Iron−Sulfur Cluster Biosynthesis: Toward an Understanding of Cellular Machinery and Molecular Mechanism†

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ABSTRACT
Iron−sulfur clusters are among the most complex metal-containing prosthetic centers in biology. Most if not all of the proteins involved in the biosynthesis of "simple" Fe−S clusters have been identified. The structural and functional chemistry of these proteins has been the subject of intense research efforts, and many of the key details are now understood in structural and mechanistic detail. The fact that Fe−S cluster-binding proteins can be reconstituted in vitro with no accessory proteins provides an important indicator of the intracellular roles for many proteins on the Fe−S cluster assembly pathway. Indeed, such proteins are more correctly viewed as carrier proteins, rather than as catalysts for the reaction, that both avoid the toxicity associated with free iron and sulfide and allow delivery at lower intracellular concentrations of these species. The IscU (or ISU) family of proteins serves a key role as scaffolding proteins on which [2Fe−2S] building blocks are assembled prior to transfer to final apo target proteins. IscU in particular exhibits highly unusual conformational flexibility that appears critical to its function.

Iron−Sulfur Cluster Proteins: Biological Relevance and Biosynthesis

Iron−sulfur cluster proteins are widely distributed in nature and can be found in anaerobic, aerobic, and photosynthetic bacteria, fungi, plants, and mammals. They are among the most ancient of metallocofactors[1] and serve a variety of biological roles, including electron transport, catalytic, structural, and sensory roles. The most common Fe−S clusters include [2Fe−2S], [3Fe−4S], and [4Fe−4S] centers where the iron ions are typically tetrahedrally coordinated by thiolate ligands of cysteine side chains with additional coordination to each iron provided by inorganic sulfides. Rubredoxin is the exception with its single iron center tetrahedrally coordinated by cysteines. Although protein ligation is typically provided by cysteine residues, there are several examples of non-cysteinyl ligands to Fe−S clusters, including histidine ligation to [2Fe−2S] and [4Fe−4S] clusters[2,3] and oxygen coordination to [4Fe−4S] clusters.[4]

Interest in the biological chemistry of Fe−S clusters has evolved from biological redox chemistry to enzymology (aconitase and mutY) and gene regulatory functions (IRP and soxR). Understanding the biosynthesis of these complex cofactors is the most recent manifestation of interest in their biological chemistry and forms part of a broader interest in the biogenesis of metal cofactors and the intracellular trafficking of metal ions and cofactors. Proteins that deliver discrete metal ions to target proteins, including copper, nickel, and manganese, constitute a growing family of metal transporters.[5−7] However, many proteins and enzymes contain complex metal cofactors that must themselves be the product of a biosynthetic assembly apparatus. Recent advances in this area include the characterization of operons that define gene products responsible for the biosynthesis of nitrogenase cofactors,[8,9] heme,[10] and iron−sulfur clusters.[11−13]

While understanding the biosynthetic mechanism for cluster formation is an important topic in its own right, it takes on added significance with the realization that many well-characterized diseases have been implicated with the inability of cells to biosynthesize iron−sulfur clusters. Typically these result from a defect in one or more of the gene products involved in either iron transport to or from the mitochondrion or the biosynthetic apparatus for cluster assembly. In particular, the inability to synthesize clusters results in deleterious effects through the absence of cluster-containing proteins in essential respiratory pathways and cytosolic metabolism.[14,15]

Cast of Characters in Iron−Sulfur Cluster Biosynthesis

Recent efforts have led to significant advances in unraveling the intricate physiological pathways responsible for intracellular metal trafficking. Such pathways ensure appropriate coordination of Cu, Ni, and Zn ions and heme to their respective protein targets,[16−19] thereby avoiding the toxic effects of free metal, especially the generation of free radicals via Fenton-like reactions that can result in the degradation of nucleic acids, proteins, and lipids. Proteins responsible for coordination and delivery of their metal cargo to target apo proteins have been termed metallochaperones[20,21] (see Comparisons with Other Metallochaperones" for a discussion of this terminology).

For bacterial Fe−S clusters biosynthesis, three separate systems have been identified, including ISC (iron−sulfur cluster),[11] NIF (nitrogen fixation),[22] and SUF (sulfur).[23] Of these systems, ISC appears to provide a general mechanism for Fe−S biosynthesis whereas NIF is solely involved in cluster maturation of nitrogenase and SUF seems to

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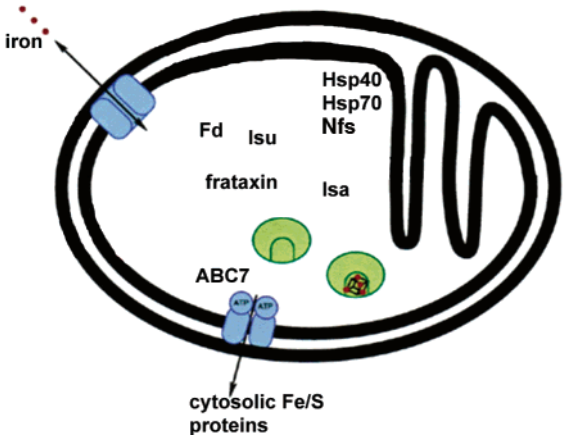

FIGURE 1. Summary of key proteins in *Hs* mitochondrial Fe−S cluster assembly and trafficking, including electron carriers (Fd), chaperones (Hsp40, Hsp70), S donors (IscS), Fe donors (frataxin), scaffolding proteins (ISU and ISA), putative cluster export proteins (ABC7), and an iron importer.

reflect a minor Fe−S biosynthesis pathway. ISC homologues have been identified in most sequenced genomes from bacteria and archeae to plants and humans[24] and thus likely represent a fundamental Fe−S cluster biosynthesis pathway. ISC-encoded proteins include the transcriptional repressor IscR,[25] a sulfur donor IscS (NFS for eukaryotes),[11] the Fe−S scaffolding proteins IscU[26,27] and IscA[28−30] (ISU and ISA, respectively, for eukaryotes), molecular chaperones[31] and a [2Fe−2S] ferredoxin.[32] Intracellular Fe−S levels in many bacteria are sensed by IscR, which in turn regulates the expression of the *isc* operon.[25] Since IscR coordinates a [2Fe−2S], it has been hypothesized that a low demand for Fe−S cluster assembly would lead to elevated levels of holo IscR and thus repression of the *isc* operon. This repression would be relieved when insufficient ISC components are available to reconstitute apo Fe−S proteins, including IscR.[25] Interestingly, an analogous regulatory protein has not yet been identified in eukaryotic cells.

In eukaryotes, ISC-encoded proteins are nuclearly transcribed but are predominantly localized within the mitochondrial matrix (Figure 1),[12,33] although there are some reports of *S. cerevisiae* ISA2 localization in the mitochondrial intermembrane space[34] and cytosolic ISU and NFS in human cell lines.[35,36] An alternative cytoplasmic Fe−S cluster maturation protein (Cfd1) has also been proposed.[37] Whether the cytosolic or intermembrane components represent an independent assembly apparatus or cluster transport roles in the case of ISU and ISA is unclear. Nevertheless, both intra- and extramitochondrial Fe−S proteins appear to depend on mitochondrial ISC components for the biosynthesis of their metallocofactors.[15,38] While studies of ISC homologues in the *nif* operon[22] have shown IscS and IscU to be the most critical proteins for Fe−S cluster assembly, the characterization of other key proteins and their functional roles in cluster assembly is less well defined and has been the subject of our own research efforts.

IscU as a [2Fe−2S] Cluster Scaffold. Initial evidence for the function of IscU (prokaryotic) [or ISU (eukaryotic)] was based on genetic and cell biology studies that implicated IscU in Fe−S cluster biosynthesis.[39,40] However, these studies could not differentiate between IscU functioning as an iron or an iron−sulfur cluster delivery protein. Given our long-standing interest in Fe−S biochemistry and mechanisms of cluster assembly and disassembly,[41−43] we initiated a program to compare and contrast the properties of IscU from a variety of sources, including human (*Hs* ISU), yeast (*Schizosaccharomyces pombe*, *Sp* ISU), and a hyperthermophilic bacterium (*Thermotoga maritima*, *Tm* IscU). In agreement with Dean and colleagues,[27] we found that IscU coordinates one reductively labile $[2Fe-2S]^{2+}$ cluster per monomeric subunit that is stabilized by substitution of a highly conserved aspartate (D37 for *Hs* ISU and *Sp* ISU, and D40 for *Tm* IscU), as judged by Mössbauer, EXAFS, EPR, and UV−vis spectroscopies.[44,45] Our studies of cluster stability and cluster transfer suggests that this highly conserved acidic residue maintains a solvent-accessible channel to the cluster.[46] IscU proteins are generally dimers,[47,48] with the exception of human ISU, which is a monomer.[44] IscU contains three highly conserved cysteines with some eukaryotic homologues carrying an additional cysteine residue. Since Fe−S clusters typically require four cysteine ligands, it seemed plausible that the three cysteine-containing IscUs coordinate one interfacial Fe−S cluster between two monomeric subunits while four cysteine-containing IscUs bind one cluster per monomer. However, site-directed mutagenesis studies,[44] iron-to-protein ratios,[44,45] and correlations of cysteine content to oligomeric state[45] indicate that one Fe−S cluster coordinates to one monomeric subunit of IscU regardless of cysteine content. Also, comparison of UV−vis absorption, near-UV−vis CD, and Mössbauer spectra are consistent with a similar Fe−S cluster environment for all IscUs studied thus far. Such data, and the similarity of CD spectra of the cluster region (300−700 nm) with those of Cys to Ser ferredoxin mutants,[26] are consistent with oxygen ligation (possibly by water) to one coordination site of the Fe−S cluster.

Data from in vitro biochemical and spectroscopic studies[27,44,45,49] coupled with results from in vivo experiments[33,39,40,50] strongly suggested that IscU is a biological scaffold for Fe−S cluster assembly. Further support for such a role came from far-UV CD[26] and NMR[48] studies, which showed essentially identical secondary structural content and similar tertiary structural and dynamic properties for apo and holo *T. maritima* IscU, i.e., unlike other low molecular weight metalloproteins, IscU retains its tertiary structure in the apo state that is preformed for Fe−S cluster coordination.

[2Fe−2S] Cluster Assembly on IscU. In order for IscU to deliver Fe−S clusters to target proteins, it must first acquire iron and sulfide and assemble these components before finally delivering intact Fe−S units to target proteins. The sulfur donor for this process is IscS, a pyridoxal 5′-phosphate-containing enzyme that converts L-cysteine to L-alanine and sulfur via an enzyme-bound cysteine

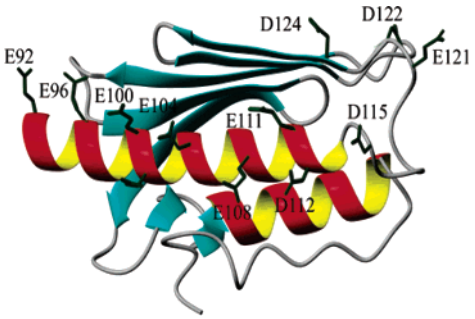

FIGURE 2. Ribbon diagram of *Hs* frataxin (PDB ID: 1EKG) showing surface carboxylates that are putative binding sites for iron ions.

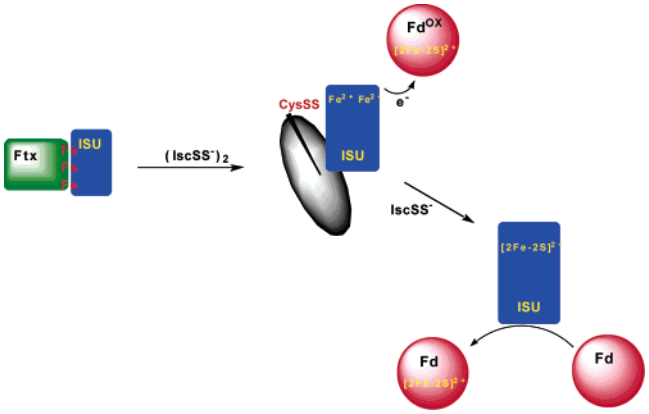

FIGURE 3. Schematic of Fe−S biosynthesis on ISU and subsequent delivery to a target ferredoxin.

persulfide intermediate.[51] However, the identity of a natural iron donor was not known until recently. Consistent with previous reports that implicated the mitochondrial protein frataxin in iron homeostasis,[52] we found that human frataxin is capable of binding up to 6−8 iron ions per protein equivalent with apparent dissociation constants between 10 and 55 μM.[53] Moreover, fluorescence quenching and isothermal titration calorimetry experiments demonstrated that frataxin complexes to one equivalent of ISU with submicromolar binding affinity but only in the presence of iron ions. Since there is no evidence for an iron-promoted structural change in frataxin,[54,55] this observation suggests that iron may assist in cross-linking frataxin to ISU, presumably by ligation to surface carboxylate residues on each protein. Frataxin clearly displays an extensive array of carboxylate residues on one face of the protein (Figure 2). We have also shown that human frataxin is able to mediate the delivery of iron to human ISU for iron−sulfur cluster biosynthesis and that iron transfer is likely to be the rate-limiting step for cluster assembly on ISU since the rate of cluster assembly is comparable to the rate of abstraction of ferrous iron from frataxin by small molecule chelators.[53] Interestingly, our in vitro data is consistent with *Saccharomyces cerevisiae in organello* and in vivo studies by Lill and colleagues in which they too proposed a role for frataxin in Fe−S biosynthesis, although the precise function was not established.[56,57] With known iron and sulfide donors in hand and by successful reconstitution of the biosynthesis system in vitro, we proposed a working model for Fe−S cluster assembly on ISU[53] (Figure 3). Initial studies led to speculation that the first step in Fe−S cluster biogenesis is IscS-mediated sulfur delivery to IscU.[58,59] Such a labeling does in fact occur, although the resulting persulfide form does not bind iron, and so we believe that this constitutes a dead-end product rather than a physiologically relevant intermediate.[60] IscU can, in fact, bind iron ions prior to sulfide delivery, while subsequent addition of sulfide (inorganic sulfide- or protein-mediated delivery) does promote cluster formation, and so we feel that the chemically more reasonable pathway is frataxin-mediated iron delivery to ISU followed by IscS-mediated sulfide delivery (Figure 3). More specifically, cluster assembly is initiated by the delivery of two iron ions to apo ISU from holo frataxin. Subsequently, ISU complexes with the persulfide form of IscS. Two IscS persulfide bonds are then reductively cleaved yielding two equivalents of inorganic sulfide for the nascent Fe−S cluster. Since frataxin homologues are encoded in the genomes of a wide variety of prokaryotic and eukaryotic organisms, such a mechanism likely represents a fundamental physiological process.[53] However, it is of some significance that not all organisms appear to encode frataxin homologues, and so another protein must serve an analogous role to frataxin.

IscU-Mediated Cluster Transfer to a Target Ferredoxin. In vivo studies and the identification of a labile [2Fe−2S] clearly implicated IscU in Fe−S cluster delivery. Further support was provided by EDC cross-linking between human and yeast ISU with ferredoxin.[45] EDC is a zero-length cross-linker that forms amide bonds between aspartate/glutamate side chains and lysine.[61] Our finding of EDC-promoted cross-linking of these two proteins is good initial evidence for a specific interaction that is substantially mediated by a complementarity of acidic and basic residues. Interestingly, formation of a cross-linked species was found to depend on ISU metal content. For example, holo ISU (both human and yeast) cross links with both apo and holo human ferredoxin (*Hs* Fd). However, apo ISU does not cross link with either apo or holo *Hs* Fd.[45] Such data is consistent with a protein complex involved in cluster transfer from holo ISU to apo Fd. Indeed, we have directly shown this cluster transfer event to occur.[46] Through the use of an ^{57}Fe-labeled cluster, [2^{57}Fe−2S], we demonstrated direct transfer of intact cluster (Figure 4) from holo *Hs* ISU to apo *Hs* Fd by Mössbauer spectroscopy.[46] Since the reaction was carried out in the presence of background natural abundance Fe^{2+}, only direct cluster transfer from ISU to Fd was detected, i.e., the transfer mechanism does not involve ISU Fe−S cluster release into solution followed by reconstitution of iron and sulfide components on ferredoxin but rather direct transfer of intact cluster between proteins.

It has been suggested that holo Fd may reduce and thus facilitate the release of IscU-bound Fe−S clusters, which is consistent with the holo−holo interactions observed by cross-linking. However, incubation of holo ISU with reduced Fd does not induce cluster release even in the presence of NADPH/Fd reductase.[46] The available data is most consistent with a mechanism in which Fd accepts

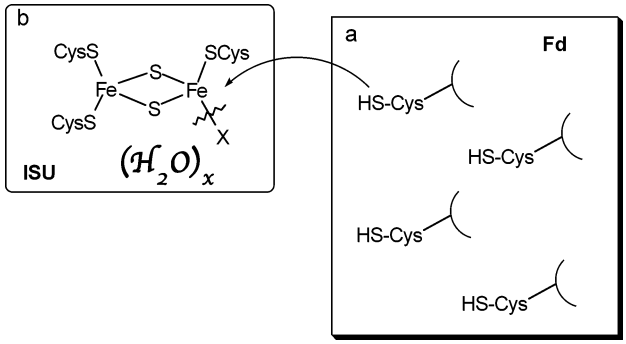

FIGURE 4. Schematic showing cluster transfer to a target Fd (a) from holo ISU (b), emphasizing the importance of a solvent-exposed and accessible cluster and the transfer of an intact cluster unit following coordination by Cys ligands on the apo target.

electrons during Fe−S cluster biosynthesis on IscU to form the stable oxidized cluster.

By use of two assay methods (native-PAGE and a coupled cytochrome *c* reduction assay),[46] we were able to perform a detailed kinetic analysis of the transfer reactions. Studies of the cluster transfer reaction between *Hs* ISU and *Hs* Fd yielded a rate constant of 540 ± 23 M^{-1} min^{-1} at 25 °C that was not significantly affected by changes in solvent viscosity.[46] Therefore, the rate-determining step is not the collision of proteins but rather the cluster transfer reaction itself. Interestingly, when the rate constant for cluster transfer from wild-type ISU is compared to that for D37A ISU, a dramatic order of magnitude decrease in rate constant was observed (74 ± 9 M^{-1} min^{-1} at 25 °C).[46] Additionally, comparison of activation parameters (ΔH^* and ΔS^*) are consistent with the notion that the Fe−S cluster of WT ISU (5.5 ± 1.0 kcal mol^{-1} and −27.7 ± 3.1 K^{-1} mol^{-1}, respectively) is significantly more solvated than that of D37A ISU (1.1 ± 0.2 kcal mol^{-1} and −46.8 ± 5.2 K^{-1} mol^{-1}, respectively). The reaction appears to be entropically controlled with solvent release from the ISU cluster binding pocket, resulting in a less negative ΔS^* for cluster transfer from WT ISU (Figure 4). Indeed, the need to displace interfacial solvent during complex formation and the influence of hydrolytic disassembly of solvent-accessible Fe−S clusters readily explains the observed differences in cluster transfer activity and cluster stability, respectively.[46] Further definition of the influence of D37 was evident from the pH profiles of cluster transfer.[46] The trends for both WT and D37A were similar with an apparent pK_a of 6.9. Such similarities suggest that D37 does not directly participate in cluster transfer by either protonation of the [2Fe−2S] or by deprotonation of the incoming cysteine nucleophile. However, the pH dependence does support solvent accessibility and general base catalysis as factors contributing to cluster transfer reactivity.[46]

Although the cluster transfer reaction that has been characterized between ISU and ferredoxin involves only [2Fe−2S] units, in vivo evidence suggests that ISU mediates the maturation of [4Fe−4S] proteins.[40] Johnson and Dean reported a [4Fe−4S] cluster-bound form of IscU that could potentially be used to reconstitute apo [4Fe−4S] targets;[27] however, there are no clearly documented examples of [4Fe−4S] cluster transfer to a target protein, while the [2Fe−2S] form of IscU is catalytically competent to promote assembly of [4Fe−4S] ferredoxins. For example, [2Fe−2S] *Tm* IscU is capable of reconstituting an apo [4Fe−4S] *Tm* Fd. Therefore, it appears that [2Fe−2S] units are suitable and sufficient building blocks for the assembly of more complex [4Fe−4S] clusters on target proteins. This is particularly relevant since more lowly evolved organisms, such as *T. maritima*, tend to have larger numbers of [4Fe−4S] carrying proteins relative to [2Fe−2S] forms. Such functional flexibility is consistent with the extent of evolutionary sequence conservation seen for IscU proteins.

It is also of some significance that IscU proteins from a number of sources (human, yeast, and bacterial cell lines) are able to transfer cluster to a target ferredoxin from a variety of sources with similar rates and efficiencies.[26,46] This suggests the retention of key recognition features on both IscU-type proteins and target and a similar cluster transfer mechanism. Structural issues are taken up next.

IscU Structure and Dynamics. At present there are no X-ray crystal structures for any IscU-type protein, while our solution studies suggest that IscU possesses unusual conformational flexibility. In particular, $^1H-^{15}N$ HSQC and CD studies of yeast and human ISU suggested that these proteins are largely unfolded in both apo and holo states in vitro. Given the greater structural rigidity found for proteins from thermophilic hosts, we were led to investigate an IscU from the hyperthermophile *T. maritima*. In fact, *Tm* IscU did display spectroscopic parameters that were more consistent with a structured tertiary fold.[26] In addition to the spectroscopic data cited, including near- and far-UV CD, chemical shift dispersion in $^1H-^{15}N$ HSQC experiments, and dynamic light scattering, we also found limited proteolytic digestion and free energy of unfolding data of *Tm* IscU to be typical for a well-folded protein.[26,47,48] However, other data were consistent with a flexible protein, such as resonance splitting, hydrogen exchange, lack of long-range NOEs, and ANS binding.[47,48] ANS is a fluorescent probe that is used to detect the presence of molten globule states.[62] Such apparently conflicting data was reconciled with a dynamic model of IscU in which the protein alternates between different structurally distinct conformations on a millisecond time scale.[47,48] While the structural data acquired thus far for *Tm* IscU are not sufficient to calculate a unique tertiary fold, we were, nevertheless, able to extract meaningful structural information[48] that is fully consistent with the subsequent structural elucidation of *Haemophilus influenzae* IscU (*Hi* IscU, PDB ID: 1Q48) by Montelione and colleagues (Figure 5). However, there are differences between IscU homologues from lowly evolved organisms, such as *T. maritima*, and those from higher organisms, such as *H. influenzae*, *E. coli*, and eukaryotes. The former contain an ∼18 amino acid insert[26] that is structured and contains an α-helix with unknown function.[48] Therefore, *Tm* IscU is composed of six α-helices and one three stranded β-sheet, whereas IscU proteins from higher organisms only possess five α-helices. Additionally, the

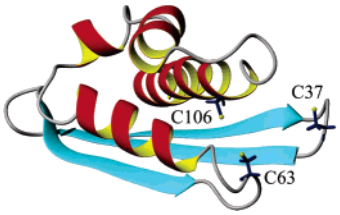

FIGURE 5. Ribbon diagram of *Hi* IscU (PDB ID: 1Q48). Residues 1−26 are not shown as they are largely unstructured, and the carboxy-terminal His-tag is also not shown. The Cys residues are labeled, and their sulfurs are colored yellow.

amino terminus of *Hi* IscU is not rigid with respect to the rest of the structure, and so this region is not shown in Figure 5. *Tm* IscU NMR data shows an α-helix within this region that is predicted to exist, based on secondary structure prediction, in all of the IscUs that we have tested, including *Hi* IscU.

IscU proteins possess three highly conserved cysteine residues that ligate the cluster. The *Hi* and *Tm* IscU structural data reveal that two of these cluster-ligating cysteines are located in loop regions within a section of IscU containing the β-sheet and are thus likely to be highly flexible. Interestingly, one side of this β-sheet is primarily composed of hydrophilic side chains and the other consists mostly of hydrophobic amino acids that form the cluster-binding pocket. The remaining cysteine cluster ligand resides in a stretch of IscU that is only three residue positions away from a consensus chaperone-binding motif.[48]

The flexibility of IscU is consistent with its in vivo function, since IscU must interact with a variety of proteins in order to synthesize and deliver cluster. For example, IscU must accept iron and sulfur from their respective donor proteins and then exchange the IscU-derived ligand set with that of the target protein. Moreover, IscU interacts with a variety of target proteins with variable tertiary folds that often do not possess significant structure in the apo state. Therefore, it is plausible that upon interaction with a partner protein, a particular IscU conformation is stabilized.

Alternate Fe−S Scaffolds. Although IscU is believed to be the most critical Fe−S scaffold of the ISC machinery, there appears to be other cluster scaffolds within this system. For instance, IscA/ISA[28,30,63] has been shown to coordinate and deliver Fe−S clusters to target proteins. The necessity of multiple scaffolds is not clear, particularly since many of the their biochemical and functional properties appear to be nearly equivalent with IscU. For example, ISA binds one reductively labile $[2Fe-2S]^{2+}$ cluster per monomeric subunit, contains three highly conserved cysteine residues that bind the Fe−S cluster, most likely possesses one non-cysteinyl ligand, and mediates cluster transfer to apo ferredoxin with a similar rate constant and affinity as ISU. However, there are some important differences, such as the fact that ISA proteins are generally tetrameric and appear to bind to different regions of Fd when transferring cluster. The significance of these differences is not currently known but may reflect different preferences for target proteins.

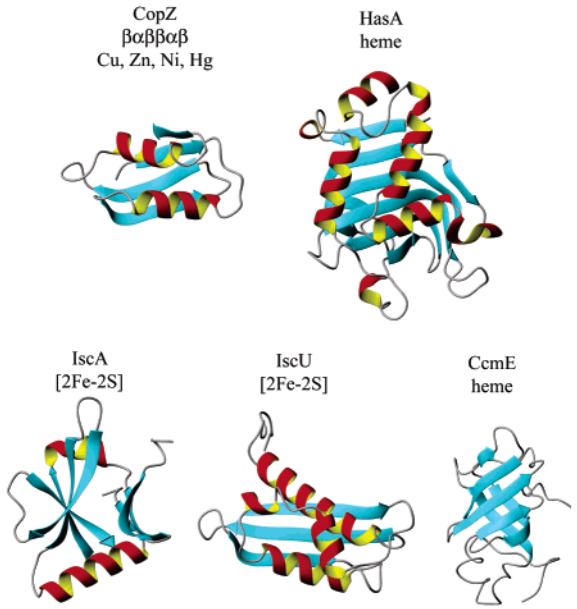

FIGURE 6. Comparison between the structural characteristics of metal carrier (CopZ) and metal cofactor scaffolding proteins (HasA, IscA, IscU, and CcmE).

Comparisons with Other "Metallochaperones". Significant advances have been made in identifying cellular metal ion carriers, termed metallochaperones. In addition to single metal-ion transporters, proteins involved in metal efflux and assembly and/or delivery of more complex metallocofactors have been investigated. Results to date show some interesting similarities that most likely reflect the common physiological roles that these proteins must fulfill. As recently discussed,[46] these characteristics include a retention of tertiary structure in the apo and holo states, increased affinity for target proteins in the holo state, and a solvent-exposed metallocofactor binding domain that incompletely ligates the metal ion, leaving an open ligation site that most likely facilitates cluster transfer via nucleophilic attack on iron by a Cys from the target protein. However, there are significant differences among those proteins that mediate the assembly and trafficking of more complex metal cofactors. While the mononuclear metallochaperones share a $\beta\alpha\beta\beta\alpha\beta$ ferredoxin-like domain[48] (represented by CopZ in Figure 6) and interact with other acceptor proteins that contain the same structural motif, more complex scaffolding proteins (such as IscU, IscA, CcmE, and HasA in Figure 6) do not contain a $\beta\alpha\beta\beta\alpha\beta$ ferredoxin-like motif. Not only are such proteins structurally distinct, they are also functionally distinct. The term ion carrier would seem more appropriate for the former class since they mediate transfer of a single metal center and function more in the role of a carrier protein. However, this is not likely to be a genuine chaperone role as typically defined.[64] The "scaffolding" proteins serve more the purpose of assembling and stabilizing a complex metal cofactor prior to delivery to a target protein. Thus far, proteins involved in Fe−S cluster assembly,[26,65] heme transport,[66,67] and a putative protein implicated in the biosynthesis of molybdenum cofactor[68] and delivery have been structurally characterized. The differences in struc-

tural content are to be expected since such proteins often participate in both the synthesis and delivery of the cofactor to a variety of distinct target proteins with distinct tertiary structures. An intriguing aspect of the functional diversity of this class of proteins is that they share all of the aforementioned biochemical characteristics without sharing a common structure. Such similarities clearly indicate the importance of these shared characteristics in metallocofactor assembly and transfer.

Concluding Remarks

The combined research efforts of a number of laboratories have resulted in a detailed model for the thermodynamic, kinetic, and structural basis for Fe−S cluster assembly on the scaffolding protein IscU and subsequent transfer to target ferredoxins. Similarities in the structures of single metal carriers are observed to contrast markedly with the greater structural diversity of protein scaffolds involved in the assembly and transport of more complex metal cofactors.

When considering the functions of these proteins it is noteworthy that Fe−S clusters (including the ISU-bound cluster) can be assembled or reconstituted in vitro with no accessory proteins under appropriate (typically higher and nonphysiological) concentration conditions. The fact that the cluster can be reconstituted in the absence of protein mediators provides an important indicator of the intracellular roles for many proteins on the Fe−S cluster assembly pathway. Indeed, such proteins are not enzymes that catalyze a multiturnover reaction and are more correctly viewed as carrier proteins, rather than as catalysts for the reaction, that both circumvent the toxicity associated with free iron and sulfide and allow delivery at lower intracellular concentrations of these species.

While our measured reaction rates cannot be directly compared with physiological rates, it is likely that the assembly apparatus as defined here is fast enough to meet cellular needs. For example, the reaction for delivery of nickel to urease by the UreD-UreE-UreG-urease complex has a reported half-life of ∼6 h.[16]

Several important themes have emerged that will keep the field busy for some time. These include further study of the chemistry of frataxin as a cellular iron donor, the functional roles of chaperone proteins in cluster assembly and transfer, and mechanisms of mitochondrial cluster export. The availability of structural data on IscU and IscA proteins (appearing at the time of writing) promises to provide molecular insight to fuel more detailed studies of function and mechanism as well as prompting deeper investigations of the unusual conformational dynamics exhibited by this class of protein. As more is learned, the similarities and differences in structural and functional properties of the various classes of metal carrier protein and metal cofactor assembly pathways will become more evident. The mechanisms of transcriptional and translational control, the manner in which cofactor biosynthesis is tied into cellular metal ion homeostasis, and the interdependence of metal regulatory mechanisms are also ripe for future study.

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