligation to give approximately tetrahedral sulfur ligation at each Fe site, see Figure 1.1 (*5*). These clusters are abbreviated as $[2Fe\text{-}2S]^n$, $[3Fe\text{-}4S]^n$ and $[4Fe\text{-}4S]^n$, respectively, where *n* is the core

oxidation state assuming a -2 oxidation state for sulfide. Depending on the extent of valence localization or delocalization for each type of cluster in each accessible redox state, the formal oxidation state on each Fe can be +2, +3 or +2.5.

Mononuclear Fe-centers such as rubredoxins and desulforedoxins with tetrahedral cysteinyl ligation are considered to be the simplest type of Fe-S centers even though they lack the inorganic sulfide. These Fe-centers provide fundamental information concerning the properties of Fe^{3+} and Fe^{2+} in a tetrahedral S coordination environment (*7*).

[2Fe-2S] clusters are the simplest type of Fe-S clusters found in nature and primarily function in electron transport. Based on structural and electronic properties of these clusters, they are considered to be the building block of almost all types of Fe-S clusters. Usually the [2Fe-2S] core is ligated by four cysteinate ligands; however, there are a few exceptions. For example, the Rieske-type [2Fe-2S] clusters have two histidyl ligands at one Fe site (*8*), the [2Fe-2S] clusters in some succinate dehydrogenases and sulfide dehydrogenases have one aspartate ligand (*9;10*), the [2Fe-2S] cluster in biotin synthase has one arginine ligand (*11*), and the [2Fe-2S] cluster in the MitoNEET protein has one histidine ligand (*12-14*). The presence of more electropositive histidine ligands as opposed to the less electropositive cysteine ligands at the reducible Fe site has a direct effect on the redox potential. The redox potentials of the all cysteinyl-ligated $[2Fe\text{-}2S]^{2+,+}$ centers range from +100 to -460 mV, whereas the Rieske-type $[2Fe\text{-}2S]^{2+,+}$ centers generally have higher potentials ranging from +380 to -150 mV. These redox potentials are reported relative to the normal hydrogen electrode (NHE).

Cubane-type [3Fe-4S] clusters can be visualized as a cubane-type [4Fe-4S] cluster that had lost one Fe atom. Based on X-ray structures, primary sequences, and *in vivo*

detection, cubane-type $[3Fe\text{-}4S]^{+,0}$ clusters have been shown to be physiologically relevant electron carriers in numerous redox enzymes and proteins (*15*). Cubane-type [4Fe-4S] clusters are one of the most common types of prosthetic groups in biology and represent the thermodynamic sink for the reaction between $Fe^{3+,2+}$, S^{2-} and thiols under strictly anaerobic conditions (*16*). Although [4Fe-4S] clusters are primarily involved in electron transfer, they also constitute the active sites of a wide range of redox and non-redox enzymes. [4Fe-4S] clusters involved in electron transfer usually have all cysteinyl ligation. However, some exceptions involve a single aspartate ligand in some ferredoxins that is proposed to play a role in gating electron transfer and a single histidine ligand in NiFe- and Fe-Fe-hydrogenases and nitrate reductases, which may play a role in coupling electron and proton transfer (*17-20*). $Fe(SCys)_4$, [2Fe-2S], [3Fe-4S] and [4Fe-4S] clusters are all commonly found in nature and are known to function as one-electron donors or acceptors (*5*). However, there are more complex clusters, such as a double-cubane [8Fe-7S] P-cluster (see Figure 1.1), which is found only in nitrogenases and has the potential to mediate two-electron transfer processes.

Biological Roles of Iron-Sulfur Clusters

Initially Fe-S clusters were believed to be involved only in mediating electron transfer, but it is now apparent that they also have a wide variety of biological roles including redox and non-redox catalysis, generating radicals, sensing small molecules in regulatory processes, and locating/repairing damaged DNA along with the electron transfer (*5*). The stability that Fe-S clusters exhibit in multiple oxidation states, along with the possibility of having at least one one-electron redox couple in the physiological

redox potential range, makes these clusters excellent donors and acceptors of electrons in a variety of biological reactions.

A wide range of enzymes utilize site-specific substrate binding and activation mediated by active site Fe-S clusters in redox and non-redox catalysis (*21*). Siroheme-[4Fe-4S] center in sulfite and nitrite reductases (Figure 1.2A) (*22;23*), the Fe-Fe-[4Fe-4S] center in Fe-hydrogenases (Figure 1.2B) (*19;24*), and the Ni-Ni-[4Fe-4S] center in acetyl-CoA synthases (Figure 1.2C) (*25-27*) are some of the examples where the [4Fe-4S] cluster is directly attached to the substrate-binding active site via a bridging cysteinyl-S, thereby facilitating rapid electron transfer to or from the substrate. In addition, there are two major classes of enzymes that utilize a [4Fe-4S] with non-cysteinyl ligation at a unique Fe site to bind and activate the substrate. The first is the Fe-S cluster-containing (de)hydratases, such as aconitase (Figure 1.2D), which bind water and substrate at a unique Fe site to facilitate water insertion or elimination (*28*). The second is the radical-SAM superfamily of Fe-S enzymes that initiate a wide range of radical reactions by binding *S*-adenosylmethionine at a unique Fe site in order to facilitate reductive cleavage to yield methioinine and the highly reactive 5'-deoxyadenosyl radical (Figure 1.2E) (*29;30*).

Furthermore, Fe-S clusters play a major part in sensing environmental or intracellular conditions to regulate gene expression at the translational or transcriptional level (*31*). Most regulatory Fe-S cluster proteins have a cluster binding domain and a DNA binding domain. Among the best characterized examples of bacterial transcriptional regulators are the fumarate-nitrate reduction (FNR) protein which undergo the cluster

conversion in the presence of O_2 and the SoxR protein which is involved in sensing oxidative stress conditions by undergoing redox chemistry at the cluster.

Diseases Related to Defects in Iron-Sulfur Biogenesis

Fe-S cluster biosynthesis is vital for many cellular processes such as the citric acid cycle (via succinate dehydrogenase and aconitase), mitochondrial respiration (via complexes I-III), and for numerous other enzymes or proteins in the mitochondria, cytosol and nucleus of mammalian cells (*32*). The biosysnthesis or repair of these clusters are governed by highly conserved sets of genes present in both eukaryotes and prokaryotes known as Fe-S cluster assembly genes (*33*). Due to the importance of Fe-S clusters, it is not surprising that disruption of Fe-S cluster biogenesis results in several human diseases.

For example, reduced expression of the frataxin gene (*34;35*), which encodes for the frataxin protein that serves as an iron donor for Fe-S cluster formation in eukaryotes, causes Friedreich's ataxia (FRDA). FRDA is a neurodegenerative disease which is characterized by cardiac failure and the death of specific neuronal cell types, including the dorsal root ganglia, which are responsible for sensory perception and maintenance of balance (*36;37*). Splicing mutations of the *iscu* gene, which encodes for a mitochondrial protein that serves as a primary scaffold for Fe-S cluster assembly, is the cause of the IscU myopathy, a disease which almost exclusively affects skeletal muscles (*38;39*).

Microcytic anemia is linked to defects in glutaredoxin Grx5, which serves as an Fe-S cluster carrier protein in Fe-S cluster biogenesis (*40;41*). This is a hematological disease characterized by deficient hemoglobin synthesis resulting in anemia (*41*). Another disease associated with impairment in the Fe-S cluster transport pathway

involving the mitochondrial ABC7 transporter is X-linked sideroblastic anemia with ataxia (XLSA/A) (*42-44*). Moreover, defects in Fe-S cluster biogenesis have also been linked to reduction of the virulence in bacterial pathogens (*45*). In addition, recent studies discovered the cause of respiratory complex 1 deficiency, which leads into mitochondrial encephalomyopathy, is mutation of the protein, NUBPL (*32;46*) (aka Ind1), which is involved in transferring Fe-S clusters to respiratory chain complex I (*32;47;48*). Furthermore, mutations in Nfu1 or BolA3 were found to specifically interfere with the activity of the Fe-S enzyme lipoate synthase leading to a disease called multiple mitochondrial dysfunctions syndrome. This enzyme plays a major role in lipoic acid modifications of some specific enzymes, such as the E2 subunits of pyruvate dehydrogenase, α-ketoglutarate dehydrogenase (*49;50*). Therefore, understanding the basic steps in Fe-S cluster biogenesis is important in order to gain molecular level insight into several human diseases.

Iron-Sulfur Cluster Assembly Systems

Three distinct types of biosynthetic machinery for Fe-S cluster biogenesis have been discovered in prokaryotes (Figure 1.3), termed the ISC (iron sulfur cluster), NIF (nitrogen fixation), and SUF (sulfur utilization factor) systems (*33;51*). Even though these three systems are used by different organisms under different conditions to synthesze Fe-S clusters, they share a common fundamental mechanism involving cysteine-desulfurase mediated Fe-S cluster assembly on a primary scaffold protein followed by intact cluster transfer to apo acceptor or carrier proteins. In addition, they share highly homologous A-type proteins (IscA, ^{Nif}IscA, and SufA) which are the subject of this dissertation and will be discussed separately below. The roles of each of the other

components of the ISC, NIF, and SUF Fe-S cluster biogenesis machineries are discussed below.

ISC System

The ISC system is involved in general Fe-S cluster biosynthesis in numerous bacteria and constitutes the heart of the mitochondrial system for Fe-S cluster biogenesis in eukaryotes (*52-56*). This system is known as the house-keeping Fe-S cluster assembly machinery due to its ability to operate under normal growth conditions. The genes encoding these Fe-S assembly proteins (IscR, IscS, IscU, IscA, HscA, HscB, and ferredoxin) are organized in a cluster *iscRSUA-hscBA-fdx (5;52)*. IscS a PLP dependent cysteine desulfurase, which acts as a sulfur donor for Fe-S cluster biosynthesis by transferring the sulfur directly to the primary Fe-S cluster scaffold protein, IscU (*57*). As discussed below, IscA has been proposed to function as an alternative scaffold protein, a carrier protein for delivery of clusters assembled on IscU, and an Fe donor for cluster assembly on IscU (*58-65*).

IscU has three conserved cysteines that serve as ligands for *de novo* biosynthesis of Fe-S clusters and has been proven to be essential for the general Fe-S cluster biogenesis in cells (*52;66*). Extensive spectroscopic and analytical characterization revealed that IscS-mediated cluster assembly on *Azotobacter vinelandii* (*Av*) IscU resulted in the sequential formation of [2Fe-2S] and [4Fe-4S] clusters under anaerobic conditions (*58;67*). Initially, the IscU homodimer assembles a single $[2Fe\text{-}2S]^{2+}$ cluster, and consequently, a second transient $[2Fe\text{-}2S]^{2+}$ cluster is assembled (*51;67*). Then two adjacent $[2Fe\text{-}2S]^{2+}$ clusters at the subunit interface undergo a two-electron reductive coupling to form a $[4Fe\text{-}4S]^{2+}$ cluster on each IscU dimer using electron donors such as

dithionite or reduced Isc Fdx (*68*). This provided evidence that one of the roles of IscFdx is to act as the reductant for this cluster conversion. Spectroscopic studies further revealed that upon O_2 exposure, the $[4Fe\text{-}4S]^{2+}$ cluster-bound form converts back to the one $[2Fe\text{-}2S]^{2+}$ cluster-bound form of IscU with the loss of one cluster (*68*).

The *iscR* gene encodes for a DNA- and cluster-binding regulatory protein IscR (iron-sulfur cluster regulator). In its cluster-bound form, IscR is a transcriptional repressor of the entire *isc* operon (*69*). HscA (heat-shock-cognate) and HscB co-chaperons and their eukaryotic homologs (Ssp1/Jac1 in yeast) play a crucial role in the efficient maturation of Fe-S cluster proteins in both prokaryotic and eukaryotic organisms (*33;55;70*). HscA and Ssq1 have been shown to interact with both the apo and cluster-bound forms of IscU. However, this interaction is highly selective as they only bind to the highly conserved LPPVK motif located adjacent to the cluster-binding site of IscU (*71-77*). HscB binds and escorts cluster-bound IscU to HscA, which greatly stimulates the ATPase activity of HscA, thereby facilitating ATP-dependent intact Fe-S cluster transfer from IscU to acceptor proteins. Using *in vitro* cluster transfer studies, Chandramouli and Johnson (*78*) demonstrated that both HscA and HscB bind independently to the [2Fe-2S] cluster-bound form of IscU, and that the trimeric complex involving [2Fe-2S]-IscU/HscA/HscB greatly enhanced the rates of cluster transfer from holo-IscU to apo-IscFdx in the presence of MgATP.

NIF System

The NIF system was the first Fe-S cluster biogenesis system to be discovered, and it is specific for the maturation of Fe-S cluster proteins involved in nitrogen fixation in organisms like *Av (33;79).* However, some non-nitrogen fixing organisms such as

Helicobacter pylori and *Campylobacter jejuni* have only the NIF system for Fe-S cluster assembly, which indicates that these organisms utilize the NIF system for the assembly of non-nitrogenase Fe-S clusters (*80*). The *nif* operon consists of genes which encoded for ^{Nif}IscA, NifS, and NifU.

^{Nif}IscA is the A-type protein homolog of IscA, whereas the NifS is the homolog of IscS. NifU is a modular homodimeric protein with three separate domains, namely, the N- and C- terminal domains and the central domain. Each of these domains can assemble Fe-S clusters. The N-terminal domain is homologous to the IscU, and it assembles a transient $[4Fe\text{-}4S]^{2+}$ cluster *via* a reductive coupling of $[2Fe\text{-}2S]^{2+}$ clusters, whereas the C-terminal domain directly assembles a transient $[4Fe\text{-}4S]^{2+}$ cluster (*81*). However, *in vitro*, both these $[4Fe\text{-}4S]^{2+}$ clusters can be transferred to apo-nitrogenase Fe protein, to form the active protein (*81;82*). Many eukaryotic and prokaryotic systems have separate proteins or domains with sequence homology to the C-terminal domain, termed NifU-like or Nfu-type proteins, and have been shown to function in Fe-S cluster biogenesis. The central domain contains a permanent redox-active $[2Fe\text{-}2S]^{2+,+}$ cluster and has sequence homology to the bacterial ferritin associated ferredoxin *(82).* NifS and NifU are essential for the assembly of the nitrogenase Fe-S centers in *Av* (*79*), and NifS has been shown to mediate the assembly of Fe-S clusters on NifU that can be transferred to nitrogenase (*57;81;82*). Disruption of the *nif* Fe-S cluster biogenesis genes in *Av* results in defects in the maturation of either the Fe-protein or the FeMo protein component of nitrogenase, and deletion of the *nifU* and *nifS*, individually or together, results in a dramatic loss of activity in both components of nitrogenase (*83*).

SUF System

The SUF system is encoded by the *sufABCDSE* operon and represents an alternative system for Fe-S cluster biogenesis in bacteria (*84*). It was first discovered in an *Escherichia coli* (*Ec*) variant in which the entire *isc* operon was deleted (*85*). Subsequently, the SUF system was found in many bacteria and archea and constitutes the heart of the Fe-S cluster biogenesis system found in plant chloroplasts, an O_2 producing organelle. This system acts as a backup system for more general ISC systems under oxidative stress and/or iron limiting conditions. SufS is a cysteine desulfurase homologous to IscS and NifS. However, the cysteine desulfurase activity of SufS is greatly increased by the complex formation with the sulfurtransferase SufE (*86;87*). Interestingly, the structural homology shown by SufE to IscU and SufU (in Gram-positive bacteria) indicate a similar sulfur transfer mechanism for SufS to SufE as with IscS to IscU and SufS to SufU (*87;88*). SufA is an A-type protein which is homologous to IscA.

In vivo studies have shown that SufB, SufC, and SufD forms a unique $\alpha\beta_2\gamma$ heterotetrameric complex, $SufBC_2D$, which has been shown to further enhance the desulfurase activity of the SufSE complex (*89*) and to assemble a [4Fe-4S] cluster on Suf B via *in vitro* cluster reconstitution (*90;91*). Furthermore, it has been demonstrated that this complex could be purified containing one equivalent of $FADH_2$ and one [4Fe-4S] cluster (*92*). Moreover, recent studies have shown that when *Ec* SufA is coexpressed *in vivo* with its cognate partner proteins from the *suf* operon (*SufBCDSE*), it purifies with a $[2Fe\text{-}2S]^{2+}$ cluster rather than with Fe-bound (*93*). Furthermore, *in vitro* and *in vivo* studies have shown that the SufBCD complex assembles clusters on SufB that can be

transferred to SufA for maturation of Fe-S proteins under oxidative stress conditions *(90;92)*. SufB is believed to be the primary scaffold of the SUF system with four highly conserved cysteine residues (*94*). The role of SufB as the primary scaffold protein in the SUF system is further supported by the evidence illustrating the capability of SufB to assemble a [4Fe-4S] cluster *in vitro* under anaerobic reconstitution conditions as well as its ability to interact with SufE and accept sulfur for Fe-S cluster assembly (*94*). SufC is an ATPase which interacts with SufB and SufD to form the SufBCD complex (*86*). Except the lack of conserved cysteines on SufD, it is homologous to SufB. These two interact with SufC to form the $SufBC_2D$ complex. Mutational studies on SufD have revealed residues near the C-terminus (414-423) that are critical for protein stabilization. Furthermore, the presence of highly conserved His^{360} was shown to be important for the function of SufD for recruiting Fe for Fe-S cluster assembly on SufB (*95*).

In addition, there are some Gram-positive bacteria, such as *T. maritima* (see Figure 1.3) and *E. faecalis*, which utilize an altered SUF system with a *sufBUSDC* operon. This system encodes for SufU rather than SufA and does not have a SufE (*66*). Even though SufU has the three conserved cysteine residues and the protein structure resembling IscU, it does not engage the co-chaperones for the maturation of Fe-S proteins due to the lack of LPPVK motif. In *Synechocystis* and many cyanobacterial species, the *suf* system has a *sufR* gene located directly upstream to the *SufBCDS* operon. The *sufR* gene encodes a transcriptional factor SufR, which functions as a transcriptional repressor of the *SufBCDS* operon and as an autoregulator for *SufR*. SufR is a homodimer with each monomer having an N-terminal helix-loop-helix DNA binding domain and an Fe-S cluster binding domain that binds a [4Fe-4S] cluster (*96*).

A-type Proteins

As indicated in Figure 1.3, all three cluster assembly systems contain A-type proteins, ^{Nif}IscA in the NIF system, IscA in the ISC system, and SufA in the SUF system. In addition eukaryotic mitochondria generally contain Isa1, a homolog of bacterial IscA, and Isa2, a unique A-type protein specific to eukaryotes with a 21 residue insert between the first and second conserved cysteines. Many bacteria including *Av* and *Ec* have a separate, stand-alone A–type protein termed ErpA (*97*).

However, despite the involvement of A-type proteins in all the bacterial Fe-S cluster biogenesis systems, as well as in eukaryotic mitochondria and chloroplasts, their specific role(s) in Fe-S cluster biogenesis remain elusive. Most organisms contain several A-type proteins, i.e. *Ec* has three A-type proteins: IscA, SufA and ErpA. *In vivo* genetic studies demonstrate that the inactivation of IscA or SufA had no effect on growth under aerobic or anaerobic conditions, whereas an IscA/SufA double mutant was found to strongly inhibit growth, but only under aerobic growth conditions, due to deficient maturation of [4Fe-4S] cluster-containing proteins (*98*). However, under aerobic conditions ErpA mutation was found to be lethal (*98*). ErpA has been shown to play a specific and essential role in the maturation of the key [4Fe-4S] cluster-containing enzymes (IspH and IspG) in the bacterial isoprenoid biosynthesis pathway (*97*). Furthermore, in *Av* which contains ^{Nif}IscA and IscA, deletion of ^{Nif}IscA had no effect on Mo-dependent diazotropic growth (*83*), whereas deletion of IscA was shown to be lethal only under elevated oxygen conditions (*99*). These *in vivo* studies point out that A-type proteins demonstrate some functional redundancy and are only required under aerobic or oxidative stress conditions.

Bacterial A-type proteins have three conserved cysteine residues in the sequence motif Cys-X_{63-65}-Cys-X-Cys (see Figure 1.4) and have been proposed to function as alternative scaffolds for assembling Fe-S clusters under oxidative stress conditions, as metallochaperones for delivering Fe to U-type primary scaffolds proteins, and as cluster chaperones for delivering preformed clusters from primary scaffold proteins to apo acceptor proteins (*59;61;62;98*). *In vivo* and *in vitro* studies have shown that the three conserved cysteine residues are critical for the above mentioned functions of A-type proteins (*62*). Crystallographic structural data is only available for the apo forms of *Ec* IscA and SufA and IscA and a [2Fe-2S] cluster-bound form of *Thermosynechococcus elongatus (Te)* IscA (*100*). In the latter, the [2Fe-2S] cluster is coordinated by two conformationally distinct IscA protomers, termed α and β. As shown in Figure 1.5, the cluster is asymmetrically coordinated by four cysteinyl residues at the subunit interface. The ligation by Cys37, Cys101, and Cys103 from the α protomer and by Cys103 from the β protomer suggests that cluster transfer is mediated by dissociation of the β protomer to facilitate interaction with the apo acceptor protein (*100*).

Evidence of a role for A-type proteins function in the delivery of Fe for Fe-S cluster assembly stems from the work of Ding and coworkers on the ability of A-type proteins to bind mononuclear Fe(III) with high affinity (*60-62;101;102*). Recombinant *Ec* IscA and SufA were purified with trace amounts of intermediate-spin Fe(III) bound to the protein (*60;62;101*). Furthermore, they showed that, in the presence of dithiothreitol (DTT) or catalytic amounts of thioredoxin reductase and thioredoxins, the incubation of either IscA or SufA with Fe(II) ion under aerobic conditions yields the same ferric-bound species. More recently, these studies were extended to the human homolog of IscA, *Hs*

IscA1, which also binds Fe(III) with high affinity. In the presence of L-cysteine, the ferric-bound *Hs* IscA1, *Ec* IscA, and *Ec* SufA were shown to be competent iron donors for cluster assembly on *Ec* IscU. These results have been confirmed and extended by studies of *Av* ^{Nif}IscA (*103*), which showed binding of both mononuclear Fe(II) or Fe(III) in a predominantly cysteinyl-ligated coordination environment. The iron in Fe(II) and Fe(III)-bound forms of ^{Nif}IscA is released as Fe(II) by L-cysteine. Moreover, both forms were competent iron donors for cluster assembly on NifU. However, they are unlikely to be specific iron donors as the rates of cluster assembly on NifU using Fe-loaded ^{Nif}IscA are the same as that observed using equivalent concentrations of free Fe(II) under comparable conditions (*103*), indicating that the rate limiting step is the release of free Fe(II) in the presence of L-cysteine . Recent *in vivo* analysis of the role of *Saccharomyces cerevisiae* (*Sc*) Isa1 and Isa2 shows that these two form an Fe-bound complex which is required for the maturation of mitochondrial [4Fe-4S] proteins including aconitase and homoaconitase (*104*). However, Isa1-Isa2 was not necessary for the generation of mitochondrial [2Fe-2S] proteins and cytosolic [4Fe-4S] proteins (*104*). Furthermore, absence of aconitase, a major mitochondrial [4Fe-4S] enzyme, and depletion of Iba57, which interacts directly with the Fe-bound Isa1-Isa2 complex and promotes Fe release, results in accumulation of iron on the Isa protein (*104*). These findings suggest that the Fe-bound Isa1-Isa2 complex and Iba57 constitute a late-acting system that is specifically dedicated to the maturation of mitochondrial [4Fe-4S] proteins (*104*). The bacterial homolog of Iba57, YgfZ, is an *E. coli* protein whose crystal structure is highly similar to aminomethyl transferase, dimethylglycine oxidase, and related tetrahydrofolate-binding enzymes (*105*). *E. coli* YgfZ is a folate-dependent protein and proposed to participate in

the synthesis and repair of Fe-S clusters (*106*). Recent evidence for its involvment in Fe-S cluster biogenesis came from the mutational studies of *ygfZ* gene, which resulted in reduced growth, increased sensitivity to oxidative stress and decreased activities of MiaB and other Fe-S enzymes (*106*). Though it seems likely that the bacterial homolog of Iba57 also plays a specific role in Fe/S protein biogenesis, its role has not yet been identified (*105*). However, a molecular level understanding of how A-type proteins facilitate [4Fe-4S] cluster maturation and whether this involves the mononuclear Fe or Fe-S cluster bound forms or both are still unresolved.

The combination of UV-visible absorption, resonance Raman and Mössbauer spectroscopic techniques has demonstrated that as purified apo-forms of these A-type proteins, such as *Ec* IscA and SufA, can function as a scaffold for cysteine desulfurase-mediated assembly of labile Fe-S clusters which can be transferred to apo-acceptor proteins such as Isc Fdx and biotin synthase in the presence of DTT (*59;65;107;108*). However, preliminary evidence involving mixing [2Fe-2S] cluster bound *Ec* IscU with apo *Ec* IscA and repurifying and assessing the cluster content of each protein after a fixed time interval, suggested that [2Fe-2S] clusters are transferred from IscU to IscA ,but not vice-versa (*64*). This raised the possibility that IscA functions as a cluster chaperone for delivery of clusters assembled on IscU rather than an alternative scaffold for cluster assembly. Furthermore, recent *in vivo* studies have shown that *Ec* SufA coexpressed with its cognate partner proteins from the *suf* operon (*SufBCDSE*), purifies with a bound [2Fe-2S]$^{2+}$ cluster (*93*). More recently, studies of *Av* ^{Nif}IscA showed that forms containing one [2Fe-2S]$^{2+}$ per homodimer and one [4Fe-4S]$^{2+}$ cluster per homodimer can be reversibly interconverted with aerobic conditions favoring the former, and anaerobic conditions in

the presence of apo- ^{Nif}IscA and dithiol reagents favoring the latter (*109*) . This suggests that the cluster composition of A-type proteins *in vivo* is dependent on cellular growth conditions and may provide a rationalization for the observation that this class of Fe-S cluster assembly proteins is only required for the maturation of [4Fe-4S] cluster-containing proteins under oxidative stress conditions (*109*).

Roles and Properties of Monothiol Glutaredoxins (Grxs) in Fe-S Cluster Biogenesis

Glutaredoxins (Grxs) have long been known to function as thiol-disulfide oxidoreductases, similar to thioredoxins (Trx) which share a similar protein fold (*110*). These small ubiquitous proteins are dependent on glutathione (GSH) and have been shown to be involved in many functions such as GSH-dependent electron donation, glutathionylation, which has a major role in protecting the cell against oxidative damage (*111-113*), cellular iron homeostasis, and Fe-S cluster biogenesis (*114;115*). Initially Grxs were divided into two classes, monthiol and dithiol, based on their active-site sequences, but recent advances have led to classification of six classes of Grxs (*116;117*). However, among these six classes, two classes have been found in most prokaryotic and eukaryotic organisms, namely, Class I Grxs with CxxC/S active sites (also known as the dithiol Grxs), and Class II Grxs with CGFS active sites (also known as the monothiol Grxs). Figure 1.6 shows the domain organization of distinct types of class II Grxs which are relevant to this study.

The monothiol Grxs with CGFS active site (GCFS Grxs) can be either single-domain or multi-domain structured. Gene knockout studies carried out on Grx5 from the yeast *S. cerevisiae* provided the initial proof that established roles for monothiol Grxs in Fe-S biogenesis. These knockout studies resulted in a mutant that exhibits increased

sensitivity to oxidative stress as a result of iron accumulation in the cell and deficient cluster assembly in at least two Fe–S cluster-containing proteins (aconitase and succinate dehydrogenase) leading to impaired respiratory growth (*118;119*). Furthermore, radiolabelled ^{55}Fe immunoprecipitation studies carried on this mutant revealed accumulation of iron on Isu1, thereby suggesting a role for Grx5 as a cluster carrier or storage protein for clusters assembled on the U-type scaffold protein in order to facilitate delivery to apo acceptor proteins (*120;121*). In addition, specific interaction observed between Grx5 and the A-type protein Isa1 in *S. cerevisiae* (*122*) as well as in both Isa1 and Isa2 in *S. pombe* further supported a role in Fe-S cluster biogenesis for monothiol Grxs (*123*). In addition, recent studies have shown regulation of Fe levels in *Sc* occurs by sensing the level of [2Fe-2S] cluster-bound monothiol Grxs in the cytosol by displacing one of the Grx monomers with a BolA-like protein (termed Fra2) to form a [2Fe-2S] cluster bound Grx3/Fra2 or Grx4/Fra2 heterodimer which interacts with the activator of ferrous transport (Aft) protein (*124*). Moreover, recent studies using [2Fe-2S] cluster-bound complexes of *Ec* Grx4 and the cytosolic homologs of *Sc*Grx3/4 with BolA-type proteins in *Schizosaccharomyces pombe* and humans indicates this is likely to be of general importance in cellular iron metabolism (*125-127*).

The *in vivo* evidence for a role of monothiol Grxs in cluster trafficking and storage is further supported by a wide variety of *in vitro* studies. Monothiol CGFS Grxs have been shown to contain bound [2Fe-2S] clusters as purified and the apo forms readily incorporate labile [2Fe-2S] clusters during cysteine desulfurase-mediated cluster assembly under anaerobic conditions in the presence of glutathione (GSH) (*128-130*). Analytical and spectroscopic studies revealed that monothiol CGFS Grxs incorporate one

subunit-bridging [2Fe-2S] cluster per homodimer with complete cysteinyl ligation (*129*). Subsequent crystallographic studies of *Ec* Grx4 revealed a subunit-bridging [2Fe-2S] cluster, which is ligated by the CGFS active-sites cysteine residues of the two Grx monomers and the cysteines of two GSHs (*131*), see Figure 1.7. Similar [2Fe-2S] cluster coordination had been previously observed in crystallographic studies of two dithiol Grxs, plant Grx C1 and Human Grx2 (*132;133*). Furthermore, the observation that the [2Fe-2S] cluster-bound form of *At* GrxS14 (a plant chloroplast monothiol Grx) is capable of rapid and efficient maturation of apo plant chloroplast ferredoxin (Fdx) *via* intact cluster transfer (*129*), along with the most recent *in vitro* studies demonstrating rapid, unidirectional and quantitative [2Fe-2S] cluster transfer from the *Av* IscU to *Av* Grx5 only in the presence of the dedicated HscA/HscB co-chaperone system and MgATP (*134*), has provided convincing evidence of a cluster carrier or storage role for monothiol Grxs.

Summary of Presented Work

The above discussion suggests that A-type proteins may serve a dual role, as an iron donor for maturation of [4Fe-4S] clusters on acceptor proteins, and as a chaperone that works together with monothiol Grxs for the delivery of clusters assembled on primary scaffold proteins to apo Fe-S proteins. Support for this dual role has recently been provided by *in vitro* studies of *Av* ^{Nif}IscA using the NIF system for Fe-S cluster biogenesis and relevant nitrogen fixation acceptor proteins (*103;109*). The objective of this work is to extend these studies to *Av* IscA using ISC proteins and relevant apo acceptor proteins, and to *At* SufA1 using the SUF proteins and relevant chloroplastic acceptor proteins. Chapter 2 reports the spectroscopic characterization of both the Fe(III)-

bound *At* SufA1 and *Av* IscA as well as [2Fe-2S] cluster-bound *At* SufA1 by UV-visible absorption, CD, EPR, and resonance Raman spectroscopies. Fe and protein assays carried on Fe(III)-bound A-type protein illustrate that both *At* SufA1 and *Av* IscA are able to bind one Fe per homodimer, and spectroscopic results indicate that they bind Fe^{3+} ions in a tetrahedral, predominantly cysteinyl-ligated coordination environment. Furthermore, Fe and protein assays carried on cluster-bound *At* SufA demonstrate that it binds one [2Fe-2S]-cluster per homodimer, and spectroscopic results indicate that [2Fe-2S] cluster on *At* SufA1 has the stable all cysteinyl-ligated coordination environment. This chapter also reports UV-visible CD studies that demonstrate rapid and quantitative transfer of a [2Fe-2S] cluster from *At* [2Fe-2S]-GrxS14 to *At* apo-SufA1. *In vitro* experiments indicate that these two classes of proteins work together in cellular Fe-S cluster trafficking.

Abbreviations

Nitrogen fixation, nif; iron sulfur cluster, isc; sulfur utilization factor, suf; pyridoxal phosphate, PLP; glutaredoxin, Grx; ferredoxin, Fdx; *Azotobacter vinelandii*, *Av*; *Arabidopsis thaliana*, *At*; *Aquifex aeolicus*, *Aa*; *Escherichia coli*, *Ec*; *Saccharomyces cerevisiae*, *Sc*; *Schizosaccharomyces pombe*, *Sp*; *Thermosynechococcus elongates*, *Te*;

References

1. Beinert, H. (2000) A tribute to sulfur, *Eur. J. Biochem. 267*, 5657-5664.

2. Beinert, H. (2000) Iron-sulfur proteins: ancient structures, still full of surprises, *J. Biol. Inorg. Chem. 5*, 2-15.

3. Wächtershäuser, G. (1992) Groundworks for an evolutionary biochemistry - the iron-sulfur world, *Prog. Biophys. Mol. Biol. 58*, 85-201.

4. Beinert, H., Holm, R. H., and Münck, E. (1997) Iron-sulfur clusters; Nature's modular, multipurpose structures, *Science 277*, 653-659.

5. Johnson, M. K. and Smith, A. D. (2005) Iron-sulfur proteins, in *Encyclopedia of Inorganic Chemistry, Second Edition* (King, R. B., Ed.) pp 2589-2619, John Wiley & Sons, Chichester.

6. Rees, D. C. and Howard, J. B. (2003) The interface between the biological and inorganic worlds: iron-sulfur metalloclusters, *Science 300*, 929-931.

7. Romao, M. J. and Archer, M. (1999) Structural versatility of proteins containing rubredoxin-type centers, in *Iron Metabolism* (Ferreira, G. C., Moura, J. J. G., and Franco, R., Eds.) pp 341-358, Wiley-VCH Verlag, Weinheim.

8. Link, T. A. (1999) The structures of Rieske and Rieske-type proteins, *Adv. Inorg. Chem. 47*, 83-157.

9. Hagen, W. R., Silva, P. J., Amorim, M. A., Hagedoorn, P. L., Wassink, H., Haaker, H., and Robb, F. T. (2000) Novel structure and redox chemistry of the prosthetic groups of the iron-sulfur flavoprotein sulfide dehydrogenase from *Pyrococcus furiosus*; evidence for a [2Fe-2S] cluster with $Asp(Cys)_{3\ ligands}$, *J. Biol. Inorg. Chem. 5*, 527-534.

10. Yankovskaya, V., Horsefield, R., Törnroth, S., Luna-Chavez, C., Miyoshi, H., Léger, C., Byrne, B., Cecchini, G., and Iwata, S. (2003) Architecture of succinate dehydrogenase and reactive oxygen species generation, *Science 299*, 700-704.

11. Berkovitch, F., Nicolet, Y., Wan, J. T., Jarrett, J. T., and Drennan, C. L. (2004) Crystal structure of biotin synthase, an *S*-adenosylmethionine-dependent radical enzyme, *Science 303*, 76-79.

12. Paddock, M. L., Wiley, S. E., Axelrod, H. L., Cohen, A. E., Roy, M., Abresch, E. C., Capraro, D., Murphy, A. N., Nechushtai, R., Dixon, J. E., and Jennings, P. A. (2007) MitoNEET is a uniquely folded 2Fe 2S outer mitochondrial membrane protein stabilized by pioglitazone, *Proc. Natl. Acad. Sci. U.S A. 104*, 14342-14347.

13. Lin, J., Zhou, T., Ye, K., and Wang, J. (2007) Crystal structure of human mitoNEET reveals distinct groups of iron sulfur proteins, *Proc. Natl. Acad. Sci. U. S. A. 104*, 14640-14645.

14. Sendra, M., Ollagnier de Choudens, S., Lascoux, D., Sanakis, Y., and Fontecave, M. (2007) The SUF iron-sulfur cluster biosynthetic machinery: Sulfur transfer from the SUFS-SUFE complex to SUFA, *FEBS Lett. 581*, 1362-1368.

15. Johnson, M. K., Duderstadt, R. E., and Duin, E. C. (1999) Biological and synthetic [Fe_3S_4] clusters, *Adv. Inorg. Chem. 47*, 1-82.

16. Rao, P. V. and Holm, R. H. (2004) Synthetic analogues of the active sites of iron-sulfur proteins, *Chem. Rev. 104*, 527-559.

17. Calzolai, L., Gorst, C. M., Zhou, Z.-H., Teng, Q., Adams, M. W. W., and La Mar, G. N. (1995) ^{1}H-NMR investigation of the electronic and molecular structure of the 4-iron cluster ferredoxin from the hyperthermophile *Pyrococcus furiosus* - identification of Asp-14 as a cluster ligand in each of the 4 redox states., *Biochemistry 34*, 11373-11384.

18. Volbeda, A., Charon, M.-H., Piras, C., Hatchikian, E. C., Frey, M., and Fontecilla-Camps, J. C. (1995) Crystal structure of the nickel-iron hydrogenase from *Desulfovibrio gigas*, *Nature 373*, 580-587.

19. Peters, J. W., Lanzilotta, W. N., Lemon, B. J., and Seefeldt, L. C. (1998) X-ray crystal structure of the Fe-only hydrogenase (Cp1) from *Clostridium pasteurianum* to 1.8 Angstrom resolution, *Science 282*, 1853-1858.

20. Bertero, M. G., Rothery, R. A., Palak, M., Hou, C., Lim, D., Blasco, F., Weiner, J. H., and Strynadka, N. C. (2003) Insights into the respiratory electron transfer pathway from the structure of nitrate reductase A, *Nat. Struct. Biol. 10*, 681-687.

21. Flint, D. H. and Allen, R. M. (1996) Iron-sulfur proteins with non-redox functions, *Chem. Rev. 96*, 2315-2334.

22. Crane, B. R., Siegel, L. M., and Getzoff, E. D. (1995) Sulfite reductase structure at 1.6 Å - Evolution and catalysis for reduction of inorganic anions, *Science 270*, 59-67.

23. Crane, B. R., Seigel, L. M., and Getzoff, E. D. (1997) Probing the catalytic mechanism of sulfite reductase by X-ray crystallography: structures of the Escherichia coli hemoprotein in complex with substrates, inhibitors, intermediates, and products, 36 ed., pp 12120-12137.

24. Nicolet, Y., Cavazza, C., and Fontecilla-Camps, J. C. (2002) Fe-only hydrogenases: structure, function and evolution, *J. Inorg. Biochem. 91*, 1-8.

25. Darnault, C., Volbeda, A., Kim, E. J., Legrand, P., Vernède, X., Lindahl, P. A., and Fontecilla-Camps, J. C. (2003) Ni-Zn-[Fe4-S4] and Ni-Ni-[Fe4-S4] clusters in closed and open a subunits of acetyl-CoA synthase/carbon monoxide dehydrogenase , *Nat. Struct. Biol. 10*, 271-279.

26. Doukov, T. I., Iverson, T. M., Seravalli, J., Ragsdale, S. W., and Drennan, C. L. (2002) A Ni-Fe-Cu center in a bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase, *Science 298*, 567-572.

27. Svetlitchnyi, V., Dobbek, H., Meyer-Klaucke, W., Meins, T., Thiele, T., Rõmer, P., Huber, R., and Meyer, O. (2004) A functional Ni-Ni-[4Fe-4S] cluster in the monomeric acetyl-CoA synthase from *Carboxydothermus hydrogenoformans*, *Proc. Natl. Acad. Sci. U. S. A. 101*, 446-451.

28. Beinert, H., Kennedy, M. C., and Stout, C. D. (1996) Aconitase as iron-sulfur protein, enzyme, and iron-regulatory protein, *Chem. Rev. 96*, 2335-2373.

29. Jarrett, J. T. (2003) The generation of 5'-deoxyadenosyl radicals by adenosylmethioine-dependent radical enzymes, *Curr. Opin. Chem. Biol. 7*, 174-182.

30. Cheek, J. and Broderick, J. B. (2001) Adenosylmethionine-dependent iron-sulfur enzymes: versatile clusters in a radical new role, *J. Biol. Inorg. Chem. 6*, 209-226.

31. Kiley, P. J. and Beinert, H. (2003) The role of Fe-S proteins in sensing and regulation in bacteria, *Curr. Opin. Microbiol. 6*, 181-185.

32. Rouault, T. A. (2012) Biogenesis of iron-sulfur clusters in mammalian cells: new insights and relevance to human disease, *Dis. Model. Mech. 5*, 155-164.

33. Johnson, D. C., Dean, D. R., Smith, A. D., and Johnson, M. K. (2005) Structure, function and formation of biological iron-sulfur clusters, *Annu. Rev. Biochem. 74*, 247-281.

34. Campuzano, V., Montermini, L., Molto, M. D., Pianese, L., Cossee, M., Cavalcanti, F., Monros, E., Rodius, F., Duclos, F., and Monticelli, A. (1996) Friedreich's ataxia:autosomal recessive disease caused by an intronic GAA triplet repeat expansion, *Science 271*, 1423-1427.

35. Babcock, M., de Silva, D., Oaks, R., Davis-Kaplan, S., Jiralerspong, S., Montermini, L., Pandolfo, M., and Kaplan, J. (1997) Regulation of mitochondrial iron accumulation by Yfh1p, a putative homlog of frataxin, *Science 276*, 1709-1712.

36. Durr, A., Cossee, M., Agid, Y., Campuzano, V., Mignard, C., Penet, C., Mandel, J. L., Brice, A., and Koenig, M. (1996) Clinical and genetic abnormalities in patients with Friedreich's ataxia, *N. Engl. J. Med. 335*, 1169-1175.

37. Martelli, A., Napierala, M., and Puccio, H. (2012) Understanding the genetic and molecular pathogenesis of Friedreich's ataxia through animal and cellular models, *Dis. Model. Mech. 5*, 165-176.

38. Kollberg, G., Tulinius, M., Melberg, A., Darin, N., Andersen, O., Holmgren, D., Oldfors, A., and Holme, E. (2009) Clinical manifestation and a new ISCU mutation in iron-sulphur cluster deficiency myopathy, *Brain 132*, 2170-2179.

39. Sheftel, A., Stehling, O., and Lill, R. (2010) Iron-sulfur proteins in health and disease, *Trends Endocrinol. Metab*.

40. Wingert, R. A., Galloway, J. L., Barut, B., Foott, H., Fraenkel, P., Axe, J. L., Weber, G. J., Dooley, K., Davidson, A. J., Schmidt, B., Paw, B. H., and Shaw, G. C. (2005)

Deficiency of glutaredoxin 5 reveals Fe-S clusters are required for vertebrate haem synthesis, *Nature 436*, 1035-1039.

41. Camaschella, C., Campanelle, A. F. L., Boschetto, L., Merlini, R., Silvestri, L., Levi, S., and Iolascon, A. (2007) The human counterpart of zebrafish *shiraz* shows sideroblastic-like microcytic anemia and iron overload., *Blood 110*, 1353-1358.

42. Maguire, A., Hellier, K., Hammans, S., and May, A. (2001) X-linked cerebellar ataxia and sideroblastic anaemia associated with a missense mutation in the ABC7 gene predicting V411L, *Br. J. Haematol. 115*, 910-917.

43. Bekri, S., Kispal, G., Lange, H., Fitzsimons, E., Tolmie, J., Lill, R., and Bishop, D. F. (2000) Human ABC7 transporter: gene structure and mutation causing X-linked sideroblastic anemia with ataxia with disruption of cytosolic iron-sulfur protein maturation, *Blood 96*, 3256-3264.

44. Allikmets, R., Raskind, W. H., Hutchinson, A., Schueck, N. D., Dean, M., and Koeller, D. M. (1999) Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A), *Hum. Mol. Genet. 8*, 743-749.

45. Nachin, L., El Hassouni, M., Loiseau, L., Expert, D., and Barras, F. (2001) SoxR-dependent response to oxidative stress and virulence of *Erwinia chrysanthemi*: the key role of SufC, an orphan ABC ATPase, *Mol. Microbiol. 39*, 960-972.

46. Calvo, S. E., Tucker, E. J., Compton, A. G., Kirby, D. M., Crawford, G., Burtt, N. P., Rivas, M., Guiducci, C., Bruno, D. L., Goldberger, O. A., Redman, M. C., Wiltshire, E., Wilson, C. J., Altshuler, D., Gabriel, S. B., Daly, M. J., Thorburn, D. R., and Mootha, V. K. (2010) High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency, *Nat. Genet. 42*, 851-858.

47. Bych, K., Kerscher, S., Netz, D. J., Pierik, A. J., Zwicker, K., Huynen, M. A., Lill, R., Brandt, U., and Balk, J. (2008) The iron-sulphur protein Ind1 is required for effective complex I assembly, *EMBO J. 27*, 1736-1746.

48. Sheftel, A. D., Stehling, O., Pierik, A. J., Netz, D. J., Kerscher, S., Elsasser, H. P., Wittig, I., Balk, J., Brandt, U., and Lill, R. (2009) Human ind1, an iron-sulfur cluster assembly factor for respiratory complex I, *Mol. Cell Biol. 29*, 6059-6073.

49. Cameron, J. M., Janer, A., Levandovskiy, V., Mackay, N., Rouault, T. A., Tong, W. H., Ogilvie, I., Shoubridge, E. A., and Robinson, B. H. (2011) Mutations in iron-sulfur cluster scaffold genes NFU1 and BOLA3 cause a fatal deficiency of multiple respiratory chain and 2-oxoacid dehydrogenase enzymes, *Am. J. Hum. Genet. 89*, 486-495.

50. Navarro-Sastre, A., Tort, F., Stehling, O., Uzarska, M. A., Arranz, J. A., Del, T. M., Labayru, M. T., Landa, J., Font, A., Garcia-Villoria, J., Merinero, B., Ugarte, M., Gutierrez-Solana, L. G., Campistol, J., Garcia-Cazorla, A., Vaquerizo, J., Riudor, E., Briones, P., Elpeleg, O., Ribes, A., and Lill, R. (2011) A fatal mitochondrial disease is associated with defective NFU1 function in the maturation of a subset of mitochondrial Fe-S proteins, *Am. J. Hum. Genet. 89*, 656-667.

51. Bandyopadhyay, S., Chandramouli, K., and Johnson, M. K. (2008) Iron-sulfur cluster biosynthesis, *Biochem. Soc. Trans. 36*, 1112-1119.

52. Zheng, L., Cash, V. L., Flint, D. H., and Dean, D. R. (1998) Assembly of iron-sulfur clusters. Identification of an *iscSUA-hscBA-fdx* gene cluster from *Azotobacter vinelandii*, *J. Biol. Chem. 273*, 13264-13272.

53. Gerber, J. and Lill, R. (2002) Biogenesis of iron-sulfur proteins in eukaryotes: components, mechanism and pathology, *Mitochondrion 2*, 71-86.

54. Lill, R. and Mühlenhoff, U. (2005) Iron-sulfur-protein biogenesis in eukaryotes, *Trends Biochem. Sci. 30*, 133-141.

55. Lill, R. and Mühlenhoff, U. (2006) Iron-sulfur protein biogenesis in eukaryotes: Components and mechanisms, *Annu. Rev. Cell Dev. Biol. 22*, 457-486.

56. Lill, R. and Kispal, G. (2000) Maturation of cellular Fe-S proteins: an essential function of mitochondria, *Trends Biochem. Sci. 25*, 352-356.

57. Zheng, L., White, R. H., Cash, V. L., Jack, R. F., and Dean, D. R. (1993) Cysteine desulfurase activity indicates a role for NIFS in metallocluster biosynthesis, *Proc. Natl. Acad. Sci. U. S. A. 90*, 2754-2758.

58. Agar, J. N., Zheng, L., Cash, V. L., Dean, D. R., and Johnson, M. K. (2000) Role of the IscU protein in iron-sulfur cluster biosynthesis: IscS-mediated assembly of a $[Fe_2S_2]$ cluster in IscU, *J. Am. Chem. Soc. 122*, 2136-2137.

59. Krebs, C., Agar, J. N., Smith, A. D., Frazzon, J., Dean, D. R., Huynh, B. H., and Johnson, M. K. (2001) IscA, an alternative scaffold for Fe-S cluster biosynthesis, *Biochemistry 40*, 14069-14080.

60. Ding, H. and Clark, R. J. (2004) Characterization of iron-binding in IscA, an ancient iron-sulfur cluster assembly protein, *Biochem. J. 379*, 433-440.

61. Lu, J., Bitoun, J. P., Tan, G., Wang, W., Min, W., and Ding, H. (2010) Iron-binding activity of human iron-sulfur cluster assembly protein hIscA1, *Biochem. J. 428*, 125-131.

62. Ding, H., Clark, R. J., and Ding, B. (2004) IscA mediates iron delivery for assembly of iron-sulfur clusters in IscU under limited "free" iron conditions, *J. Biol. Chem. 279*, 37499-37504.

63. Tan, G., Lu, J., Bitoun, J. P., Huang, H., and Ding, H. (2009) IscA/SufA paralogues are required for the [4Fe-4S] cluster assembly in enzymes of multiple physiological pathways in Escherichia coli under aerobic growth conditions, *Biochem. J. 420*, 463-472.

64. Ollagnier-de-Choudens, S., Sanakis, Y., and Fontecave, M. (2004) SufA/IscA: reactivity studies of a class of scaffold proteins involved with [Fe-S] cluster assembly, *J. Biol. Inorg. Chem. 9*, 828-838.

65. Ollagnier-de-Choudens, S., Mattioli, T., Takahashi, Y., and Fontecave, M. (2001) Iron-sulfur cluster assembly. Characterization of IscA and evidence for a specific functional complex with ferredoxin, *J. Biol. Chem. 276*, 22604-22607.

66. Riboldi, G. P., Verli, H., and Frazzon, J. (2009) Structural studies of the Enterococcus faecalis SufU [Fe-S] cluster protein, *BMC. Biochem. 10*, 3.

67. Agar, J. N., Krebs, B., Frazzon, J., Huynh, B. H., Dean, D. R., and Johnson, M. K. (2000) IscU as a scaffold for iron-sulfur cluster biosynthesis: Sequential assembly of [2Fe-2S] and [4Fe-4S] clusters in IscU, *Biochemistry 39*, 7856-7862.

68. Chandramouli, K., Unciuleac, M.-C., Naik, S., Dean, D. R., Huynh, B. H., and Johnson, M. K. (2007) Formation and properties of [4Fe-4S] clusters on the IscU scaffold protein, *Biochemistry 46*, 6804-6811.

69. Schwartz, C. J., Giel, J. L., Patschkowski, T., Luther, C., Ruzicka, F. J., Beinert, H., and Kiley, P. J. (2001) IscR, an Fe-S cluster-containing transcription factor, represses expression of *Escherichia coli* genes encoding Fe-S cluster assembly proteins, *Proc. Natl. Acad. Sci. U. S. A. 98*, 14895-14900.

70. Ayala-Castro, C., Saini, A., and Outten, F. W. (2008) Fe-S cluster assembly pathways in bacteria, *Microbiol. Mol. Biol. Rev. 72*, 110-125.

71. Cupp-Vickery, J. R., Peterson, J. C., Ta, D. T., and Vickery, L. E. (2004) Crystal structure of the molecular chaperone HscA substrate binding domain complexed with the IscU recognition peptide ELPPVKIHC, *J. Mol. Biol. 342*, 1265-1278.

72. Hoff, K. G., Silberg, J. J., and Vickery, L. E. (2000) Interaction of the iron-sulfur cluster assembly protein IscU with the Hsc66/Hsc20 molecular chaperone system of *Escherichia coli*, *Proc. Natl. Acad. Sci. U. S. A. 97*, 7790-7795.

73. Hoff, K. G., Ta, D. T., Tapley, T. L., Silberg, J. J., and Vickery, L. E. (2002) Hsc66 substrate specificity is directed toward a discrete region of the iron-sulfur cluster template protein IscU, *J. Biol. Chem. 277*, 27353-27359.

74. Hoff, K. G., Cupp-Vickery, J. R., and Vickery, L. E. (2003) Contributions of the LPPVK motif of the iron-sulfur template protein IscU to interactions with the Hsc66-Hsc20 chaperone system, *J. Biol. Chem. 278*, 37582-37589.

75. Mayer, M. P., Brehmer, D., Gassler, C. S., and Bukau, B. (2001) Hsp70 chaperone machines, *Adv. Prot. Chem. 59*, 1-44.

76. Silberg, J. J. and Vickery, L. E. (2000) Kinetic characterization of the ATPase cycle of the molecular chaperone Hsc66 from *Escherichia coli*, *J. Biol. Chem. 275*, 7779-7786.

77. Silberg, J. J., Tapley, T. L., Hoff, K. G., and Vickery, L. E. (2004) Regulation of the HscA ATPase reaction cycle by the co-chaperone HscB and the iron-sulfur cluster assembly protein IscU, *J. Biol. Chem. 279*, 53924-53931.

78. Chandramouli, K. and Johnson, M. K. (2006) HscA and HscB stimulate [2Fe-2S] cluster transfer from IscU to apoferredoxin in an ATP-dependent reaction, *Biochemistry 45*, 11087-11095.

79. Frazzon, J. and Dean, D. R. (2003) Formation of iron-sulfur clusters in bacteria: An emerging field in bioinorganic chemistry, *Curr. Opin. Chem. Biol. 7*, 166-173.

80. Olson, J. W., Agar, J. N., Johnson, M. K., and Maier, R. J. (2000) Characterization of the NifU and NifS Fe-S cluster formation proteins essential for the viability of *Helicobacter pylori*, *Biochemistry 39*, 16213-16219.

81. Smith, A. D., Jameson, G. N. L., Dos Santos, P. C., Agar, J. N., Naik, S., Krebs, C., Frazzon, J., Dean, D. R., Huynh, B. H., and Johnson, M. K. (2005) NifS-mediated assembly of [4Fe-4S] clusters in the N- and C-terminal domains of the NifU scaffold protein, *Biochemistry 44*, 12955-12969.

82. Yuvaniyama, P., Agar, J. N., Cash, V. L., Johnson, M. K., and Dean, D. R. (2000) NifS-directed assembly of a transient [2Fe-2S] cluster within the NifU protein, *Proc. Natl. Acad. Sci. U. S. A. 97*, 599-604.

83. Jacobson, M. R., Cash, V. L., Weiss, M. C., Laird, N. F., Newton, W. E., and Dean, D. R. (1989) Biochemical and genetic analysis of the *nifUSVWZM* cluster from *Azotobacter vinelandii*, *Mol. Gen. Genet. 219*, 49-57.

84. Takahashi, Y. and Tokumoto, U. (2002) A third bacterial system for the assembly of iron-sulfur clusters with homologs in archaea and plastids, *J. Biol. Chem. 277*, 28380-28383.

85. Balk, J. and Lobréaux, S. (2005) Biogenesis of iron-sulfur proteins in plants, *Trends Plant Sci. 10*, 324-331.

86. Loiseau, L., Ollagnier-de-Choudens, S., Nachin, L., Fontecave, M., and Barras, F. (2003) Biogenesis of Fe-S cluster by the bacterial Suf system. SufS and SufE form a new type of cysteine desulfurase, *J. Biol. Chem. 278*, 38352-38359.

87. Ollagnier-de-Choudens, S., Lascoux, D., Loiseau, L., Barras, F., Forest, E., and Fontecave, M. (2003) Mechanistic studies of the SufS-SufE cysteine desulfurase: evidence for S transfer from SufS to SufE, *FEBS Lett. 555*, 263-267.

88. Goldsmith-Fischman, S., Kuzin, A., Edstrom, W. C., Benach, J., Shastry, R., Xiao, R., Acton, T. B., Honig, B., Montelione, G. T., and Hunt, J. F. (2004) The SufE sulfur-acceptor protein contains a conserved core structure that mediates interdomain interactions in a variety of redox protein complexes, *J. Mol. Biol. 344*, 549-565.

89. Outten, F. W., Wood, M. J., Muñoz, F. M., and Storz, G. (2003) The SufE protein and the SufBCD complex enhance SufS cysteine desulfurase activity as part of a sulfur transfer pathway for Fe-S cluster assembly in *E. coli*, *J. Biol. Chem. 278*, 45713-45719.

90. Chahal, H. K., Dai, Y., Saini, A., Ayala-Castro, C., and Outten, F. W. (2009) The SufBCD Fe-S scaffold complex interacts with SufA for Fe-S cluster transfer, *Biochemistry 48*, 10644-10653.

91. Wollers, S., Layer, G., Garcia-Serres, R., Signor, L., Clemancey, M., Latour, J. M., Fontecave, M., and Ollagnier de Choudens, S. (2010) Iron-sulfur (Fe-S) cluster assembly, *J. Biol. Chem. 285*, 23331-23341.

92. Saini, A., Mapolelo, D. T., Chahal, H. K., Johnson, M. K., and Outten, F. W. (2010) SufD and SufC ATPase activity are required for iron acquisition during in vivo Fe-S cluster formation on SufB, *B 49*, 9402-9412.

93. Gupta, V., Sendra, M., Naik, S. G., Chahal, H. K., Huynh, B. H., Outten, F. W., Fontecave, M., and Ollagnier de Choudens, S. (2009) Native *Escherichia coli* SufA, coexpressed with SufBCDSE, purifies as a [2Fe-2S] protein and acts as an Fe-S transporter to Fe-S target enzymes, *J. Am. Chem. Soc. 131*, 6149-6153.

94. Layer, G., Gaddam, S. A., Ayala-Castro, C. N., Ollagnier-de-Choudens, S., Lascoux, D., Fontecave, M., and Outten, F. W. (2007) SufE transfers sulfur from SufS to SufB for iron-sulfur cluster assembly, *J. Biol. Chem. 282*, 13342-13350.

95. Wada, K., Sumi, N., Nagai, R., Iwasaki, K., Sato, T., Suzuki, K., Hasegawa, Y., Kitaoka, S., Minami, Y., Outten, F. W., Takahashi, Y., and Fukuyama, K. (2009) Molecular dynamism of Fe-S cluster biosynthesis implicated by the structure of the SufC(2)-SufD(2) complex, *J. Mol. Biol. 387*, 245-258.

96. Shen, G., Balasubramanian, R., Wang, T., Wu, Y., Hoffart, L. M., Krebs, C., Bryant, D. A., and Golbeck, J. H. (2007) SufR coordinates two $[4Fe\text{-}4S]^{2+,1+}$ clusters and functions as a transcriptional repressor of the *sufBCDS* operon and an autoregulator of *sufR* in cyanobacteria, *J. Biol. Chem. 282*, 31909-31919.

97. Loiseau, L., Gerez, C., Bekker, M., Ollagnier-de-Choudens, S., Py, B., Sanakis, Y., Teixeira, M., Fontecave, M., and Barras, F. (2007) ErpA, an iron-sulfur (Fe-S) protein of the A-type essential for respiratory metabolism in *Escherichia coli*, *Proc. Natl. Acad. Sci. U. S. A. 104*, 13626-13631.

98. Vinella, D., Brochier-Armanet, C., Loiseau, L., Talla, E., and Barras, F. (2009) Iron-sulfur (Fe/S) protein biogenesis: phylogenomic and genetic studies of A-type carriers, *PLoS. Genet. 5*, e1000497.

99. Johnson, D. C., Unciuleac, M.-C., and Dean, D. R. (2006) Controlled expression and functional analysis of iron-sulfur cluster biosynthetic components within *Azotobacter vinelandii*, *J. Bacteriol. 188*, 7551-7561.

100. Morimoto, K., Yamashita, E., Kondou, Y., Lee, S. J., Arisaka, F., Tsukihara, T., and Nakai, M. (2006) The asymmetric IscA homodimer with an exposed [2Fe-2S] cluster suggests the structural basis of the Fe-S cluster biosynthetic scaffold, *J. Mol. Biol. 360*, 117-132.

101. Lu, J., Yang, J., Tan, G., and Ding, H. (2008) Complementary roles of SufA and IscA in the biogenesis of iron-sulfur clusters in *Escherichia coli*, *Biochem. J. 409*, 535-543.

102. Ding, H., Harrison, K., and Lu, J. (2005) Thioredoxin reductase system mediates iron binding in IscA and iron-delivery for the iron-sulfur cluster assembly in IscU, *J. Biol. Chem. 280*, 30432-30437.

103. Mapolelo, D. T., Zhang, B., Naik, S. G., Huynh, B. H., and Johnson, M. K. (2012) Spectroscopic and Functional Characterization of Iron-Bound Forms of Azotobacter vinelandii NifIscA, *Biochemistry 51*, 8056-8079.

104. Mühlenhoff, U., Richter, N., Pines, O., Pierik, A. J., and Lill, R. (2011) Specialized function of yeast Isa1 and Isa2 in the maturation of mitochondrial [4Fe-4S] proteins, *J. Biol. Chem. 286*, 41205-41216.

105. Gelling, C., Dawes, I. W., Richhardt, N., Lill, R., and Muhlenhoff, U. (2008) Mitochondrial Iba57p is required for Fe/S cluster formation on aconitase and activation of radical SAM enzymes, *Mol. Cell. Biol. 28*, 1851-1861.

106. Waller, J. C., Ellens, K. W., Hasnain, G., Alvarez, S., Rocca, J. R., and Hanson, A. D. (2012) Evidence that the folate-dependent proteins YgfZ and MnmEG have opposing effects on growth and on activity of the iron-sulfur enzyme MiaB, *J. Bacteriol. 194*, 362-367.

107. Wu, G., Mansy, S. S., Hemann, C., Hille, R., Surerus, K. K., and Cowan, J. A. (2002) Iron-sulfur cluster biosynthesis: Characterization of *Schizosaccharomyces pombe* Isa1, *J. Biol. Inorg. Chem. 7*, 526-532.

108. Wollenberg, M., Berndt, C., Bill, E., Schwenn, J. D., and Seidler, A. (2003) A dimer of the FeS cluster biosynthesis protein IscA from cyanobacteria binds a [2Fe2S] cluster between two protomers and transfers it to [2Fe2S] and [4Fe4S] apo proteins, *Eur. J. Biochem. 270*, 1662-1671.

109. Mapolelo, D. T., Zhang, B., Naik, S. G., Huynh, B. H., and Johnson, M. K. (2012) Spectroscopic and Functional Characterization of Iron-Sulfur Cluster-Bound Forms of Azotobacter vinelandii NifIscA, *Biochemistry 51, 8071-8084*.

110. Rouhier, N., Couturier, J., Johnson, M. K., and Jacquot, J. P. (2010) Glutaredoxins: roles in iron homeostasis, *Trends Biochem. Sci. 35*, 43-52.

111. Fernandes, A. P. and Holmgren, A. (2004) Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system, *Antioxid. Redox. Signal. 6*, 63-74.

112. Herrero, E. and de la Torre-Ruiz, M. A. (2007) Monothiol glutaredoxins: a common domain for multiple functions, *Cell. Mol. Life Sci. 64*, 1518-1530.

113. Rouhier, N., Lemaire, S. D., and Jacquot, J. P. (2008) The role of glutathione in photosynthetic organisms: emerging functions for glutaredoxins and glutathionylation, *Annu. Rev. Plant Biol. 59*, 143-166.

114. Lillig, C. H., Berndt, C., and Holmgren, A. (2008) Glutaredoxin systems, *Biochim. Biophys. Acta 1780*, 1304-1317.

115. Rouhier, N., Couturier, J., Johnson, M. K., and Jacquot, J. P. (2010) Glutaredoxins: roles in iron homeostasis, *Trends Biochem. Sci. 35*, 43-52.

116. Couturier, J., Jacquot, J. P., and Rouhier, N. (2009) Evolution and diversity of glutaredoxins in photosynthetic organisms, *Cell Mol. Life Sci. 66*, 2539-2557.

117. Rouhier, N., Couturier, J., and Jacquot, J. P. (2006) Genome-wide analysis of plant glutaredoxin systems, *J. Exp. Bot. 57*, 1685-1696.

118. Rodríguez-Manzaneque, M. T., Tamarit, J., Bellí, G., Ros, J., and Herrero, E. (2002) Grx5 is a mitochondrial glutaredoxin required for the activity of iron/sulfur enzymes, *Mol. Biol. Cell 13*, 1109-1121.

119. Rodríguez-Manzaneque, M. T., Ros, J., Cabrito, I., Sorribas, A., and Herrero, E. (1999) Grx5 glutaredoxin plays a central role in protection against protein oxidative damage in *Saccharomyces cerevisiae*, *Mol. Cell. Biol. 19*, 8180-8190.

120. Mühlenhoff, U., Gerber, J., Richhardt, N., and Lill, R. (2003) Components involved in assembly and dislocation of iron-sulfur clusters on the scaffold protein Iscu1p, *EMBO J. 22*, 4815-4825.

121. Alves, R., Herrero, E., and Sorribas, A. (2004) Predictive reconstruction of the mitochondrial iron-sulfur cluster assembly metabolism. II. Role of glutaredoxin Grx5, *Proteins:Struct. Func. Bioinform. 57*, 481-492.

122. Vilella, F., Alves, R., Rodriguez-Manzaneque, M. T., Belli, G., Swaminathan, S., Sunnerhagen, P., and Herrero, E. (2004) Evolution and cellular function of monothiol glutaredoxins: involvement in iron-sulphur cluster assembly, *Comp Funct. Genomics 5*, 328-341.

123. Kim, K. D., Chung, W. H., Kim, H. J., Lee, K. C., and Roe, J. H. (2010) Monothiol glutaredoxin Grx5 interacts with Fe-S scaffold proteins Isa1 and Isa2 and supports Fe-S assembly and DNA integrity in mitochondria of fission yeast, *Biochem. Biophys. Res. Commun. 392*, 467-472.

124. Li, H. and Outten, C. E. (2012) Monothiol CGFS Glutaredoxins and BolA-like Proteins: [2Fe-2S] Binding Partners in Iron Homeostasis, *Biochemistry 51*, 4377-4389.

125. Li, H., Mapolelo, D. T., Dingra, N. N., Naik, S. G., Lees, N. S., Hoffman, B. M., Riggs-Gelasco, P. J., Huynh, B. H., Johnson, M. K., and Outten, C. E. (2009) The yeast iron regulatory proteins Grx3/4 and Fra2 form heterodimeric complexes containing a [2Fe-2S] cluster with cysteinyl and histidyl ligation, *Biochemistry 48*, 9569-9581.

126. Li, H., Mapolelo, D. T., Dingra, N. N., Keller, G., Riggs-Gelasco, P. J., Winge, D. R., Johnson, M. K., and Outten, C. E. (2011) Histidine 103 in Fra2 is an iron-sulfur cluster ligand in the [2Fe-2S] Fra2-Grx3 complex and is required for in vivo iron signaling in yeast, *J. Biol. Chem. 286*, 867-876.

127. Li, H., Mapolelo, D. T., Randeniya, S., Johnson, M. K., and Outten, C. E. (2012) Human glutaredoxin 3 forms [2Fe-2S]-bridged complexes with human BolA2, *Biochemistry 51*, 1687-1696.

128. Picciocchi, A., Saguez, C., Boussac, A., Cassier-Chauvat, C., and Chauvat, F. (2007) CGFS-type monothiol glutaredoxins from the cyanobacterium *Synechocystis* PCC6803 and other evolutionary distant model organisms possess a glutathione-ligated [2Fe-2S] cluster, *Biochemistry 46*, 15018-15026.

129. Bandyopadhyay, S., Gama, F., Molina-Navarro, M. M., Gualberto, J. M., Claxton, R., Naik, S. G., Huynh, B. H., Herrero, E., Jacquot, J.-P., Johnson, M. K., and Rouhier, N. (2008) Chloroplast monothiol glutaredoxins as scaffold proteins for the assembly and delivery of [2Fe-2S] clusters, *EMBO J. 27*, 1122-1133.

130. Li, H., Mapolelo, D. T., Dingra, N. N., Naik, S. G., Lees, N. S., Hoffman, B. M., Riggs-Gelasco, P. J., Huynh, B. H., Johnson, M. K., and Outten, C. E. (2009) The yeast iron regulatory proteins Grx3/4 and Fra2 form heterodimeric complexes containing a [2Fe-2S] cluster with cysteinyl and histidyl ligation, *Biochemistry 48*, 9569-9581.

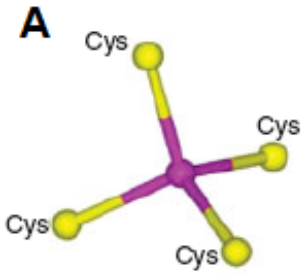
131. Iwema, T., Picciocchi, A., Traore, D. A., Ferrer, J. L., Chauvat, F., and Jacquamet, L. (2009) Structural basis for delivery of the intact [Fe_2S_2] cluster by monothiol glutaredoxin, *Biochemistry 48*, 6041-6043.

132. Johansson, C., Kavanagh, K. L., Gileadi, O., and Oppermann U. (2007) Reversible sequestration of active site cysteines in a 2Fe-2S-bridged dimer provides a mechanism for glutaredoxin 2 regulation in human mitochondria, *J. Biol. Chem. 282*, 3077-3082.

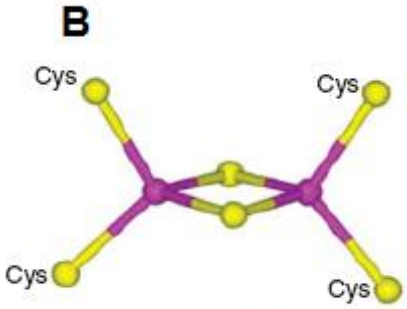
133. Rouhier, N., Unno, H., Bandyopadhyay, S., Masip, L., Kim, S. K., Hirasawa, M., Gualberto, J. M., Lattard, V., Kusunoki, M., Knaff, D. B., Georgiou, G., Hase, T., Johnson, M. K., and Jacquot, J. P. (2007) Functional, structural, and spectroscopic characterization of a glutathione-ligated [2Fe-2S] cluster in poplar glutaredoxin C1, *Proc. Natl. Acad. Sci. U. S. A. 104*, 7379-7384.

134. Shakamuri, P., Zhang, B., and Johnson, M. K. (2012) Monothiol Glutaredoxins Function in Storing and Transporting [Fe_2S_2] Clusters Assembled on IscU Scaffold Proteins, *J. Am. Chem. Soc. 134*, 15213-15216.

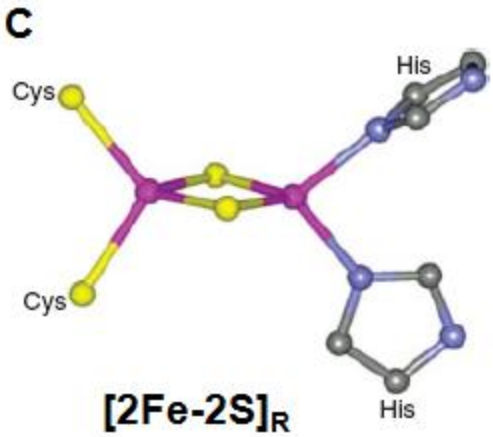
Figure 1.1: Structures of Fe-S centers as determined by X-ray crystallography. Structures are taken from the coordinates deposited in the Protein Data Bank: A. Fe Rd, PDB ID# 18RXN, rubredoxin from *Desulfovibrio vulgaris*; B. [2Fe-2S], PDB ID# 1FRD, *Anabaena* pcc 7120 Fd; C. $[2Fe\text{-}2S]_R$, PDB ID# 1JM1, *Sulfolobus acidocaldaius* Rieske protein; D. [3Fe-4S], PDB ID# 6FDR, *Azotobacter vinelandii* FdI; E. [4Fe-4S], PDB ID# 6FDR, *Azotobacter vinelandii* FdI. Color code: purple, Fe; yellow, S; grey, C; violet, N. Adapted from reference 5.



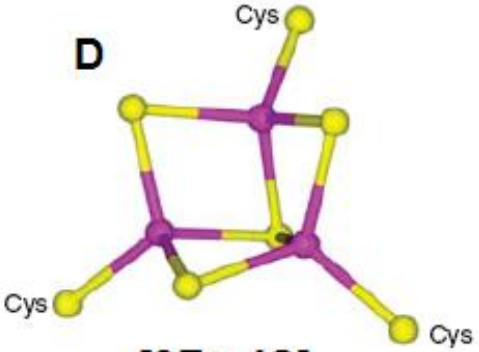
Fe Rd



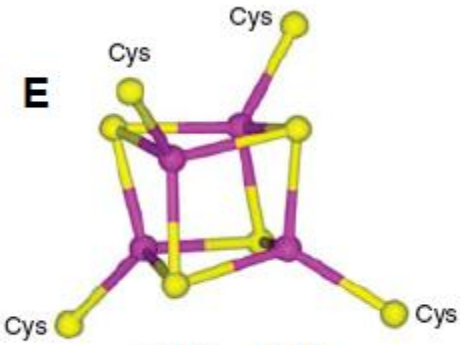
[2Fe-2S]



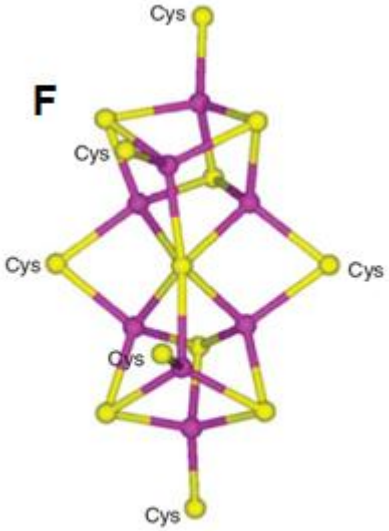
$[2Fe\text{-}2S]_R$



[3Fe-4S]

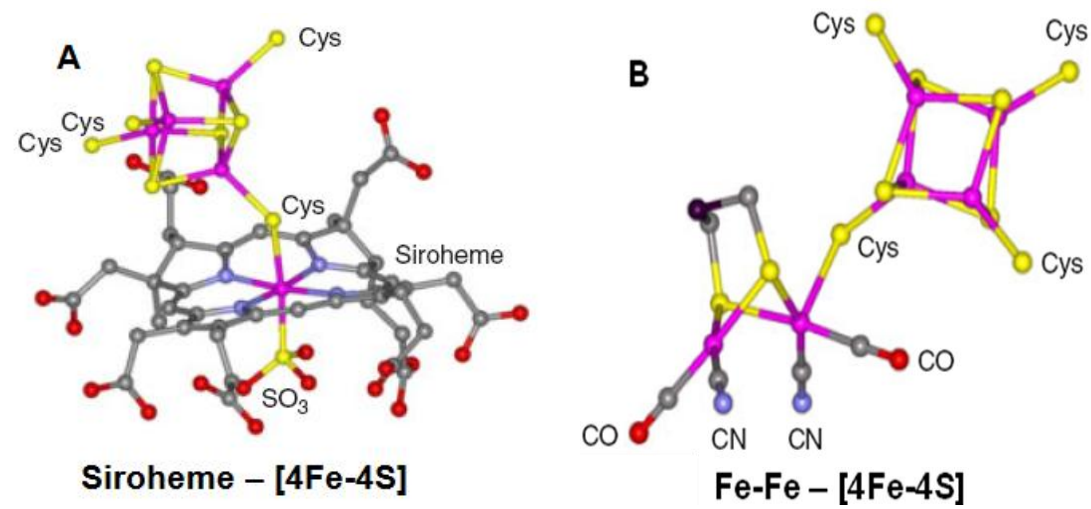


[4Fe-4S]



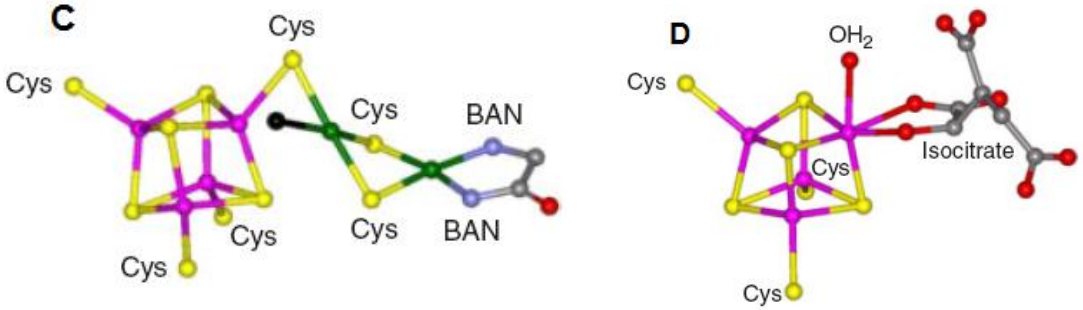
$[8Fe\text{-}7S]^N$

Figure 1.2: Structures of Fe-S centers primarily involved in substrate binding and activation, as determined by X-ray crystallography. Structures are taken from coordinates deposited in the Protein Data Bank: (A) siroheme-[4Fe-4S], sulfite-bound form of sulfite reductase from *E. coli*; (B) Fe-Fe-[4Fe-4S], Fe-hydrogenase from *Desulfovibrio vulgaris*, PDB ID# 1HFE;PDB ID # 2GEP; (C) Ni-Ni-[4Fe-4S], acetyl-CoA synthase from *Carboxydothermus hydrogenoformans*, PDB ID# 1RU3; (D) [4Fe-4S] -isocitrate, isocitrate-bound form of porcine heart aconitase, PDB ID#7ACN; (E) [4Fe-4S]-SAM, SAM-bound form of HemN from *E. coli*, PDB ID# 1OLT. Color code: magenta, Fe; yellow S; gray, C; red, O; green, Ni; black, unknown low Z atom (O/C/N). Unlabeled S atoms correspond to bridging sulfides and BAN indicates backbone amide N. Adapted from reference 5.



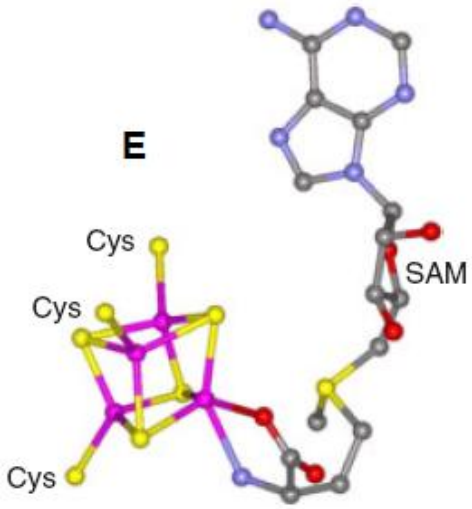
Siroheme – [4Fe-4S]

Fe-Fe – [4Fe-4S]



Ni-Ni – [4Fe-4S]

[4Fe-4S] + isocitrate



[4Fe-4S] + SAM

Figure 1.3: Schematic representation of the three distinct Fe-S cluster biogenesis operons in prokaryotic organisms. Genes producing homologous protein products with the same function have the same color. Adapted from reference 33

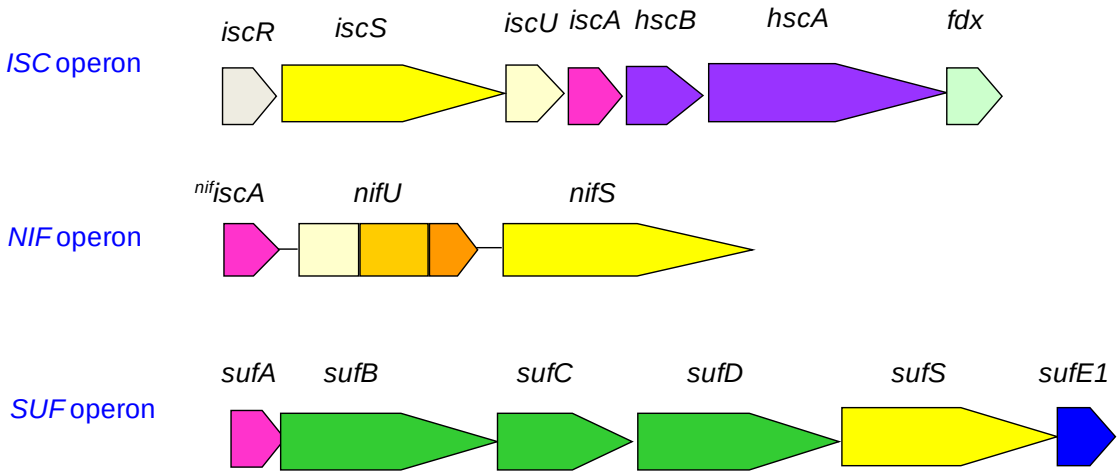
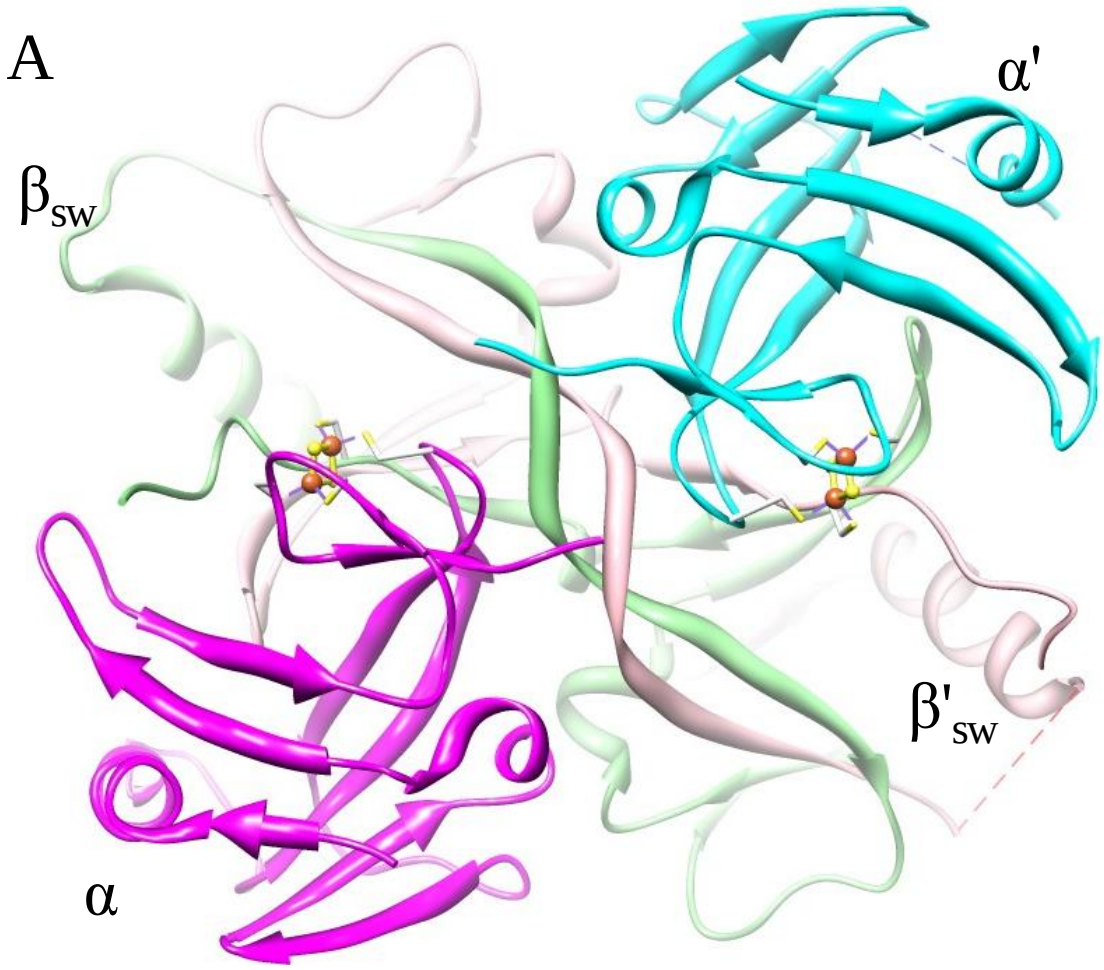

ISC operon
iscR
iscS
iscU
iscA
hscB
hscA
fdx
NIF operon
nifiscA
nifU
nifS
SUF operon
sufA
sufB
sufC
sufD
sufS
sufE1

Figure 1.4: Primary sequences alignment of selected A-type proteins. Te, *Thermosynechococcus elongatus*; At, *Arabadopsis thaliana*; Ec, *Escherichia coli*; Av, *Azotobacter vinelandii*. The conserved cysteine residues proposed to be critical for the roles of A-type proteins in Fe-S cluster biogenesis are shown in red. Other highly conserved residues are shown in blue.


Te IscA 1 MVELTPAAIQELERLQTHGVRRGQAAILRIQV 32
Av IscA 1 MAVTMTEAAARHIRRSLDGRGKGEGIRLGV 30
Ec IscA 1 MSITLSDSAAARVNTFLANRGKGF-GLRLGV 30
At SufA 1 MEGLKPAISLSENALKHLSKMRSERGE-DLCLRIGV 35
Av NifIscA 1 MITLTESAKSAVTRFISSTGKPIAGLRIRV 30
C1
Te IscA 33 QPSECGDWRYDLALVAEPKPTDLLTQSQGWTIAIAAEAAELLRGLR 78
Av IscA 31 RTSGCSGLAYVLEFVDEVASEDQVFESHGVKVIVDPKSLVYLDGTE 76
Ec IscA 31 RTSGCSGMAYVLEFVDEPTPEDIVFEDKGVKVVVDGKSLQFLDGTQ 76
At SufA 36 KQGGCSGMSYTMDFENRANARPDDSTIEYQGFTIVCDPKSMLFLFG 81
Av NifIscA 31 EGGGCSGLKYSLKLEEAGAEDDQLVDCDGITLLIDSASAPLLDGVT 76
C2 C3
Te IscA 79 --VDYIEDLMGGAFRFHNPNASQTCGCGMAFRVSRS 112
Av IscA 77 --LDFVREGLNEGFKFNNPNVRGECGCGESFNI 107
Ec IscA 77 --LDFVKEGLNEGFKFTNPNVKDECGCGESFHV 107
At SufA 82 KQLDYSDALIGGGFSFSNPNATQTCGCGKSFAAEM 116
Av NifIscA 77 --MDFVESMEGSGFTFVNPNATNSCGCGKSFAC 107

Figure 1.5: Crystal structure of [2Fe-2S] cluster-bound *Thermosynechococcus elongatus* IscA, at 2.5 Å resolution, PDB ID# 1X0G. A. Ribbon view of the domain-swapped $\alpha\beta_{sw}$ $\alpha'\beta'_{sw}$ tetramer as crystallized. B. Coordination environment of the [2Fe-2S] cluster illustrating the asymmetric ligation. The cluster is coordinated by three cysteine residues from α protomer and one cysteine residue from β protomer. Each protomer has a different color and the S of the Cys residues are shown in yellow. The Fe and bridging sulfides of the [2Fe-2S] clusters are shown as orange and yellow balls, respectively. Adapted from reference 100.


A
α'
β$_{sw}$
β'$_{sw}$
α

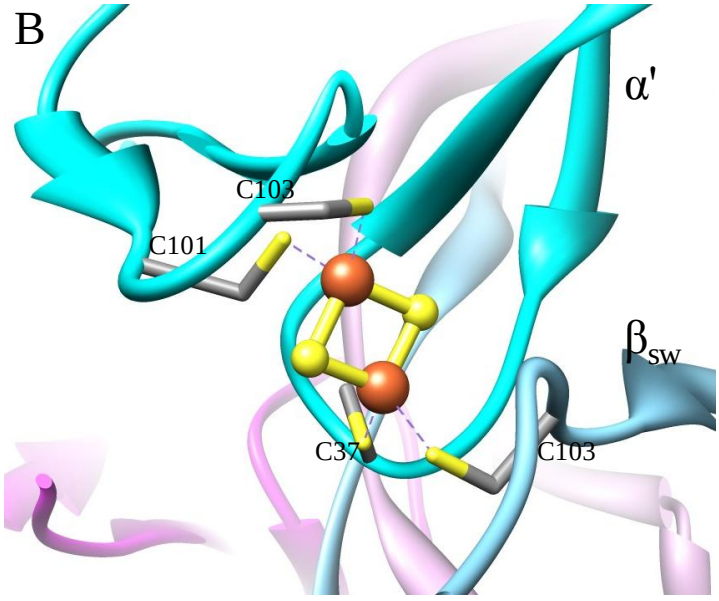

B
α'
C103
C101
β$_{sw}$
C37
C103

Figure 1.6: Schematic representation of classification and domain organization of class II glutaredoxins (Grxs) in different photosynthetic organisms (terrestrial plants, algae, cyanobacteria) and other organisms such as Ec, *Escherichia coli*; Hs, *Homo sapiens*; Sc, *Saccharomyces cerevisiae*. Adapted from reference 117.

Class II

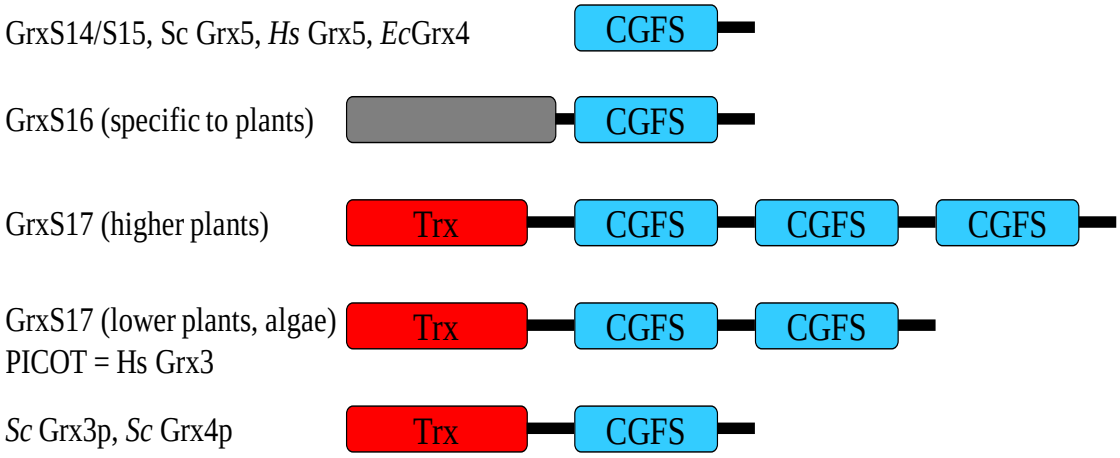
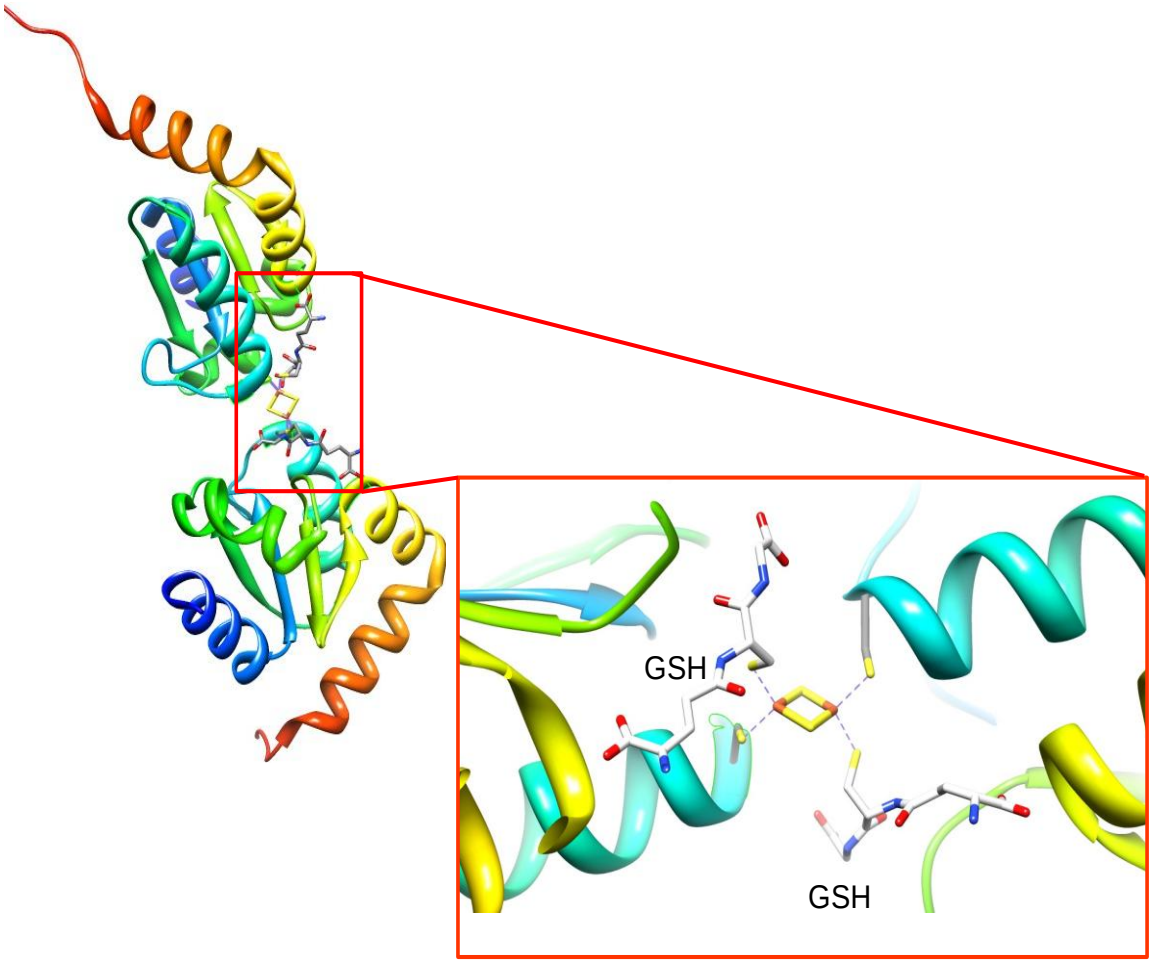

GrxS14/S15, Sc Grx5, *Hs* Grx5, *Ec*Grx4
CGFS
GrxS16 (specific to plants)
CGFS
GrxS17 (higher plants)
Trx
CGFS
CGFS
CGFS
GrxS17 (lower plants, algae)
PICOT = Hs Grx3
Trx
CGFS
CGFS
Sc Grx3p, *Sc* Grx4p
Trx
CGFS

Figure 1.7: Crystal structure of monothiol Grx taken from Protein Data Bank. (A) *E. coli* monothiol Grx4. PDB ID# 2WCI. (B) Coordination environment of the [2Fe-2S] cluster illustrating the complete cysteinyl ligation. Structure is colored by protomer. The active site cysteine residues, the [2Fe-2S] cluster and the GSH are shown in sticks using the following color code: Orange, Fe; yellow, S; grey, C; blue, N; red, O. Adapted from the reference 129.


GSH
GSH

CHAPTER 2

POTENTIAL ROLES OF A-TYPE PROTEINS AS IRON DONORS AND IRON-SULFUR CLUSTER CARRIERS IN Fe-S CLUSTER BIOSYNTHESIS

Introduction

Iron-sulfur (Fe-S) clusters are ubiquitous protein cofactors involving iron and bridging acid-labile sulfide (*1-3*). However, they are very versatile in their functions and required to maintain fundamental life processes such as photosynthesis, respiration, nitrogen fixation, DNA repair and various metabolic pathways (*2;4-8*). While the initial work conducted on Fe-S cluster biosynthesis mainly focused on bacterial, yeast, and mammalian systems, information concerning the biosynthesis of Fe-S clusters in plants has recently emerged (*7;9;10*). Plant cells contain both mitochondria and chloroplasts, which have separate Fe-S cluster biosynthesis machineries (*7;11*). Operons containing highly conserved genes whose gene products are involved in Fe-S cluster biogenesis were originally identified in prokaryotes and this led to the discovery of homologous proteins in eukaryotic chloroplasts and mitochondria (*7;12-14*). Hence Fe-S cluster biogenesis is a highly conserved process throughout biology (*12*).

Among these Fe-S biosynthesis systems, the *nif* system which is dedicated to nitrogenase maturation in azototrophic bacteria was the first to be discovered (*12*). Subsequently more general purpose Fe-S cluster assembly systems, the *isc* and *suf* systems, were discovered (*12;15;16*). In many bacteria, the *isc* system is known as the house keeping Fe-S cluster assembly machinery and is utilized under normal growth

conditions (*17*), whereas the *suf* system operates as a backup system which utilize under iron limitation and oxidative stress conditions (*18-20*). The *isc* system constitutes the heart of the Fe-S cluster biogenesis system found in the eukaryote mitochondrial system (*13;21-24*), whereas the *suf* system constitutes the heart of the Fe-S cluster biogenesis system found in many archaea as well as cyanobacteria and plant chloroplasts, an O_2 producing organelle (*7;13*).

The Fe-S cluster assembly process carried out by these systems can be separated into two stages, first, *de novo* assembly of the cluster on a primary scaffold protein and second, the insertion of preformed clusters into target apo-proteins. During the first stage, sulfur is mobilized from cysteine by a pyridoxal-dependent cysteine desufurase, i.e., NifS, IscS, SufS, to the primary scaffold protein, i.e., IscU, NifU, SufBCD, where it combines with iron to form an Fe-S cluster. The nature of the immediate iron donor for Fe-S cluster assembly remains unclear in spite of several *in vivo* and *in vitro* studies, but eukaryotic frataxin and the bacterial homologue, CyaY, have been implicated as the iron donors for the i*sc* system (*13;25;26*). The second stage of Fe-S cluster assembly, transfer of the cluster to a target apo-protein requires molecular chaperones and ATP to facilitate cluster release from IscU, as well as the use of specific carrier proteins. A-type proteins (e.g. IscA, ^{Nif}IscA and SufA), Nfu (related to NifU) and monothiol glutaredoxins (Grxs) have been proposed as possible carrier proteins (*27-32*).

A-type proteins (IscA, SufA, ^{Nif}IscA, ErpA, Isa1, and Isa2) are a class of small of proteins with about 110 amino acids residues and three highly conserved cysteine residues in the sequence motif Cys-X_n-Cys-X-Cys (n = 60-80), which was shown to be critical for the function of these proteins (*33;34*). They have been proposed to function as

alternate scaffold proteins (*11;33;35-37*), Fe-carriers (*38-40*), or cluster carriers that mediate the transfer of preformed clusters from primary scaffold proteins to acceptor proteins (*27;35;36*). Even though these proteins are highly conserved from bacteria to yeast (*33*), plants (*11*) and humans (*37*), their precise role in Fe-S cluster assembly remains unclear, in part due to the functional redundancy exhibited by A-type proteins. Studies have shown that mutation of IscA or SufA in *Escherichia coli* has only mild effects on cell growth while mutation of both IscA and SufA results in a null growth phenotype under aerobic conditions (*41*). Furthermore, the *E. coli iscA/sufA* double mutant was shown to affect the assembly of [4Fe-4S] clusters in dihydroxyacid dehydratase (involved in branched chain amino acid biosynthesis), ThiC of the thiamine biosynthesis pathway, aconitase B of the citric acid cycle, and endonuclease III of the DNA base excision repair pathway under aerobic growth conditions (*42*). However, this double mutant had little or no effect on the [2Fe-2S] clusters of the redox transcriptional factor SoxR, the siderophore-iron reductase FhuF, and ferredoxin. Furthermore, under anaerobic growth conditions, [4Fe-4S] and [2Fe-2S] cluster assembly in *E. coli* were not affected in the *iscA/sufA* double mutant.

Recent studies on *Azotobacter vinelandii* (*Av*) ^{Nif}IscA illustrate that these A-type proteins are able to bind mononuclear $Fe^{2+,3+}$ (*43*), as well as accept clusters formed on primary scaffold proteins and reversibly convert between [2Fe-2S] and [4Fe-4S] cluster-bound forms in response to cellular redox status and/or oxygen levels (*44*). In addition *in vivo* studies on *S. cerevisiae* reveal that the Fe-bound Isa1/Isa2/Iba57 complex constitutes a late-acting system that is specifically dedicated to the maturation of mitochondrial [4Fe-4S] proteins (*45*). These studies indicate that these A-type proteins are likely to be

involved in the maturation or repair of [4Fe-4S] clusters in mitochondrial proteins (*45-47*) and in bacterial proteins under aerobic growth or oxidative stress conditions (*42;48*).

The objective of this work is to extend these studies to the chloroplastic SufA1 from *Arabidopsis thaliana* (*At*) and *Av* IscA, in order to investigate the possible role(s) of A-type proteins. This work focuses on characterizing Fe(III)-bound forms of *At* SufA1 and *Av* IscA as well as the [2Fe-2S] cluster-bound form of *At* SufA1. Furthermore, evidence is presented for rapid, intact and quantitative cluster transfer from the [2Fe-2S] cluster-bound form of *At* GrxS14, a choroplastic monothiol glutaredoxin with a CGFS active site (CGFS Grx), to *At* SufA1 using UV-visible CD spectroscopy. CGFS Grxs are proposed to function in the storage or transfer of Fe-S clusters and are widely present in all organisms (*28;49-52*). In yeast (*Saccharomyces cerevisiae*), studies on CGFS Grx knockouts have suggested a role for these proteins in Fe-S cluster biogenesis (*53;54*). The *grx5* gene mutation is accompanied by iron accumulation on Isu1(eukaryotic homologue of IscU), implying a role for Grx5 in facilitating the transfer of pre-assembled clusters on the U-type scaffold protein to apo-acceptor proteins (*29;30*). This evidence were further supported by more recent *in vitro* studies, which demonstrate rapid, unidirectional and quantitative [2Fe-2S] cluster transfer from *Av* IscU to *Av* Grx5 only in the presence of the dedicated HscA/HscB co-chaperone system and Mg-ATP (*55*). Moreover, the physical interactions observed in CGFS Grx5 with Isa1(eukaryotic homologue of IscA) in *S. cerevisiae* (*50*) as well as with both Isa1 and Isa2 in *S. pombe* (*56*) indicate that this CGFS Grxs and A-type proteins might act together in Fe-S cluster biogenesis. The results presented in this work, coupled with the *in vivo* evidence for interaction between CGFS

Grxs and A-type proteins (*50;56*), indicate that these two classes of proteins work together in cellular Fe-S cluster trafficking.

Materials and Methods

Materials: Materials used in this work were of reagent grade and purchased from Fisher Scientific, Sigma-Aldrich Chemical Co, Invitrogen, or VWR International, unless otherwise stated.

Expression of A-type Proteins: A plasmid containing *At* SufA1 and *Av* IscA was transformed into *E.coli* BL21 (DE3) and protein expression was induced according to the following procedure. The *At* SufA1 and *Av* IscA cells were grown in LB media at 37 °C containing 1 mg/mL ampicillin until OD_{600} reached 0.6 - 0.8. Then protein expression was induced by the addition of isopropyl 1-thio-β-D-galactopyranoside (IPTG) to a final concentration of 0.8 mM. The cells were allowed to grow for 5 additional hours at 35 °C before harvesting by centrifugation. All the cells were stored at -80 °C.

Purification of At SufA1 and *Av IscA Proteins:* The overexpressed protein was purified aerobically. During aerobic purification, cells (~15 g) were resuspended in 100 mM Tris-HCl, 2 mM DTT, pH 7.8 (buffer A) with 10 μg/mL phenylmethyl sulphonyl fluoride (PMSF), 15 μg/mL DNase, and 5×10^{-3} μg/mL RNase. The cells were lysed by sonication on ice and the cell debris were removed by the centrifugation at 17000 rpm for 1 hour at 4 °C. Soluble proteins were then precipitated with 40% of ammonium sulfate saturation and centrifuged as above. The resulting pellet was resuspended in buffer A and loaded onto a 25 mL Q-Sepharose anion exchange column, equilibrated with the buffer A. Elution of the protein of interest was achieved with a 0-1 M NaCl gradient in 100 mM Tris-HCl, 2 mM DTT pH 7.8. Furthermore, based on the SDS-PAGE (sodium dodecyl

sulfate polyacrylamide gel electrophoresis) analysis the fractions containing the protein of interest were collected and, concentrated down to 3 mL using a YM-10 Amicon and loaded onto a Superdex-75 size exclusion column, which was equilibrated with 100 mM Tris-HCl, 150 mM NaCl, pH 7.8. The purity of this fraction was estimated to be 95% by SDS-PAGE analysis. Expression and purification of *At* GrxS14, a CGFS Grx in *A. thaliana* chloroplasts, was carried out as previously described (*28*).

Chemical Analyses: Concentrations of all the proteins were determined using the Bio-Rad DC protein assay with bovine serum albumin as a standard (Roche) according to the microscale modified procedure of Brown *et al.* (*57*). All concentrations are based on protein monomer unless otherwise stated. Iron concentrations were determined after $KMnO_4$/HCl protein digestion as described by Fish (*58*), using a 1000 ppm atomic absorption iron standard to prepare standard solutions of known Fe concentrations (Fluka). Metal analyses of as purified SufA1 and IscA samples was carried out by Dr. Sunil Kumar using the ICP-MS in Dr. Michael Adams's laboratory at the University of Georgia.

Spectroscopic Methods: Samples for all spectroscopic investigations were prepared under an argon atmosphere in a glovebox (Vacuum Atmospheres, Hawthorne, CA) at oxygen levels < 1 ppm. UV-visible absorption spectra were recorded under anaerobic conditions in septum-sealed quartz 1 mm cuvettes or small-volume 1 cm cuvettes at room temperature, using a Shimadzu UV-3101 PC scanning spectrophotometer fitted with a TCC-260 temperature controller. CD spectra were recorded under anaerobic conditions in the same cuvettes using a JASCO J-715 spectropolarimeter (Jasco, Easton, MD). Resonance Raman spectra were recorded at 17

K as previously described (*59*), using a Ramanor U1000 spectrometer (Instruments SA, Edison, NJ) coupled with a Sabre argon ion laser (Coherent, Santa Clara, CA), with 17 µL frozen droplets of sample mounted on the cold finger of a Displex Model CSA-202E closed cycle refrigerator (Air Products, Allentown, PA). X-band (~ 9.6 GHz) EPR spectra were recorded using a ESP-300E spectrometer (Bruker, Billerica, MA), equipped with an ER-4116 dual mode cavity and an ESR-900 helium flow cryostat (Oxford Instruments, Concord, MA).

Preparation of Fe-bound A-type Proteins: All sample preparation procedures were carried out under strictly anaerobic conditions inside a Vacuum Atmospheres glove box under argon (< 2 ppm O_2), unless otherwise noted. Fe(III)-bound A-type protein was prepared according to a previously reported method (*43*), where A-type protein (1.4 mM) in 100 mM Tris-HCl buffer, pH 7.8, with 150 mM NaCl with 100 mM Tris(hydroxypropyl)phosphine (THP) was titrated with ferric ammonium citrate at room temperature (Figure 2.1). Fe(III)-loaded A-type protein was then passed through a 5.0 mL desalting column to remove any adventitiously bound iron. The fraction containing the Fe-bound A-type protein was then concentrated by ultrafiltration using an Amicon YM-10 membrane. Fe(III)-bound A-type protein was quantified for both Fe and protein before being spectroscopically characterized.

Cysteine desulfurase-mediated Fe-S Cluster Assembly on SufA1: All steps were carried out anaerobically under argon in a glove box. In order to cleave the disulfides or polysulfides, as-purified apo- *At* SufA1 was treated with 40 mM tris(2-carboxyethyl)phosphine (TCEP), a reducing agent that cleaves disulfide bonds, for 30 min in 100 mM Tris-HCl buffer at pH 7.8 (buffer B). Excess TCEP was removed by

repeated concentration/dilution cycles with buffer B containing 150 mM NaCl (10 mL 4 times) in a YM-10 Amicon ultrafiltration device. Subsequently, the TCEP-treated *At* SufA1 (1 mM) was incubated with ferrous ammonium sulfate (FAS) (4 mM) and L-cysteine (16 mM) and a catalytic amount of cysteine desulfurase (6.27 µM) in the same buffer for 45 min in an ice bath. The time course of cluster reconstitution was monitored over time using UV-visible absorption and CD spectroscopy in septum-sealed 1 mm quartz cuvettes at room temperature. Protein concentrations of SufA1 and NifS are reported as monomers. The reconstitution mixture was loaded onto two concatenated 5 mL High-trap Q-Sepharose columns (GE Healthcare) previously equilibrated with buffer 100 mM Tris-HCl buffer, pH 7.8. Finally, the protein was eluted with a 0-1 M NaCl gradient in buffer 100 mM Tris-HCl buffer, pH 7.8 to purify cluster-bound SufA1 from excess reagents. The [2Fe-2S] cluster-bound form of SufA1 eluted between 0.45 and 0.55 M NaCl and was concentrated using a YM-10 Amicon ultrafiltration membrane.

Holo GrxS14 to apo-SufA1 cluster transfer: [2Fe-2S] cluster-bound *At* GrxS14 (100 µL, 0.058 mM in [2Fe-2S] cluster concentration) was added into 548 µL of 100 mM Tris-HCl buffer, pH 7.8 with 5 mM DTT. Fe-S cluster transfer from [2Fe-2S] cluster-bound *At* Grx S14 was initiated by adding apo- *At* SufA1 (150 µL, 0.120 mM in apo-SufA1 protein monomer), pre-treated with TCEP, and was monitored anaerobically for 50 min using CD spectrometry in septum-sealed small-volume 1 cm cuvette at room temperature. Reactions were carried out in buffer 100 mM Tris-HCl buffer, pH 7.8, with 150 mM NaCl and [2Fe-2S] cluster-bound GrxS14 was prepared as described previously (*28*).

Results

Fe(III)-binding on A-type Proteins: Contrary to the reports by Ding *et al.* for *E. coli* IscA and SufA (*40;62*), *Av* IscA and *At* SufA1 were not purified under aerobic conditions in the Fe(III)-bound form according to ICP-MS analysis (< 0.03 Fe/monomer as purified). However, as purified *At* SufA1 and *Av* IscA exhibit a 320 nm band in the UV-visible absorption spectrum which has been attributed to the Fe(III)-bound forms of *E. coli* IscA and SufA (*41;60*). Furthermore, the addition of EDTA or DTT did not decrease the intensity of the 320 nm band. Addition of an alternative, non-thiol-based disulfide/polysulfide cleaving reagent, tris(hydroxypropyl)phosphine (THP), however, resulted in substantial loss of the 320 nm band suggesting that this band originates from polysulfides that cannot be accessed or reduced by DTT.

However, both *At* SufA1 and *Av* IscA were found to bind Fe(III) in the presence of THP. Figure 2.1 shows a titration of *At* SufA1 (Figure 2.1A) and *Av* IscA (Figure 2.1B) with ferric ammonium citrate under aerobic conditions in the presence of 100 mM THP. For both proteins, the inserts indicate tight binding with a stoichiometry of ~0.5 Fe/monomer. Moreover, the Fe(III)-bound form of *At* SufA1 and *Av* IscA obtained from this titration can be purified without losing iron, again demonstrating tight binding of Fe(III) to these A-type proteins. Iron and protein determinations indicate that after removal of excess Fe, both *At* SufA1 and *Av* IscA contain 0.50 ±0.2 Fe per *At* SufA1 or *Av* IscA monomer, indicating 1.0 ±0.4 Fe per *At* SufA1 or *Av* IscA dimer. Figure 2.2 shows the UV-visible absorption and CD spectra of repurified Fe(III)-bound *At* SufA1 (Figure 2.2A) and *Av* IscA (Figure 2.2B). UV-visible absorption spectra of the Fe(III)-bound *At* SufA1 and *Av* IscA look similar to that of Fe(III)-bound *Av* ^{Nif}IscA with broad

CysS-to-Fe(III) charge transfer bands centered near 320, 440 and 520 nm (*43*). As illustrated in Figure 2.3, UV-visible CD spectra of Fe(III)-bound *At* SufA1 and *Av* IscA are also similar to that of Fe(III)-bound *Av* ^{Nif}IscA (*43*).

Electronic ground state properties of Fe(III)-bound A-type Proteins: The ground state properties of the Fe(III)-bound form of *At* SufA1 (Figure 2.4A) and *Av* IscA (Figure 2.4B) were investigated by EPR spectroscopy. In both cases the spectra exhibit an isotropic resonance at g = 4.3, which originates from a trace contaminant of adventitiously bound high spin (S = 5/2) Fe(III). However, the dominant low-field component is a broad resonance centered at g = 5.5 for *At* SufA1 and at g = 5.2 for *Av* IscA. Both are indicative of an intermediate spin (S = 3/2) Fe(III) center with the former very similar to that observed for Fe(III)-bound ^{Nif}IscA, rhombic intermediate spin Fe(III) centers (S = 3/2 and E/D ~ 0.33) (*43*), and the latter more similar to the less well-defined intermediate spin Fe(III) centers reported in *E. coli* IscA/SufA and human IscA1 (*41;60*).

Vibrational properties of Fe(III)-bound A-type Proteins: Resonance Raman was used to investigate Fe-S stretching modes in Fe(III)-bound SufA1 using visible excitation into S(Cys)-to-Fe(III) charge transfer bands. Figure 2.5 shows a comparison of the resonance Raman spectra of Fe(III)-bound *At* SufA1 and *Av* ^{Nif}IscA with that of *Clostridium pasteurianum* C6D rubredoxin in the Fe-S stretching region. The spectra of Fe(III)-bound *At* SufA1 and *Av* ^{Nif}IscA are clearly very similar, with the sharp intense bands at 333/338 cm^{-1} and the weaker broad band at 397 cm^{-1} assigned to symmetric and asymmetric Fe-S(Cys) stretching modes of two or three ligated cysteine residues (*43*). The broad and weak band at 296 cm^{-1} is assigned to the S-C-C bending mode of the coordinated cysteines. Support for these assignments comes from the similarity to the

spectra observed for Cys-to-Asp rubredoxin variants that are coordinated by three cysteines and one monodentate or bidentate aspartate, although the asymmetric Fe-S stretching mode is clearly split into two well-resolved and bands in this case. The data therefore indicate that the coordination environment of Fe(III)-bound *Av* ^{Nif}IscA is 4- or 5-coordinate with only two or three cysteinate ligands. 5-coordinate with two of three cysteinate ligands seems most likely, since the vast majority of intermediate spin Fe(III) complexes are 5-coordinate (*43*).

Cysteine desulfurase-mediated Fe-S cluster assembly on SufA1: Cysteine-desulfurase mediated cluster assembly on SufA1 was initiated by adding a catalytic amount of cysteine-desulfurase to a mixture containing TCEP-treated *At* SufA1, FAS and L-cysteine and was monitored anaerobically for 50 min using UV-visible absorption and CD spectroscopies. The UV-visible absorption spectrum of Fe-S cluster-reconstituted *At* SufA1, after purification to remove excess reagents, is shown in Figure 2.6A. The absorption spectrum shows a pronounced shoulder centered at 320 nm and a band at 415 nm with low-energy shoulders centered at 460 and 540 nm, see Figure 2.6A, which are characteristic bands corresponding to S-to-Fe charge-transfer transitions of a [2Fe-2S] cluster (*61-63*). Furthermore, in the visible CD, positive bands centered at 348, 456, 522, and 625 nm and negative bands centered at 318, 393 and 563 nm were also observed, see Figure 2.6B. As previously reported, these are the characteristic bands of a [2Fe-2S] cluster (*63-65*). Fe and protein analysis data indicate that the reconstituted *At* SufA1 contain 0.62 ± 0.05 Fe per SufA1 monomer. Assuming all the Fe in the sample is present as $[2Fe\text{-}2S]^{2+}$ clusters, the Fe and protein data should correspond to 0.62 ± 0.05 $[2Fe\text{-}2S]^{2+}$ clusters per homodimeric SufA1. Recent studies of repurified reconstituted *Av*

^{Nif}IscA reported 0.79 ± 0.10 $[2Fe\text{-}2S]^{2+}$ clusters per homodimeric ^{Nif}IscA (*43*) indicating that *At* SufA1 has slightly less [2Fe-2S] clusters per homodimer. Figure 2.7 illustrates the similarities in UV-visible CD spectra of [2Fe-2S] cluster-bound *At* SufA1 and *Av* ^{Nif}IscA.

Vibrational properties of [2Fe-2S] cluster-bound At SufA1: In order to characterize the vibrational properties of the [2Fe-2S] cluster in *At* SufA1, the resonance Raman spectrum of [2Fe-2S] cluster-bound SufA1 in the Fe-S stretching region (240 - 450 cm^{-1}), was obtained using 457.9, 487.9 and 514.5 nm laser excitation, see Figure 2.8. The spectra obtained for the Fe-S stretching region are very similar to those previously reported for the [2Fe-2S] cluster-bound form of ^{Nif}IscA, comprising an intense band at 290 cm^{-1} and additional bands at 338, 358, 396, and 421 cm^{-1} (Figure 2.9) (*63*). Furthermore, the similarities in the Fe-S stretching frequencies and resonance Raman spectra for the [2Fe-2S] center in *At* SufA1 to structurally well-characterized all-cysteine ligated [2Fe-2S] centers in simple ferredoxins, such as *S. oleracea* ferredoxin, bovine adrenodoxin, and *P. putida* putidaredoxin (*66*), strongly suggest analogous cluster ligation in *At* SufA1. This is in accord with the crystallographic data for $[2Fe\text{-}2S]^{2+}$ cluster-bound *Thermosynechococcus elongatus* IscA (*67*), which shows an all cysteinyl ligated [2Fe-2S] cluster bound at the interface of an asymmetric homodimer, by the three conserved cysteines from one subunit and the C-terminal conserved cysteine of the other subunit.

In vitro cluster transfer from [2Fe-2S] cluster-bound At GrxS14 to apo- At SufA1: In order to investigate the role of chloroplastic SufA1 and GrxS14 in cellular Fe-S cluster transfer or trafficking, cluster transfer experiments from one of the two monothiol glutaredoxins in plant chloroplasts GrxS14 to SufA1 were conducted. The visible CD

spectrum of [2Fe-2S] cluster GrxS14 is very intense and quite distinct compared to that of [2Fe-2S] cluster SufA1 (Figure 2.10). Thus visible CD was used to monitor the cluster transfer reaction. Figure 2.11 shows simulated spectra corresponding to 0-100% cluster transfer in 10% increments based on the spectra of the individual [2Fe-2S] cluster-bound proteins. Figure 2.12 shows the observed UV-visible CD time course of cluster transfer from *At* GrxS14 to *At* SufA1. The CD spectrum taken at zero time (red line) corresponds to [2Fe-2S] cluster-bound GrxS14 with 5 mM DTT before the addition of apo-SufA1. Other UV-visible CD spectra (gray lines) were recorded at 3, 6, 9, 12, 15, 18 and 21 min after adding apo-SufA1 in a 1:1 ratio based on [2Fe-2S] cluster concentration of the individual proteins (50 μM in apo-SufA1 protein homodimer and 50 μM in GrxS14 [2Fe-2S] cluster concentration). Based on the simulated CD spectra (see Figure 2.11) for a stoichiometric cluster transfer reaction, it is evident that the transfer is >95% within the first 3 min and is complete within 6 min. In order to obtain a more precise measure the rate of cluster transfer, CD intensity was monitored at 348 nm as function of time. This yielded a second-order rate constant of ~50,000 $M^{-1}min^{-1}$, see Figure 2.13.

Discussion

A-type proteins involved in Fe-S cluster biogenesis are found in all domains of life. However, the specific function(s) of these A-type proteins in mitochondrial, chloroplastic, and bacterial Fe/S cluster biogenesis are only now starting to emerge. Recent studies suggest that A-type proteins may serve a dual role. First, as an iron donor either for assembling Fe-S clusters on primary scaffold proteins (IscU, NifU, and SufB) (*38-40*) or for the *de novo* assembly or repair of [4Fe-4S] clusters on acceptor proteins

(*43;45*). Second, as a cluster carrier to deliver clusters assembled on primary scaffolds to apo-Fe-S proteins (*27;36;44*).

The results presented in this work indicate that both *At* SufA1 and *Av* IscA are able to bind mononuclear Fe(III) in an intermediate spin state (S = 3/2), with a predominantly cysteinate-ligated coordination environment similar to that characterized in Fe(III)-bound *Av* ^{Nif}IscA (*43*). Previous studies on *E. coli* IscA, SufA and human IscA (hIscA1) have also shown that A-type proteins can bind iron that can be used for Fe-S cluster assembly on the IscU scaffold protein in the presence of L-cysteine (*60;68*) However, the recent studies of iron-bound *Av* ^{Nif}IscA suggest that the release of iron in the presence of L-cysteine does not require specific interaction between the primary scaffold and iron-bound A-type protein (*43*). Rather, L-cysteine specifically facilitates the release of Fe(II) that can be used in cysteine desulfurase-mediated cluster assembly. Due to cellular toxicity of free Fe(II), via Fenton chemistry, it seems unlikely that a non-specific Fe-donor functions as the Fe donor to IscU. Moreover, this conclusion is supported by *in vivo* studies using *S. cerevisiae* which showed that deletion of Isa1 and Isa2 resulted in a small increase in cluster loaded Isu1 (*45*). However, these *in vivo* studies did clearly demonstrate the presence of an Fe-bound Isa1/Isa2/Iba57 complex that required for the maturation of mitochondrial [4Fe-4S] clusters, but not [2Fe-2S] clusters, and thereby constitutes a late-acting system that is specifically dedicated to the assembly or repair of mitochondrial [4Fe-4S] proteins (*45*). Iba57 is a tetrahydrofolate-dependent protein that appears to be specifically required for release of Fe from Fe-bound Isa1/Isa2 to facilitate assembly or [4Fe-4S] clusters on acceptor proteins. Homologs of Iba57, e.g. *E. coli* YgfZ, are found in almost all bacteria, and have been proposed to participate in

the synthesis and repair of Fe-S clusters (*69*). The YgfZ/Iba57 family of enzymes has a crystal structure analogous to those of aminomethyl transferase, dimethylglycine oxidase, and related tetrahydrofolate-binding enzymes (*47*). In *E. coli*, mutation of *ygfZ* gene resulted in reduced growth, increased sensitivity to oxidative stress and decreased activities of MiaB and other Fe-S enzymes implying a role in Fe/S protein biogenesis or repair under oxidative stress conditions (*69*). However, the involvement of this class of proteins with Fe(III)-bound IscA has not yet been identified in bacteria (*47*).

In addition to a role for Fe-bound A-type proteins in the maturation of [4Fe-4S] clusters on acceptor proteins, there is a substantial body of *in vitro* evidence that they may also play a role in trafficking [2Fe-2S] and [4Fe-4S] clusters. For example, recombinant A-type proteins have been purified in [2Fe-2S] and [4Fe-4S] cluster-bound forms (*70;71*), recent studies of ^{Nif}IscA have shown that A-type proteins can cycle between [2Fe-2S] and [4Fe-4S] cluster-bound forms in response to cellular conditions (*44*), see Figure 2.14, and ^{Nif}IscA, IscA and SufA have all been shown to accept Fe-S clusters from their respective scaffolds, NifU (*44*), IscU (*27*) and the SufBCD complex (*72*). Furthermore, A-type proteins have been shown to transfer [4Fe-4S] or [2Fe-2S] clusters to acceptor apo-proteins such as the nitrogenase Fe protein (*44*) and Fdx (*70*).

A cluster trafficking role for *At* SufA1 in chloroplasts is also indicated by the work presented herein. Although *At* SufA1 is purified as an apo protein under aerobic conditions, *in vitro* cysteine desulfurase-mediated cluster reconstitution under anaerobic conditions yields a stable [2Fe-2S] cluster-bound form. Although *At* SufA1 is unlikely to act as a primary scaffold in chloroplasts, our observation that apo *At* SufA1 can rapidly accept [2Fe-2S] clusters from *At* GrxS14 via intact cluster transfer, suggests a viable

mechanism for cluster incorporation. Indeed this observation contributes to an emerging hypothesis that monothiol Grxs and A-type proteins are partners in cluster trafficking in all organisms (*73*). For example, a similar unidirectional, rapid and intact [2Fe-2S] cluster transfer from *Av* Grx-nif (a *nif*-specific monothiol Grx) to ^{Nif}IscA has been observed and there is *in vivo* yeast two-hybrid and bimolecular fluorescence complementation evidence for a direct interaction between mitochondrial monothiol Grxs and Isa proteins in yeast (*50;56*). Moreover, the recent observation that IscU rapidly transfers [2Fe-2S] clusters to monothiol Grxs in bacteria in the presence of the dedicated HscA/HscB co-chaperone system and ATP (*55*) suggests that monothiol Grxs are primary [2Fe-2S] cluster storage and carrier proteins in bacteria. Hence our current working hypothesis is that A-type proteins receive [2Fe-2S] clusters from [2Fe-2S] cluster-bound forms of monothiol Grxs, see Figure 2.14

The above discussion implicates a role for both Fe-bound and Fe-S cluster bound forms of A-type proteins in the maturation or repair of [4Fe-4S] clusters in mitochondrial proteins (*45-47*) and in bacterial or chloroplast proteins under aerobic growth or oxidative stress conditions (*42;48*). *In vitro* studies have demonstrated intact transfer of [4Fe-4S] clusters on bacterial primary scaffold proteins and A-type proteins to apo-acceptor proteins, only under strictly anaerobic conditions (*44;74-76*). However, since the [4Fe-4S] clusters on these donor proteins are extremely oxygen sensitive, it seems that maturation of [4Fe-4S] cluster containing proteins via direct transfer of a [4Fe-4S] cluster is only applicable under anaerobic conditions, see Figure 2.14. Hence an alternative mechanism is required for the maturation or repair of [4Fe-4S] clusters under aerobic or oxidative stress conditions.

Our current working hypothesis for this alternative mechanism is schematically depicted in Figure 2.14 (*73*). In large part, this mechanism is based on two observations. First, the early work of Beinert and Kennedy which indicated the presence of cysteine persulfides in the apo forms of oxygen-sensitive [4Fe-4S] cluster-containing proteins such as aconitase that had been generated by oxygen exposure (*77*). Second, recent *in vitro* studies of the oxygen-sensing fumarate nitrate reduction (FNR) regulatory protein which is present in many facultative anaerobes (*78*). Resonance Raman and mass spectrometry have revealed that the oxygen-sensing mechanism involves [4Fe-4S] cluster degradation to yield a [2Fe-2S] cluster ligated by two cysteines and two cysteine persulfides. A similar cluster transformation is also observed in other oxygen sensitive [4Fe-4S] cluster-containing proteins such as members of the radical *S*-adenosylmethionine family of enzymes (*78*). Moreover, this cluster transformation can be reversed by addition of Fe(II) in the presence of a dithiol reagent. Hence under aerobic conditions, we propose that the [2Fe-2S] cluster-bound form of an A-type protein is generated by cluster transfer from a monothiol Grx, see Figure 2.14. Under anaerobic conditions, in the presence of a cellular dithiol reagent, the [2Fe-2S] cluster-bound form of the A-type protein can be converted to a [4Fe-4S] cluster bound form which can effect maturation of [4Fe-4S] cluster-containing acceptor proteins via intact [4Fe-4S] cluster transfer, see Figure 2.14. Under aerobic or oxidative stress conditions, the Fe-bound A-type protein can directly repair oxygen-damaged [4Fe-4S] clusters that have been degraded to the level of a [2Fe-2S] cluster with two cysteine persulfide ligands in the presence of a cellular dithiol reagent. Alternatively, if the [4Fe-4S] cluster is degraded to an apo form containing cysteine persulfides, the above step is preceded by transfer of a

[2Fe-2S] cluster from the A-type proteins to yield a [2Fe-2S] cluster with two cysteine persulfide ligands. This scheme is presented as a working hypothesis that is consistent with the available data and provides the basis for further experimentation.

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References

1. Beinert, H. (2000) A tribute to sulfur, *Eur. J. Biochem. 267*, 5657-5664.

2. Beinert, H. (2000) Iron-sulfur proteins: ancient structures, still full of surprises, *J. Biol. Inorg. Chem. 5*, 2-15.

3. Wachtershauser, G. (1992) Groundworks for an evolutionary biochemistry: the iron-sulphur world, *Prog. Biophys. Mol. Biol. 58*, 85-201.

4. Beinert, H., Holm, R. H., and Münck, E. (1997) Iron-sulfur clusters; Nature's modular, multipurpose structures, *Science 277*, 653-659.

5. Johnson, M. K. and Smith, A. D. (2005) Iron-sulfur proteins, in *Encyclopedia of Inorganic Chemistry, Second Edition* (King, R. B., Ed.) pp 2589-2619, John Wiley & Sons, Chichester.

6. Rees, D. C. and Howard, J. B. (2003) The interface between the biological and inorganic worlds: iron-sulfur metalloclusters, *Science 300*, 929-931.

7. Balk, J. and Lobréaux, S. (2005) Biogenesis of iron-sulfur proteins in plants, *Trends Plant Sci. 10*, 324-331.

8. Balk, J. and Pilon, M. (2011) Ancient and essential: the assembly of iron-sulfur clusters in plants, *Trends Plant Sci. 16*, 218-226.

9. Kessler, D. and Papenbrock, J. (2005) Iron-sulfur cluster biosynthesis in photosynthetic organisms, *Photosynth. Res. 86*, 391-407.

10. Pilon, M., Abdel-Ghany, S. E., Van, H. D., Ye, H., and Pilon-Smits, E. A. (2006) Biogenesis of iron-sulfur cluster proteins in plastids, *Genet. Eng (N. Y.) 27*, 101-117.

11. Abdel-Ghany, S., Ye, H., Garifullina, G. F., Zhang, L., Pilon-Smits, E. A., and Pilon, M. (2005) Iron-sulfur cluster biogenesis in chloroplasts. Involvement of the scaffold protein CpIscA, *Plant Physiol. 138*, 161-172.

12. Johnson, D. C., Dean, D. R., Smith, A. D., and Johnson, M. K. (2005) Structure, function and formation of biological iron-sulfur clusters, *Annu. Rev. Biochem. 74*, 247-281.

13. Lill, R. and Mühlenhoff, U. (2006) Iron-sulfur protein biogenesis in eukaryotes: Components and mechanisms, *Annu. Rev. Cell Dev. Biol. 22*, 457-486.

14. Ayala-Castro, C., Saini, A., and Outten, F. W. (2008) Fe-S cluster assembly pathways in bacteria, *Microbiol. Mol. Biol. Rev. 72*, 110-125.

15. Fontecave, M. and Ollagnier-de-Choudens, S. (2008) Iron-sulfur cluster biosynthesis in bacteria: Mechanisms of cluster assembly and transfer, *Arch. Biochem. Biophys. 474*, 226-237.

16. Bandyopadhyay, S., Chandramouli, K., and Johnson, M. K. (2008) Iron-sulfur cluster biosynthesis, *Biochem. Soc. Trans. 36*, 1112-1119.

17. Takahashi, Y. and Nakamura, M. (1999) Functional assignment of the ORF2-*iscS*-*iscA*-*hscB*-*hscA*-*fdx*-ORF3 gene cluster involved in the assembly of Fe-S clusters in *Escherichia coli*, *J. Biochem. 126*, 917-926.

18. Takahashi, Y. and Tokumoto, U. (2002) A third bacterial system for the assembly of iron-sulfur clusters with homologs in archaea and plastids, *J. Biol. Chem. 277*, 28380-28383.

19. Outten, F. W., Djaman, O., and Storz, G. (2004) A *suf* operon requirement for Fe-S cluster assembly during iron starvation in *Escherichia coli*, *Mol. Microbiol. 52*, 861-872.

20. Fontecave, M., Ollagnier-de-Choudens, S., Py, B., and Barras, F. (2005) Mechanisms of iron-sulfur cluster assembly: the SUF machinery, *J. Biol. Inorg. Chem. 10*, 713-721.

21. Zheng, L., Cash, V. L., Flint, D. H., and Dean, D. R. (1998) Assembly of iron-sulfur clusters. Identification of an *iscSUA-hscBA-fdx* gene cluster from *Azotobacter vinelandii*, *J. Biol. Chem. 273*, 13264-13272.

22. Gerber, J. and Lill, R. (2002) Biogenesis of iron-sulfur proteins in eukaryotes: components, mechanism and pathology, *Mitochondrion 2*, 71-86.

23. Lill, R. and Mühlenhoff, U. (2005) Iron-sulfur-protein biogenesis in eukaryotes, *Trends Biochem. Sci. 30*, 133-141.

24. Lill, R. and Kispal, G. (2000) Maturation of cellular Fe-S proteins: an essential function of mitochondria, *Trends Biochem. Sci. 25*, 352-356.

25. Yoon, T. and Cowan, J. A. (2003) Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins, *J. Am. Chem. Soc. 125*, 6078-6084.

26. Layer, G., Ollagnier-de-Choudens, S., Sanakis, Y., and Fontecave, M. (2006) Iron-sulfur cluster biosynthesis. Characterization of *Eschericia coli* CyaY as an iron donor for the assembly of [2Fe-2S] clusters in the scaffold IscU, *J. Biol. Chem. 281*, 16256-16263.

27. Ollagnier-de-Choudens, S., Sanakis, Y., and Fontecave, M. (2004) SufA/IscA: reactivity studies of a class of scaffold proteins involved with [Fe-S] cluster assembly, *J. Biol. Inorg. Chem. 9*, 828-838.

28. Bandyopadhyay, S., Gama, F., Molina-Navarro, M. M., Gualberto, J. M., Claxton, R., Naik, S. G., Huynh, B. H., Herrero, E., Jacquot, J.-P., Johnson, M. K., and Rouhier, N. (2008) Chloroplast monothiol glutaredoxins as scaffold proteins for the assembly and delivery of [2Fe-2S] clusters, *EMBO J. 27*, 1122-1133.

29. Mühlenhoff, U., Gerber, J., Richhardt, N., and Lill, R. (2003) Components involved in assembly and dislocation of iron-sulfur clusters on the scaffold protein Iscu1p, *EMBO J. 22*, 4815-4825.

30. Alves, R., Herrero, E., and Sorribas, A. (2004) Predictive reconstruction of the mitochondrial iron-sulfur cluster assembly metabolism. II. Role of glutaredoxin Grx5, *Proteins:Struct. Func. Bioinform. 57*, 481-492.

31. Nishio, K. and Nakai, M. (2000) Transfer of iron-sulfur cluster from NifU to apoferredoxin, *J. Biol. Chem. 275*, 22615-22618.

32. Ollagnier-de-Choudens, S., Mattioli, T., Takahashi, Y., and Fontecave, M. (2001) Iron-sulfur cluster assembly. Characterization of IscA and evidence for a specific functional complex with ferredoxin, *J. Biol. Chem. 276*, 22604-22607.

33. Jensen, L. T. and Culotta, V. C. (2000) Role of *Saccharomyces cerevisiae* Isa1 and Isa2 in iron homeostasis, *Mol. Cell. Biol. 20*, 3918-3927.

34. Kaut, A., Lange, H., Diekert, K., Kispal, G., and Lill, R. (2000) Isa1p is a component of the mitochondrial machinery for maturation of cellular iron-sulfur proteins and requires conserved cysteine residues for function, *J. Biol. Chem. 275*, 15955-15961.

35. Wollenberg, M., Berndt, C., Bill, E., Schwenn, J. D., and Seidler, A. (2003) A dimer of the FeS cluster biosynthesis protein IscA from cyanobacteria binds a [2Fe2S] cluster between two protomers and transfers it to [2Fe2S] and [4Fe4S] apo proteins, *Eur. J. Biochem. 270*, 1662-1671.

36. Vinella, D., Brochier-Armanet, C., Loiseau, L., Talla, E., and Barras, F. (2009) Iron-sulfur (Fe/S) protein biogenesis: phylogenomic and genetic studies of A-type carriers, *PLoS. Genet. 5*, e1000497.

37. Cozar-Castellano, I., del Valle, M. M., Trujillo, E., Arteaga, M. F., Gonzalez, T., Martin-Vasallo, P., and Avila, J. (2004) hIscA: a protein implicated in the biogenesis of iron-sulfur clusters, *Biochim. Biophys. Acta 1700*, 179-188.

38. Ding, H., Clark, R. J., and Ding, B. (2004) IscA mediates iron delivery for assembly of iron-sulfur clusters in IscU under limited "free" iron conditions, *J. Biol. Chem. 279*, 37499-37504.

39. Ding, B., Smith, E. S., and Ding, H. (2005) Mobilization of the iron centre in IscA for the iron-sulfur cluster assembly in IscU, *Biochem. J. 389*, 797-802.

40. Yang, J., Bitoun, J. P., and Ding, H. (2006) Interplay of IscA and IscU in biogenesis of iron-sulfur clusters, *J. Biol. Chem. 281*, 27956-27963.

41. Lu, J., Yang, J., Tan, G., and Ding, H. (2008) Complementary roles of SufA and IscA in the biogenesis of iron-sulfur clusters in *Escherichia coli*, *Biochem. J. 409*, 535-543.

42. Tan, G., Lu, J., Bitoun, J. P., Huang, H., and Ding, H. (2009) IscA/SufA paralogues are required for the [4Fe-4S] cluster assembly in enzymes of multiple physiological pathways in Escherichia coli under aerobic growth conditions, *Biochem. J. 420*, 463-472.

43. Mapolelo, D. T., Zhang, B., Naik, S. G., Huynh, B. H., and Johnson, M. K. (2012) Spectroscopic and Functional Characterization of Iron-Bound Forms of *Azotobacter vinelandii* NifIscA, *Biochemistry 51, 8056-8070*.

44. Mapolelo, D. T., Zhang, B., Naik, S. G., Huynh, B. H., and Johnson, M. K. (2012) Spectroscopic and Functional Characterization of Iron-Sulfur Cluster-Bound Forms of *Azotobacter vinelandii* NifIscA, *Biochemistry 51, 8071-8084*.

45. Mühlenhoff, U., Richter, N., Pines, O., Pierik, A. J., and Lill, R. (2011) Specialized function of yeast Isa1 and Isa2 in the maturation of mitochondrial [4Fe-4S] proteins, *J. Biol. Chem. 286*, 41205-41216.

46. Mühlenhoff, U., Gerl, M. J., Flauger, B., Pirner, H. M., Balser, S., Richhardt, N., Lill, R., and Stolz, J. (2007) The iron-sulfur cluster proteins Isa1 and Isa2 are required for the function but not the de novo synthesis of the Fe/S clusters of biotin synthase in *Saccharomyces cerevisiae*, *Eukaryot. Cell 6*, 495-504.

47. Gelling, C., Dawes, I. W., Richhardt, N., Lill, R., and Muhlenhoff, U. (2008) Mitochondrial Iba57p is required for Fe/S cluster formation on aconitase and activation of radical SAM enzymes, *Mol. Cell. Biol. 28*, 1851-1861.

48. Johnson, D. C., Unciuleac, M.-C., and Dean, D. R. (2006) Controlled expression and functional analysis of iron-sulfur cluster biosynthetic components within *Azotobacter vinelandii*, *J. Bacteriol. 188*, 7551-7561.

49. Rouhier, N., Lemaire, S. D., and Jacquot, J. P. (2008) The role of glutathione in photosynthetic organisms: emerging functions for glutaredoxins and glutathionylation, *Annu. Rev. Plant Biol. 59*, 143-166.

50. Vilella, F., Alves, R., Rodriguez-Manzaneque, M. T., Belli, G., Swaminathan, S., Sunnerhagen, P., and Herrero, E. (2004) Evolution and cellular function of monothiol glutaredoxins: involvement in iron-sulphur cluster assembly, *Comp Funct. Genomics 5*, 328-341.

51. Molina-Navarro, M. M., Casas, C., Piedrafita, L., Bellí, G., and Herrero, E. (2006) Prokaryotic and eukaryotic monothiol glutaredoxins are able to perform the functions of Grx5 in the biogenesis of Fe/S clusters in yeast mitochondria, *FEBS Lett. 580*, 2273-2280.

52. Iwema, T., Picciocchi, A., Traore, D. A., Ferrer, J. L., Chauvat, F., and Jacquamet, L. (2009) Structural basis for delivery of the intact [Fe_2S_2] cluster by monothiol glutaredoxin, *Biochemistry 48*, 6041-6043.

53. Rodríguez-Manzaneque, M. T., Ros, J., Cabrito, I., Sorribas, A., and Herrero, E. (1999) Grx5 glutaredoxin plays a central role in protection against protein oxidative damage in *Saccharomyces cerevisiae*, *Mol. Cell. Biol. 19*, 8180-8190.

54. Rodríguez-Manzaneque, M. T., Tamarit, J., Bellí, G., Ros, J., and Herrero, E. (2002) Grx5 is a mitochondrial glutaredoxin required for the activity of iron/sulfur enzymes, *Mol. Biol. Cell 13*, 1109-1121.

55. Shakamuri, P., Zhang, B., and Johnson, M. K. (2012) Monothiol Glutaredoxins Function in Storing and Transporting [Fe(2)S(2)] Clusters Assembled on IscU Scaffold Proteins, *J. Am. Chem. Soc. 134*, 15213-15216.

56. Kim, K. D., Chung, W. H., Kim, H. J., Lee, K. C., and Roe, J. H. (2010) Monothiol glutaredoxin Grx5 interacts with Fe-S scaffold proteins Isa1 and Isa2 and supports Fe-S assembly and DNA integrity in mitochondria of fission yeast, *Biochem. Biophys. Res. Commun. 392*, 467-472.

57. Brown, R. E., Jarvis, K. L., and Hyland, K. J. (1989) Protein measurement using bicinchoninic acid: elimination of interfering substances, *Anal. Biochem. 180*, 136-139.

58. Fish, W. W. (1988) Rapid colorimetric micromethod for the quantitation of complexed iron in biological samples, *Meth. Enzymol. 158*, 357-364.

59. Drozdzewski, P. M. and Johnson, M. K. (1988) A simple anaerobic cell for low temperature Raman spectroscopy, *Appl. Spectrosc. 42*, 1575-1577.

60. Ding, H. and Clark, R. J. (2004) Characterization of iron-binding in IscA, an ancient iron-sulfur cluster assembly protein, *Biochem. J. 379*, 433-440.

61. Agar, J. N., Krebs, B., Frazzon, J., Huynh, B. H., Dean, D. R., and Johnson, M. K. (2000) IscU as a scaffold for iron-sulfur cluster biosynthesis: Sequential assembly of [2Fe-2S] and [4Fe-4S] clusters in IscU, *Biochemistry 39*, 7856-7862.

62. Dailey, H. A., Finnegan, M. G., and Johnson, M. K. (1994) Human ferrochelatase is an iron-sulfur protein, *Biochemistry 33*, 403-407.

63. Krebs, C., Agar, J. N., Smith, A. D., Frazzon, J., Dean, D. R., Huynh, B. H., and Johnson, M. K. (2001) IscA, an alternative scaffold for Fe-S cluster biosynthesis, *Biochemistry 40*, 14069-14080.

64. Fu, W., Morgan, T. V., Mortenson, L. E., and Johnson, M. K. (1991) Resonance Raman studies of the [4Fe-4S] to [2Fe-2S] cluster conversion in the iron protein of nitrogenase, *FEBS Lett. 284*, 165-168.

65. Agar, J. N., Zheng, L., Cash, V. L., Dean, D. R., and Johnson, M. K. (2000) Role of the IscU protein in iron-sulfur cluster biosynthesis: IscS-mediated assembly of a [Fe_2S_2] cluster in IscU, *J. Am. Chem. Soc. 122*, 2136-2137.

66. Fu, W., Drozdzewski, P. M., Davies, M. D., Sligar, S. G., and Johnson, M. K. (1992) Resonance Raman and magnetic circular dichroism studies of reduced [2Fe-2S] proteins, *J. Biol. Chem. 267*, 15502-15510.

67. Morimoto, K., Yamashita, E., Kondou, Y., Lee, S. J., Arisaka, F., Tsukihara, T., and Nakai, M. (2006) The asymmetric IscA homodimer with an exposed [2Fe-2S] cluster suggests the structural basis of the Fe-S cluster biosynthetic scaffold, *J. Mol. Biol. 360*, 117-132.

68. Lu, J., Bitoun, J. P., Tan, G., Wang, W., Min, W., and Ding, H. (2010) Iron-binding activity of human iron-sulfur cluster assembly protein hIscA1, *Biochem. J. 428*, 125-131.

69. Waller, J. C., Ellens, K. W., Hasnain, G., Alvarez, S., Rocca, J. R., and Hanson, A. D. (2012) Evidence that the folate-dependent proteins YgfZ and MnmEG have opposing effects on growth and on activity of the iron-sulfur enzyme MiaB, *J. Bacteriol. 194*, 362-367.

70. Gupta, V., Sendra, M., Naik, S. G., Chahal, H. K., Huynh, B. H., Outten, F. W., Fontecave, M., and Ollagnier de Choudens, S. (2009) Native *Escherichia coli* SufA, coexpressed with SufBCDSE, purifies as a [2Fe-2S] protein and acts as an Fe-S transporter to Fe-S target enzymes, *J. Am. Chem. Soc. 131*, 6149-6153.

71. Zeng, J., Geng, M., Jiang, H., Liu, Y., Liu, J., and Qiu, G. (2007) The IscA from *Acidithiobacillus ferrooxidans* is an iron-sulfur protein which assemble the [Fe_4S_4] cluster with intracellular iron and sulfur, *Arch. Biochem. Biophys. 463*, 237-244.

72. Chahal, H. K., Dai, Y., Saini, A., Ayala-Castro, C., and Outten, F. W. (2009) The SufBCD Fe-S scaffold complex interacts with SufA for Fe-S cluster transfer, *Biochemistry 48*, 10644-10653.

73. Mapolelo, D. T., Zhang, B., Randeniya, S., Albetel, A. N., Li, H., Outten, C. E., Couturier, J., Rouhier, N., and Johnson, M. K. (2012) Monothiol glutaredoxins and A-type proteins: Partners in Fe-S cluster trafficking, *Dalton Trans.*, submitted.

74. Dos Santos, P. C., Smith, A. D., Frazzon, J., Cash, V. L., Johnson, M. K., and Dean, D. R. (2004) Iron-sulfur cluster assembly: NifU-directed activation of the nitrogenase Fe-protein, *J. Biol. Chem. 279*, 19705-19711.

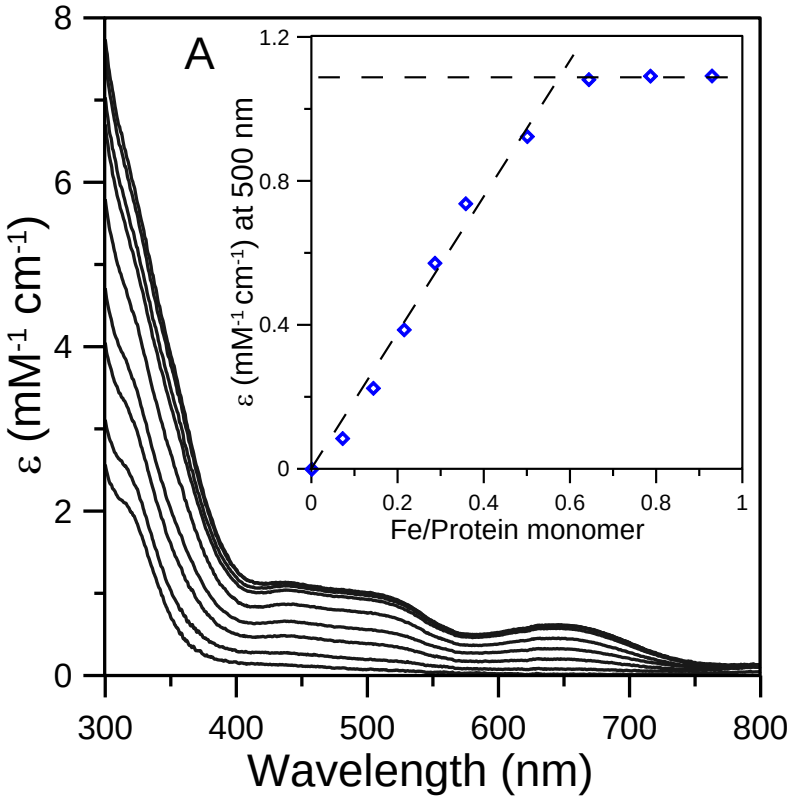
75. Smith, A. D., Jameson, G. N. L., Dos Santos, P. C., Agar, J. N., Naik, S., Krebs, C., Frazzon, J., Dean, D. R., Huynh, B. H., and Johnson, M. K. (2005) NifS-mediated assembly of [4Fe-4S] clusters in the N- and C-terminal domains of the NifU scaffold protein, *Biochemistry 44*, 12955-12969.

76. Unciuleac, M.-C., Chandramouli, K., Naik, S., Mayer, S., Huynh, B. H., Johnson, M. K., and Dean, D. R. (2007) *In vitro* activation of apo-aconitase using a [4Fe-4S] cluster-loaded form of the IscU [Fe-S] cluster scaffolding protein, *Biochemistry 46*, 6812-6821.

77. Kennedy, M. C. and Beinert, H. (1988) The state of cluster SH and S^{2-} of aconitase during cluster interconversion and removal, *J. Biol. Chem. 263*, 8194-8198.

78. Zhang, B., Crack, J. C., Subramanian, S., Green, J., Thomson, A. J., Le Brun, N. E., and Johnson, M. K. (2012) Reversible cycling between cysteine persulfide-ligated [2Fe-2S] and cysteine-ligated [4Fe-4S] clusters in the FNR regulatory protein, *Proc. Natl. Acad. Sci. U. S. A 109*, 15734-15739.

Figure 2.1: Fe(III) binding to *At* SufA1 (A) and *Av* IscA (B) monitored by UV−visible absorption spectroscopy. *At* SufA1 (1.4 mM) / *Av* IscA (1.6 mM) was titrated with ferric ammonium citrate under anaerobic conditions in 100 mM Tris/HCl buffer, pH 7.8, in the presence of 100 mM THP. The insets show plots of the extinction coefficient at 500 nm as a function of the Fe(III)/SufA1 monomer or Fe(III)/IscA monomer ratio. All ε values are based on the concentration of SufA/IscA monomer.


A
ε (mM-1 cm-1)
Wavelength (nm)
ε (mM-1 cm-1) at 500 nm
Fe/Protein monomer

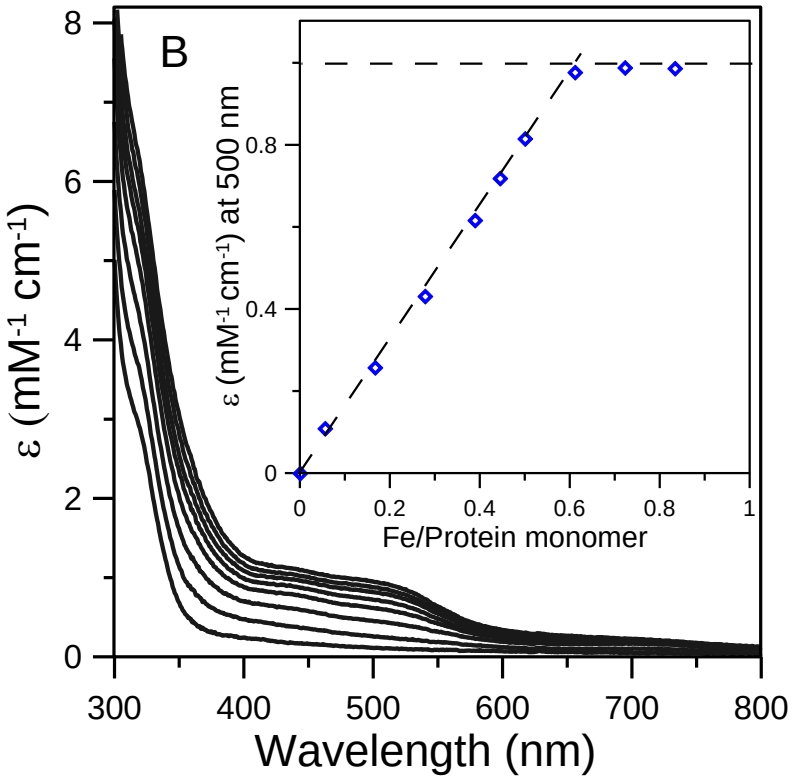

B
ε (mM-1 cm-1)
Wavelength (nm)
ε (mM-1 cm-1) at 500 nm
Fe/Protein monomer

Figure 2.2: UV–visible absorption and CD spectra of purified Fe(III)-bound *At* SufA1 (A) and Fe(III)-bound *Av* IscA (B). Spectra were recorded under anaerobic conditions in sealed 0.1 cm cuvette in 100 mM Tris-HCl buffer with 250 mM NaCl at pH 7.8. All ε values are based on the concentration of A-type protein monomer and Δε values are based on the Fe concentration.

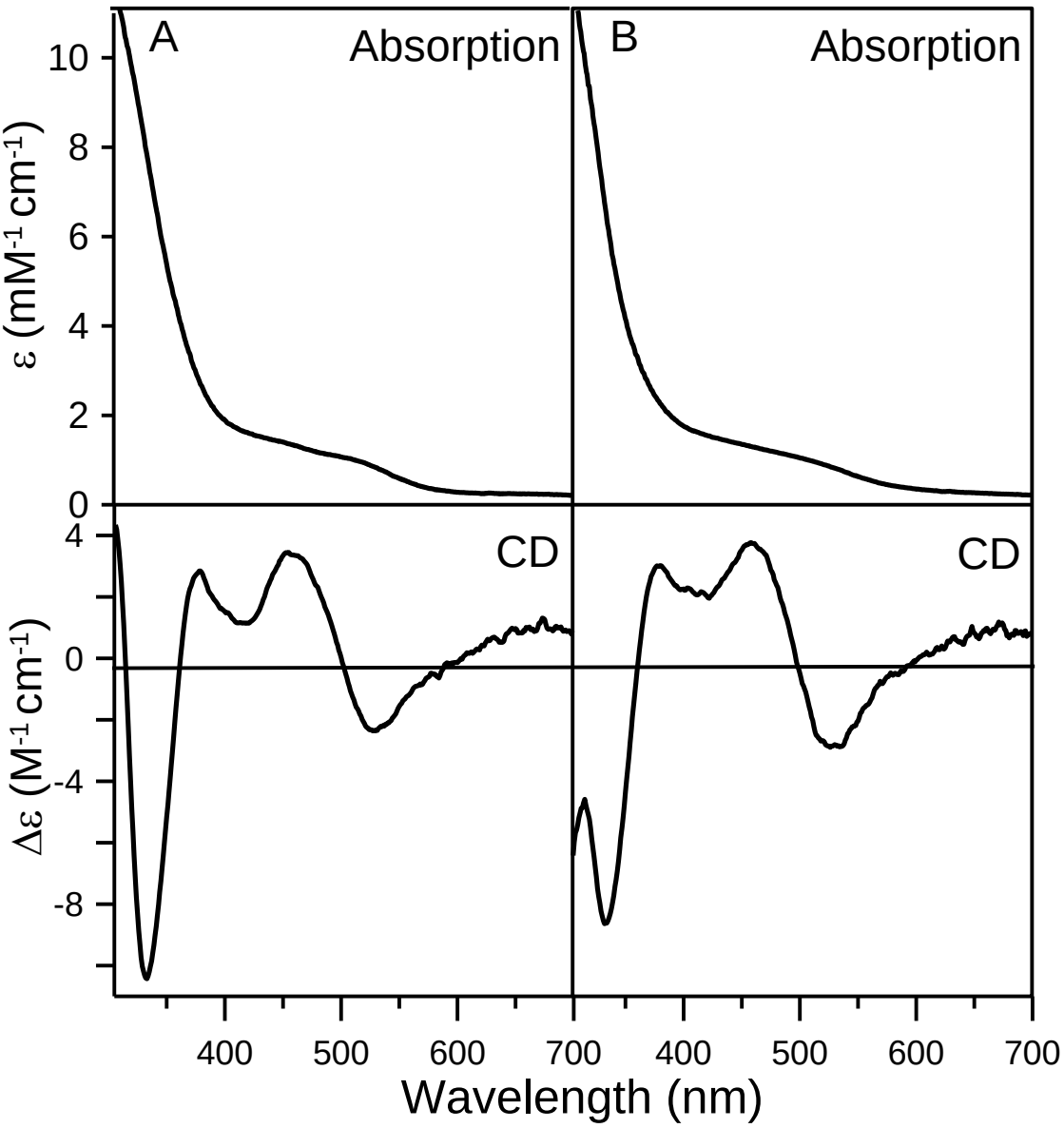

A
Absorption
B
Absorption
CD
CD
ε (mM-1 cm-1)
Δε (M-1 cm-1)
10
8
6
4
2
0
4
0
-4
-8
400
500
600
700
400
500
600
700
Wavelength (nm)

Figure 2.3: Comparison of the UV-visible CD spectra of the Fe(III)-bound *Av* ^{Nif}IscA (black line), *At* SufA1 (red line) and *Av* IscA (blue line). Spectra were recorded under anaerobic conditions in sealed 0.1 cm cuvette in 100 mM Tris-HCl buffer with 250 mM NaCl at pH 7.8. All $\Delta\varepsilon$ values are based on the Fe concentrations.

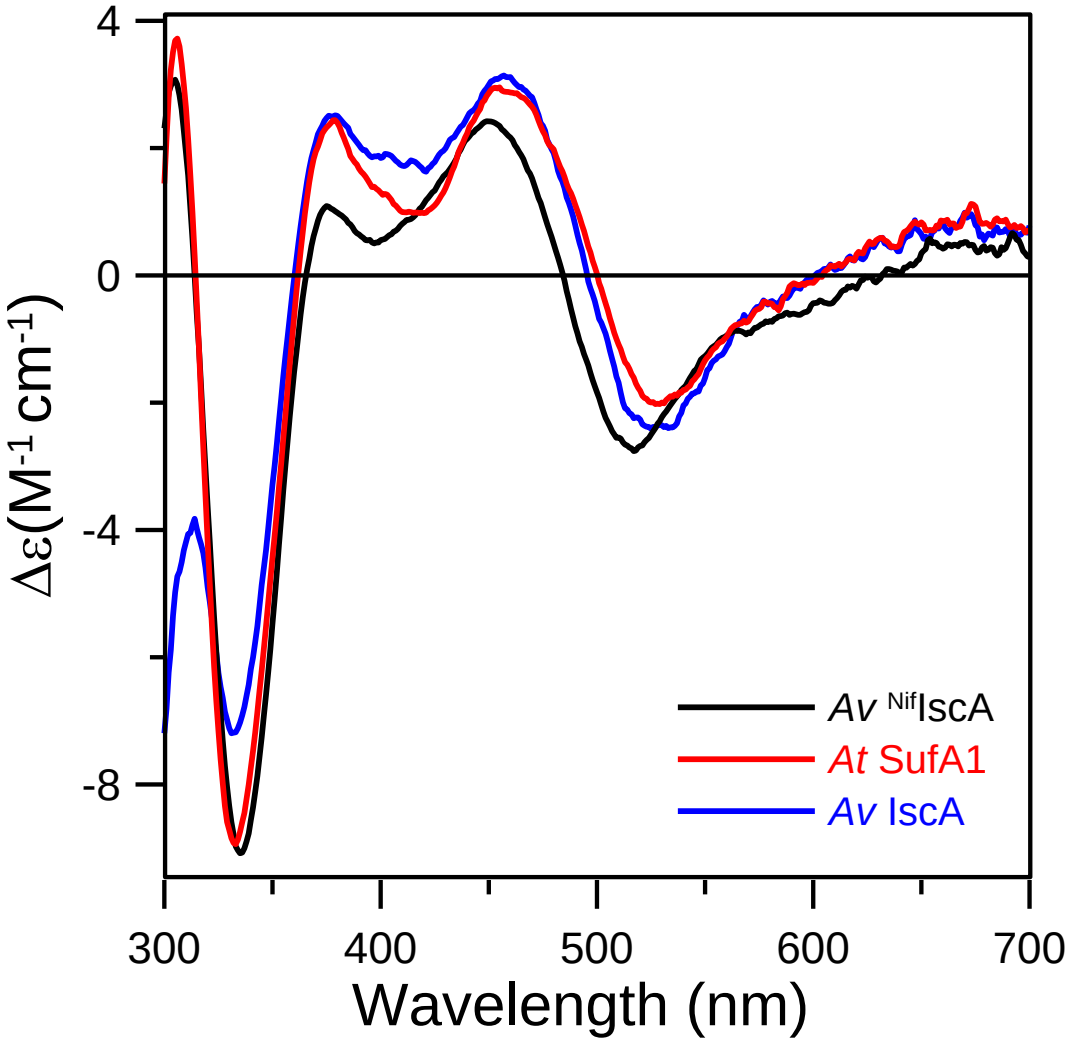
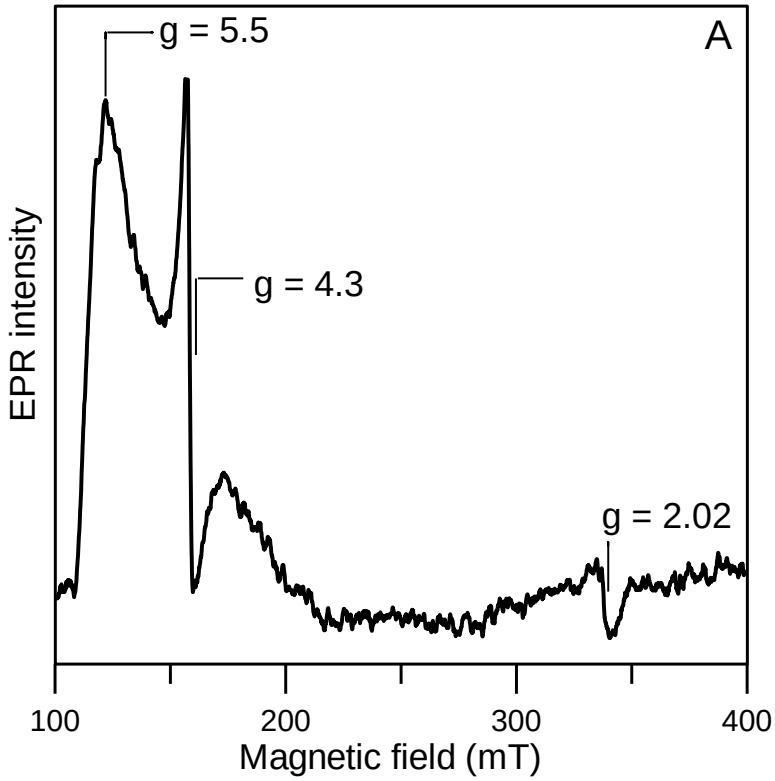

4
0
-4
-8
Δε(M-1 cm-1)
300
400
500
600
700
Wavelength (nm)
Av NifIscA
At SufA1
Av IscA

Figure 2.4: X-band EPR spectra of Fe(III)-bound *At* SufA1 and *Av* IscA. (A) EPR spectrum of Fe(III) bound SufA1 (1.30 mM) recorded at 4.5 K and a microwave frequency of 9.60 GHz, with modulation amplitude of 0.65 mT and microwave power of 20 mW. (B) EPR spectrum of Fe(III) bound IscA (1.38 mM) recorded at 5.0 K and a microwave frequency of 9.60 GHz, with modulation amplitude of 0.65 mT and microwave power of 20 mW.


A
g = 5.5
g = 4.3
g = 2.02
EPR intensity
100
200
300
400
Magnetic field (mT)

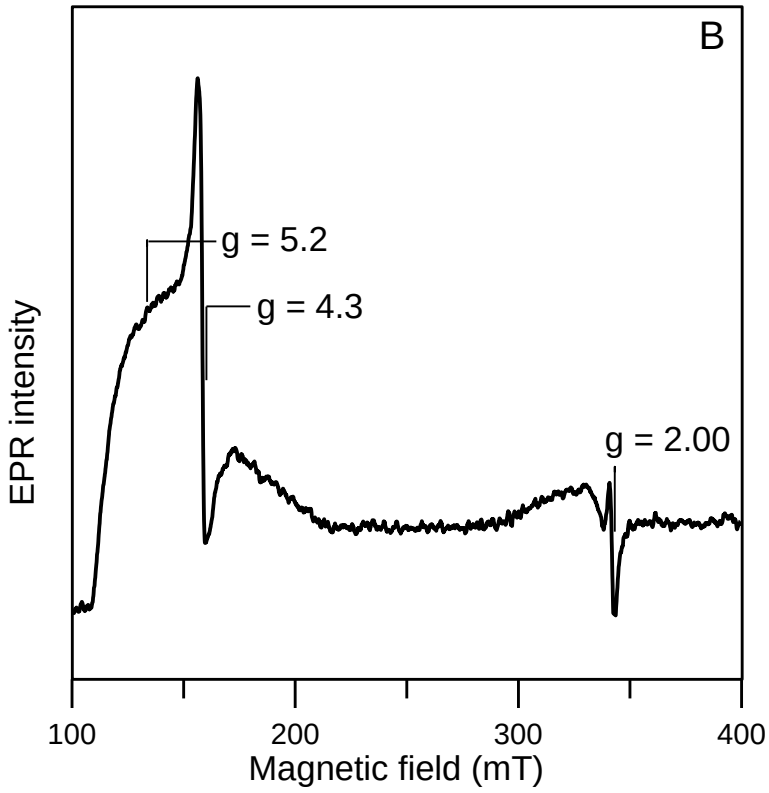

B
g = 5.2
g = 4.3
g = 2.00
EPR intensity
100
200
300
400
Magnetic field (mT)

Figure 2.5: Comparison of the resonance Raman spectra of *C. pasteurianum* C6D rubredoxin, Fe(III)-bound ^{Nif}IscA, and Fe(III)-bound SufA1. Spectra were recorded at 17 K with 458-nm excitation, using samples that were 3-4 mM in protein monomer. Each spectrum is the sum of 100 scans, with each scan involving photon counting for 1 s every 0.5 cm^{-1} with a spectral bandwidth of 7 cm^{-1}. Raman bands originating from the frozen buffer solution have been subtracted from both spectra.

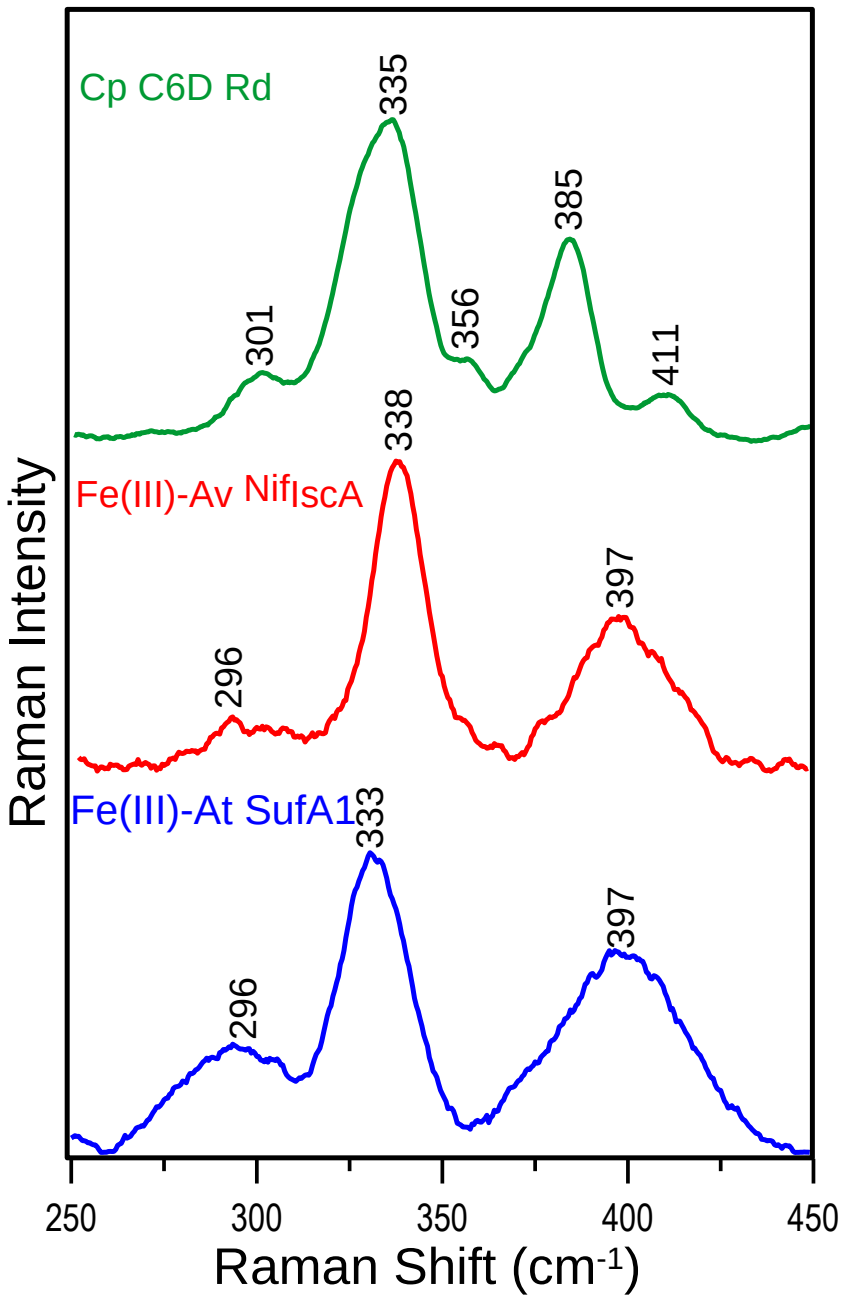

Cp C6D Rd
335
385
301
356
411
Fe(III)-Av NifIscA
338
397
296
Fe(III)-At SufA1
333
397
296
Raman Intensity
250
300
350
400
450
Raman Shift (cm-1)

Figure 2.6: UV–visible absorption and CD spectra of purified [2Fe-2S] cluster-bound form of *At* SufA1. Spectra were recorded under anaerobic conditions in sealed 0.1 cm cuvette in 100 mM Tris-HCl buffer with 250 mM NaCl at pH 7.8. ε values are based on the concentration of *At* SufA protein monomer where as Δε values are based on [2Fe-2S] cluster concentrations.

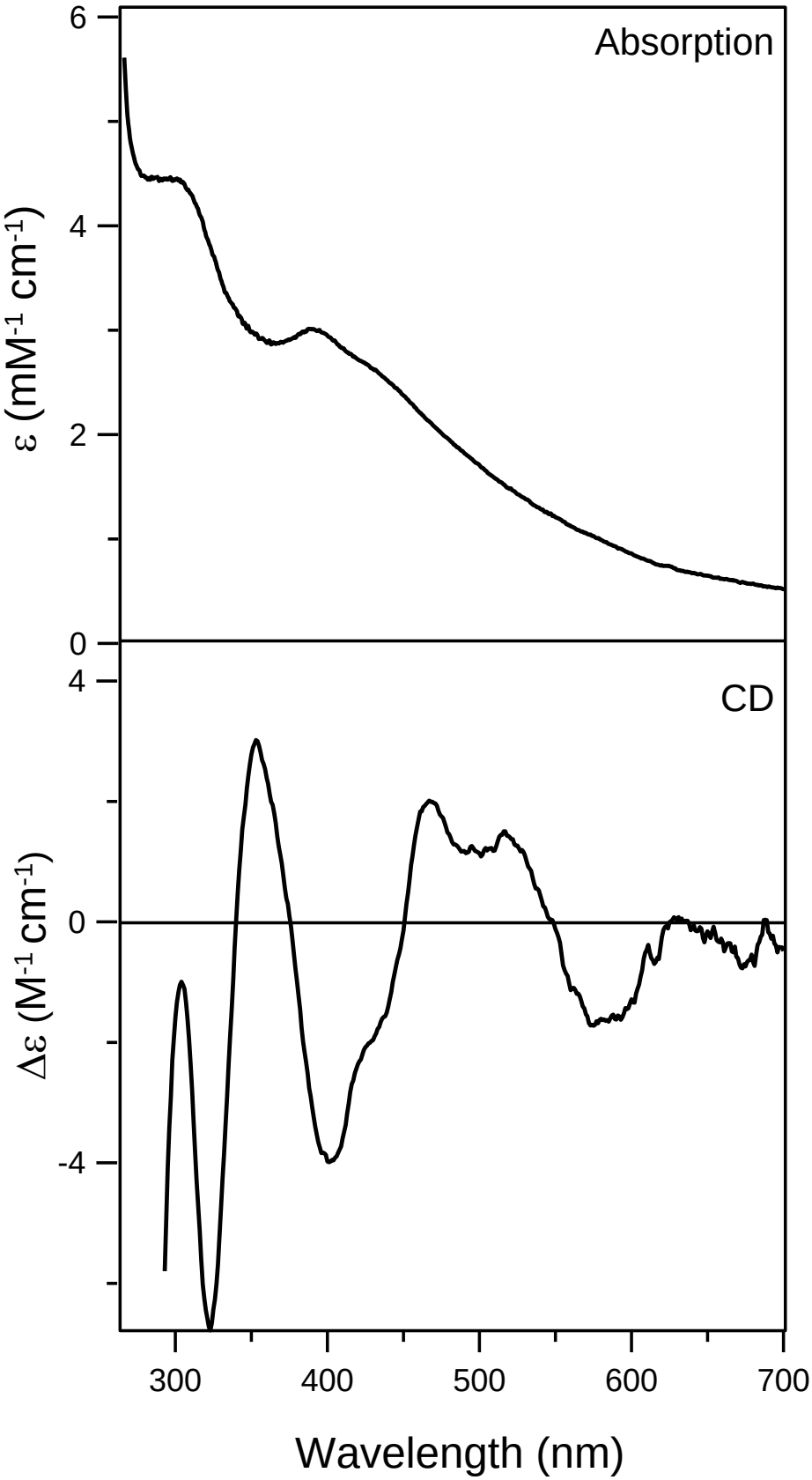

Absorption
ε (mM-1 cm-1)
6
4
2
0
CD
Δε (M-1 cm-1)
4
0
-4
300
400
500
600
700
Wavelength (nm)

Figure 2.7: Comparison of the UV−visible CD spectra of [2Fe-2S] cluster-bound *Av* ^{Nif}IscA (black line) and *At* SufA1 (red line). Spectra were recorded under anaerobic conditions in sealed 0.1 cm cuvette in 100 mM Tris-HCl buffer with 250 mM NaCl at pH 7.8. All the Δε values are based on [2Fe-2S] cluster concentrations.

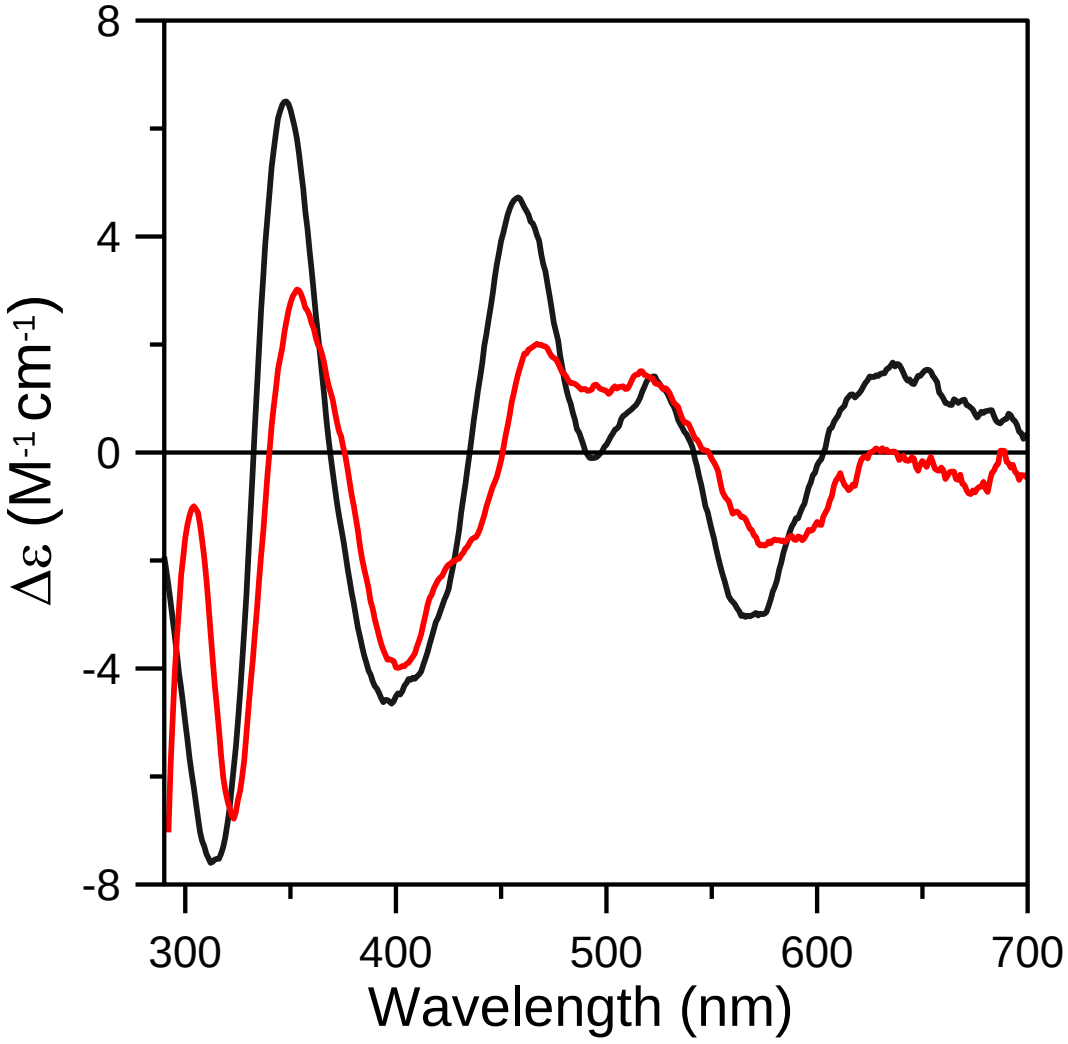

8
4
0
-4
-8
Δε (M-1 cm-1)
300
400
500
600
700
Wavelength (nm)

Figure 2.8: Resonance Raman spectra of purified [2Fe-2S] cluster-bound form of *At* SufA1. Raman spectra were recorded at 17 K with 457.9, 487.9 and 514.5 nm laser excitation with ~ 200 mW laser power at the sample. The sample (~ 2 mM in *At* SufA1 monomer) was in 100 mM Tris-HCl buffer, pH 7.8 containing ~ 0.45 M NaCl. Each spectrum is a sum of 100 scans, with each scan involving counting photons for 1 s at 0.5 cm^{-1} increments, with 6 cm^{-1} spectral resolution. Bands due to lattice modes of ice from frozen buffer in sample have been subtracted from all spectra.

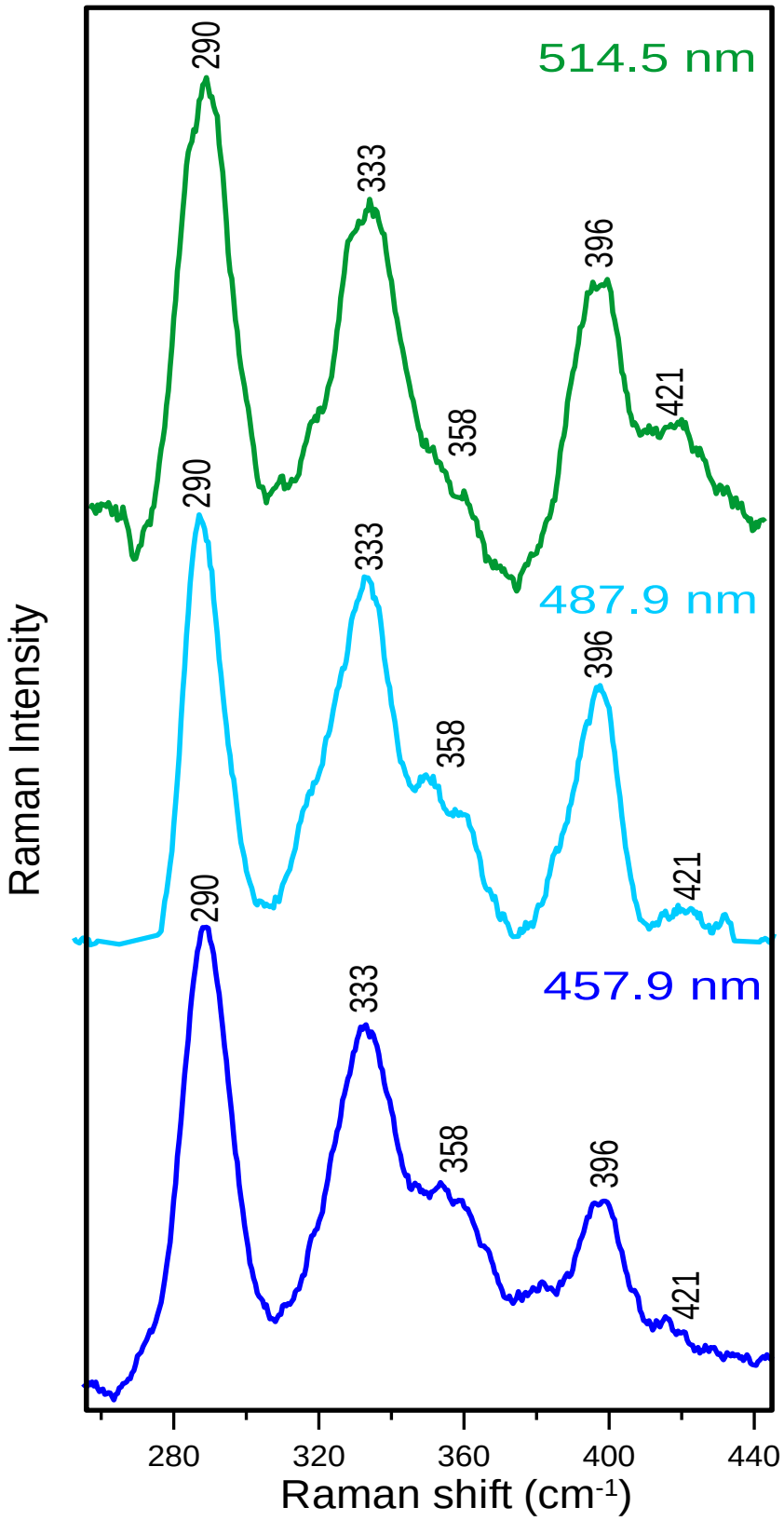

514.5 nm
290
333
358
396
421
487.9 nm
290
333
358
396
421
457.9 nm
290
333
358
396
421
Raman Intensity
280
320
360
400
440
Raman shift (cm-1)

Figure 2.9: Comparison of resonance Raman spectra of purified [2Fe-2S] cluster-bound form of *At* SufA1 (red) with [2Fe-2S] cluster-bound form of ^{Nif}IscA (black). Raman spectra were recorded at 17 K with 457.9, 487.9 and 514.5 nm laser excitation with ~ 200 mW laser power at the sample. The samples (~ 2 mM in *At* SufA1 monomer and ~ 4 mM in *Av* ^{Nif}IscA monomer) were in 100 mM Tris-HCl buffer, pH 7.8 containing ~ 0.45 M NaCl. Each spectrum is a sum of 100 scans, with each scan involving counting photons for 1 s at 0.5 cm^{-1} increments, with 6 cm^{-1} spectral resolution. Bands due to lattice modes of ice from frozen buffer in sample have been subtracted from all spectra.

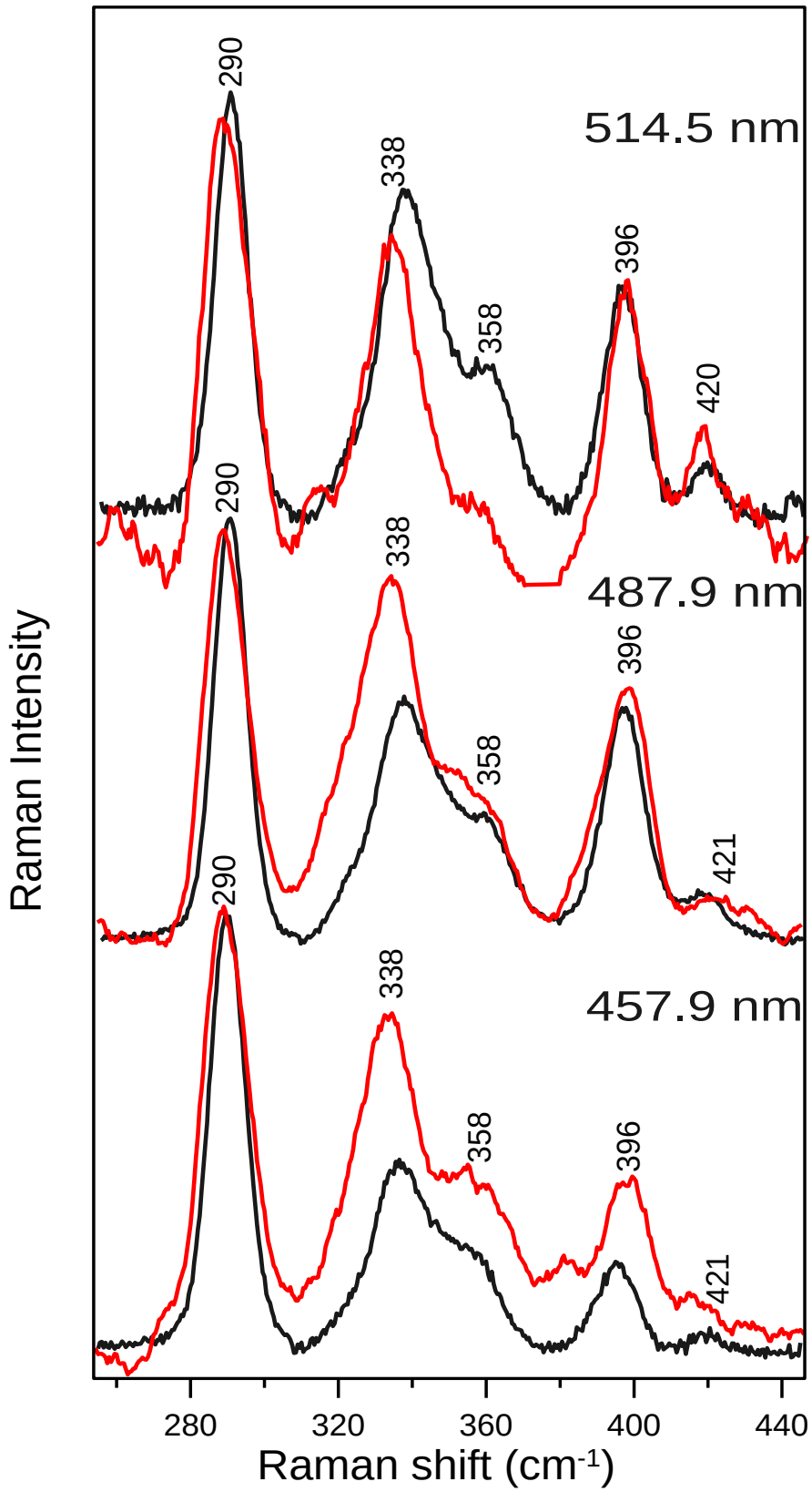

514.5 nm
487.9 nm
457.9 nm
290
338
358
396
420
421
Raman Intensity
280
320
360
400
440
Raman shift (cm-1)

Figure 2.10: Comparison of the UV−visible CD spectra of the [2Fe-2S] cluster-bound *At* SufA1 (blue line) with [2Fe-2S] cluster-bound *At* GrxS14 (red line). Spectra were recorded under anaerobic conditions in sealed 0.1 cm cuvette in 100 mM Tris-HCl buffer with 250 mM NaCl at pH 7.8. Δε values are based on [2Fe-2S] cluster concentrations.

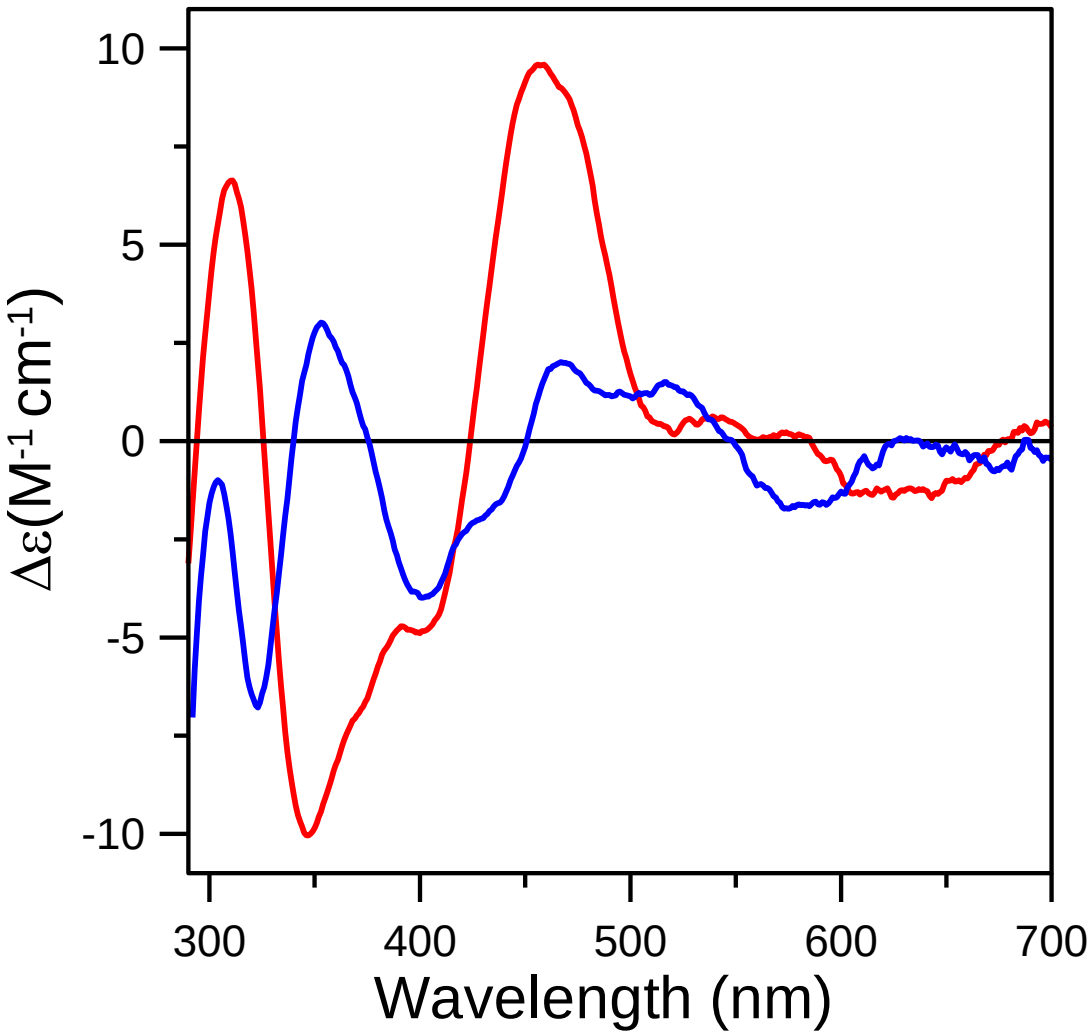

10
5
0
-5
-10
Δε(M-1 cm-1)
300
400
500
600
700
Wavelength (nm)

Figure 2.11: Simulated CD spectra corresponding to quantitative [2Fe-2S] cluster transfer from [2Fe-2S] GrxS14 to apo-SufA1. The red and blue lines correspond to [2Fe-2S] cluster-bound GrxS14 and SufA1, respectively, whereas the thin grey lines correspond to 10-90% intact cluster transfer in 10% increments. Spectra were recorded under anaerobic conditions in sealed 0.1 cm cuvette in 100 mM Tris-HCl buffer with 250 mM NaCl at pH 7.8. $\Delta\varepsilon$ values are based on the [2Fe-2S] cluster concentration.

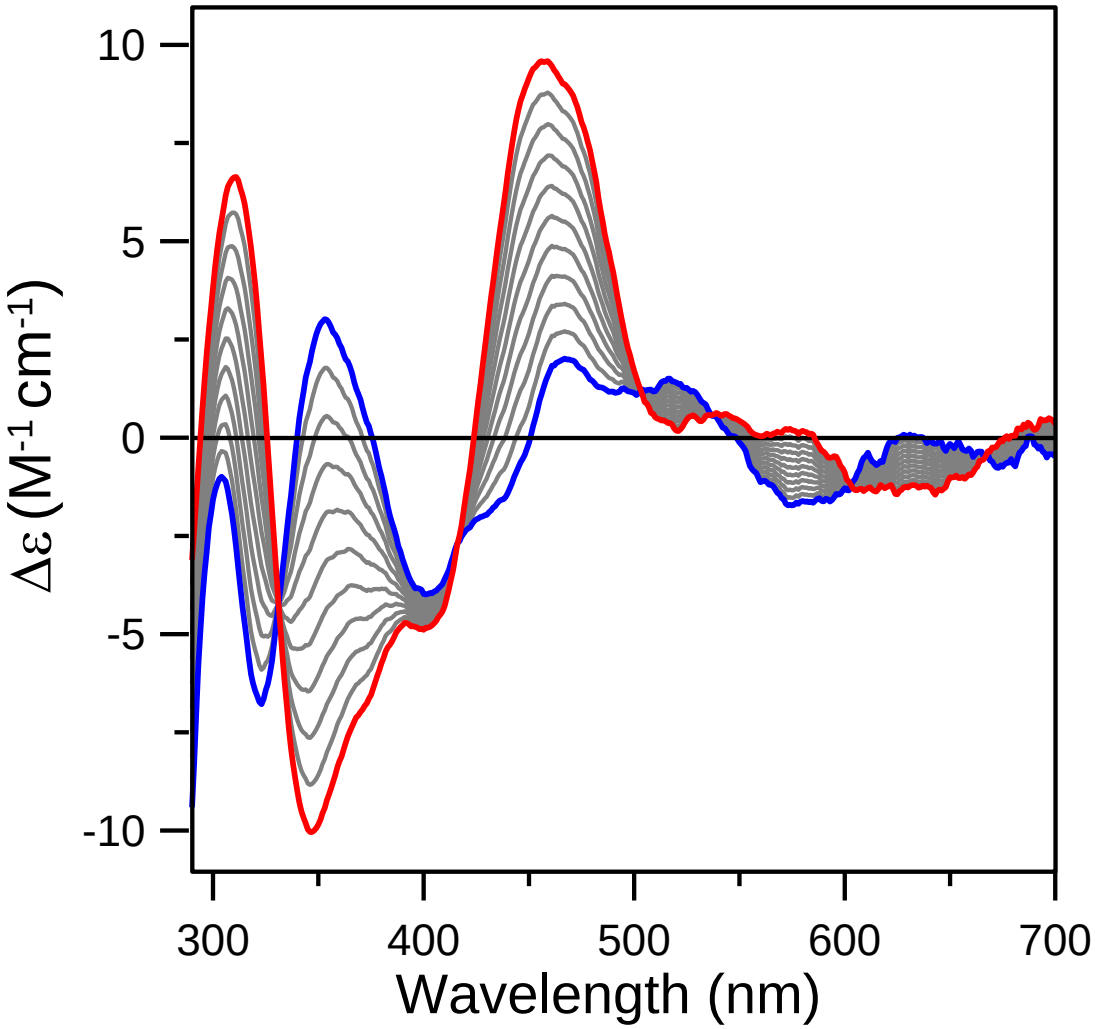

10
5
0
-5
-10
Δε (M-1 cm-1)
300
400
500
600
700
Wavelength (nm)

Figure 2.12: Time course of cluster transfer from *At* [2Fe-2S] GrxS14 to apo-*At* SufA1 in the presence of 5 mM DTT monitored by UV-visible CD spectroscopy at room temperature. The red spectra correspond to [2Fe-2S] GrxS14 in the presence of DTT whereas the time course of cluster transfer is shown by thin-line grey spectra. CD spectra were recorded at 3, 6, 10, 14, 18, 22, 26, 30, 40, 50 and 60 min after the addition of apo-SufA to the reaction mixture. Spectra were recorded under anaerobic conditions in sealed 1 cm cuvette in 100 mM Tris-HCl buffer with 250 mM NaCl at pH 7.8. $\Delta\varepsilon$ values are based on the initial [2Fe-2S] cluster concentration on GrxS14.

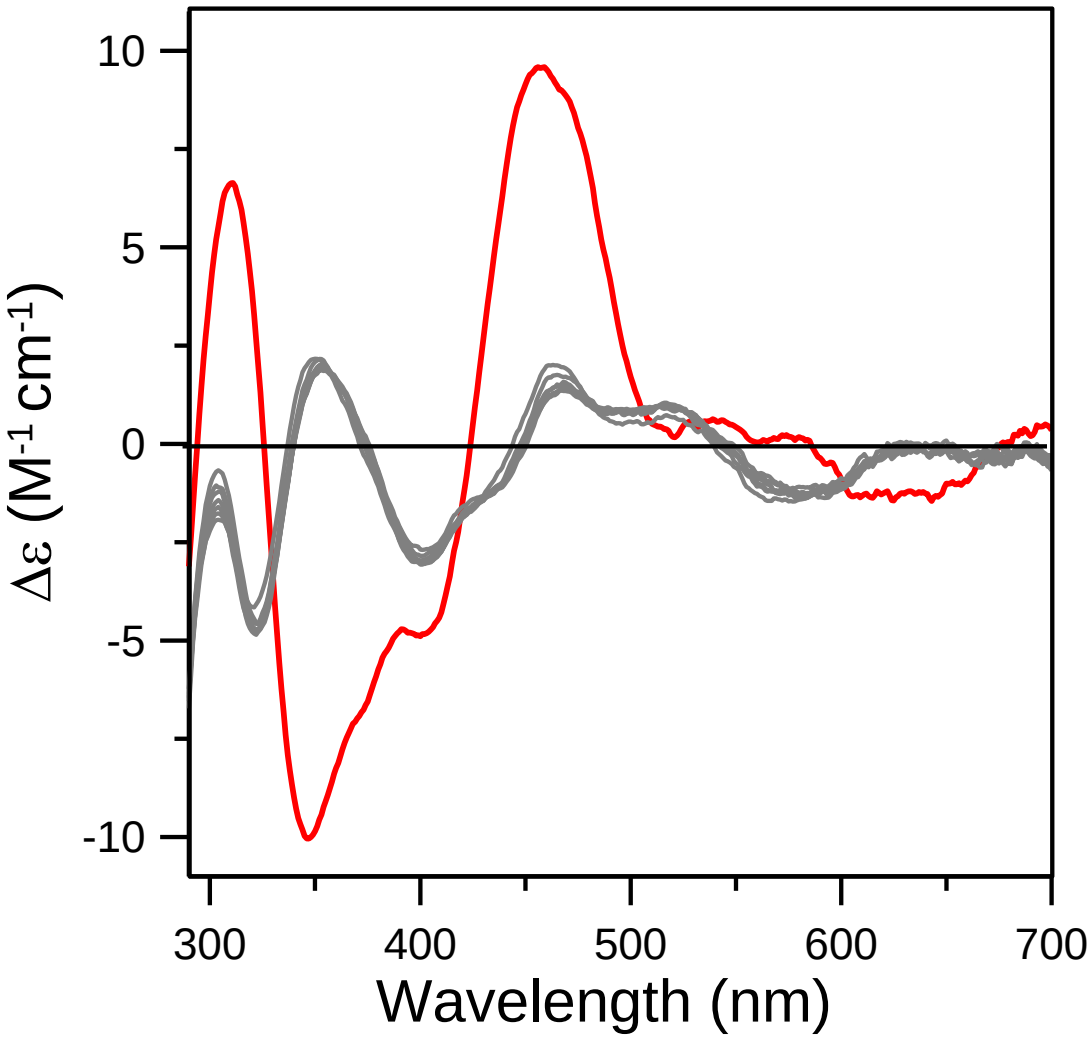

10
5
0
-5
-10
Δε (M-1 cm-1)
300
400
500
600
700
Wavelength (nm)

Figure 2.13: Kinetic analysis of [2Fe-2S] cluster transfer from holo-*At* GrxS14 to apo-*At* SufA1 monitored by CD spectroscopy. The conditions are given in Figure 2.12, and the data points correspond to the extent of [2Fe-2S] SufA1 formation as a function of time, as determined by the CD intensity at 348 nm. Solid line corresponds to the best fit to second-order kinetics based on the initial [2Fe-2S] cluster concentration on GrxS14 (0.050 mM) and the initial concentration of dimeric apo-SufA1 (0.050 mM) with a rate constant of 50,000 M^{-1} min^{-1}.

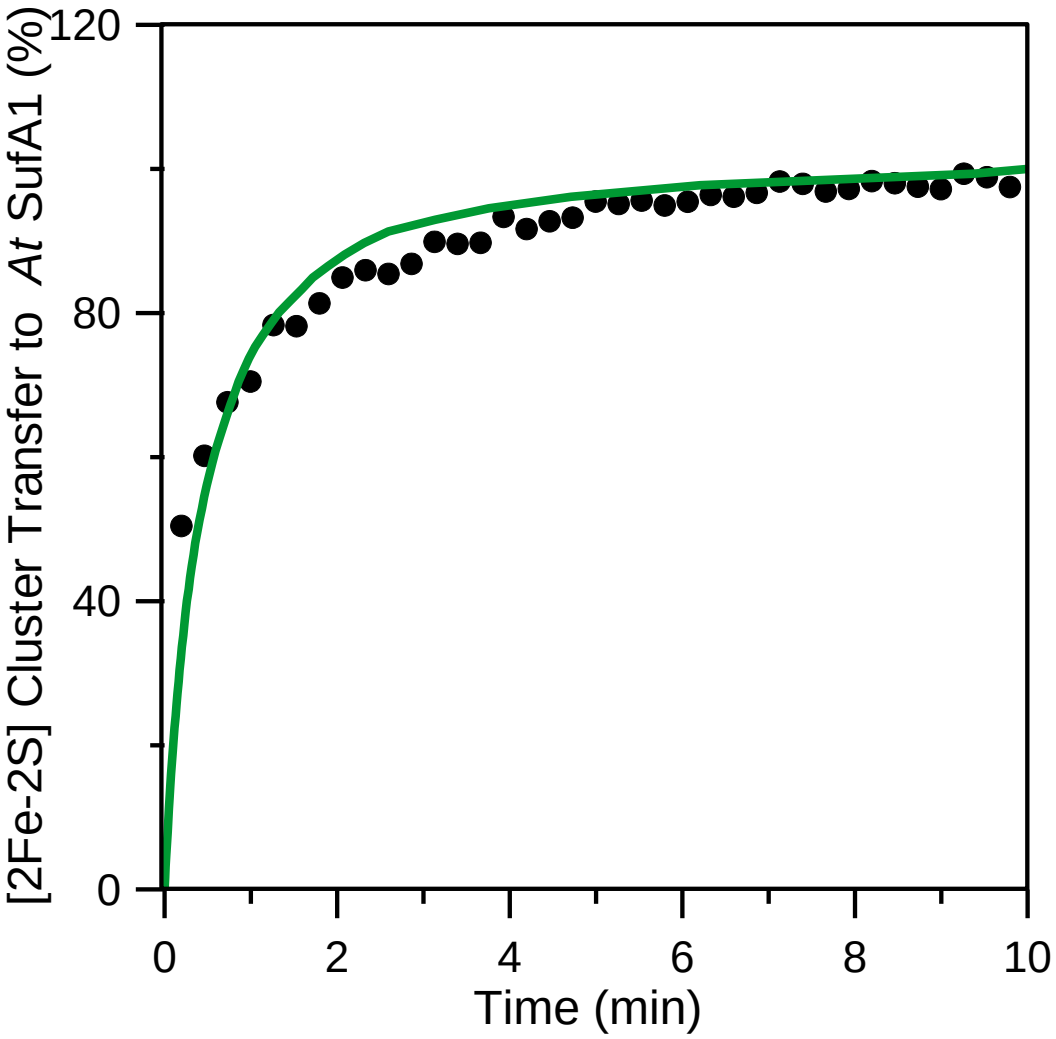
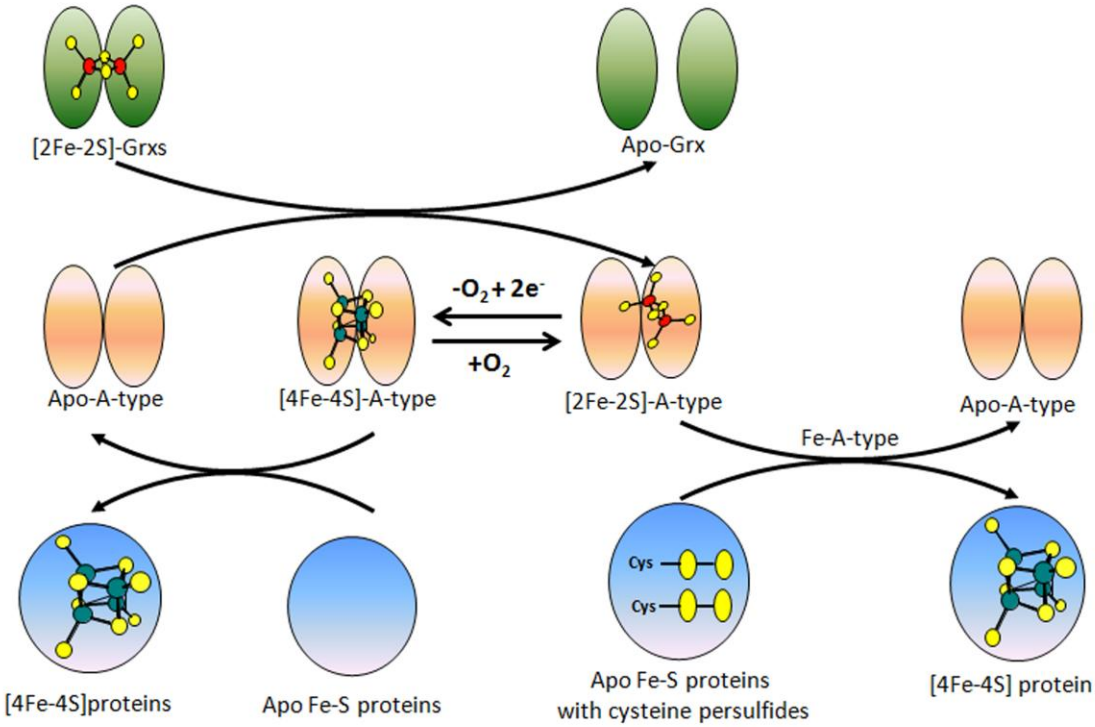

[2Fe-2S] Cluster Transfer to At SufA1 (%)
120
80
40
0
0
2
4
6
8
10
Time (min)

Figure 2.14: Schematic proposal for the role of A-type proteins in cluster trafficking leading to the maturation or repair of [4Fe-4S] cluster-containing proteins, see text for details.


[2Fe-2S]-Grxs
Apo-Grx
Apo-A-type
[4Fe-4S]-A-type
-O2 + 2e-
+O2
[2Fe-2S]-A-type
Apo-A-type
Fe-A-type
[4Fe-4S]proteins
Apo Fe-S proteins
Cys
Cys
Apo Fe-S proteins
with cysteine persulfides
[4Fe-4S] protein