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Review Article

Metallocluster transactions: dynamic protein interactions guide the biosynthesis of Fe–S clusters in bacteria

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Iron–sulfur (Fe–S) clusters are ubiquitous cofactors present in all domains of life. The chemistries catalyzed by these inorganic cofactors are diverse and their associated enzymes are involved in many cellular processes. Despite the wide range of structures reported for Fe–S clusters inserted into proteins, the biological synthesis of all Fe–S clusters starts with the assembly of simple units of 2Fe–2S and 4Fe–4S clusters. Several systems have been associated with the formation of Fe–S clusters in bacteria with varying phylogenetic origins and number of biosynthetic and regulatory components. All systems, however, construct Fe–S clusters through a similar biosynthetic scheme involving three main steps: (1) sulfur activation by a cysteine desulfurase, (2) cluster assembly by a scaffold protein, and (3) guided delivery of Fe–S units to either final acceptors or biosynthetic enzymes involved in the formation of complex metalloclusters. Another unifying feature on the biological formation of Fe–S clusters in bacteria is that these systems are tightly regulated by a network of protein interactions. Thus, the formation of transient protein complexes among biosynthetic components allows for the direct transfer of reactive sulfur and Fe–S intermediates preventing oxygen damage and reactions with non-physiological targets. Recent studies revealed the importance of reciprocal signature sequence motifs that enable specific protein–protein interactions and consequently guide the transactions between physiological donors and acceptors. Such findings provide insights into strategies used by bacteria to regulate the flow of reactive intermediates and provide protein barcodes to uncover yet-unidentified cellular components involved in Fe–S metabolism.

Introduction

Fe–S clusters are ancient cofactors proposed to have played a critical role in the emergence of life on Earth [1–3]. The chemistries catalyzed by these inorganic cofactors are diverse, ranging from simple one-electron transfer to thermodynamically unfavorable reactions like dinitrogen reduction [4,5], and more complex organic rearrangements like those promoted by Fe–S/SAM radical enzymes [6]. This array of chemical functionalities is attributed to different types of clusters, the attachment of organic ligands and heteroatoms, and the protein environment assisting these reactions. Despite the vast catalog of biologically occurring Fe–S cofactors, it is presumed that all Fe–S cofactors are initially pre-assembled from simple building blocks of 2Fe–2S or 4Fe–4S units. For example, the assembly of simple Fe–S clusters also serves as the starting point for the synthesis of more complex metallostructures such as the FeMo cofactor contained within nitrogenase, the H-cluster of hydrogenase, and the A- and C-clusters of acetyl-CoA/CO dehydrogenase [7,8]. In bacterial systems, the synthesis of Fe–S units is a tightly regulated process and involves specialized pathways. The general synthetic scheme for

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the assembly of 2Fe–2S or 4Fe–4S units follows three major events: (1) the activation of sulfur, (2) the assembly of clusters, and (3) the transfer of these units to final acceptor proteins or to intermediates involved in the synthesis of complex metalloclusters (Figure 1).

The inventory of proteins containing Fe–S clusters and their associated functions continues to expand. Reported cluster-binding motifs from genetic and protein databases assist in the identification and classification of Fe–S proteins [9–11]. However, the type and number of clusters coordinate by certain proteins in their physiologically active forms are not easily predicted by computational analysis. Functional assignment of Fe–S clusters through experimental characterization also proves to be a challenging endeavor. Fe–S clusters are prone to react with O_2 at atmospheric concentrations, resulting in damage or loss of clusters upon isolation of their associated proteins under aerobic conditions. *In vitro* attempts to reconstitute apo-proteins may lead to distinct cluster nuclearity or stoichiometry [12–15]. In addition, synthesis and insertion of certain types of Fe–S centers requires specific cluster biosynthetic enzymes, which are not always available in host species used during heterologous expression [8,16]. Thus, the isolation of proteins with damaged or absence of clusters fails to assign the correct metal cofactors corresponding to their cellular functions. Despite experimental challenges, hundreds of proteins containing Fe–S clusters have been identified that serve as a variety of functions in metabolism. The bacterial model organism *Escherichia coli* is expected to contain at least 140 distinct Fe–S proteins [17,18]. Likewise, other well-studied bacteria such as *Azotobacter vinelandii* and *Salmonella enterica* also encode a large number of genes with Fe–S cluster-binding motifs (129 and 113, respectively) [19]. It is remarkable that the metabolism of most bacterial species has retained a large number of Fe–S proteins across evolution, despite unfavorable conditions for the synthesis and use of these cofactors such as the rise of oxygen concentrations and the decrease in Fe availability throughout Earth's history.

Besides the total number of Fe–S proteins reported to date, another remarkable feature about biological Fe–S clusters is the ability of certain organisms to synthesize large quantities of these cofactors to sustain growth under certain nutritional and physiological conditions. For example, when fixing nitrogen, *A. vinelandii* produces high level of the Fe–S enzyme, nitrogenase (5–12% of the total protein content) [20–22]. Considering the cellular concentration of both nitrogenase components and their associated clusters, it is estimated that cells route close to 630 μM of Fe only for the synthesis of nitrogenase metallocofactors (calculated based on the cellular concentration of nitrogenase MoFe and Fe proteins [22]). In this case, the high cluster content is not only detected through spectroscopic methods and enzymatic assays, but also by visual assessment of the dark brown color of nitrogenase-containing cells. The abundant accumulation of nitrogenase metalloclusters has, in fact, guided the initial studies leading to the discovery on how simple Fe–S clusters are assembled in bacteria [16]. Early genetic studies mapping nitrogen fixation genes led to the discovery of NifS and NifU enzymes involved in the synthesis of the initial Fe–S units of nitrogenase metalloclusters (Figure 2B) [23]. Biochemical characterization of these proteins established two general paradigms in the biosynthesis of simple Fe–S clusters: the involvement of a sulfur-activating enzyme and the assembly of clusters onto a scaffold protein (Figure 1). To date, all studied Fe–S biosynthetic pathways, regardless of composition and phylogenetic origin, include at least

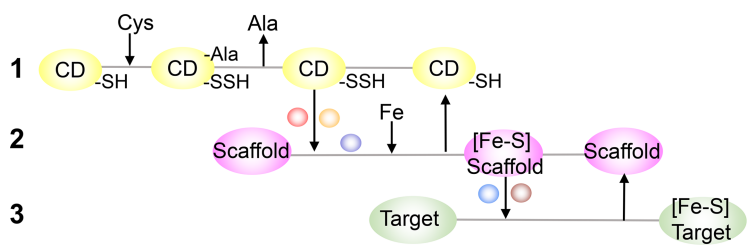

Figure 1. Major steps involving the biological synthesis of simple Fe–S clusters.

(**1**) Sulfur activation by a cysteine desulfurase (CD, yellow) leads to the formation of alanine and sulfur transfer to the scaffold (pink). In some cases, sulfur transfer is mediated by a transferase (orange) or regulated by an accessory protein (red). (**2**) Assembly of Fe–S cluster on protein scaffolds may require reductant (lilac) for the reduction in the persulfide sulfur and/or construction of clusters. (**3**) Cluster transfer events to target proteins (green) may occur directly with the assistance of chaperone proteins (blue), or indirectly via cluster carrier proteins or biosynthetic intermediates in the synthesis of complex metalloclusters (brown).

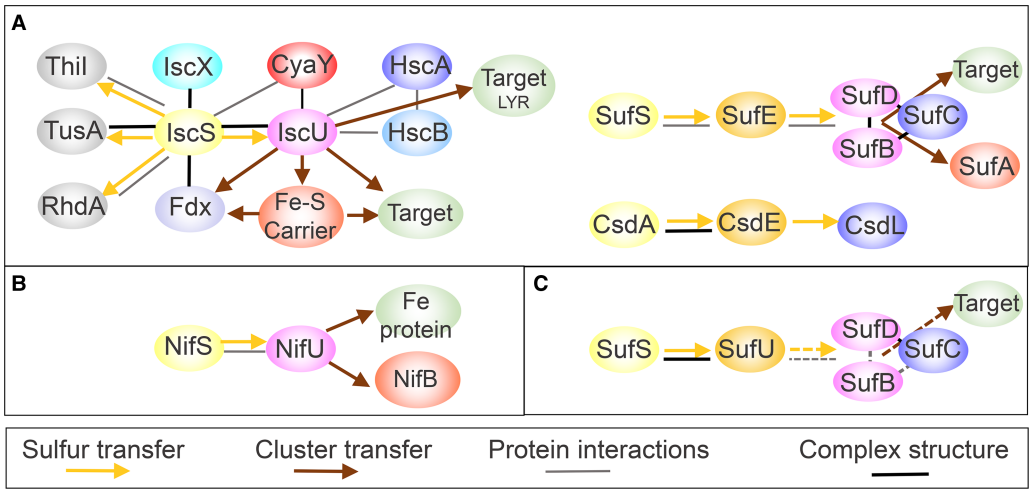

Figure 2. Network of protein interactions involving the biosynthesis of Fe–S clusters in bacteria.

Examples of Fe–S cluster biosynthetic systems found in Gram-negative bacteria including *E. coli* (**A**), in selected diazotrophic bacteria containing the specialized Nif system involved in cluster formation for nitrogenase (**B**), and in Gram-positive bacteria including *B. subtilis* (**C**). Sulfur transfer events (yellow arrow) from the cysteine desulfurase (yellow) occur directly to a sulfur acceptor scaffold (pink) or indirectly via a sulfurtransferase (orange). Cysteine desulfurases also interact and transfer sulfur to additional acceptors in the biosynthesis of thio-cofactors other than Fe–S clusters (gray). Fe–S cluster transfer events (brown arrow) can be achieved from the scaffold directly to an apo-target acceptor (green) or indirectly through an Fe–S cluster carrier or cluster biosynthetic protein (brown). Protein interactions shown through crystallography or NMR structure of protein complexes are indicated with a solid thick line. Direct protein interactions showed through *in vitro* binding, cross-linking, co-purification, and/or yeast two-hybrid experiments are shown with a gray line. Predicted interactions that have not yet been demonstrated experimentally are indicated with a dotted line and arrows.

two components supporting these two paradigms. The NifS–NifU system represents the minimum toolbox in which the cysteine desulfurase NifS is responsible for mobilization and the subsequent transfer of sulfur to its concomitant scaffold protein [24], NifU, where the synthesis of clusters takes place [25,26]. Variations of this two-component system include additional proteins mediating sulfur transfer events, serving as reducing agents, and assisting in the transfer and insertion of nascent clusters to downstream acceptors [19,27–30] (Figure 2).

Because Fe–S proteins are critical for life-sustaining reactions, enzymes promoting the synthesis of Fe–S clusters are likewise essential in nearly all bacteria [5,18,31–34]. The biogenesis of Fe–S clusters is an oxygen-sensitive process that involves reactive pathway intermediates. These systems generate and carry intermediates directly from one protein to another. That is, specific protein–protein interactions among biosynthetic components dictate the flow of reaction intermediates and protect reactive species during the synthesis and delivery of these cofactors to their final targets. Interestingly, sequence identity elements controlling interactions among biosynthetic proteins, while critical to the function of these components, are often located remotely from the active site of these enzymes. More recently, many studies have targeted the specificity of Fe–S cluster biosynthetic enzymes through identification of conformational states adopted by these individual biosynthetic components and have begun to map the dynamic protein complexes (Figure 2).

Interactions between sulfur donors and acceptors

The free amino acid L-cysteine is the sulfur source in the biogenesis of Fe–S clusters. All Fe–S cluster biosynthetic systems studied to date contain a phylogenetic and mechanistic related sulfur-activating enzyme, L-cysteine:acceptor sulfurtransferase (EC 2.8.1.7), known as a cysteine desulfurase. Members of this enzyme family are active as homodimers harboring a pyridoxal-5′-phosphate (PLP) cofactor within each active site monomer [35]. Sulfur transfer reactions proceed through formation of a persulfide intermediate covalently attached to the active site cysteine, which is a strictly conserved and essential residue in all active members of the cysteine desulfurase group. The reaction catalyzed by these enzymes is initiated upon the binding of the substrate to the PLP cofactor. Electronic rearrangement of the Cys–PLP adduct allows the nucleophilic attack

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of active site cysteine thiolate group onto the substrate cysteine thiol. This event results in the cleavage of the carbon–sulfur bond from the substrate and the formation of the persulfide intermediate (Figure 1). In the proposed ping-pong kinetic mechanism, the first product of the reaction, alanine, is released prior to the transfer of the sulfur to an acceptor molecule [36]. While the chemical steps leading to the formation of the enzyme persulfide are thought to be common to all enzymes participating in Fe–S cluster biogenesis, the transfer of sulfur to the acceptor molecule is specific to each cysteine desulfurase, i.e. inter- and intra-species cross-reactivity between cysteine desulfurases and acceptor molecules from different types of Fe–S cluster biosynthetic systems is either limited or non-existent [35,37–41]. Transient protein complexes are formed between donor (cysteine desulfurase) and acceptors (intermediate sulfurtransferase or scaffold protein) during each reaction cycle [26,36,42–45]. Therefore, it is proposed that donor–acceptor pairs contain reciprocal sequence signatures that allow for productive interactions and successful sulfur transfer reactions. Kinetic analyses of cysteine desulfurases showed that the turnover rate of these enzymes may be increased up to 200-fold in the presence of physiological sulfur acceptors [36,46]. This effect is attributed to the acceleration of the second step of the reaction — the transfer of persulfide sulfur to the acceptor molecule and therefore resetting the enzyme for the subsequent catalytic cycle.

The interaction between cysteine desulfurase and sulfur acceptors dictates their specific roles in sulfur metabolism. In addition to serving as the sulfur-activating enzyme for the biogenesis of Fe–S clusters, cysteine desulfurases also participate as S-donors in the formation of several other thio-cofactors. In most organisms, a single, general cysteine desulfurase serves as the hub of sulfur mobilization and delivery to various pathways [35]. In *A. vinelandii*, *E. coli*, *S. enterica*, and many other Gram-negative bacteria, inactivation of the gene encoding the master cysteine desulfurase IscS leads to severe growth defects and phenotypes associated with not only impaired activity of Fe–S enzymes, but also lack of thionucleosides and auxotrophy for thiamin, nicotinic acid, and branched-chain amino acids [32,47–50]. Structural and mutagenesis studies have attempted to map sequence elements between IscS and interacting proteins TusA, ThiI, IscU, IscX, and CyaY (bacterial frataxin homolog) (Figure 2A). These analyses showed that sulfur acceptors bind to the cysteine desulfurase in a distinct mode that, in some cases, allows ternary complexes to form [45,50,51]. For example, binding to IscX [52–54] did not prevent interaction with the sulfur acceptor IscU, suggesting that both proteins play regulatory roles. In contrast, the overlapping regions shown in ThiI and TusA binding indicate mutually exclusive events (Figure 3).

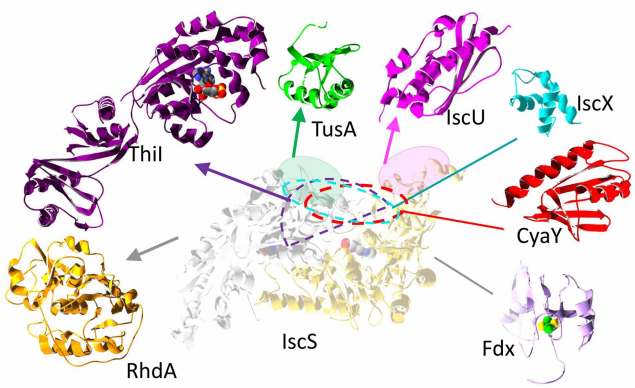

Figure 3. IscS interacts with a suite of sulfur acceptors with diverse folds.

Sulfur transfer events to RhdA (PDB 1H4K), ThiI (PDB 2C5S), TusA (PDB 3LVJ), and IscU (PDB 3LVL) are indicated with an arrow. Interacting proteins known to regulate sulfur transfer IscX (PDB 2BZT), CyaY (PDB 1SOJ), and Fdx (PDB 3AH7) are connected with a solid line. Structural location of IscS regions making protein–protein contact with interacting proteins based on the crystal structure of IscS–IscU (PDB 3LVL) and IscS–TusA (PDB 3LVJ) are shown in shaded pink and green, respectively. IscS regions that have been shown through mutagenesis studies to be involved in binding with ThiI, IscX, and CyaY are circled in dashed purple, blue, and red, respectively. RhdA and Fdx are known partners of IscS and interact near the active site cysteine residue; however, the specific interaction surfaces are less understood. Although it is anticipated that both active sites within the dimer carry equivalent interactions, for simplicity purposes, the figure shows only interactions affecting the monomer shown in yellow.

A recent *in vivo* analysis showed that a strain of *E. coli* carrying a deletion of *tusA* had more Fe–S clusters [55], supporting the model stating that TusA and IscU compete to bind to the sulfur donor, IscS. The general involvement of IscS in several biochemical pathways, not limited to Fe–S cluster synthesis, stems from its ability to interact with a suite of sulfur acceptors with diverse folds and functions (Figure 3). Although the identity of many sulfur partners has been determined [35,56], IscS likely interacts with other, as yet-unidentified proteins. How IscS and other cysteine desulfurases interact and restrict the clientele of sulfur acceptors remains a topic of investigation.

In addition to IscS, some bacteria contain additional cysteine desulfurases partaking specialized roles in Fe–S metabolism (Figure 2). These enzymes require the presence of dedicated sulfur acceptors that direct sulfur flow for the synthesis of clusters under specific conditions or targeted pathways. In the *E. coli* Suf system, the sulfur-mobilization reaction involves the cysteine desulfurase SufS, which functions in a similar capacity as IscS. However, its physiological role depends on the availability of its dedicated sulfur acceptor SufE [57,58]. Serving as an intermediate sulfurtransferase, SufE interacts with SufS and the cluster scaffold SufBCD when mediating protected sulfur transfer reactions [59,60]. Communication between each monomer of the SufS dimer affects its affinity for SufE and, consequently, its catalytic efficiency [61]. Overexpression of the Suf system, but not SufS only, can partially replace the functions of IscS possibly due to the overlapping roles of the Suf system in Fe–S cluster synthesis but not the ability of SufS to interact directly with IscS partners [29]. *E. coli* also encodes a third cysteine desulfurase, CsdA, and its sulfur acceptor CsdE [30,42], both of which are phylogenetic and structurally related to SufS and SufE. Contrary to expectations, overexpression of CsdA, but not CsdAE, rescues the activity of certain Fe–S enzymes in strains lacking both SufS and IscS [62]. Presumably, high expression levels of CsdA allows for cross-reactivity towards SufE, suggesting that transfer from SufE to the SufBCD scaffold is highly specific, and CsdE is not able to fulfill this role. Lack of complementation within sulfur acceptors highlights the specificity of subsequent sulfur transfer reactions involving downstream biosynthetic proteins. In *A. vinelandii*, the cysteine desulfurase NifS transfers sulfur specifically to NifU for the assembly of Fe–S clusters during aerobic nitrogen fixation (Figure 2B) [25,26]. Neither NifS nor NifS–NifU can replace IscS, not even when expressed at high levels [16,37,63]. Thus, specialized cysteine desulfurases cannot replace the multifunctional IscS enzyme.

Specific interactions between S-donors and S-acceptors are also observed in bacterial species lacking IscS, but expressing multiple cysteine desulfurases. Genomic analysis of Gram-positive bacteria shows the presence of several cysteine desulfurase genes [19,35]. Their chromosomal location and neighboring genes give insights into the identities of sulfur acceptor proteins and their respective S-transfer pathways. Worth noting, despite the high degree of sequence similarity between *E. coli* IscS and each cysteine desulfurase from *Bacillus subtilis*, interspecies reactivity has not been observed [40]. Likewise, orthologous sulfur acceptors are not functional when individually expressed [39,64]. However co-expression of *B. subtilis* cysteine desulfurases along with their sulfur acceptor counterparts is able to recover defects associated with selected pathways in *E. coli ΔiscS* strains. That is, sequence signatures contained within S-donor and S-acceptors restrict protein interactions and consequently their involvement in cofactor biosynthesis.

Another distinct feature of sulfur transfer reactions in Gram-positive bacteria is that each species appears to have only one system for Fe–S cluster biosynthesis [1,19]. In *B. subtilis*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis*, the general assembly of Fe–S clusters is performed by a modified Suf system which is essential for survival of these species (Figure 2C), but distinct from the one identified in *E. coli* [34,65,66]. The cysteine desulfurase SufS in Gram-positive species specifically transfers sulfur to SufU, a zinc-dependent sulfur acceptor protein that enhances SufS' catalytic turnover rate nearly 200-fold and is proposed to serve as an intermediate S-carrier to the proposed SufBCD scaffold [1,28,36,67]. The tight binding of zinc to this sulfur acceptor (K_a ~10^{17}) limits SufU's role as an intermediate in sulfur mobilization, mirroring the function of SufE, that also enhances the rate of sulfide production nearly a 100-fold in the *E. coli* Suf system [61]. Dynamic complex formations enabling sulfur transfer events between SufS and SufU are also specific [43]. Bidirectional cross-complementation using *E. coli* and *B. subtilis* knockout strains showed the requirement for correct donor–acceptor pair for functionality in sulfur transfer reactions. Interspecies functional replacement was only achieved upon co-expression of *E. coli* SufSE or *B. subtilis* SufSU pairs [40]. Curiously, the partnership of the cysteine desulfurase SufS with SufU or SufE appears to be mutually exclusive; *sufE* and *sufU* genes tend to not occur in the same species. Although both proteins are phylogenetically unrelated and display less than 20% sequence identity, they share equivalent roles as sulfur acceptors in Fe–S cluster biogenesis [67] and both are indispensable sulfurtransferases directing sulfur flow from SufS to SufBCD.

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Protein interactions enabling the synthesis of Fe–S clusters

The construction of Fe–S clusters on the scaffold requires the acquisition of Fe from an unknown source and the transfer of sulfur directly from a cysteine desulfurase or indirectly via a sulfurtransferase (Figure 1). The order of events, S first or Fe first, is not well understood. Scaffold proteins are competent sulfur acceptors independently of their metal coordination [60,68,69], but they are also able to bind Fe independently of their sulfuration state [70,71]. Cysteine residues residing within scaffold proteins can participate in persulfide sulfur transfer. In this reaction scheme, sulfur remains in the S^0 redox state and no electron source is required during sulfur transfer. Conversely, following an Fe first model, sulfur transfer to a nascent Fe–S cluster must be coupled with the reduction in S^0 to S^{2-} (the redox state of S in the cluster). For the synthesis of a $[2Fe–2S]^{2+}$ cluster, the oxidation of Fe^{2+} (reactant) to Fe^{3+} (within the cluster) can serve as the source of two electrons requiring the input of additional two reducing equivalents, whereas the synthesis of a $[4Fe–4S]^{2+}$ cluster requires the input of six additional reducing equivalents. In the Isc system, ferredoxin (Fdx) is proposed to serve as the electron donor during the formation of Fe–S clusters [72,73]. Reduced Fdx can promote the reductive coupling of pre-formed $[2Fe–2S]^{2+}$ into $[4Fe–4S]^{2+}$ cluster onto IscU [74]. It has also been shown that Fdx can form a transient complex with IscS during cluster assembly on IscU [75,76]. The network of dynamic protein interactions shows that Fdx binding to IscS displaces CyaY and accelerates the rate of cluster assembly (Figure 3). In the proposed model, reduced Fdx provides electrons for the reduction in S^0 to S^{2-} during formation of a cluster on IscU [77]. Intriguingly, CyaY has an inhibitory effect on the formation of Fe–S clusters on IscU in bacterial species, whereas its eukaryotic ortholog frataxin (Fxn) exhibits the opposite effect via the acceleration of Fe–S cluster assembly on the human and yeast IscU [78–80]. Mutagenic analyses of both bacterial and eukaryotic IscU sequences showed that a single residue within IscU is responsible for these distinct functionalities [81]. Bacterial IscU sequences contain a conserved isoleucine residue (*E. coli* IscU Ile108) responsible for CyaY's inhibitory effect, whereas eukaryotic IscU contains a methionine residue at the equivalent position (*S. cerevisiae* IscU Met141). Substitution of these residues Ile108Met in *E. coli* IscU or Met141Ile in *S. cerevisiae* reverts the effect of frataxin in cluster biogenesis [82,83]. The formation and displacement of transient complexes involving IscS–CyaY, IscS–Fdx, IscS–IscU, and IscU–CyaY provides another example for the requirement of specific sequence tags within biosynthetic protein for controlling the flow of intermediate species during the synthesis and trafficking of Fe–S clusters.

Formation of transient complexes during the synthesis of Fe–S clusters is also observed among components of the Suf system. Fe–S cluster assembly is proposed to occur at the dimer interface between SufB and SufD [84]. These proteins are phylogenetically and structurally related to each other, but distinct from IscU. Yet, both SufBD and IscU hold equivalent functions in cluster biogenesis. An additional component of this system, the ATPase SufC, is proposed to use ATP binding and hydrolysis to induce conformational changes on the SufBD proteins and assists in the assembly and/or delivery steps [85]. Distinct quaternary structures of SufBCD proteins have been observed upon isolation of $SufB_2C_2$, $SufD_2C_2$, and $SufBC_2D$ complexes [86–88]. This series of conformation states is proposed to represent different stages of the biosynthetic process enabling sulfur transfer events from SufE to SufB, the recruitment of iron from SufD, and the assembly of clusters on SufBD.

Interactions between Fe–S cluster donors and acceptors

Labile clusters formed on the scaffold protein are directly shuttled to acceptor proteins or indirectly transferred via cluster carrier proteins (Figure 1). The transfer of clusters from scaffolds to target proteins is faster and more efficient *in vitro* than their *in situ* construction onto final acceptors, an observation of which provides kinetic support for the scaffold hypothesis [89,90]. Additionally, chaperone proteins can further accelerate the direct cluster transfer step in cluster biogenesis. In the Isc system, the chaperone HscA and co-chaperone HscB enhance the rate of cluster transfer more than 20-fold [91]. HscB interacts with IscU through its C-terminal domain and with HscA through its J-domain. While interaction with HscB alone impairs cluster transfer from IscU to ferredoxin [92], IscU–HscB complex can interact with HscA through a signature LPPVK sequence motif contained within IscU. The formation of this dynamic ternary complex enhances HscA's rate of ATP hydrolysis that in turn induces a conformational change on IscU from a structured (S) to a disordered (D) state, ultimately leading to cluster release to the acceptor protein [93]. HscB has also been shown to interact with acceptor proteins through a LYR sequence motif within certain proteins such as the succinate dehydrogenase β-subunit (SdhB) [94–96]. This motif occurs as variations of (LIV)(YF)(RK) tripeptide and it is found near the N-terminus end of the cluster-binding motif. Based on this network of protein contacts, HscB is proposed

to recognize LYR motifs within target proteins and direct cluster transfer from IscU to their final destinations. Interestingly, the presence of a canonical HscB appears to occur in species that also encode IscU sequences containing an LPPVK motif and SdhB sequences containing LYR motifs. The apparent co-occurrence of these proteins and their associated sequence motifs highlights a co-evolutionary requirement for a network of protein interactions enabling guided transfer of clusters during this biosynthetic process. Supporting this observation, sequence analysis of Gram-positive species, which lack both *iscU* and *hscB*, fails to identify LYR motifs at equivalent positions within SdhB. In *B. subtilis*, *S. aureus*, *M. tuberculosis* along with other Gram-positive bacteria, the proposed scaffold SufBCD complex is highly distinct in sequence and structure when compared with IscU. Although this complex also contains an ATPase (SufC) that drives conformational changes in the scaffold and perhaps performs a role analogous to that of HscBA, it is a reasonable assumption that alternate motifs are used to guide cluster transfer in these species.

Cluster carriers can also mediate the insertion of Fe–S clusters into final acceptor proteins (Figure 2). Many cluster carriers have been described including A-type (SufA, IscA, ErpA), U-type (Nfu, NufA), and Grx-type (Grx4, Grx5) [97,98]. These proteins readily accept nascent clusters from scaffold proteins and traffic these reactive species effectively to final destinations. Typically, the rate of cluster transfer by cluster carriers is faster than the transfer rates observed from scaffolds to final targets. The requirement for cluster delivery by a carrier protein can be pinpointed to a particular cluster recipient, as in the case of ErpA requirement for IspH/G cluster insertion [99]. In other cases, carriers are required under conditions of high demand for Fe–S clusters such as oxidative stress or iron starvation [87,100,101]. In most cases though, these proteins can perform overlapping roles; phenotypes associated with defects upon inactivation of individual carriers may be suppressed upon overexpression of other carriers. Such cross-reactivity in cluster delivery by carrier proteins suggests that, although this late biosynthetic step requires protein mediated-delivery, the degree of sequence requirement is not critical as the intact delivery of its cargo to the final destination.

Perspective on how direct transactions guide Fe–S cluster metabolism in bacteria

Fe–S clusters are critical components of several biochemical processes. These cofactors as well as their biosynthetic intermediates are prone to oxidation and damage. Unlike most metabolic pathways, intermediates in Fe–S metabolism do not dissociate into solution at each biosynthetic step, but instead are channeled from one biosynthetic enzyme to another. Given the inherent reactivity of pathway intermediates, enzymes participating in the formation and delivery of Fe–S clusters directly interact to transfer their products to the subsequent biosynthetic components. The presence of specific sequence motifs serves as protein barcodes allowing for direct transactions and restricting the clientele of acceptor molecules. Such biochemical strategy is interpreted as a mode of protection of reactive species and regulation of the flow of reaction intermediates. Identification of reciprocal protein motifs within donor and acceptors will provide a mechanistic understanding for how these interactions guide productive reactions and will allow for the discovery of yet-unidentified cellular targets.

Abbreviations

CD, cysteine desulfurase; Fdx, ferredoxin; Fe–S, iron–sulfur; PLP, pyridoxal-5′-phosphate; SdhB, succinate dehydrogenase β-subunit.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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