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Review

Iron/sulfur proteins biogenesis in prokaryotes: Formation, regulation and diversity ☆

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ABSTRACT

Iron/sulfur centers are key cofactors of proteins intervening in multiple conserved cellular processes, such as gene expression, DNA repair, RNA modification, central metabolism and respiration. Mechanisms allowing Fe/S centers to be assembled, and inserted into polypeptides have attracted much attention in the last decade, both in eukaryotes and prokaryotes. Basic principles and recent advances in our understanding of the prokaryotic Fe/S biogenesis ISC and SUF systems are reviewed in the present communication. Most studies covered stem from investigations in *Escherichia coli* and *Azotobacter vinelandii*. Remarkable insights were brought about by complementary structural, spectroscopic, biochemical and genetic studies. Highlights of the recent years include scaffold mediated assembly of Fe/S cluster, A-type carriers mediated delivery of clusters and regulatory control of Fe/S homeostasis *via* a set of interconnected genetic regulatory circuits. Also, the importance of Fe/S biosynthesis systems in mediating soft metal toxicity was documented. A brief account of the Fe/S biosynthesis systems diversity as present in current databases is given here. Moreover, Fe/S biosynthesis factors have themselves been the object of molecular tailoring during evolution and some examples are discussed here. An effort was made to provide, based on the *E. coli* system, a general classification associating a given domain with a given function such as to help next search and annotation of genomes. This article is part of a Special Issue entitled: Metals in Bioenergetics and Biomimetics Systems.

1. Introduction

Iron/sulfur (Fe/S) clusters are thought to rank among the most ancient and versatile inorganic cofactors found in all kingdoms of life [1,2]. Thanks to their chemical versatility, Fe/S centers can act as catalysts or redox sensors and are predicted to be used by a large number of protein species (over 150 in *Escherichia coli* and 50 in *Mycobacterium tuberculosis* [3,4]). Likewise, Fe/S proteins are found to participate in diverse biological processes such as respiration, central metabolism, DNA repair or gene regulation [5–8].

The most common types of Fe/S clusters are the rhombic [2Fe – 2S] and cubic [4Fe – 4S] types, which possess either ferrous (Fe^{2+}) or ferric (Fe^{3+}) iron and sulfide (S^{2-}). In a vast majority of proteins, cysteine residues coordinate the iron ions of the Fe/S cluster, but histidinyl residues can also function as ligands [1].

Although Fe/S clusters formation can be achieved spontaneously in vitro with inorganic iron and sulfur sources [9], the in vivo situation is more complex and requires so-called Fe/S biogenesis systems. These latter systems were identified in both prokaryotes and eukaryotes. Basic principles and key molecular actors required for the building of a Fe/S cluster are depicted in Fig. 1. Briefly, a cysteine desulfurase produces sulfur from L-cysteine, a scaffold provides a molecular platform allowing iron and sulfur to meet and form a cluster, and a carrier delivers a cluster to the terminal apotarget. The source of iron remains uncertain and multiple origins have been proposed such as frataxin, which will be discussed below in a dedicated section.

Additional factors can join this basic assembly line (Table 1). The number of Fe/S biogenesis systems varies depending upon the organism. Three systems have been identified in bacteria, namely NIF, ISC and SUF systems, the two latter systems being conserved in eukaryotes [10–13]. NIF, first discovered in the nitrogen-fixing bacterium *Azotobacter vinelandii*, is dedicated to maturation of nitrogenase [14,15]. In contrast, the ISC and SUF systems permit the maturation of all Fe/S proteins in the cell. Components homologous to ISC are found in mitochondria, and those homologous to SUF are present in the chloroplasts [16–18].

In the present review, we present the basic principles and most recent findings on how ISC and SUF systems carry out Fe/S cluster biogenesis in prokaryotes. A most significant part of this review deals with characteristics and roles of components assisting Fe/S biogenesis. Because *E. coli* has the two conserved systems, ISC and SUF, it offers a unique opportunity to investigate the interplays between the two systems and to look into an organism that takes advantage of redundancy to accommodate growth conditions and/or target specificity. An attempt was also made to describe the features of Fe/S biogenesis beyond

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model organisms and we aimed at giving a flavor of what biodiversity could make soon available to research in the field. Last, special attention was given to bacterial pathogens and the use they make of Fe/S biogenesis systems. For readers interested in the eukaryotic systems, an excellent and comprehensive review is presented in the same series [18].

2. Mechanism of Fe/S cluster assembly

2.1. The ISC system

The ISC-mediated assembly of Fe/S cluster is mediated by a five-protein complex (Fig. 2), wherein a rich choreography of controlled protein–protein interactions and associated conformational changes are taking place (Fig. 3). Biochemical in vitro studies have shown that IscU is able to act both as a sulfur and iron acceptor, to promote the assembly of the Fe/S cluster, and to transfer it to apotargets [19–27]. Moreover, in vivo, a dominant-negative allele that has a highly conserved aspartate residue changed to an alanine ($IscU^{D39A}$) was trapped with the sulfur donor IscS, in a non-covalent, non-dissociating complex that contains a Fe/S cluster [28]. These studies showed that cluster assembly and release could be uncoupled, and that Fe/S assembly on the scaffold occurred without dissociation of the IscU–IscS complex. These conclusions were later substantiated by in vitro Fe/S transfer assay and structural analysis (see below) [28–30]. Together, these studies provided convincing evidence that IscU acts as a scaffold.

IscS, a pyridoxal-5′-phosphate (PLP)-dependent enzyme, catalyzes the production of sulfur from L-cysteine [31]. The sulfur is transiently bound in the form of a persulfide to an active-site cysteine (Cys328) of IscS and is subsequently transferred to the scaffold IscU [20,21,24,32].

The crystal structure of the *E. coli* apoIscU–IscS complex was obtained by X-ray crystallography showing each IscU molecule interacts with one subunit of the IscS dimer leading to a 2:2 stoichiometry [33]. The catalytic Cys328 residue of IscS was located in a disordered region, but was estimated to be too far from any of the three Fe/S Cys ligands (Cys37, Cys63 and Cys106) of IscU for sulfur transfer, unless movement of the loop occurred [33]. Such movement was likely trapped in the structure of the holoIscU–IscS complex from *Archeoglobus fulgidus* recently characterized. This complex includes the $IscU^{D39A}$ allele that contains a stable [2Fe–2S] cluster (see above), and the IscS active site containing loop that is well ordered and points at the cluster-binding site of IscU [30]. Remarkably, the catalytic Cys residue of IscS is used as a ligand of the [2Fe–2S] cluster together with the three Cys residues of IscU. This structural analysis shows that the D39 residue plays a critical role in the dissociation of the IscU–IscS complex. Its modification to alanine prevents release of the cluster just formed, hence yields a dominant negative allele [30].

NMR methods provided information on the apoIscU–IscS complex, which were quite divergent from those obtained with X-ray crystallography. In fact, NMR investigations of IscU protein have repeatedly pointed to a highly dynamic structure. Early observations with the *Thermotoga maritima* enzyme suggested a “molten globule” type of the structure, whereas the Zn-bound IscU from *Haemophilus influenzae* was largely structured [34,35]. In line with these studies, recent investigations by NMR analysis have proposed that *E. coli* apoIscU could exist in two slowly inter-converting conformational states: one disordered (D) and one structured (S). Importantly, the dynamics of the S/D inter-conversion could be modified by adding Zn^{2+} in the medium, which favored the S state, or by the D39A mutation in IscU [36]. These results suggest that the D state of apoIscU could be the primary substrate for IscS, which would then be converted to an S state that stabilizes the [2Fe–2S] cluster form [37]. Another actor in Fe/S assembly, ferredoxin, has been proposed to participate in the reductive coupling of two $[2Fe–2S]^{2+}$ clusters to form a single $[4Fe–4S]^{2+}$ cluster on IscU [26].

For the Fe/S cluster release process, IscU interacts with two other partners, HscA and HscB, members of the DnaK/DnaJ chaperones/co-chaperones families, respectively [38]. The presence of the specialized HscA and HscB greatly increases the cluster transfer rate in an ATP-dependent manner [39–42]. HscA recognizes a specific LPPVK sequence motif of IscU and its interaction with the scaffold protein is regulated by the co-chaperone HscB, whose interaction with IscU involves hydrophobic residues [43–47]. A model for the mechanism by which the chaperone facilitates cluster release was recently proposed, based on the spectroscopic and kinetic properties of Fe/S transfer of IscU mutants [48]. In this model, among the conformational isomers of IscU2 [2Fe–2S] having different [2Fe–2S] cluster affinity, the chaperone binds and stabilizes an isomer with low [2Fe–2S] cluster affinity, thereby favoring the release of the Fe/S cluster from IscU [48].

As mentioned above, these series of studies have led to picture that the assembly and release steps act as a highly concerted interplay between different conformational states of the scaffold, chaperone and target as depicted in Fig. 3 [37,49]. As we described below, Fe/S carrier proteins are likely to act at a step between cluster assembly on the scaffold and transfer to the target, but these carrier proteins were not included in the Fig. 3 as in vitro Fe/S transfer can occur in their absence.

2.2. The SUF system

The SUF-mediated assembly of Fe/S clusters requires two sub-complexes constituted by the SufBCD and the SufSE proteins, respectively (Fig. 2). The SufBCD complex can bind and transfer a

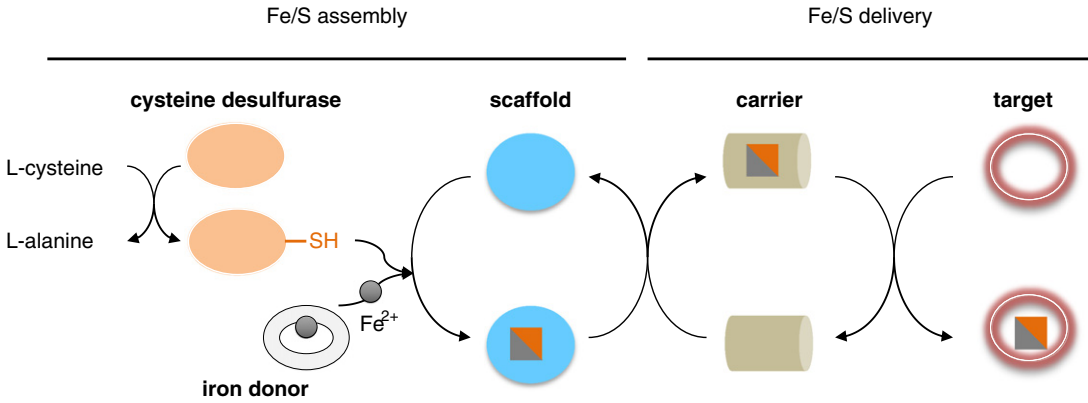

Fig. 1. General principles of Fe/S cluster biogenesis. The Fe/S cluster assembles on a scaffold protein, which receives sulfur from a cysteine desulfurase and iron from an as yet non identified source. Then, the pre-formed Fe/S cluster is transferred to a carrier protein, which delivers it to the final apotarget.

Table 1
Components involved in Fe/S biosynthesis. InterPro (IPR) domain number, biochemical activity, cofactor, motif or functionally important residues are given for each component. Shape and colour of each component are the same as those used in Fig. 7.

	Domain	Description	Cofactor	Motif or functionally important residues	*E. coli* representative
	IPR000192	Aminotransferase, class V cysteine desulfurase; provides sulfur from L-cysteine.	PLP	Cys328/364	IscS, SufS
	IPR003808	Relays sulfur from cysteine desulfurase to scaffold.		Cys51	SufE
	IPR002871	U-type scaffold protein, receives sulfur and iron, builds Fe/S clusters.	Fe/S	Cys37, Cys63, Cys106 99-LPPVK-103	IscU
	IPR001041	2Fe–2S ferredoxin-type domain; provides electrons in Fe/S cluster synthesis.	Fe/S	Cys42, Cys48, Cys51, Cys87	Fdx
	IPR010236	DnaK-like chaperone; ressembles DnaK, but belongs to a separate clade. Involved in Fe/S cluster release from U-type scaffold protein.	ATP		HscA
	IPR001623 IPR009073	DnaJ-like co-chaperone, acts together with the DnaK-like chaperone.			HscB
	IPR000825 (UPF0051)	Fe/S scaffold protein, receives sulfur and iron, builds Fe/S clusters. In complex with IPR011542 and IPR010230.	$FADH_2$ Fe/S		SufB
	IPR011542 (UPF0051)	Interacts with IPR000825 and IPR010230.			SufD
	IPR010230	ATPase of the ATP-binding cassette family (IPR003439).	ATP	Walker A (G34-S41) P loop-Walker B-D loop (E163–D177)	SufC
	IPR000361	A-type Fe/S carrier, receives and delivers Fe/S clusters.	Fe/S	CX61-65CXC	IscA, SufA, ErpA
	IPR000361	Protein interacting domain.			NfuA N-ter
	IPR001075	Nfu-type Fe/S carrier, receives and delivers Fe/S clusters.	Fe/S	149-CXXC-152	NfuA C-ter
	IPR0025669 IPR0019591	AAA (ATPase Associated with diverse cellular Activites) domain; ATPase-like; ParA/MinD family. Binds and transfers Fe/S clusters.	Fe/S ATP	283-CXXC-286 Walker A (V112-S122) Ser182	Mrp
	IPR014434	Monothiol glutaredoxin, binds and transfers Fe/S clusters.	Fe/S GSH	Cys30	GrxD
	IPR002908	Frataxin/CyaY family.	Iron		CyaY
	IPR007500	Domain of unknown function at the N-ter of proteins involved in cell wall development and nitrous oxide proctection.			YtfE
	IPR012312	Haemerythrin/HHE cation-binding motif. This domain binds iron but can bind other metals in related proteins.	Di-iron	6xHis	YtfE
	IPR007457	Fe(II) trafficking protein.		Cys7	YggX
	IPR010242	Transcription factor HTH, rrf2-type.	Fe/S	92-CX5CX5CX2H-107	IscR
	IPR006222 IPR017703	Glycine cleavage T-protein. YgfZ/GcvT conserved site. Folate and tetrahydrofolate binding protein. Unknown function in Fe/S cluster biogenesis.		226-KGCYxGQE-233	YgfZ

[4Fe–4S] cluster to apoprotein [50,51]. In the SufBCD complex, SufB is described as the scaffold since it binds a [4Fe–4S] cluster [50,51]. SufB interacts with SufD, which is a SufB paralog, and with SufC, a soluble ATPase similar to those found associated with ABC transporters [52–54]. SufD was proposed to be involved in iron entry into the complex, and the ATPase activity of SufC is essential but its role is unclear (see below) [55].

The SufSE heterodimeric complex serves as the sulfur donor for Fe/S cluster assembly. SufS, the homolog of IscS, is the cysteine desulfurase, which mobilizes the sulfur from L-cysteine [56]. The homodimeric SufE protein interacts with SufS and greatly enhances its cysteine desulfurase activity [54,57]. X-ray analysis and site-directed mutagenesis established that a persulfide is produced on Cys364 of SufS and then the sulfur is transmitted to Cys51 of SufE. Likewise, SufE was defined as a sulfur transfer protein acting early in the assembly step [54,57]. SufE exhibits a structure similar to that of IscU, but lacks sequence features required to bind a cluster and to interact with HscA/B [58]. Despite that they can be isolated as sub-complexes, SufSE and SufBCD must interact, at least in vitro, as the cysteine desulfurase activity of SufSE is greatly stimulated by the presence of SufBCD [54,59]. Further investigations demonstrated that in vitro, SufC is necessary for the interaction between SufB and the SufSE complex [59]. From all of these studies, it was proposed that the sulfur transfer occurs from SufS to SufE and then from SufE to SufB.

Biochemical analyses established that the SufBCD complex exists predominantly as a $SufBC_2D$ stable complex, but that other subcomplexes between SufB, SufC and SufD components also form [51]. For instance, X-ray crystallography analyses indicated the existence of a $SufC_2D_2$ complex, where the catalytic pocket of SufC is remodeled as to facilitate ATP binding and hydrolysis [60,61]. Moreover, recent in vitro studies have investigated the role of the $SufB_2C_2$ sub-complex in maturation of the [2Fe–2S] ferredoxin [62]. It was shown that $SufB_2C_2$ is more efficient than $SufBC_2D$ to assemble de novo Fe/S clusters on the ferredoxin [62]. These results suggest that, among the different SuBCD complexes, the $SufB_2C_2$ species acts as the final scaffold [62].

A noteworthy and yet unexplained feature of the whole $SufBC_2D$ complex concerns its capacity to bind one equivalent of $FADH_2$ [51]. Such a flavin-based cofactor could serve as an electron donor to mobilize ferric iron from ferric citrate, ferritins or CyaY-Fe^{3+} (see below) to provide iron for Fe/S cluster assembly on SufB.

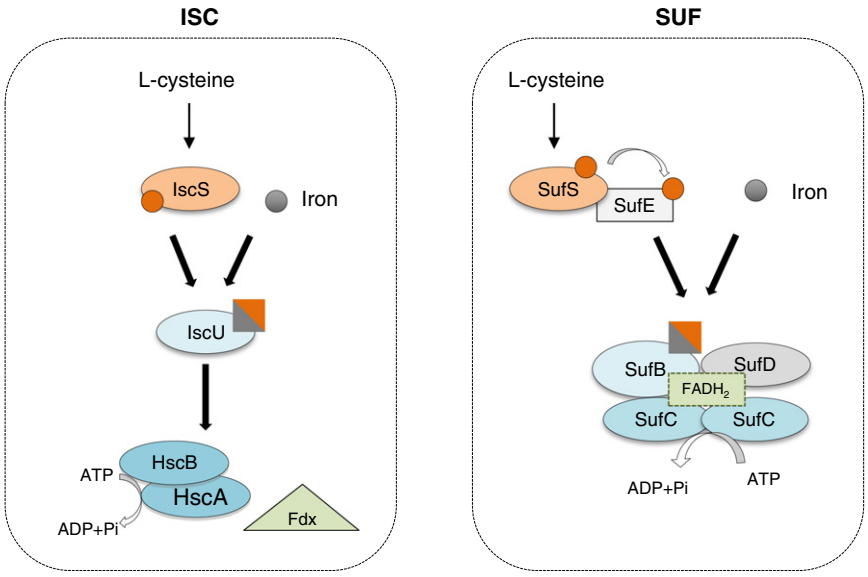

Fig. 2. A model of Fe/S assembly in *E. coli*. The two systems, ISC and SUF, are depicted. For each system, the components assisting the different steps are indicated. ISC and SUF components carrying out similar function are shown with the same colour. Building of the cluster arises on the scaffolds, IscU and SufB. Release of the cluster is catalyzed by the HscBA co/chaperone in the ISC system, whereas this step remains to be clarified within the SUF system. The role of Fdx in ISC remains a matter of discussion as it was proposed to act at the release step in prokaryotes, and in an earlier step in the homologous eukaryotic ISC system [18]. The cofactor $FADH_2$ within the $SufBC_2D$ complex is likely to provide electrons, but its role in Fe/S cluster assembly is not understood.

2.3. A potential hybrid—CsdAE–SufBCD-system

E. coli contains a third cysteine desulfurase, named CsdA, which shares 45% sequence identity with SufS [56,63]. The *csdA* gene is located upstream of *csdE* (formerly *ygdK*), which shares 35% sequence identity with *sufE* [64]. Crystallography and NMR analyses revealed structural conservation between CsdE and SufE [58,64]. CsdA interacts with CsdE, which enhances the CsdA cysteine desulfurase activity resulting in sulfur transfer to Cys61 of CsdE [65,66]. All of these data point to shared properties between CsdA–CsdE and SufS–SufE complexes. Besides the intrinsic features of CsdAE as a sulfur producing system, the *csdA–csdE* genes were found to act as a multicopy suppressor of the *iscS* mutation for deficiencies in vitamin B1 and nicotinic acid [65]. Importantly, this CsdAE-mediated multicopy suppressor effect depended upon the presence of the SufBCD complex. It was proposed that CsdAE recruits the SufBCD scaffold suggesting a third Fe/S cluster assembly system in *E. coli* [66]. Consistent with this hypothesis, characterization of the CsdE interactome revealed a subset of Fe/S cluster-containing proteins, such as NuoF, NuoI, which are Fe/S proteins of the NADH dehydrogenase-I complex, ErpA, an A-type carrier protein involved in Fe/S cluster delivery [67]. Further investigation is needed to clarify conditions under which this potential hybrid—CsdAE–SufBCD–Fe/S assembly complex operates.

3. The delivery step

Once a cluster has been built on a scaffold, it must be transferred to the apoproteins, the total number of which is estimated to be around 150 in *E coli* [3]. Multiple factors have been proposed to intervene in this delivery step but the so-called A-type proteins have received most of the attention [8].

3.1. The canonical A-type carriers (ATC)

E. coli contains three A-type proteins, namely IscA, SufA, and ErpA. Structural genes of the two former belong to the *isc* and *suf* operons, while the *erpA* gene is localized away on the chromosome and forms a single transcription unit [68]. On the basis of in vitro studies, A-type proteins were initially described as alternative scaffold proteins. Indeed, an Fe/S cluster could be reconstituted on A-type proteins by using IscS, L-cysteine and ferric salt [69]. Moreover, the A-type-bound cluster could be transferred directly to a series of apoproteins such as ferredoxin, a [2Fe–2S] protein, and BioB, a [4Fe–4S] protein [69–71]. Lastly, A-type proteins were found to bind Fe/S clusters *via* three conserved cysteine residues, as it was observed for the IscU scaffold [70,72,73]. However, the role of A-type proteins as scaffolds was not satisfying for many reasons. First, they were not found to interact with the cysteine desulfurase proteins, in contrast to IscU [33]. Second, mutations in *iscA* or *sufA* had no phenotype and overexpression of *iscA* or *sufA* failed to suppress an *iscU* mutation [74–76]. Last, in vitro studies revealed that the Fe/S cluster was transferred from IscU to IscA (or from SufBCD to SufA) but the reverse was not possible [50,71]. In contrast, a new competing hypothesis emerged that A-type proteins were iron donors [75,77–79]. Such a hypothesis was attractive considering the lack of identified iron source but appear to be inconsistent with the capacity of the A-type proteins to bind an Fe/S cluster in vivo, as observed with the "as isolated" IscA proteins of *Thermosynechococcus elongatus* and *Acidithiobacillus ferrooxidans* [73,80]. Moreover, the *E. coli* "as isolated" SufA contained a [2Fe–2S] cluster when it was purified from cells co-expressing higher than normal levels of the whole SUF system [81]. As a consequence, a third hypothesis was put forward, which postulated that A-type proteins are "Fe/S cluster carriers", that is, proteins that could capture pre-formed Fe/S clusters and deliver them to downstream apotargets [68,81,82].

IscA and SufA were shown to be functionally redundant and essential as the double *iscA sufA* mutant was not viable under aerobiosis [82]. ErpA was also found to be essential under the same conditions [68]. Lethality of *iscA sufA* and of *erpA* mutants was interpreted as reflecting a reduced maturation of the essential isoprenoid synthesizing enzymes IspG/H. Moreover, the presence of functional SufB or IscU was found to be required for the function of these A-type proteins, thereby demonstrating that scaffolds (IscU, SufB) and A-type proteins do not fulfill the same function in the Fe/S biogenesis process [82]. Taken together, these results led to the proposal that A-type proteins form an Fe/S transfer relay and thus they were named A-type carriers (ATCs). In this view, clusters travel from IscU or SufB scaffolds, where they are built, to the

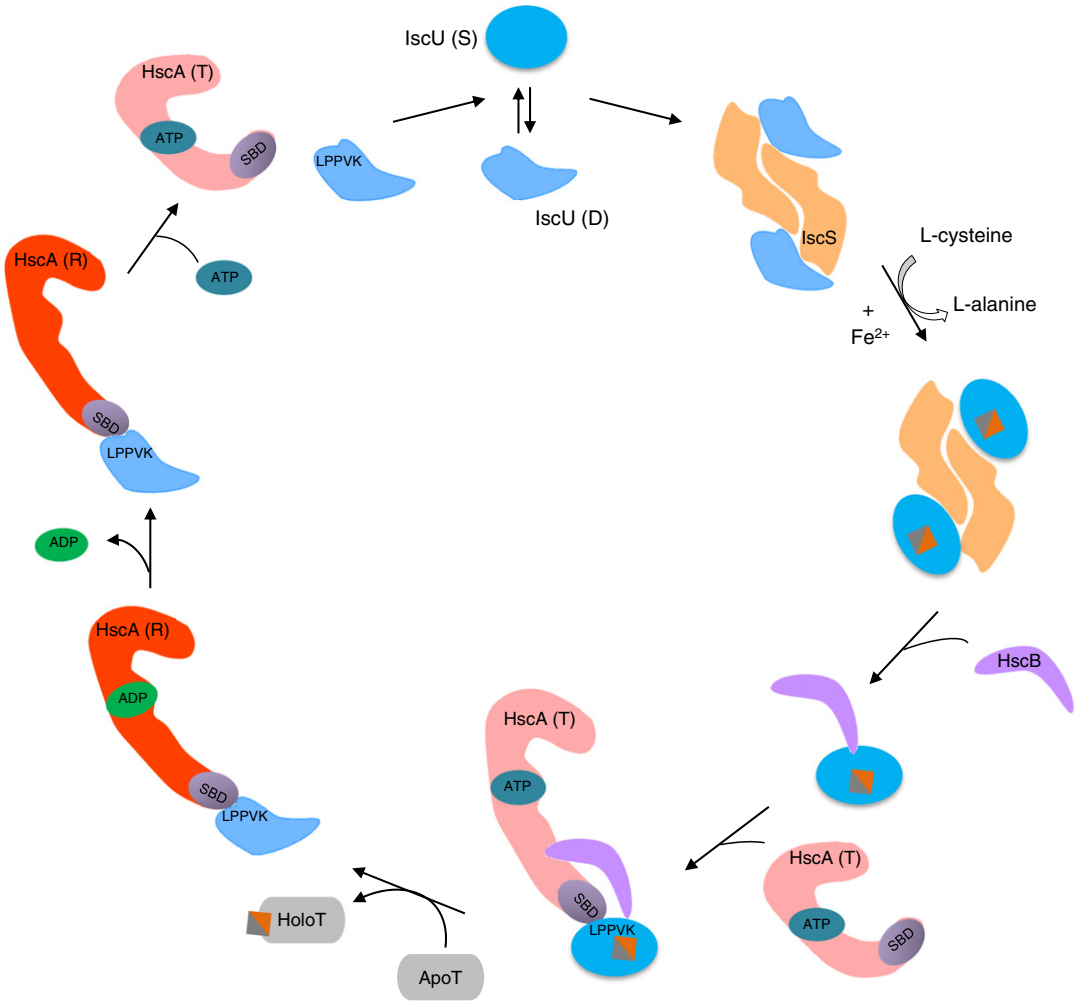

Fig. 3. The interplay between the scaffold and the chaperone within the ISC system. All components involved in the assembly and the release steps are predicted to arise in different conformations. IscU occurs either in a disordered (D) or structured (S) state. In its D state, IscU interacts with the IscS dimer. Conversion of IscU to the S state is accompanied by Fe/S cluster formation. IscU under its S state has little affinity for IscS, and HscB can displace it easily. HscB targets the ATP-bound HscA chaperone, which is in a tense state (T), to holoIscU leading to a temporary HscA/ATP/HscB/holoIscU complex. The substrate binding domain (SBD) of HscA binds the conserved sequence motif ^{99}LPPVK^{103} of IscU. Then, the apoform of an acceptor protein (apoT) could attack the Fe/S cluster of IscU, leading to ATP hydrolysis and conversion of S state to D state of IscU. Thus, the ADP-bound form of HscA could stabilize the D state of IscU, which releases the Fe/S cluster. Then, exchange of ADP with ATP leads to a conformational change of HscA, which releases apoIscU under its D state, ready to engage in a new cycle of interaction with IscS. Adapted from Bonomi et al. [48] and Kim et al. [37,49].

ATCs IscA or SufA then to ErpA, which transfers them to the final apotargets IspG/H (Fig. 4). This model was sufficient to account for the maturation of other Fe/S proteins such as hydrogenases, formate-nitrate reductases and NsrR, the nitric oxide stress sensing transcriptional repressor [76,83,84]. Interestingly, the maturation of IscR did not appear to follow the same scheme [76,85,86]. ApoIscR was found to be maturated by several pathways, one of them being ATC-independent as in vitro and in vivo studies suggested that the SufB scaffold could directly transfer a Fe/S cluster to apoIscR (Fig. 4) [76]. In fact, genetic analysis showed that Fe/S cluster trafficking could use different routes (defined by the type of Fe/S biogenesis factors recruited) as a function of the growth conditions (Fig. 4). The choice of one route over the other is predicted to be dictated by intrinsic features of the apotarget being matured, such as stability of its Fe/S cluster and its cellular concentration, by its affinity with components of the Fe/S cluster biosynthesis system, and by the overall cellular demand for Fe/S clusters under the conditions considered. It is a fact that some of these variables will be strongly influenced by the growth conditions. For instance, in *A. vinelandii*, IscA was required for the maturation of [4Fe–4S] proteins under high oxygen concentration only [87]. Similarly, in *E. coli*, ErpA is required for IspG/H maturation under aerobic conditions, but can be replaced by IscA under anaerobiosis [82].

Recently, the issue of whether ATCs are Fe/S carriers or Fe donors received specific attention from the Johnson's lab [88,89]. Hence, both the Fe/S bound and Fe bound species of the *A. vinelandii* ^{Nif}IscA were submitted to a thorough spectroscopic and functional characterization. The conclusion favored the notion that ATCs function as Fe/S carriers as ^{Nif}IscA was found to accept a cluster from [4Fe–4S] cluster-bound ^{Nif}IscU and to deliver it to the aponitrogenase [88]. In addition, Mapolelo, Johnson and coworkers indicated that ^{Nif}IscA has high affinity for Fe^{3+}, which could revive the debate about IscA acting as a Fe donor for Fe/S cluster assembly. However, they pointed out the fact that the release of Fe^{2+} from Fe^{3+} bound-IscA requires L-cysteine, which serves already as a substrate for sulfur acquisition [89]. As a consequence, IscA should be considered as a Fe/S carrier protein rather than a Fe donor. The possibility that IscA could exploit its Fe-binding capacity to repair damaged clusters or helps convert a [2Fe–2S] cluster to a [4Fe–4S] cluster remains an open question (see below).

Another important issue raised by this spectroscopic study relates to the type of clusters ATCs carry. In vivo analyses suggested that ATCs are required for the maturation of [4Fe–4S] proteins [76,90,91]. An early study showed that the *E. coli* IscA binds either a [2Fe–2S] or a [4Fe–4S] cluster [71]. Hence the question arose of whether the [4Fe–4S]-bound ATC could originate from the [2Fe–2S]-bound ATC

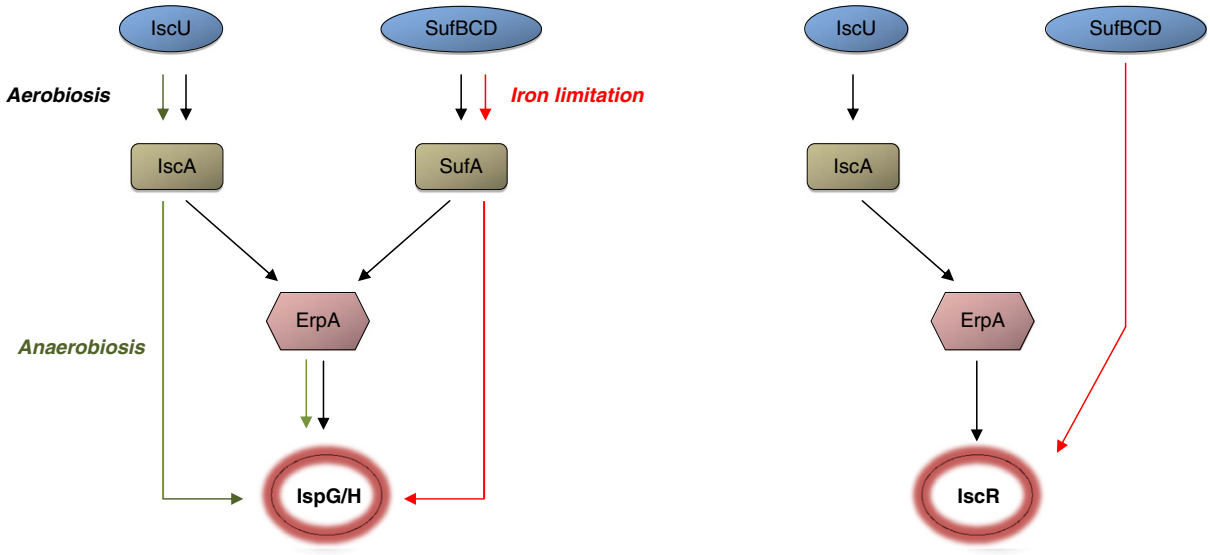

Fig. 4. A model of Fe/S cluster delivery from scaffold to apotargets. The figure is meant to illustrate two features of the way proteins get their clusters in vivo: (i) a same apoprotein can get its clusters from different "routes" probably as a function of the growth conditions, and (ii) different proteins might exhibit different maturation maps. Colour code for environmental conditions is as follows: black arrows: aerobiosis, green arrows: anaerobiosis, red arrows: iron limitation. Left: maturation of IspG/H, two [4Fe–4S] cluster-containing enzymes catalysing the production of isoprenoïds. Right: maturation of the IscR transcriptional regulator.

via a reductive coupling mechanism, and this was recently demonstrated to be the case by the Johnson's lab [89]. In this context, it is worth considering that ferredoxin had been found earlier to interact with the [2Fe–2S] cluster-bound IscA, and therefore, could act as an electron donor for this reductive coupling mechanism [69,88]. However, an in vitro study of the O_2-sensing regulator FNR in *E. coli* suggested another mechanism for the [2Fe–2S] to [4Fe–4S] cluster conversion [92]. Indeed, it was demonstrated that, under aerobic conditions, a [2Fe–2S] cluster could be ligated with two cysteine persulfide ligands. In this case, the generation of a [4Fe–4S] cluster from the [2Fe–2S] cluster could be made possible by incorporation of two Fe^{2+} ions [92]. In such a model, the putative iron binding capacities of ATCs could conceivably be exploited by the cell [89].

3.2. The atypical ATCs: scaffolds or true carriers?

The Nfu domain was among the first to be identified in the protein databases as an ubiquitous domain found in a large number of different proteins [93]. Briefly, the Nfu domain can occur as a single polypeptide like in cyanobacteria where it was concluded to be a scaffold [94,95]. In eukaryotes, the Nfu domain underwent a series of duplications and/or fusions which led to the occurrence of multiple multi-domains chloroplastic or mitochondrial proteins. Others variants include fusion of the Nfu domain to entities such an apurinic endonuclease.

A recent phylogenomic analysis revealed that Nfu containing proteins could be organized into 4 different classes, each exhibiting different domains organization [93].

E. coli synthesizes the NfuA type, which is the most represented class I. It consists of a fusion between a degenerated ATC domain (ATC*), i.e. lacking the three cysteine residues that ligand the Fe/S cluster, and an Nfu domain [93,96]. The C-terminal Nfu domain was found to contain a [4Fe–4S] cluster, whereas the degenerated ATC* N-terminal domain interacted physically with NuoG, an Fe/S protein of the NADH dehydrogenase-I complex, and with aconitase B [93,96]. Moreover, it was shown that NfuA can acquire a Fe/S cluster from both the IscU and $SuBC_2D$ scaffolds, indicating that this Fe/S carrier works with both the ISC and SUF systems. Because NfuA is important for *E. coli* and *A. vinelandii* to sustain growth under stress conditions [96,97], a possibility is that NfuA has evolved such as to ensure the maturation of some more fragile Fe/S proteins by interacting directly with them, presumably rendering the Fe/S cluster transfer more efficient under stress conditions. Interestingly, the degenerated ATC* domain was found to enhance Fe/S cluster transfer efficiency from NfuA to apotarget [93].

Another factor is Mrp (also called ApbC). Mrp is an ATPase, that binds and transfers an Fe/S cluster in vitro [98–100]. Mrp is a member of the ATPase ParA-like family, which shares sequence similarity with eukaryotic proteins involved in Fe/S cluster biogenesis, such as Nbp35 and Cfd1 [100–102]. In *Salmonella enterica*, ApbC was proposed to be a scaffold as an increased *apbC* gene dosage could suppress *iscU* mutations [99]. However, the archaeal ApbC/Nbp35 homolog was described as a carrier rather than a scaffold [100]. Its actual role in the *E. coli* context, its targets and how it cooperates with the ISC and SUF systems remain to be investigated.

A drop in the activity of some Fe/S enzymes is observed in a yeast strain deleted of the mitochondrial monothiol glutaredoxin *grx5*, and in mutants defective for glutathione (GSH) biosynthesis in *S. enterica* and *Saccharomyces cerevisiae* [103–106]. In addition, the deletion of *grxD*, the gene encoding the monothiol glutaredoxin of *E. coli*, is synthetic lethal with mutations in the *isc* operon [107]. Together, this indicates a link between monothiol glutaredoxins, glutathione and Fe/S cluster homeostasis. In vitro studies showed that monothiol glutaredoxins form a homodimer bridged by a [2Fe–2S] cluster with two GSH molecules providing sulfur atoms as ligands [108–111]. Further in vitro characterization showed that monothiol glutaredoxins receive the [2Fe–2S] cluster from the ISC scaffold and transfer it to apoferredoxin [109,111–113]. These characteristics qualify the monothiol glutaredoxins for being carrier proteins in the [2Fe–2S] cluster trafficking. Additionally, monothiol glutaredoxins and GSH impact on iron metabolism [111,114–123]. This is illustrated by the fact that an *E. coli* grxD mutant is hypersensitive to an iron chelator, and that in *S. cerevisiae*, the cytosolic monothiol glutaredoxins controls the Fe regulon [111,117–122]. Thus, in addition to its role as Fe/S cluster carriers, the monothiol glutaredoxins could also influence Fe/S clusters biogenesis *via* their link to the iron and GSH metabolism.

4. CyaY: a possible iron donor protein

CyaY is the bacterial homolog of the eukaryotic frataxin, a highly conserved protein with homologs in almost all known organisms [124]. In humans, frataxin deficiency causes various metabolic disturbances and was found to be responsible for Friedreich's ataxia, an autosomal recessive neurodegenerative disease [125–127]. Frataxin deficiency results in the

accumulation of mitochondrial iron and a loss of Fe/S protein activity [124,128]. The CyaY protein was shown to rescue a yeast frataxin (Yfh1) deletion mutant [129]. Moreover, the deletion of *cyaY* in *E. coli* has been shown to not affect iron homeostasis [130]. Although a *cyaY* mutant in *Salmonella enterica* does not exhibit any significant Fe/S or iron related phenotypes, unless combined with other specific lesions, an *E. coli cyaY* mutant exhibited reduced amounts of complex I and II, most likely due to reduced biogenesis of their Fe/S cluster-containing proteins [131, 132]. Recently, a new type of structural homolog of frataxin was reported in *Bacillus subtilis*, the YdhG protein. YdhG displays the known properties of frataxin (i.e. iron binding, interaction with the scaffold protein) and its inactivation showed a severe growth phenotype and a decrease in Fe/S protein activity [133,134].

The role of CyaY has been further investigated in vitro. CyaY interacts with the IscU–IscS complex in vitro [33,135] and was shown to bind iron specifically in a stable form, but with very unusual properties compared with typical iron binding proteins [136,137]. Indeed, CyaY can bind iron but relatively weakly compared to ferritins, and this iron-binding does not involve histidine or cysteine, the conserved residues usually found in typical iron-binding proteins [136,138]. Finally, studies with *E. coli* proteins reported that CyaY could act as an inhibitor of Fe/S cluster assembly by slowing down the activity of the cysteine desulfurase IscS [135,139,140]. However, the results were somehow contradicted by the fact that eukaryotic frataxin was found to accelerate Fe/S cluster assembly by stimulating Nfs1/Isd11 cysteine desulfurase activity [141]. Despite the difference whether it is activating or inhibiting [142], the fact was that these studies pointed to a role of frataxin in sulfur production rather than iron delivery. Thus, all of these data converge to an involvement of CyaY in Fe/S cluster biogenesis, but its precise contribution remains to be sorted out.

5. Regulation of Fe/S homeostasis in *E. coli*

One of the primary roles of Fe/S biogenesis regulation is to maintain, rebuild and possibly repair the pool of Fe/S cluster-containing proteins in fluctuating environmental conditions. In *E. coli*, the regulator IscR lies at the core of Fe/S homeostasis regulation but additional regulators are also involved such as Fur, OxyR and possibly NsrR (Fig. 5) [8,143,144].

IscR is a [2Fe–2S] transcriptional regulator encoded by the first gene of the *iscRSUA-hscBA-fdx* operon [145]. Mutagenesis studies have shown that IscR contains three cysteines (Cys92, Cys98 and Cys104) likely required for the Fe/S cluster ligation, a feature expected of most [2Fe–2S] cluster proteins that have cysteinyl (Cys) ligation [146]. However, recent data from the Kiley' lab have reported an unusual ligation scheme in IscR, since a histidine residue (His107), in addition to the three cysteines, is essential for [2Fe–2S] cluster ligation [147]. This atypical His residue ligand might render the cluster less stable under specific conditions, a feature that could be connected with its sensor role.

Genome-wide transcription profiling showed that IscR regulates expression of at least 40 genes in *E. coli*, among which are the *isc* and *suf* operons [148]. Most of the other genes encode Fe/S proteins such as the periplasmic hydrogenases (HyaA, HybO) and Fe/S carriers (ErpA, NfuA) [68,96]. IscR regulates also some non Fe/S protein encoding genes such as the *fim* and *flu* operons, involved in surface structure, and several operons whose function remains unknown, suggesting an important role of IscR in cell homeostasis [148]. Mutagenesis analyses have revealed the existence of two classes of IscR-binding sites, namely the Type 1 and Type 2 [148]. It was demonstrated that holoIscR (i.e. containing its [2Fe–2S] cluster) binds the Type 1 site with a higher affinity than apoIscR, whereas both the holo and apoform bind similarly to the Type 2 site [146,148].

Both in vitro and in vivo studies have shown that IscR, in its holoform, represses its own expression as well as that of the rest of the *isc* operon by binding to the Type 1 sites in its promoter [86,145,147,148]. Then, under conditions unfavorable for Fe/S maturation of IscR, repression of the *isc* operon is alleviated (see below). This mechanism allows IscR to sense Fe/S homeostasis, assuming that IscR ranks among the poorest Fe/S substrates of the ISC machinery, and in this way can instruct the cell about the equilibrium between the Fe/S cluster demand and its capacity to respond to it. This view has been recently supported by the fact that under aerobiosis, overexpression of the [4Fe–4S] protein FNR, which amplified the overall cellular Fe/S cluster demand, led to the induction of the *isc* operon [86].

In addition to its regulation of the ISC system, IscR also participates in the regulation of the *suf* operon [148–150] (Fig. 5). It was shown that IscR in its non-mature, apoform, activates expression of the *suf* operon which exhibits a Type 2 DNA-binding site [146]. Thus, the prediction is that, under conditions that favor accumulation of apoIscR, expression of both the *isc* and *suf* operons will be induced and the cell accumulates both Fe/S biogenesis sytems. Such conditions comprise iron limitation and oxidative stress (Fig. 5) [149–151].

Interestingly, these two stress signals are transduced in part to the *suf* and *isc* operons via additional regulators, Fur, RyhB and OxyR. Fur, the iron sensing regulator, controls SUF synthesis directly as Fur-Fe^{2+} binds to the *suf* promoter and represses its expression (Fig. 5). Fur controls ISC synthesis indirectly via a small non-coding RNA, RyhB (Fig. 5). RyhB, whose synthesis is under the negative control of Fur, base-pairs at the level of the *iscS* Shine Dalgarno sequence of the *iscRSUA* polycistronic mRNA. This pairing leads to the degradation of the 3′ part mRNA, containing *iscSUA*, while the 5′ part, containing *iscR*, is stabilized and actively translated [152]. The consequence is that apoIscR formation is favored and synthesis of the SUF system enhanced as the apoIscR form will activate *suf* operon expression. Thus, Fur/RyhB will allow switching from the ISC to the SUF system when iron becomes limiting. The challenge for the future will be to predict the outcome of the interplays between the two Fur/RyhB and IscR controlled circuits. Presumably, relevant variables will concern iron concentration, whether the system goes from high-to-poor iron (or the reverse) and the kinetics of the response of each operon. Last, oxidative stress is sensed by OxyR, which acts as an activator of *suf* operon expression (Fig. 5) [153]. Oxidative stress is also likely to be sensed by IscR as it might destabilize the Fe/S cluster and by Fur as iron will become limiting. Here, also, the interplay between OxyR and the other regulators will be of great interest to investigate further.

In addition, the following physiological and biochemical studies suggest that under stress conditions, cells switch from the ISC to the SUF system rather than accumulate both systems: (i) the SUF system is more efficient to collect iron under limiting conditions; evidences were provided for this with the *Dickeya dadantii* model [53], (ii) the ISC system likely depends upon a ferredoxin/ferredoxin reductase system like in the case of mitochondria, whereas the SUF system might depend upon an iron-free flavin reductase system (see above), hence allowing better adaptation to iron poor conditions [51], (iii) the scaffold IscU is a potential entry site for poisoning the ISC system by hydrogen peroxide and possibly metals (see below) [154,155] and (iv) recent work by the Outten's lab reported that the SufS-SufE complex appears to be more resistant to H_2O_2 as compared with the IscU–IscS complex [156].

6. Diversity of Fe/S biogenesis systems

6.1. Cellular diversity

Since their discovery, Fe/S biosynthesis machineries have received much attention because of their role in essential cellular processes. Even though homologs of the *E. coli* ISC and SUF systems are found in many organisms, it is interesting to note that some species possess an unusual pattern of Fe/S cluster assembly systems.

Most Gram-positive bacteria carry only a *suf* operon, which exhibits some differences with the *E. coli* operon (Fig. 6). Indeed, it comprises a *sufU* gene, no *sufE* and the *sufA* gene is elsewhere in the

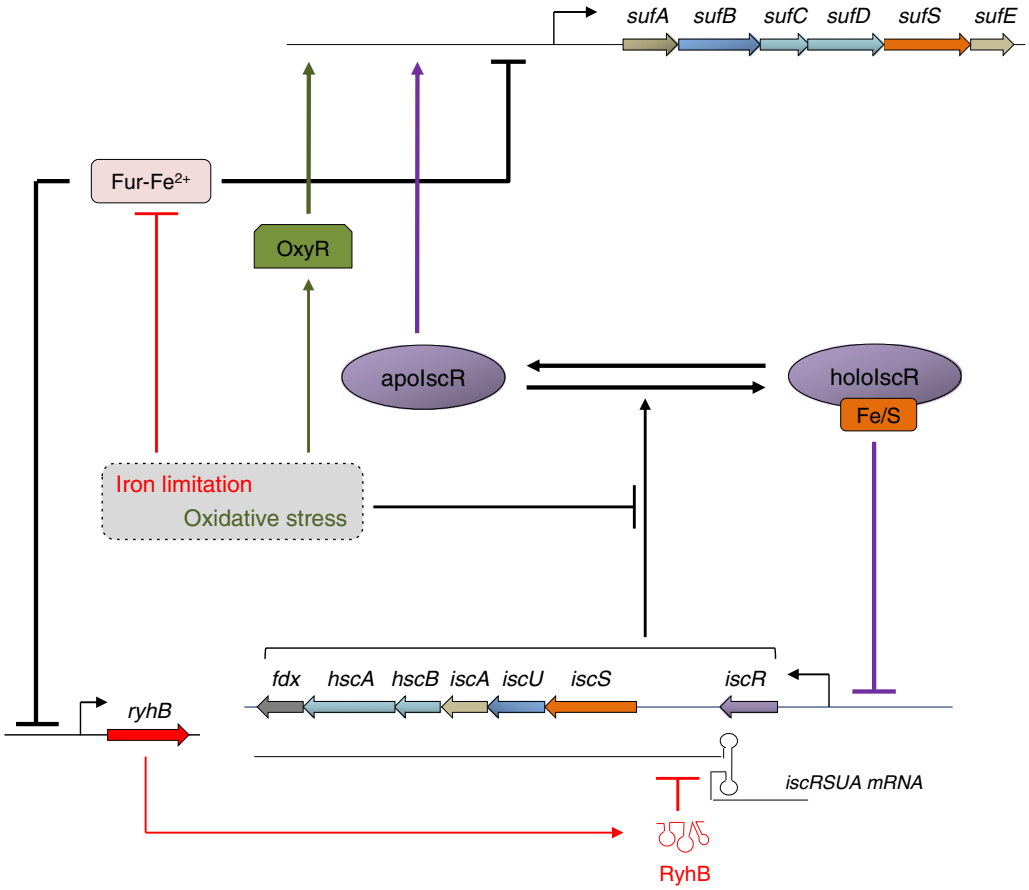

Fig. 5. A model of the genetic regulation of Fe/S homeostasis in *E. coli*. The *suf* (top) and *isc* (bottom) operons are depicted. The *suf* operon expression is shown to be under the control of Fur, OxyR and IscR regulators. The *isc* operon expression is shown to be under the influence of IscR and of the small non coding RNA, RyhB. Under normal conditions, the IscR regulator is matured by the ISC system and represses its expression to give rise to an autoregulatory circuit that senses the Fe/S state of the cell. The *ryhB* gene is not expressed under such conditions. Under iron limitation, the *suf* operon is activated in two ways: (i) Fur repression is alleviated, and (ii) the *ryhB* gene is expressed, the IscSUA mRNA, its target, degraded, the IscSUA proteins not synthesized and the IscR regulator converted to its apoform, which activates the expression of the *suf* operon. Under oxidative stress, OxyR activates the expression of the *suf* operon. Under such conditions, the IscSUA system is thought to be non-functional and apoIscR formation favoured. Moreover, oxidative agents might directly damage the Fe/S cluster bound to IscR. As over-expression of the SUF system was reported to enable IscR maturation, one might predict that under stress conditions, the ISC system will be completely shut down.

chromosome [157,158]. First identified in *Thermotoga maritima*, SufU is an IscU-like protein, which lacks the LPPVK motif essential for the IscU–HscA interaction [157]. This is consistent with the absence of *hscA* in these genomes. Functional, enzymological and structural studies established that SufU is essential for growth in synthetic defined media in *B. subtilis* [158]. SufU, which receives sulfur from the SufS cysteine desulfurase, acts as a scaffold allowing maturation of multiple Fe/S proteins both in vivo and in vitro [158–161]. Interestingly, in addition to the SufU scaffold, most Gram-positive genomes also encode the SufBCD scaffold complex. This scaffold redundancy could help the bacterium to grow under different environmental conditions. By analogy with the *E. coli* system, one could predict that SufU might work under normal growth conditions, while the SufBCD complex would operate under stress conditions. Last, *B. subtilis* was shown to also have one ATC and Nfu.

Cyanobacteria, like *Synechocystis* spp. have a SUF system that is slightly different from the one found in *E. coli* as it lacks *sufA* and has a *sufR* gene, that encodes a Fe/S cluster-containing transcriptional repressor of the *suf* operon (Fig. 6) [162,163]. This cyanobacterium has also an unusual set of *isc* genes scattered around the genome with two IscS-like cysteine desulfurases, but neither *hscB* nor *iscU* [164]. Another variation of the theme is found in the pathogen *M. tuberculosis*, which contains only a simplified *sufBCDS* operon [165]. Interestingly, SufB contains an intein sequence, whose splicing is required for SufB to interact with SufC and SufD proteins [166].

Archaea show an "unorthodox" situation. Archaea are well known to make use of Fe/S proteins, yet phylogenetic analyses indicated that known Fe/S biogenesis factors are only found in few Archaea species, probably acquired by lateral gene transfer [82]. In these cases, one finds SUF scaffold SufBCD (or a reduced version including SufC and one SufB/D homolog) and the Mrp/ApbC Fe/S carrier [167]. Whether these two elements, a scaffold and a carrier, constitute the minimal Fe/S biosynthesis pathway in Archaea remains to be demonstrated. The fact that no ISC system can be found in Archaea supports its emergence in eubacteria. Unexpectedly, cysteine desulfurase encoding genes are often missing and a very recent paper argues that in the methanogenic *Methanococcus maripaludis*, exogenous sulfide, rather than cysteine, is the source of sulfur for Fe/S cluster [168]. Although limited, these observations clearly set the stage for an exciting avenue of research in Fe/S biosynthesis in Archaea.

6.2. Molecular diversity

Fe/S biogenesis factors often exhibit a multi-domain structure. For instance, a simple search of available databases revealed that the cysteine desulfurase domain is found into 98 different types of multi-domain proteins that differ in the nature of the associated domain. Similarly, the U-type domain is found in 14 different types of multi-domain proteins. For example, *A. vinelandii* NifU possesses three domains (U-type scaffold, ferredoxin and Nfu-type carrier). Also, Fe/S carrier modules were

themselves sites of an intense evolutionary engineering. The case of NfuA, resulting from a fusion of two functional modules accompanied by the loss of the Fe/S cluster binding property of the N-terminal module (ATC*) is only one example. Other examples include the so-called Dsr proteins from sulfur oxidizing bacterium *Allochromatium vinosum*, which only contains a degenerated ATC domain (ATC*) [169]. Other cases include a protein with three domains, an N-terminal monothiol glutaredoxin domain, a central degenerated ATC domain and a rhodanese sulfurtransferase domain. Some interesting new associations of Fe/S related domains can be found in proteins from organisms whose Fe/S biogenesis has not yet been studied. For instance, in *Neorickettsia sennetsu*, *Halogeometricum borinquense* DSM 11551 and *Crocosphaera watsonii*, a cysteine desulfurase domain is fused to IscR, a U-type scaffold and a Nfu-type Fe/S carrier, respectively (Fig. 7). Analysis of the various combinations between Fe/S biogenesis domains might help in understanding their role in Fe/S biogenesis pathways. It might also provide biochemists with new type of enzyme to study. A limited series of the most unorthodox cases are given in Fig. 7 to illustrate the evolutive tinkering that took place during Fe/S biogenesis evolution.

7. Fe/S biogenesis and environmental stresses

7.1. Fe/S and oxidative stress

Bacteria are exposed to reactive oxygen species (ROS) produced intracellularly by normal aerobic metabolism or in the external environment [170]. Accumulation of ROS-dependent damage is a threat for the cell as they target macromolecules such as DNA, lipids or proteins. Studies by the group of Imlay and Fridovich showed how ROS and Fe/S clusters are intimately connected. Interestingly, Fe/S clusters seem to be both the sources and the targets of ROS. Superoxide and hydrogen peroxide can destabilize solvent-exposed labile $[4Fe-4S]^{2+}$ clusters such as those found in dehydratases. In these clusters, one of the four iron atoms is solvent-exposed and is loosely bound to a water molecule and to the enzyme's substrate [171,172]. Superoxide or hydrogen peroxide can enter the active site, subsequently oxidize and convert the $[4Fe-4S]^{2+}$ cluster into a $[3Fe-4S]^{+}$ state or possibly beyond to an apoprotein form [154,172]. This yields an inactive enzyme and accounts for the fact that exposure of *E. coli* to oxidative stress leads to inhibition of those biosynthetic and metabolic pathways employing $[4Fe-4S]^{2+}$ cluster-containing dehydratases. Recent studies demonstrated that in vivo submicromolar concentration of hydrogen peroxide is sufficient for damaging clusters of relevant dehydratases when ROS-scavenging enzymes are absent [173]. Yet, inhibition of Fe/S cluster-containing dehydratases is only one part of ROS-mediated damage. The other part comes from the fact that oxidation of the cluster releases the once solvent exposed iron atom, which can react with H_2O_2 and generate hydroxyl radicals that cause DNA damages [170]. An essential question pertains to the fate of those dehydratases that have been damaged by oxidation. As illustrated by a study with fumarase A, the $[3Fe-4S]^{+}$ product formed from the degradation of the $[4Fe-4S]^{2+}$ cluster was found to be repaired in vivo [174]. Presumably, a component acting as a ferrous iron donor would be sufficient in this case to repair the cluster. Identity of such repair factors remains elusive but YtfE, YggX or Fe-bound ATCs have been proposed to serve this function [89,170,175,176]. A different result was observed in the study of isopropyl malate dehydratase, where the $[4Fe-4S]^{2+}$ cluster was actually damaged in vivo beyond the $[3Fe-4S]^{+}$ state to $[2Fe-2S]$ cluster or its total loss [154]. Hence, in this case, in addition to iron, sulfur atoms need to be supplied to repair the cluster. In principle, both the ISC and the SUF systems could act to repair this type of damage. However, the ISC system is sensitive to oxidative stress and recently, the SUF system was shown to allow *E. coli* to repair damaged Fe/S clusters [154]. That the SUF system is endowed with a repair function is fully consistent with its in vivo expression pattern (see above).

7.2. Fe/S and metal stress

Recently, Fe/S cluster-containing enzymes were identified as being the primary targets of toxic metals and likely the cause of their toxicity.

Copper exposure was found to inhibit cellular processes similar to those observed when cells are subjected to oxidative stress, e.g. auxotrophy to branched amino acids and inhibition of a series of Fe/S cluster-containing dehydratases [177]. Curiously, similar defects were observed under anaerobiosis, rendering the oxidative stress connection unlikely. In vitro analysis of fumarase damaged by copper suggested that inhibition was due to a loss of cluster iron atoms which was supported by the finding that no mixed cluster containing copper could be identified [177]. Copper seemed to alter only those

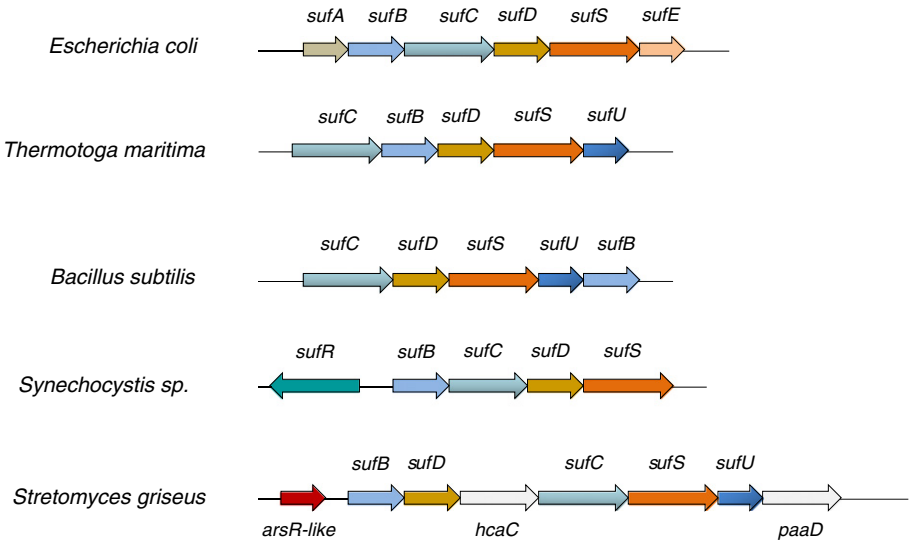

Fig. 6. Schematic representation of the *suf* locus in different bacterial species. Genes having similar functions are colour-coded. The *suf* locus of *S. griseus* comprises "non-*suf*" genes predicted to encode a transcriptional regulator (ArsR), and enzymes involved in the catabolism of propionate (HcaC) and phenyl acetate (PaaD), two pathways relying on Fe/S cluster-containing proteins.

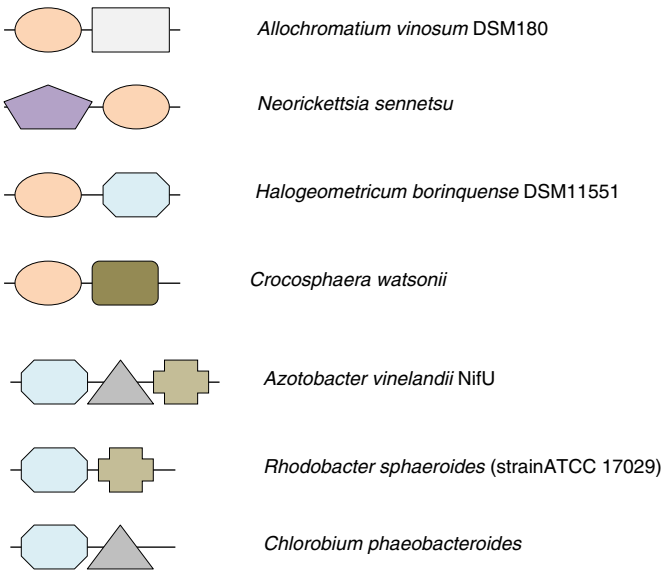

Fig. 7. Examples of multi-domain Fe/S biogenesis components found in different bacterial species. Shape and colour of each component are the same as those used in Table 1.

proteins that have solvent-exposed clusters, e.g. dehydratases in *E. coli*, or labile clusters, e.g. the *B. subtilis* scaffold SufU [178]. In contrast to the results observed with copper, cobalt appeared unable to directly attack clusters [179]. In fact, cobalt seemed to react with degraded or transiently formed clusters. Interestingly, the last category includes clusters bound on the IscU scaffold and SufA. Moreover, a mixed iron-cobalt-sulfur complex formed on the cobalt exposed IscU scaffold was transferred to target apoproteins. It was therefore proposed that cobalt toxicity was due in part to the poisoning of the whole Fe/S biosynthesis process [155,179].

Genetic analyses uncovered a complex interdependence between cobalt or copper stresses and iron homeostasis, which might eventually contribute to the deleterious effect of these metals on Fe/S homeostasis [155]. Hence, genes known to be under the control of the global iron regulator Fur (e.g. *suf*, *feoB*, *fhuH*) were induced in cobalt-treated *E. coli* cells [179,180]. Studies of the response to copper in *B. subtilis* revealed a similar up-regulation of iron and sulfur acquisition pathways, including several Fur-dependent genes [178]. Yet, the levels of intracellular total iron were decreased only 2-fold in cobalt-treated cells and not modified at all in those treated with copper [178,181]. Hence, copper- and cobalt-treated bacteria behave as if they have been misinformed of the iron status and they react as iron-starving cells. Two other similarities emerge between the way *E. coli* or *B. subtilis* respond to cobalt or copper treatment. One similarity is that both bacteria induce the Fe/S biogenesis systems, which is consistent with the fact that Fe/S enzymes are altered by both metals. This might support a model in which iron limitation is caused by the futile attempt of stressed cells to repair the cellular pool of metal-damaged Fe/S enzymes. The other similarity is that transcriptomic analyses of cobalt-treated *E. coli* and copper-treated *B. subtilis* failed to reveal an oxidative stress response, which is consistent with the notion that cells subjected to these metal stresses do not perceive any oxidative stress [178,180].

Because they are of increasing importance in environmental studies, toxicity of several metalloids is also being examined. Fe/S enzymes are also targets of cadmium, aluminium or tellurite [182–185]. Note that in this last case, oxidative stress appears to be involved in toxicity as superoxide is released by the detoxifying enzyme tellurite reductase and could be responsible for Fe/S damage. In support of these data, recent in vitro investigations have demonstrated that other metals, such as silver, mercury and zinc, also target exposed Fe/S clusters leading to the inactivation of Fe/S cluster-containing enzymes [186]. It is important to note that the effect of mercury, silver, cadmium and zinc on Fe/S clusters correlates with their thiophilicity (i.e. their sulfur-binding ability). Indeed, the greater the affinity of the metal for sulfur, the more likely it will damage Fe/S clusters [186].

8. The unexpected phenotypes of Fe/S biogenesis mutants

Besides their defect in the activity of Fe/S proteins, *isc* and *suf* mutants exhibit unexpected phenotypes, presumably reflecting the importance and the pleiotropic role of Fe/S cluster biogenesis in the cell. Hereafter, we list some of these defects and discuss the possible connection with Fe/S biogenesis.

8.1. Fe/S and antibiotics

Recently, Fe/S clusters were put at the forefront of antibiotic toxicity [187–189]. In fact, it was proposed that the bactericidal antibiotics stimulate cell respiration leading to the formation of endogenous superoxide. Then, the production of superoxide would degrade Fe/S clusters, resulting in available ferrous iron that can be oxidized by Fenton reaction to produce hydroxyl radicals. The formation of hydroxyl radical will then damage DNA and proteins, leading to cell death [187,188]. Studies have investigated the potential link between Fe/S biogenesis and the decrease in the rate of cell killing by antibiotics, which led to quite confusing results. For instance, it was reported in *E. coli* that an *iscS* mutant was more resistant to fluoroquinolones and aminoglycosides than the parent strain [189,190], but others reported that deletion of *iscU*, *hscA*, *hscB*, *fdx* in *E. coli* and of *nfuA* in *Pseudomonas aeruginosa*, confer a hypersensitivity to several antibiotic families [191,192]. Obviously, a lot of uncertainties and unsolved observations have to be further investigated to fully understand the relationship between antibiotics and Fe/S biogenesis.

8.2. Fe/S and phage resistance

E. coli iscU, *hscA* or *hscB* mutants deficient in Fe/S cluster synthesis were found to be hypersensitive to lambda phage infection [193,194]. The molecular explanation is likely to come from the fact that IscU and TusA compete for sulfur acquisition from IscS [33]. TusA is part of the TUS pathway which is involved in tRNA thiolation, a modification required during phage development [193,194]. Accordingly, a *tusA* mutant was shown to be more resistant to lambda infection because of reduced tRNA modification efficiency and, as a consequence, a defect in an associated frame-shift reprogramming control [194]. Thus, inactivation of *iscU*, *hscB* or *hscA* increases sulfur flux through the TUS pathway, resulting in an increased tRNA thiolation and, as a consequence, a “phage hypersensitivity” phenotype. Taken together, these observations illustrate how alteration in the Fe/S cluster biosynthesis process can impact a sulfur-dependent process.

8.3. Fe/S and cell surface properties

An unexpected phenotype of Fe/S biogenesis mutants relates to their modified cell surface properties such as mucoidy, motility and biofilm formation. *E. coli sufS* and *sufC* mutants are extremely mucoidy. Also *csdA* mutant was found to be affected in motility [66]. Moreover, other studies have suggested a key role of IscR in controlling the expression of type I fimbriae in *E. coli*. In fact, biofilm formation was significantly enhanced in the Δ*iscR* strain, in contrast with deletions of the *iscS*, *iscU* or *iscA* genes, which led to a decrease in biofilm formation [195]. It was proposed that apoIscR was able to induce expression of *fimE*, the gene encoding for the recombinase, which leads to the repression of the *fim* operon [195]. Thus, these observations suggest an as yet unraveled link between Fe/S homeostasis and cell surface properties.

9. Fe/S clusters biosynthesis systems in bacterial pathogenesis

Given the central role of Fe/S biogenesis systems in bacterial metabolism, it is expected that they will be of importance for pathogens during their infection cycle. A need for understanding mechanisms that control Fe/S homeostasis in pathogens is reinforced by the fact that these bacteria face, most often, iron starvation and oxidative stress, two conditions that are unfavorable for Fe/S cluster assembly and delivery.

Dickeya dadantii is a Gram-negative pathogen that causes soft-rot disease in a great variety of plants, and its genome encodes all four, ISC, SUF, CSD and NIF, systems [196,197]. The *sufA* and *sufC* mutants exhibited reduced ability to cause maceration of chicory leaves [198]. Surprisingly, the *sufC* mutant was found to be virulent against *Arabidopsis thaliana* whereas its ability to cause systemic invasion was altered in *Saintpaulia ionantha* [197,198]. An interpretation was that iron limitation is more severe in *S. ionantha* as compared with *A. thaliana* [197]. Inactivation of the ISC system led to a drastic attenuation of virulence in *A. thaliana* [197]. These results illustrate how *D. dadantii* makes use of both the ISC and the SUF systems to maintain an efficient level of Fe/S clusters biogenesis during the infection of various hosts. In contrast, the NIF system has no role in *D. dadantii* virulence [197].

Shigella flexneri, a facultative intracellular pathogen, which spends an important part of its life cycle within the epithelial cells lining the human colon, possesses both the ISC and SUF systems [199]. The *isc* mutant was unable to form plaques on Henle cells monolayers because the strain was non-invasive. In contrast, the *suf* mutant formed wild-type plaques. Expression of the *suf* and *isc* operons increased when *S. flexneri* was within Henle cells, presumably because of iron limitation [199,200].

Acinetobacter baumannii, one of the most prevalent species associated with human infections, possess the ability to form biofilms and to persist in a wide variety of environments. *A. baumannii* has the ISC system only. Screening a mutant library for derivatives impaired in their ability to grow under iron-chelated conditions led to the identification of the NfuA ortholog [201]. This gene was critical for the ability of *A. baumannii* to persist in the presence of human epithelial cells [201].

P. aeruginosa is an opportunistic human pathogen, which also possesses only the ISC system [202,203]. Deletion of the transcriptional regulator *iscR* leads to hyper susceptibility to peroxides and, virulence attenuation in *Drosophila* and mouse models [203]. Interestingly, the *iscR* mutant exhibited a reduced activity of the catalase KatA, a key factor for pathogenicity [203].

M. tuberculosis, the causative agent of tuberculosis, is a facultative intracellular pathogen that can live inside macrophages. In the last years, interest has grown in the so-called WhiB-like [4Fe–4S] cluster-containing transcriptional regulators that are present in Mycobacteria and other species. In *M. tuberculosis*, there are 7 of them. WhiB2, WhiB3, WhiB6 and WhiB7 might play an important role in the capacity of *M. tuberculosis* to develop within the host as they are, respectively, regulators of cell division, lipid metabolism, oxidative stress, and antibiotic resistance [4,204–206]. Last, WhiB5 is involved in immunomodulation as shown by the cytokine profile and immunocytochemistry [207]. Although WhiB5 is not required to grow inside macrophages, pathogenicity of *whiB5* mutant was severely affected during chronic infection. Transcriptomic analysis suggested WhiB5 to regulate the type VII secretion system [207]. *M. tuberculosis* contains the SUF system only, which is essential for survival [165]. As *suf* is absent in the human genome, it could be exploited as an attractive potential antibacterial target.

10. Conclusion

The last decade has led to the discovery that Fe/S cluster formation is catalyzed by multi-protein complexes. Biochemical, biophysical and structural analyses were all instrumental in providing us with a deeper understanding of the properties of most Fe/S biogenesis factors. Importantly though, the biological significance of those features exhibited in vitro by isolated components has yet to be assessed in the cellular context. Conversely, genetic analysis was successful in identifying Fe/S biogenesis factors, providing a map of maturation pathways or defining regulatory circuits. However, the properties of mutants altering Fe/S biosynthesis can be complex to interpret given the central role of some of the Fe/S biogenesis factors or regulators such as IscS or IscR, or the involvement of Fe/S enzymes in basic processes such as respiration or the TCA cycle. In vitro reconstitution experiments are necessary to support in vivo predictions from genetic analyses. Collaborative in vivo and in vitro studies carried out in several laboratories have contributed to the success of studies to elucidate the assembly step mediated by the IscU–IscS complex or the role of ATCs. These studies were given attention in the present review as in these two cases, structural, spectroscopic and genetic approaches, reached a unifying view. By using this multi-disciplinary approach, future studies should help clarify the role of frataxin and that of accessory components, such as Mrp, YtfE, GrxD or YggX.

Progress has been made in two exciting areas of research: the control of Fe/S cluster homeostasis and the Fe/S cluster delivery step. Although they will benefit from in vitro analysis, these two themes will gain more from systems-level approaches. The question of Fe/S cluster delivery aims at defining, for each apoprotein, which "route" is being used by clusters from their site of assembly to their final destination, knowing that maturation of the same apoprotein can include several routes and the choice of a route can be controlled by growth conditions. Development of proteomic approaches to determine the Fe/S cluster proteins whose maturation is affected in mutants lacking various Fe/S biogenesis factors will be a challenge for future studies. The question of regulation of Fe/S homeostasis will also constitute another challenge. Indeed, besides the important issues such as the specificity of the signal relayed by each regulator or the interplay between different regulators, the influence of the overall cellular demand might be a criterion of great importance, which will benefit from global methods to measure Fe/S cluster biogenesis. In both of these issues, delivery and regulation, it is likely that important advances will be made by taking global approaches. As a most telling illustration, a recent systems-level study suggests that the half-life of IscS is controlled by FtsH, a membrane bound protease involved in degradation of aberrant proteins [208]. Remarkably, the level of the eukaryotic IscU protein was shown to be controlled by the Lon-type protease [209]. The importance of this protein quality control system on Fe/S cluster biosynthesis is an exciting new venue to consider in the mechanisms allowing Fe/S cluster homeostasis.

Ironically, the early noted resemblances between *E. coli* (or *A. vinelandii*) and mitochondrial ISC systems, or else between *E. coli* and chloroplastic SUF led to the view of a great conservation between prokaryotic and eukaryotic systems. In essence, this remains true, but it is a fact that genome surveys reveal a greater diversity of the Fe/S biogenesis systems than expected. Rather, it is more prudent to entertain the possibility that there are new systems that remain to be discovered. In this context, the question of how the Archaea, most of which lack any identifiable ISC or SUF systems, assemble and deliver their clusters is evidently a distinct but equally exciting area of research for the future.

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