

Protein interactions in the biological assembly of iron–sulfur clusters in *Escherichia coli*: Molecular and mechanistic aspects of the earliest assembly steps

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Abstract

This contribution focuses on the earliest steps of the assembly of FeS clusters and their insertion into acceptor apoproteins, that call for transient formation of a 2Fe₂S cluster on a scaffold protein from sulfide and iron salts. For the sake of simplicity, this report is essentially limited to the *Escherichia coli* *isc*-encoded proteins and does not take into account agents that modulate the enzymatic synthesis of sulfide by protein in the same operon or the redox events associated with both sulfide generation and conversion of 2Fe₂S structures in clusters of higher nuclearity. Therefore, the results discussed here are based on chemical reconstitution systems using inorganic sulfide, ferric salts, and excess thiols. This simplification offers the possibility to address some mechanistic issues related to the role of protein/protein interaction as for modulating: (a) the rate of cluster assembly on scaffold proteins; (b) the stability of the cluster on the scaffold protein; and (c) the rate of transfer to acceptor apoproteins as also influenced by the acceptor concentration. The emerging picture highlights the mechanistic versatility of the systems, that is discussed in terms of the capability of such an apparently simple combination of proteins to cope with various physiological situation. The hypothetical mechanism presented here may represent an additional way of modulating the rate and outcome of the overall process while avoiding potential toxicity issues.

KEYWORDS

chaperones, cluster assembly and transfer, co-chaperones, iron–sulfur clusters, IscA, IscU, scaffold proteins

Abbreviations: ATP, adenosine triphosphate; CD, circular dichroism; Cys, cysteine; Eu, eukariots; FeS, iron sulfur; WT, wild type; TRIS, tris (hydroxymethyl)aminomethane.

1 | INTRODUCTION

Sulfide-bridged structures containing iron and other metals are considered among the earliest point of contact between inorganic chemistry and living beings. This led

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several scholars to bring forward hypotheses about a possible role of the positively charged surface of iron sulfides similar to those still present in “black smokers” on the ocean bottom in the origin of metabolic pathway¹ and of the earliest enclosed structures.^{2,3} Later on in evolution, the essential portion of these inorganic assembly were incorporated into proteins, that survived pretty much unchanged during the transition of life on our planet from its anaerobic beginning to the mostly aerobic environment of today.³

Iron–sulfur [FeS] clusters in proteins proved to provide extreme versatility and were essentially conserved through evolution.^{4,5} The role of FeS proteins in a myriad of intracellular processes has been discussed in a number of authoritative reviews, and is too vast a topic to be discussed here. However, it remains worth underscoring that iron–sulfur clusters may exert their activity through a variety of mechanisms covering the whole range from serving as mere “structuring agents” from being implied in single-electron redox processes, and from orienting proper binding of the substrate in specific enzymes to act as overall sensors of metal concentration in the cell as related to the conditional stability of iron ligation.⁵

Among the supporting evidence brought forward to support the “iron–sulfur hypothesis” of the origin of life is the relative ease of assembly of elemental clusters of the Fe_nS_n type ($n = 2, 4$) from appropriate mixtures of simple ingredients: iron salts, thiols (including water soluble ones), and a source of sulfide. The sulfide in chemical assembly of Fe_nS_n clusters could come from a variety of sources: elemental sulfur, to be reduced to sulfide by excess thiols in these (chemical) system through trisulfide or persulfide intermediates; sulfide itself; and the persulfide formed in the catalytic cycle of a number of sulfurtransferases, including rhodanese (thiosulfate/cyanide sulfurtransferase) and 3-mercaptopyruvate sulfurtransferase.⁶ The formally zero-valent sulfur in a persulfide moiety may be reduced to sulfide through various mono- and dithiols, including those common in a cellular environment, such as glutathione and lipoate/lipoamide. The biological function of these almost ubiquitous enzymes was puzzling back then (and still is, to some extent), so that the idea of their involvement in sulfide generation seemed to solve the concerns arising from the well documented cytotoxicity of sulfide.

Roughly 25 years ago, all of the guesswork in this area came to an abrupt end with the discovery of dedicated operons for FeS assembly in a number of microorganisms, where specialized proteins were clustered in a single gene to ensure that most of the required proteins were expressed in response to appropriate stimuli.^{7,8} Regardless of prokaryotic or eukaryotic origin, these operons invariably included a pyridoxal 5'-phosphate (PLP)-dependent cysteine sulfurtransferase that binds

sulfur in the cysteine substrate as a cysteine-bound persulfide in each subunit⁹ and a ferredoxin-like redox protein (oddly, a 2Fe2S protein itself!).^{10–13} The isc-encoded ferredoxin-like FeS protein is assumed to be required either for the reduction of the nominally zero-valent sulfur in a persulfide or for redox-dependent interconversion among 2Fe2S and 4Fe-4S clusters.^{14–19} In particular, the mitochondrial cluster assembly system reportedly relies on a single and specific ferredoxin isoform for carrying out the cluster interconversion function.²⁰ Other common proteins in these operons included small-sized “scaffold” proteins (iscU and IscA in *E. coli*^{21,22}) and specialized chaperone/cochaperone systems (HscA and HscB in *E. coli*.^{23,24} Scaffold proteins were thought to “host” a transient form of the cluster, prior to its delivery to the acceptor apoprotein, whereas the highly specific chaperone/cochaperone system encoded in these genes was supposed to be involved in “squeezing” the cluster out of the scaffold proteins in a way that may control the rate (and the fate) of the cluster delivery process. There is substantial agreement on the sequence of events in this biosynthetic route, but there are a number of open issues that have been either neglected or addressed only partially. For the sake of brevity, most of what discussed in what follows will take into account the components of the *E. coli* cluster assembly system, encoded by the single *isc* (iron sulfur cluster assembly) operon. An alignment of the structural genes in the *isc* operon is given in Figure 1, and it also provides a general—and necessarily simplified—scheme of the role of individual proteins in the cluster assembly/transfer process. Significant simplifications in the lower part of the scheme proposed here involve the origin of the electrons required for reduction of sulfur to sulfide, as each of them may come not only from both external sources such as ferredoxin but also from iron(II) itself (in single-electron transfer events) or from the excess thiols often present in these mixtures (in a bi-electronic process). In this frame, it should be noted that all the chemical reconstitution experiments mentioned in what follows were carried out by using Fe(III) salts, with iron(III) being complexed in a tetrahedral ferric tetrathiolate complex by the various thiols used in these experimental mixtures.⁶

The scheme in Figure 1 also indicates that three different species of cluster-loaded IscU (holoIscU)—characterized by a different stability of the ligation of the newly formed cluster they are hosting—may serve as a donor to potential acceptors. This specific aspect will be discussed in detail in the last section of this contribution.

This report plans to shed some light on yet unresolved details of the assembly pathway for Fe_nS_n structures, with a particular focus on: (a) the meaning of the co-existence of multiple scaffold proteins, often located on the same operon;

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sp|P0AAC8|ISCA_ECOLI MSITL--SD--SAAARVNTFLANRGKGFGLR-LGVRTSGSGMAYVLEFV
sp|P0ACD4|ISCU_ECOLI MAYSEKVIDHYENPRNVGSFDNN-DENVGSGMVGAPACG-DVMKQLQIKVN
*: : * . . .*:* * . . . * :* . :* . * :.

sp|P0AAC8|ISCA_ECOLI DEPTPEDIVFEDKGVKVVVDGKSL--QFLDGTQLDFVKEGLNEG--FKFT
sp|P0ACD4|ISCU_ECOLI DEGIIEDARFKTYGCGSAIASSSLVTEWVKGKSLDEAQAQIKNTDIAEELE
** ** *: * .: .** :.:.*.** .: * . :.

sp|P0AAC8|ISCA_ECOLI NPNVKDECGCGESFHV-----
sp|P0ACD4|ISCU_ECOLI LPPVKIHCSILAEDAIAKAAIADYKSKREAK
* ** .* . . :

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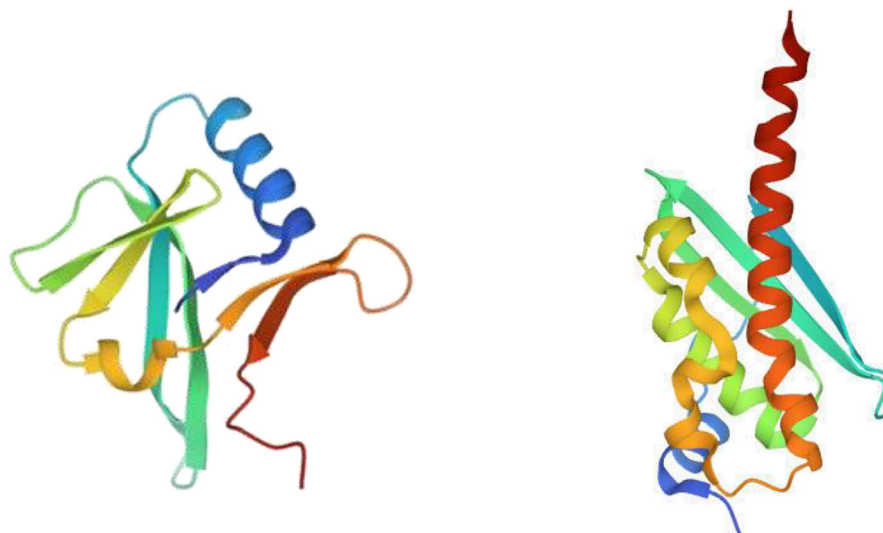


FIGURE 1 A comparison of the sequence (top) and structure of *Escherichia coli* IscU (bottom, right) and IscA (bottom, left). Structures are from PDB entries 1S98 (IscA) and 3LVL (IscU)

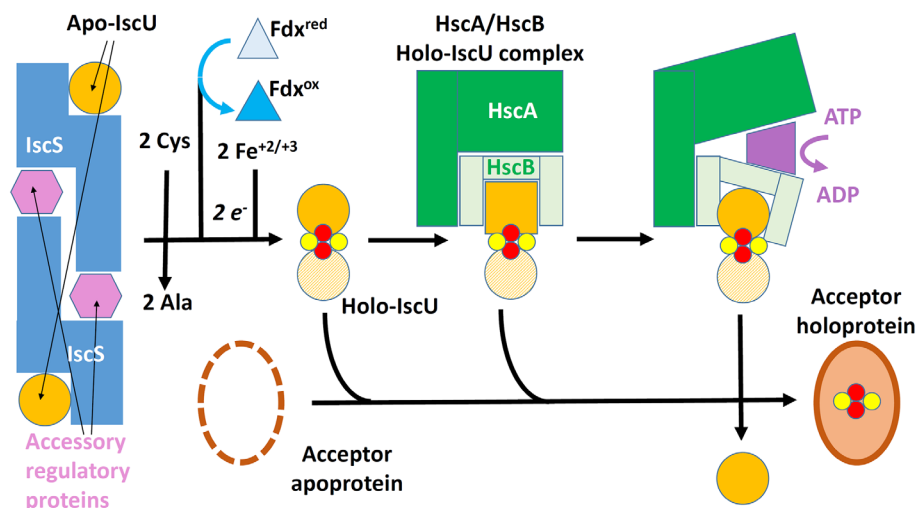
(b) the key role of structural flexibility in the scaffold proteins, and of its modulation by partner other components of the system; (c) the possible connection between chemical and mechanistic features of the cluster-producing machinery and the requirements of a given cell or cell compartments under various physiological or pathological conditions.

2 | CLUSTER ASSEMBLY ON SCAFFOLD PROTEINS

As shown in Scheme 1, there are two small proteins in the *isc* operon (IscA and IscU) that act as “scaffolds” during the assembly process, mediating the final transfer of a pre-formed cluster to an acceptor apoprotein. An alignment of the sequences of *E. coli* IscA and IscU is shown in the upper part of Figure 1 and indicates that both proteins contain three cysteines, in remarkably different sequences. The cysteine-containing sequences in both *E. coli* IscA and IscU differ substantially from motifs commonly involved in stable ligation of clusters of various

nuclearity to iron–sulfur proteins. Substantial differences in the overall folding of the two proteins were already evident in early structural studies carried out both on crystals and by nuclear magnetic resonance (NMR).^{23,25,26} However, regardless of approach, parts of the structure remained unresolved, including the cysteine-containing regions at the C-terminus end of both proteins, unless these “wobbly” structural regions were locked in a fixed position by ligation of redox-inactive and thiophylic metals other than iron, such as zinc or mercury. No structure of *E. coli* scaffold proteins with a bound iron–sulfur cluster has been determined so far, whereas a few structures of scaffold proteins with bound binuclear clusters from other micro-organisms have appeared, as discussed here below.

Early studies on the *E. coli* IscU were consistent with formation of a 2Fe2S cluster at the interface between monomers in an IscU dimer^{14,21} and in a IscA dimer,²⁷ a hypothesis that was confirmed by subsequent studies on site-directed mutants of putative cluster ligands in IscU.²⁸ Also, these same studies indicated that the formation of a



SCHEME 1 Structure of the *isc* operon in *Escherichia coli* (top) and a highly simplified and partially hypothetical scheme depicting the role of individual proteins in the iron-sulfur cluster assembly/cluster transfer processes in Prokaryotes (bottom)

cluster showing the signature CD signals of cluster-loaded IscU was complete after addition of one equivalent of sulfide/(mol IscU monomer), and that intermonomers coordination of a 2Fe₂S cluster was most likely involving facing pairs of thiols, on Cys63 and Cys106 of each monomer. However, several studies pointed out that this straightforward view of the 2Fe₂S cluster binding to holo-IscU was not explaining some of the spectroscopic properties of the *E. coli* holoprotein.¹⁵ Evidence for the existence and hypotheses on the possible significance of multiple forms of cluster-loaded IscU—with different spectroscopic properties—has also been presented,^{29–31} and will be discussed in what follows, along with the evidence of a role of the *isc*-encoded co-chaperone HscB in generating forms of holo-IscU with different cluster-donating ability.

In a trimeric IscU from *Aquiflex aeolicus*,³² only one of the subunits harbors a 2Fe₂S cluster through three of the five cysteine residues in the sequence (the remaining pair being involved in an intramolecular disulfide in all subunits). The crystal structure of a dimeric IscU from the thermophilic archaeon *Methanotheroxobacterium thermophilum* revealed a single 2Fe₂S cluster in each subunit, with coordination of the iron atoms through three Cys residues and Asp 40.³³ As for IscA, the protein from the cyanobacterium *Thermosynechococcus elongatus*—which carries three cysteine residues as the *E. coli* IscA—reveals one partially exposed 2Fe-2S cluster coordinated by two conformationally distinct protomers, with asymmetric cysteinyl ligation by Cys37, Cys101, Cys103 from one protomer, and Cys103 from the other protomer.³⁴

In consideration of the structural differences discussed above, it is not surprising that IscA and IscU differ in their ability to interact with other protein components of the system. At difference with IscA, IscU was the only one capable of binding to the sulfurtransferase IscS (Scheme 1), as a nine-residue sequence in IscU is

specifically recognized by the *isc*-encoded chaperone HscA.^{24,35,36} Binding of this sequence—that includes Cys106, one of the ligands involved in cluster ligation in *E. coli* IscU—results in a 20-fold increase in the ATPase activity of HscA. Stabilization of the IscU folding was also found to occur upon interaction with the *isc*-encoded co-chaperone HscB.³¹ Also, formation of the IscU-HscB complex prevents binding of IscU to IscS.³⁷ In this frame, it seems worthwhile underscoring that IscA does not interact with either HscA or HscB.

IscU and IscA also differ in their ability to act as “catalysts” in the multiple-turnover synthesis of 2Fe₂S structures and of their insertion into a model acceptor apoprotein in the absence of other protein interactors (i.e., when sulfide and iron being supplied as inorganic salts, and reducing power—although not formally required—was provided by a large excess of 2-mercaptoethanol).³⁸ These studies took advantage of the intense and characteristic visible-range CD signal originating upon incorporation of the cluster into the acceptor apoprotein. These studies confirmed formation of a 2Fe₂S cluster with the appropriate (although weak) spectroscopic signature on IscU and the absence of any signal in equally treated IscA, and indicated that IscU was by far a better “enzyme” than IscA, with a 20-fold higher turnover number at low molar ratios between the scaffold protein and the acceptor apoprotein. In short, as of today, the role of IscA in the system remains pretty much elusive, in spite of the mandatory role of its orthologs in mitochondria.¹⁷ A possible function of IscA as a possible “iron-chaperone” in some steps of the assembly process was suggested since the earliest studies on these proteins, but still lacks fully convincing experimental support.

The simplified assembly system used in the “multiple turnover” studies on IscU and IscA mentioned above was based on the use of inorganic salts as the suppliers of cluster atoms. This approach allowed—among others—to neglect the redox steps involved in sulfide generation

from the zero-valence state in which sulfur is bound to IscS (and likely transferred to either IscU and IscA), and to address in more detail the potential impact of agents capable of perturbing the structural flexibility of IscU, that appears a mandatory requisite for this protein. This was already suggested in early stability studies, that pointed to a specific role of the non-ligand Glu39 as for imparting flexibility to the structure of IscU.

As for the relevance of structural flexibility to cluster assembly on IscU, the data in Figure 2 compare the time course of appearance of the signature CD signal of a 2Fe2S cluster on wild-type and D39A apoIscU during chemical reconstitution in the presence of various components affecting the structural flexibility of apoIscU.³⁹ The highest rates of cluster formation are observed when the protein structure is maintained in a “flexible” unfolded state (the D-state) by stabilizing this particular conformation through hydrophobic interactions with the styrene surface of latex nanoparticles.^{40,41} Conversely, the lowest reconstitution rates are observed when apoIscU is “locked” in a compact structured state (the S-state) when forming an equimolar complex with the co-chaperone HscB.³¹ The relatively high reconstitution rates observed for the structurally stable D39A IscU relate to its stability in the holoform,⁴² and are insensitive to the presence of HscB.

A direct interaction of HscB with the apoform of IscU was also demonstrated by NMR studies, and found to

stabilize the rigid and folded S-state of apoIscU.³⁶ Conversely, the disordered conformational D-state of IscU was reported to be the initial substrate for the cysteine desulfurase IscS, converting to a structured S-state once a cluster assembled. Thus, forms of IscU with different structural stability were assumed to play alternate roles in their function as scaffolds during FeS biosynthesis.^{26,29–31,36}

Interestingly, the decreased ability of IscU allows assembly of a 2Fe2S cluster wherein the “S”-form was independent of the sulfide source. Inhibition of cluster assembly in the same experimental conditions reported in Figure 2 was observed with both inorganic sulfide and IscS + cysteine + thiols, suggesting that structural rigidity may impair steps of the cluster assembly process other than uptake by IscU of chemically added sulfide or of the persulfide on the active site cysteine in IscS.

As a matter of fact, the current model for the IscS/IscU complex calls for binding of a single IscU apoprotein near the active persulfide-transferring site located at each end of the IscS dimer, along with a number of associated proteins involved into modulating the activity of the sulfurtransferase. However, this mode of binding of IscU implies that only one atom of sulfur can only be transferred from IscS to IscU through a persulfide exchange process, that is intrinsically limited to a single-atom transfer. Therefore, either the single persulfurated form of IscU re-binds to IscS (before or after reduction of the persulfide to sulfide) to acquire the second sulfur atom required for cluster assembly, or two single-persulfurated forms of IscU interact to generate a transient dimer with the stoichiometrically required amount of reducible sulfur. Please note that—for the sake of simplicity—iron incorporation and reduction of the persulfide to sulfide are not discussed here. Based on available biosynthetic data with a number of IscU mutants²⁸ and on several physical and analytical measurements on cluster-loaded IscU,³⁸ the “dimer” hypothesis seems to remain the most reasonable one. Of course, as supported by some of the available crystal structures and by some spectroscopic measurements, it is possible that the all-cysteine coordinated 2Fe2S cluster formed at the interface between IscU monomers—as hypothesized above—may undergo further ligand exchange in the conditions used for crystallization or for some spectroscopic studies. Additional support for the “dimer” hypothesis also came from cluster transfer/release studies, as illustrated in the next subsection.

In all the studies on cluster assembly on IscU, very little attention has been paid to the timing of incorporation of iron into the nascent IscU holoprotein, quite at contrast with the very detailed knowledge available on the mechanism of action of IscS and on the modulation of its sulfurtransferase activity by “accessory”

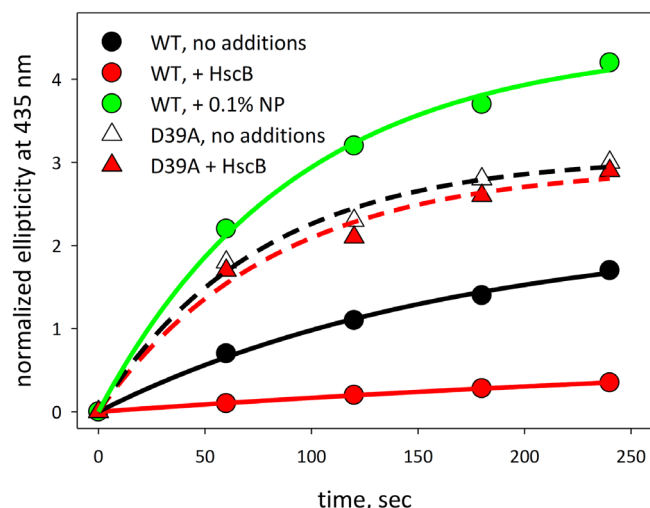


FIGURE 2 Initial time course of cluster assembly on wt and mutated IscU, in the presence/absence of HSCB and of styrene (latex) nanoparticles. The formation of holoIscU was monitored by following changes in the CD signal after the addition of 0.1 mM Li_2S to 30–45 μM apoIscU monomer (WT, circles; D39A, triangles) in 0.1 M Tris-HCl, pH 8.0, containing 5 mM dithiothreitol and 0.1 mM ferric ammonium citrate and 0.1% (w/v) of polystyrene nanoparticles (NP, 20 nm nominal diameter³⁹). When present, HscB was equimolar to the ApoIscU monomer³¹

proteins, including frataxin as well as ISD11 and its orthologs.^{9,43} Most of the studies on this topic have been driven by the search of a possible “iron chaperone” protein, that has become some sort of Holy Graal of inorganic biochemistry. Several proteins have been tested and/or suggested as a possible source of iron for cluster assembly on IscU, including IscA and frataxin, among others, but no conclusive evidence has been brought forward for a significant physiological role of any of these proteins.

In this frame, a point worth further investigation is the ability of IscU to bind individual divalent cations, such as the thiophilic zinc and mercury, that have been used to stabilize the IscU folding in various structural studies.⁴⁴ Iron has never been studied in this context, although binding of iron to proteins with all-cysteine ligation of the metals has been shown to drive protein refolding even in the presence of high chaotrope concentrations.^{45,46} Conversely, iron substitution by thiophilic metals other than mercury—requiring that bound iron is in the Fe²⁺ state—has been shown to facilitate unfolding of the same proteins,⁴⁷ as well as that of proteins with a more complex structure or with metal ligands other than cysteine.^{48–51} Although further investigation on either the interaction of IscS with iron-loaded IscU or that of persulfide-loaded IscU with either iron or sulfide (in the presence/absence of physiological reductants) could advance current knowledge on the sequence of events involved in the earliest steps of the assembly process, these studies may be complicated by either the stability of the involved species as well as by the absence of easily monitorable spectroscopic signals allowing to monitor rates and products of assumedly rapid phases of the overall process.

3 | MODULATING THE RATE OF CLUSTER TRANSFER TO ACCEPTOR PROTEINS AND CLUSTER NUCLEARITY

As discussed in previous reports^{28,38,52,53} and summarized in Table 1, the rate observed for multiple turnover cluster transfer from IscU to the *isc*-encoded apoferritin typically used in these studies as the acceptor protein was much higher than those observed for transfer from a pre-assembled IscU holoprotein, isolated after a 2Fe₂S cluster was assembled on it by using either IscS and cysteine or inorganic sulfide. However, the addition of the *isc*-encoded chaperone (HscA) and co-chaperone (HscB) resulted in significant acceleration of the rate of cluster transfer from holo-IscU to a suitable acceptor apoprotein in the presence of Mg/ATP, as also reported in Table 1. Table 1 also includes evidence of sensibly impaired cluster transfer from holo-IscU prepared in the presence of equimolar amounts of the co-chaperone, HscB. The HscB-dependent impairment in the rate of cluster transfer from holo-IscU was insensitive to the addition of ATP alone or of HscA alone, but transfer rates measured upon further addition of HscA and ATP to the system were as high as those observed for holo-IscU prepared in the absence of HscB.³¹

From a mechanistic standpoint, formation of a ternary complex between holo-IscU, HscA, and HscB is required to activate subsequent conformational changes in the complex, that are driven by ATP hydrolysis and lead to a much accelerated release of the IscU-bound cluster (Table 1) through a transient perturbation of the holo-IscU structure.^{52,53} According to a subsequent

TABLE 1 Initial rates of cluster transfer at various scaffold/acceptor molar ratios in the presence/absence of chaperones/co-chaperones/ATP and rates of “catalytic” cluster assembly on the acceptor apoprotein under turnover conditions

Experiment type	IscU, μM	Acceptor, μM	Additional components	Molar ratio acceptor/IscU	Initial rate of cluster transfer ^a , (Mol holoprotein) (Mol IscU) ⁻¹ h ⁻¹	Reference
Cluster transfer from pre-formed IscU-(2Fe ₂ S)	180	100	None	0.55	0.08	53
	42	45	None	1.00	0.32	31
	42	45	HscB, 44 μM	1.08	0.11	31
	180	100	HscA, 300 μM HscB, 300 μM ATP, 2 mM	0.55	1.77	53
	15	16	HscA, 16 μM HscA, 16 μM ATP, 2 mM	1.06	2.22	31
“Catalytic” cluster formation from ApoIscU	11	72	None	6.54	12.6	38
	11	36	None	3.27	4.81	
	11	12	None	1.09	0.60	

detailed study²⁸ involving a number of mutants of the ligand cysteines and of residues involved in protein stability in IscU, evidence was provided for ATP-dependent ligand exchange, the key step for facilitated release of the cluster from the IscU holoprotein bound to the HscA/HscB complex. In particular, a hypothesis is brought forward in which combined action of HscA/HscB/ATP leads to a movement of the region including Cys106 on one of the IscU subunits, that is replaced by Cys37 as a ligand, generating a strong instability in the cluster coordination and facilitating transfer of the 2Fe2S cluster from the scaffold protein to any suitable apoprotein acceptor.

Whatever modification that is induced in holoIscU by the combined action of the HscA/HscB/ATP system, none of these interactors had an effect on cluster nuclearity.⁴⁹ As reported since the earliest days of iron-sulfur chemistry and discussed in the Introduction, the interconversion between 2Fe2S and 4Fe4S cubane-type structures (that represent a sort of “thermodynamic sink” for the process) involves redox event. In chemical systems, the required reductant is often provided by thiols present in the reaction mix. In biological system, the current view implies that isc-encoded ferredoxin represents the source of electrons for dimerization of 2Fe2S clusters into a 4Fe4S structure, that is concomitantly taken up by a suitable apoprotein, such as aconitase, that represents the most commonly used acceptor in these studies.^{16,17,19,20} However, the nature of interactors involved in the generation of reduced ferredoxin for this particular step is still waiting for clarification.

4 | WHY ALL THIS COMPLEXITY FOR A “CHEMICALLY SPONTANEOUS” PROCESS?

Once again, for the sake of simplicity, here we are going to consider only evidence coming from assembly/transfer studies in which inorganic sulfide and ferric salts were used for cluster formation. In this context, all the otherwise relevant considerations on the physiological significance of modulators of the desulfurase activity of IscS in both eukaryotes and prokaryotes^{43,54} do not need to be taken into account. To further simplify our tackling of this issue, it seems convenient to break down this point into separate steps, namely: (a) the assembly of a 2Fe2S cluster on apo-IscU; (b) the transfer of the preformed 2Fe2S cluster hosted on IscU; and (c) the ability of IscU to insert a 2Fe2S cluster into apoproteins under turnover conditions.

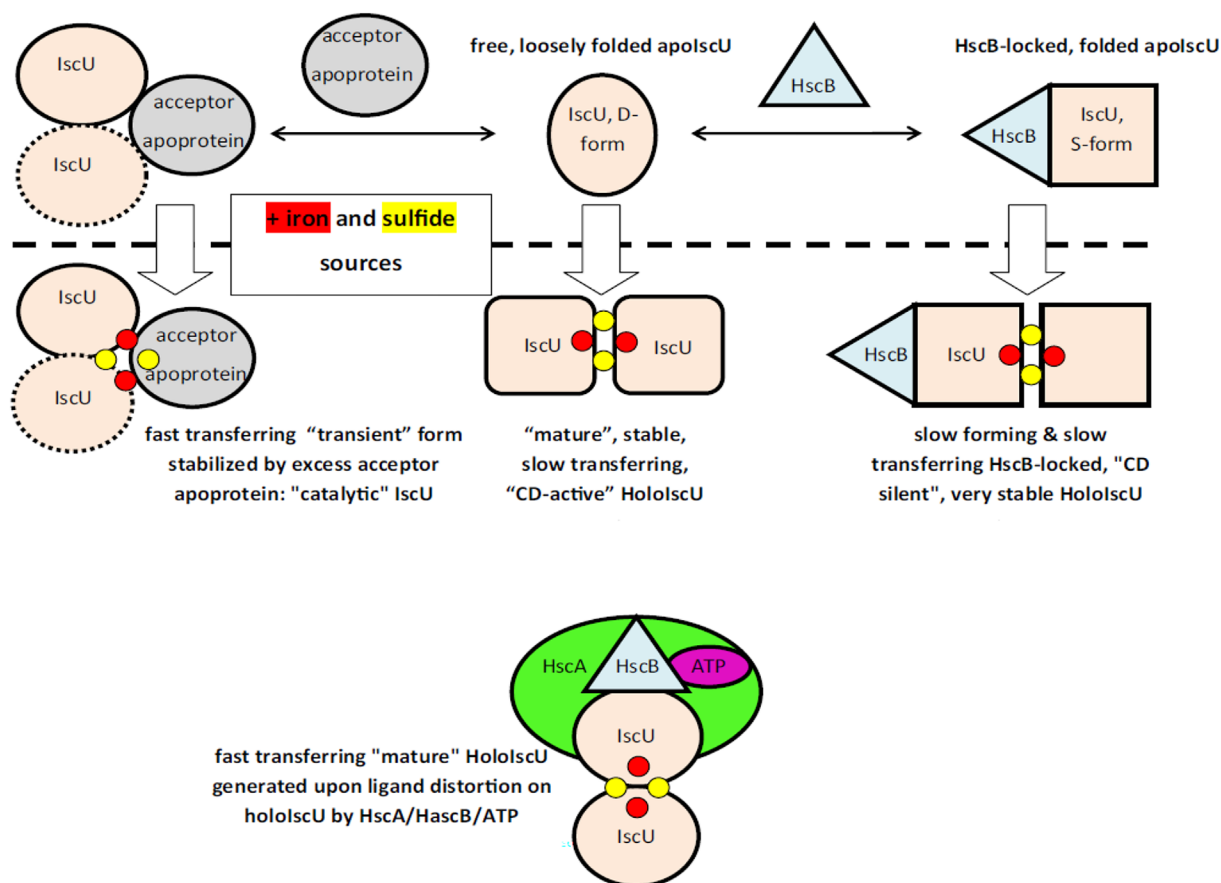
As discussed above, HscB appears to be the main modulator of the structural flexibility of IscU and, henceforth,

of the rate of assembly of a 2Fe2S cluster on the scaffold protein. As summarized in the upper part of Scheme 2, the HscB/IscU interaction may occur independently of the presence/absence of a 2Fe2S cluster on IscU, and may allow to modulate the cluster assembly rate in both chemical²⁸ and enzymatic systems^{27,28,38} as well as the stability of the cluster ligation on holo-IscU. As also inferred from Scheme 2, it seems possible that the relative ratio between HscB and an apoprotein acceptor may dictate whether IscU acts as a “catalyst” for cluster assembly rather than a “reservoir” of pre-made clusters, ready for transfer when acceptor proteins and/or accessory proteins are present. Unfortunately, in spite of the biological relevance of the HscB/IscU interaction and of its conservation through evolution,⁵⁵ structural evidence for the various HoloIscU forms present in Scheme 1 and in Scheme 2 is almost nil, as the current information is essentially restricted to a limited set of spectroscopic data.^{28,31,38}

Of course, as highlighted in Table 1 and in the lower part of Scheme 2, the chaperone activity of HscA/ATP markedly improves the rate of cluster transfer from the very stable cluster-loaded holo-IscU/HscB complex (see Table 1). It may be hypothesized that the HscB/HoloIscU complex may form in the absence of acceptor apoproteins (with the added benefit of keeping toxic intracellular iron in a bound form).⁵⁶ Thus, the HscB/HoloIscU complex may serve as a reservoir of assembled 2Fe2S clusters, to be released to acceptor proteins (when present) at rates modulated by the ATPase activity of HscA.

However, it should be noted that all the proteins mentioned here reportedly work without being capable of altering cluster nuclearity. Therefore, even in the simple prokaryotic cell considered here, accessory additional proteins may be required when the synthesis and insertion of clusters of higher nuclearity is necessary, as demonstrated for the mitochondrial system²⁰ as well as in studies on synthetic clusters⁶ and on isolated proteins.^{16,17,19}

In other words, if one were to frame this hypothetical picture in a cellular context, it could be said that in conditions when the synthesis of apoproteins is very fast, the “catalytic” function of IscU may be prevalent, as fast apoprotein synthesis requires rapid supply of clusters and calls for IscU working under high turnover conditions. Possible effects of the chaperone/cochaperone (HscA/HscB) seem to be immaterial under such conditions. Along the same line of reasoning, conditions where there is modest apoprotein synthesis allow the - relatively slow - formation of the “mature” form of holo-IscU in a process that is made even slower by the presence of HscB. HscB may also impair the interaction of IscU with the sulfurtransferase IscS in the steps required for conversion of cysteine sulfur into cluster sulfide.⁵⁵ The slow rate of cluster release from the HscB/holo-IscU complex



SCHEME 2 Availability of an acceptor apoprotein and formation of complexes with HscB modulate the cluster assembly/release process on IscU

(Table 1) suggests a possible "buffering" function for the complex. However, should a burst in apoprotein synthesis occur, the HscB-IscU bound cluster may undergo controlled ligand distortion by the action of HscA+ATP. The ensuing structural changes will turn a quite stable "reservoir" of 2Fe₂S clusters into a much less stable species, capable of ensuring that cofactor insertion may take place at a rate high enough to meet cellular biosynthetic requirements.

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