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Review

Fe-S biogenesis by SMS and SUF pathways: A focus on the assembly step

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ABSTRACT

Fe—S clusters are prosthetic groups present in all organisms. Proteins with Fe—S centers are involved in most cellular processes. ISC and SUF are machineries necessary for the formation and insertion of Fe—S in proteins. Recently, a phylogenetic analysis on more than 10,000 genomes of prokaryotes have uncovered two new systems, MIS and SMS, which were proposed to be ancestral to ISC and SUF. SMS is composed of SmsBC, two homologs of SufBC(D), the scaffolding complex of SUF. In this review, we will specifically focus on the current knowledge of the SUF system and on the new perspectives given by the recent discovery of its ancestor, the SMS system.

1. Introduction

Iron-sulfur clusters (Fe—S) are polynuclear combinations of iron and sulfur atoms and are among the most conserved cofactors in prokaryotes and eukaryotes. As an example, *Escherichia coli* synthesizes more than 180 different proteins containing Fe—S clusters [1]. Fe—S cluster binding proteins are involved in a wide variety of biological processes, allowing redox chemistry and Lewis acid-type catalysis, from respiration to photosynthesis, and from metabolism to regulation of gene expression or genomic stability [2–6]. The most common Fe—S cluster types are [2Fe—2S] and cubane [4Fe—4S] clusters containing ferric iron (Fe^{3+}) or ferrous iron (Fe^{2+}) and sulfide (S^{2-}) [3]. Usually, the thiolate of cysteine coordinates with the iron ion of the cluster [7–9], but histidine [10–13] and arginine [14] (nitrogen coordination) as well as aspartic acid [15], glutamic acid [16] and tyrosine [17] (oxygen coordination) can also act as Fe—S clusters ligands [18]. Occasionally, cofactors, water molecules or enzyme substrates are also involved in the coordination [3]. The most widely accepted hypothesis for the appearance of Fe—S clusters on Earth is their spontaneous assembly in the anaerobic environment of early life, which was rich in ferrous iron and sulfide [19].

Fe—S proteins have been exploited during evolution for their versatile biochemical properties and multiprotein systems catalyzing Fe—S cluster formation and delivery emerged in the Last Universal Common Ancestor (LUCA) [20]. To date, five Fe—S biogenesis systems that catalyse Fe—S cluster acquisition by apo-proteins have been described, namely NIF, ISC, SUF, MIS and SMS. Biogenesis of Fe—S clusters by these systems proceeds in two main steps: (i) the Fe—S cluster assembly step, during which a cysteine desulfurase/sulfurtransferase system removes sulfur from L-cysteine and transfers it to a scaffold protein, which also receives Fe and electrons to build an Fe—S cluster, (ii) the Fe—S cluster delivery step, during which the Fe—S cluster is transferred to apoprotein cellular targets either directly or *via* carrier proteins targeting apo forms of Fe—S proteins [4–6,21–27]. In this review, we will restrict ourselves to the assembly step catalyzed by the SUF and its ancestor SMS systems. We will cover mostly the biochemical, structural and mechanistic aspects of it as the genetic aspects have been the subject of several excellent reviews [6,23,28–30]. We will not discuss the SUF system from plants which is covered elsewhere in this issue.

2. From SMS to SUF: A brief evolutionary point of view

Exhaustive homology searches along with genomic context and phylogeny analysis of 10,865 archaeal and bacterial genomes, representative of the diversity of prokaryotes, led to the discovery of SMS [20]. SMS was proposed to be present in LUCA, the last universal common ancestor, and is conserved in Archaea and a restricted set of Bacteria [20]. SMS was the ancestral minimal system, which subsequently went through different events of complexification,

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incorporating additional functions yielding eventually SUF (Fig. 1). Constraints that guided the evolution from SMS to SUF are likely to be associated with increasing levels of oxygen in the atmosphere. In the present situation, SUF is the most distributed, by far, in the bacterial world although some clades contain SMS. Reciprocally in Archaea, SMS is more frequently found than SUF [20]. Presumably, the current distribution of SMS and SUF has been shaped by horizontal gene transfers, gene losses, matching with cellular proteome and ecological adaptations such as the emergence of aerobic lifestyles. Noticeably, some archaeal SMS systems were transferred to a few anaerobic bacteria, while some bacterial SUF systems were transferred to aerobic archaea.

The SUF machinery from *E. coli* is the most extensively studied and serves as a model for Gram negative bacteria. It is encoded by the *sufABCDSE* operon that comprises: a homodimeric cysteine desulfurase, SufS, a sulfurtransferase, SufE, an Fe—S assembly complex, SufB, SufC, SufD, wherein SufC is an ATPase, and a Fe—S carrier, SufA (Fig. 1) [6,31]. The SUF system, or a variant of it, also occurs in archaea and cyanobacteria, as well as in many Gram-positive pathogenic (*Staphylococcus aureus*, *Mycobacterium tuberculosis* and *Enterococcus faecalis*) and nonpathogenic (*Bacillus subtilis*) bacteria where it is the sole Fe—S assembly system. Both the SMS and the SUF systems can be found in parasites, such as *Blastocystis* and *Plasmodium falciparum*, respectively.

3. The gram-negative SUF system

3.1. Sulfur mobilization by the SufS-SufE proteins

Inorganic sulfide encountered in Fe—S clusters results from L-cysteine desulfurization by the SufS-SufE system (Fig. 2), whose current structural and functional knowledge derives mainly from the Gram negative *E. coli* system.

3.1.1. Biochemical, biophysical and enzymological analyses

SufS is a dimer in solution while SufE can form monomers at low protein concentrations and homodimers at higher protein concentrations [32]. SufE and SufS proteins can associate as shown by two-hybrid and affinity chromatography with a 1:1 stoichiometry for SufS:SufE interaction [32,33]. A dissociation constant of 0.26 μM was determined by ITC [34].

SufS is a pyridoxal 5′-phosphate (PLP) containing cysteine desulfurase that mobilizes sulfur from the L-cysteine substrate, acting as the initial step in the sulfur mobilization pathway for the synthesis of Fe—S clusters (Fig. 2). L-Cysteine desulfuration reaction gives rise to a persulfide intermediate bound to the conserved Cys_{364}, located in a short and rigid loop of the protein [35]. The persulfide (Cys_{364}-SSH) is protected from external oxidants/reductants due to key structural elements (see below) [36,37].

Recent enzymatic and biophysical studies have provided significant insights into the SufS cysteine desulfuration mechanism with the direct observation of intermediates, revealing functional roles of conserved active site residues [23]. From these studies a roughly complete view of the reaction is now available (Fig. 3). PLP begins in an internal aldimine conformation with an absorption feature of 422 nm with a binding mode with the Lys226 base [23,38]. After L-cysteine binding, the PLP cysteine form the ketoenamine and enolimine tautomers of the external Cys-aldimine, with absorbance features at 424 nm and 343 nm respectively. Cys-aldimine is transformed to Cys-ketimine that absorbs at 351 nm, likely through a spectroscopically undetected quinonoid species, which would be predicted to absorb around 500 nm. After generation of the Cys-ketimine, the cysteine desulfurase mechanism proceeds by Cys364 attacking the Cys-ketimine sulfhydryl to break the CεS bond, resulting in the formation of an Ala-eneamine species and the persulfide Cys364-SSH [23]. Following formation of Ala-eneamine, the intermediates Ala-ketimine, Ala-quinonoid, and the Ala-aldimine are likely produced, although there is a lack of spectroscopic and structural data illustrating these intermediates [39]. Finally, regeneration of the PLP internal aldimine species with Lys226 returns the enzyme to its resting state ready for another mechanistic cycle. This work has highlighted the crucial role of His123 in a) the stabilization of the ketoenamine and enolimine tautomers through π-π stacking interactions with PLP, b) Cys-ketimine- Ala-eneamine transformation acting as an acid-base catalyst (deprotonating the thiol group of Cys364) [23].

Cysteine desulfuration by SufS is inefficient due to the sluggish attack of Cys_{364} to C—S bond of L-cysteine substrate [39], but it is accelerated 8 to 150-fold by binding of SufE, depending on the excess of SufE added and the reducing agent used [32,33,38,40] (Table 1). This stimulation is due to the transpersulfidation between SufS-Cys_{364}-SSH and the conserved Cys_{51} of SufE (Fig. 2) [32,41]. SufE can accept *in vitro* up to 4 sulfur atoms on the Cys_{51} residue [38,41]. The limited reduction of the persulfide on SufE by thiols emphasizes that the transpersulfidation reaction is confined and largely excluded from solvent [38]. SufS exhibits a ping-pong mechanism for cysteine desulfuration [38] using SufE as a co-substrate [38,40]. Interestingly, the rate of sulfur transfer from SufS to SufE depends on the sulfuration state of the SufE. When SufE is in its thiolated form, it promotes nucleophilic attack on the terminal sulfur persulfide of SufS, thereby increasing the turnover rate. However, when SufE is in its persulfurated form, there is a decrease in the catalytic turnover rate, suggesting that sulfur accumulation on SufE

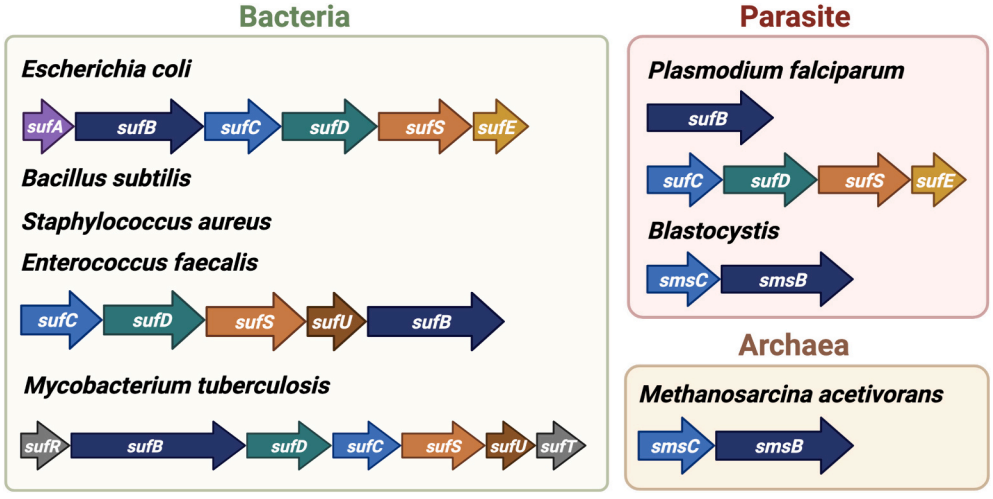

Fig. 1. Schematic representation of the *sms* and *suf* locus in archaea, bacteria and parasites. Genes having homologous sequences or similar functions are colour-coded.

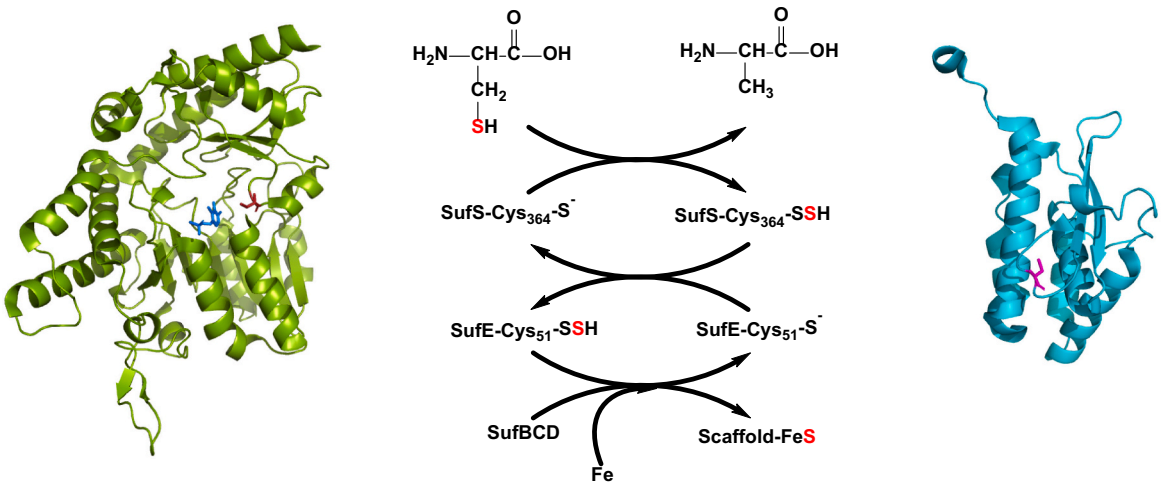

Fig. 2. Sulfur mobilization by SufS-SufE system. L-cysteine desulfurization mechanism involving SufS and SufE (middle); structure of SufS (left) showing PLP cofactor (blue) and catalytic Cys_{364} residue (red) (PDB: 1jf9); structure of SufE (right) showing conserved sulfur acceptor Cys_{51} residue (PDB: 1mzg).

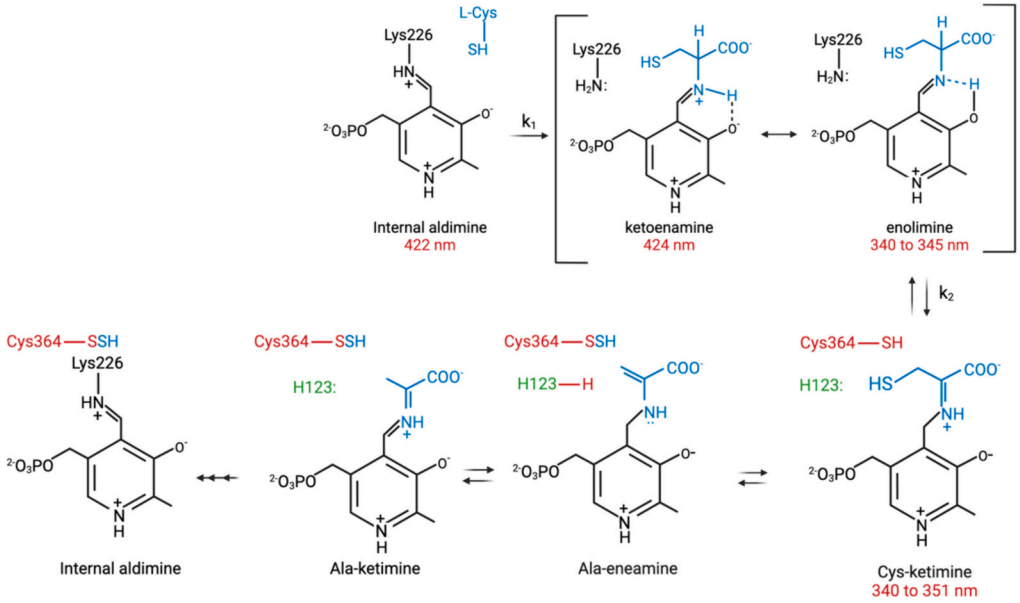

Fig. 3. Scheme for the mechanism of SufS cysteine desulfurase. PLP is in black, substrate cysteine is blue, active site Cys364 is red, and His123 acid/base catalyst in green. Adapted from [23].

Table 1
Cysteine desulfurase activity of SufS enzymes in the presence of their sulfurtransferase (SufE or SufU).

Organism	Sulfurtransferase	Cysteine desulfurase enhancement	References
Escherichia coli	SufE	40–200 fold	[38,40]
Erwinia chrysanthemi	SufE	40 fold	[33]
Bacillus subtilis	SufU	35–200	[69,76,97]
Staphylococcus aureus	SufU	1.5 fold	[72]
Enterococcus faecalis	SufU	37 fold	[71]
Mycobacterium tuberculosis	SufU	180 fold	[70]
Plasmodium falciparum	SufE	17 fold	[91]

negatively regulates SufS responsiveness, thereby preventing futile cycling [38]. In the presence of $SufBC_2D$ complex, SufE can further transfer sulfur from its Cys_{51}-SSH to the complex on the SufB subunit [42], allowing SufE to initiate another cycle of catalysis. The persulfide on SufB (within SufBCD) is used for Fe—S cluster assembly when suitable Fe ions and electrons are available (Fig. 2).

3.1.2. *Structural analyses*

The crystal structure of SufS shows that the catalytic Cys_{364} is located in a short and rigid loop (Fig. 2**)** and that the persulfide is protected from solvent [35–37,43]. The SufE X-ray structure shows that Cys_{51} is located at the tip of a loop, where its side-chain is buried from solvent exposure in a hydrophobic cavity (Fig. 2) [44]. Therefore, to make the trans-persulfidation reaction possible, from SufS Cys_{364} to SufE Cys_{51}, conformational changes in SufS and SufE are necessary. Hydrogen-Deuterium-Exchange mass spectrometry (HDX-MS) experiments on SufS-SufE combined with X-ray crystallography of SufS (wild-type and variants) have provided information on conformational changes in both

proteins, that help to dissect the sulfur mobilization mechanism by SufS-SufE. SufE binds near the SufS active site and interacts *via* two peptides (peptide 38–56 (containing Cys_{51}) and peptide 66–83), which undergo conformational changes upon complex formation [34]. An important one concerns the Cys_{51} loop whose solvent accessibility is increased upon SufS binding [34]. The increase of dynamics and solvent accessibility of the Cys_{51} loop in SufE improves the use of Cys_{51} as sulfur acceptor and the binding to SufS [45]. Dynamic changes in SufS were also observed both in active site regions (peptides 225–236 and 356–366) and in peptides covering the dimer interface (88–100 and 243–255) [34,45,46]. The cross talk between these two regions of SufS was demonstrated by mutagenesis of the dimer-interface residues followed by kinetics measurements and X-ray structures of mutants [36]. Indeed, these experiments with interface protein variants revealed that movements of the dimer interface induce a global rotation of the SufS monomers and a movement of the beta-hairpin (aa 254–272) of one monomer away from Cys_{364}-SSH of the other SufS monomer, yielding a more open conformation of the active site [36]. In 2020, a structure of *E. coli* SufS was solved as a dimer (PDB: 6uy5), both monomers being in a persulfide form [47]. In comparison with previous structures of *E. coli* SufS, the two monomers are rotated relative to their resting state, some electrostatic interactions at the dimer interface are lost, and the beta-hairpin is rotated into an open position. These observations have highlighted the role of the beta-hairpin in the sulfur mobilization mechanism, especially in the active site accessibility containing the persulfide. More recently, structural analyses suggested a more complex regulation of the beta-hairpin dynamics by adjacent conserved structural elements (α_6 helix, a glycine-rich loop preceding the beta-hairpin and a cis-proline residue following the beta-hairpin) [47]. With the beta-hairpin they form the "beta-latch motif" which plays a role in forming a close approach of SufS-SufE complex to promote persulfide transfer [37]. The beta-latch motif is broadly conserved in the structures of type 2 cysteine desulfurases from different organisms [47]. In conclusion, our knowledge on the sulfur mobilization has greatly advanced in the recent years and a schematized representation is given in Fig. 4. Nonetheless, resolving the SufS-SufE X-ray structure, with snapshots after reaction with L-cysteine substrate, should unravel the precise sequence of events of this complex mechanism.

3.2. *Fe—S cluster assembly by the SufBC$_2$D scaffold*

Our understanding of the SufBC$_2$D scaffold derives specifically from studies on *E. coli* system described hereafter.

3.2.1. *General features of the isolated SufB, SufC and SufD proteins*

SufB is a soluble protein that exists in solution as a mixture of monomer, dimer, trimers that are not very stable [42 and Blanc et al. unpublished results]. There are no structures of *E. coli* SufB alone, but the structure predicted by alpha-fold (AF-P7752-F1) shows five alpha helices and three beta-strands. There are several regions with a low scoring prediction (amino-acids 1 to 30, 94 to 119, 126 to 140, 227 to 233 and 255 to 260) (Fig. 5). As purified, SufB is devoid of any cofactor. After *in vitro* reconstitution with iron and sulfide under reducing conditions, SufB can assemble transiently a [2Fe—2S] and/or [4Fe—4S] cluster(s) [42,48]. SufB primary sequence contains a N-terminal $C_{96}XXC_{99}XXXC_{103}$ motif, which could qualify as Fe—S binding site [42]. However, a variant lacking all three cysteine residues can still assemble a Fe—S cluster *in vitro* after chemical reconstitution and a functional role for these residues was not supported by mutagenesis analysis [49]. Since SufB protein has a great tendency to form aggregates, this has precluded further structural and biochemical studies.

SufD is a paralog of SufB (17 % identity and 37 % similarity), deriving from a duplication of an ancestral SufB sequence [19,20]. SufD contains no known predicted motifs and no cofactor or prosthetic group. Several observations *in vivo* tend to support a link between SufD and iron [50–52], yet *in vitro* study showing that SufD binds iron is lacking. SufD is stable as-purified and homogeneous in its dimeric form. Its structure (PDB number: 1VH4) displays a fattened right-handed beta-helix of nine turns with two strands per turn; the N- and C-termini form helical subdomains (Fig. 5). Homodimerization of SufD doubles the length of the beta-helix (to 80 Å) and two highly conserved residues, Pro_{347} and His_{360}, interact at the dimer interface [53]. Interestingly, Tyr_{374}, Arg_{378}, Gly_{379}, Ala_{385} and Phe_{393} are highly conserved residues in the C-terminal subdomain and all these residues are conserved in SufB.

SufC is mainly monomeric in solution and possesses an ATPase activity that requires Mg [23,54]. Structure of the monomeric form (PDB 2D3W, Fig. 5) shows that SufC has two domains, including a helical domain specific to ABC ATPases containing an ABC signature motif and a catalytic alpha/beta domain that contains the nucleotide-binding

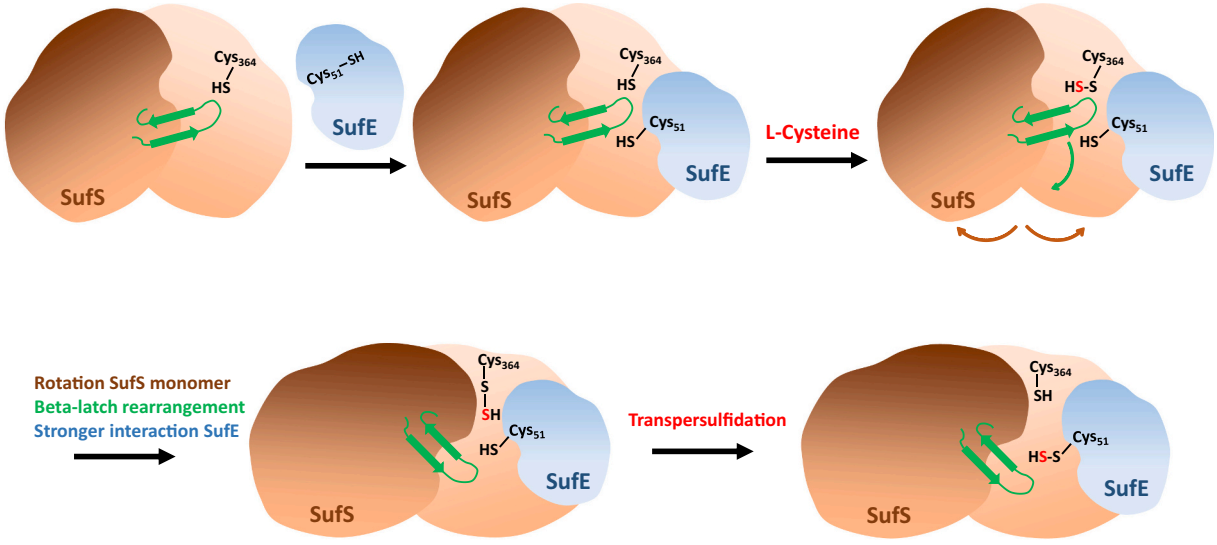

Fig. 4. Proposed mechanism of sulfur mobilization by SufS-SufE. SufE interacts with SufS leading to solvent accessible Cys_{51} loop. Persulfuration of Cys_{364} of SufS triggers monomers rotation, beta-latch (in green) rearrangement (open conformation of beta-hairpin) resulting in a stronger interaction between SufS and SufE. Transpersulfidation between Cys_{364}-SSH and Cys51 can occur. The illustration comprises a SufS dimer with a Cys_{364}-SSH located on one SufS monomer and the beta-hairpin in the adjacent monomer. For sake of clarity, only one SufE monomer is represented, but a symmetric situation may occur.

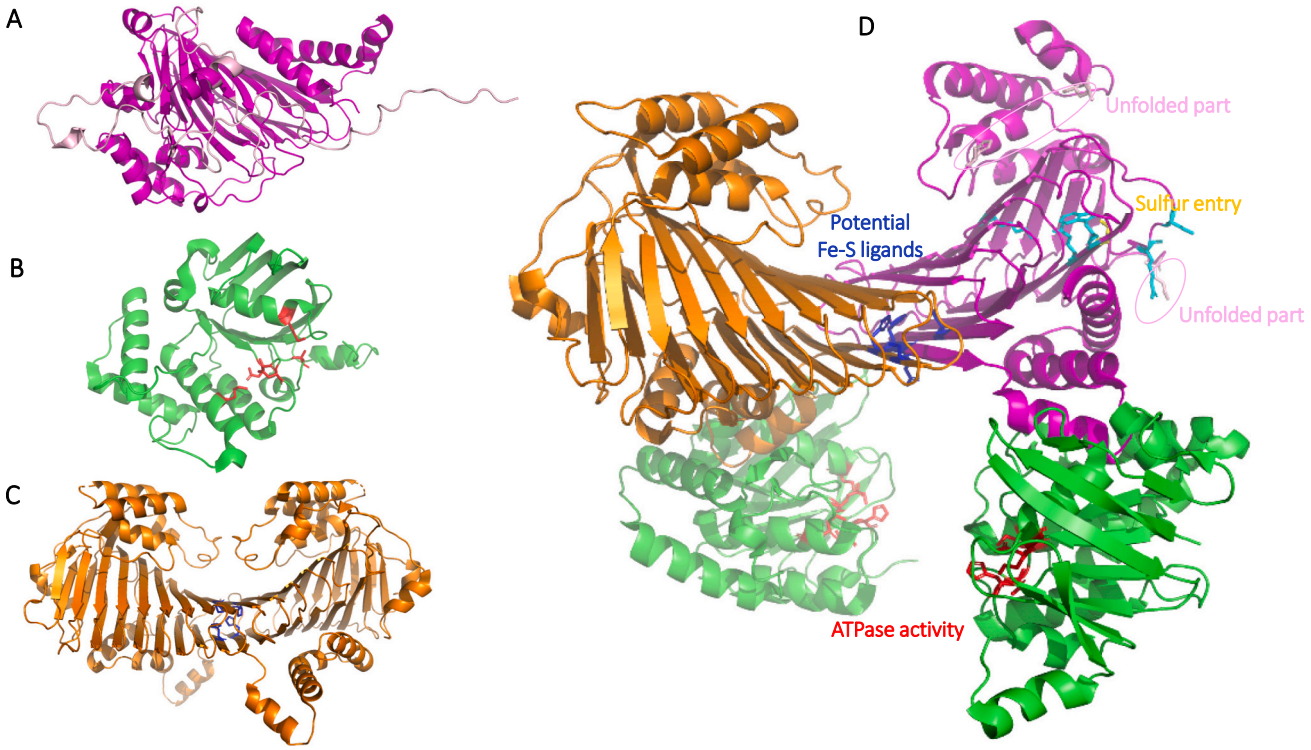

Fig. 5. Structures of SufB, SufC, SufD and SufBC$_2$D and functionally critical residues. (A): Predicted structure of SufB (AF-P77522-F1) with unfolded parts (lightpink); (B) structure of SufC monomer (2D3W) with important residues for ATPase activity (in red); (C) Structure of the SufD homodimer with interface interacting residues (in blue); (D) structure of SufBC$_2$D (5AWF) showing unfolded parts (lightpink) in SufB, important residues for SufC ATPAse activity (in red), potential Fe—S ligands in SufB and SufD (in blue), sulfur entry (in yellow), residues involved in sulfur trafficking in SufB (in cyan).

Walker A and Walker B motifs [55]. These two domains are connected by a Q-loop that contains a strictly conserved glutamate residue (Glu$_{171}$) [55]. An atypical nucleotide residue binding conformation at the end of the Walker B motif forms a 310 helix, which is not observed in other ABC ATPases. Due to this 3_{10} helix, the conserved Glu$_{171}$ involved in ATP hydrolysis is flipped out and although this unusual conformation is unfavorable for ATP hydrolysis, it is stabilized by several interactions around the 3_{10} helix [55]. Glu$_{171}$ and Asp$_{173}$ form salt-bridges with a Lys$_{152}$ and several water molecules that form a strong hydrogen bond network. Moreover, a significant displacement occurs at a linker region between the ABC alpha/beta domain and the alpha-helical domain (displacement of the Q-loop) [55]. The linker conformation is stabilized by hydrophobic interactions between conserved residues around the Q loop. The ATPase activity of SufC is low but significantly enhanced by 180-fold when associated with SufB and five-fold with SufD [54]. SufC Lys$_{40}$, Lys$_{152}$, Glu$_{171}$, Asp$_{173}$ and H$_{203}$ residues were identified as important for ATPase activity [52,54,56]. Still, the role of ATP hydrolysis remains unclear as it is dispensable for *in vitro* Fe—S binding and transfer, yet essential *in vivo* [51,57,58]. At least, as-purified inactive His$_6$-SufBC$_2$D-SufC (Lys$_{40}$Arg) variant displays an 8-fold reduction of iron content relative to the wild-type His$_6$-SufBC$_2$D opening the possibility that the ATPase activity is necessary for iron acquisition [52].

3.2.2. *Biochemical and spectroscopic properties of the SufBC$_2$D complex*

SufBC$_2$D is the predominant complex formed after aerobic purification from *E. coli* cells expressing SufABCDSE proteins [32,59]. As purified this complex contains no iron and no sulfide. When purified anaerobically, it contains one flavin (FADH$_2$) per complex [52,59]. After chemical reconstitution, the aerobically purified SufBC$_2$D complex binds either a [2Fe—2S] or a [4Fe—4S] cluster [52,59,60]. It has been suggested for a long time that the cluster is localized on SufB subunit since, *in vitro*, SufB protein (not SufC or SufD) assembles an Fe—S cluster (either [2Fe—2S] or [4Fe—4S]) resembling that of the SufBC$_2$D complex [42,48]. However, anaerobically isolated His$_6$-SufBC$_2$D complex possesses a Fe—S cluster with optical spectroscopic features assigned as a [2Fe—2S] and/or a linear [3Fe—4S] cluster [52]. All spectroscopic studies performed on SufB alone or SufBC$_2$D could not determine the nature of the cluster ligands, even though Mössbauer spectroscopy likely suggested thiolate coordination [42]. Moreover, SufBC$_2$D bound Fe—S cluster ([2Fe—2S] or [4Fe—4S]) can be transferred to apotargets proteins such as aconitase, SufA or ferredoxin [52,59,60]. Apo-SufBC$_2$D complex (containing no cofactor) displays an ATPase activity [56] and also enhances the SufSE activity between 2 and 10-fold by being the final sulfur acceptor [32,38,42]. Mass spectrometry and biochemical studies showed that SufBC$_2$D complex, in the presence of SufSE and L-cysteine, accumulates nearly 5 sulfur atoms per complex, located within the SufB subunit [42,49].

3.2.3. *Structural analysis of the SufBC$_2$D complex*

Two structures of SufBC$_2$D were solved by crystallography, one free of metal and cofactor (PDB number: 5AWF, 2.96 Å, Fig. 5), one containing 2 mercuric ions (PDB 5AWG). One Hg^{2+} is coordinated by Cys$_{405}$ of SufB subunit, the other by Cys$_{358}$ of SufD subunit [56]. For both structures, the core domains of SufB and SufD consist of long strands arranged in a helical architecture. These domains are associated by antiparallel beta-strands to form a new heterodimeric structure [56]. By using a disulfide crosslinking approach, it was shown that SufC can form dimers in the presence of ATP [56]. The structures of SufB and SufD are similar, with an N-terminal helical domain, a central domain consisting of a right-handed parallel beta-helix, and a C-terminal helical domain that contains the SufC binding site [56]. The N-terminal part of the central beta-helix domain of SufB and the SufB-SufD interface are connected by a tunnel within the central domain of the beta-helix of SufB through which the sulfur moiety is transferred from SufB Cys$_{254}$ to SufB Cys$_{405}$ [49]. SufC subunits bind to SufB and SufD subunits *via* a conserved interaction called the “transmission interface” [56]. These are

spatially separated (more than 40 Å) with their ATP binding motifs facing each other [56]. SufC-SufB and SufC-SufD sub-complexes have almost identical structures. The structure of the SufD subunit in the $SufBC_2D$ complex was almost identical to that of the previously reported SufD homodimer crystallized alone [53], even though some structural difference was observed around the interaction site with SufC. In the $SufBC_2D$ complex, the catalytic pocket of SufC is subject to a reorganization suitable for ATP binding and hydrolysis. In particular, the salt bridge observed in monomeric SufC between Glu_{171} and Lys_{152} is cleaved in the complex, causing the Glu_{171} side chain to rotate toward the ATP-binding pocket, and the His_{203} residue is shifted by approximately 4 Å toward Glu_{171} in the complex [55,56]. There is some evidences that a physical interaction of SufC protomers exists, forming a head to tail dimer, in the presence of ATP using a disulfide crosslinking approach [56]. SufC dimerization is proposed to result in enormous structural changes of the SufB–SufD heterodimer, leading to exposure of Cys_{405} of SufB inside the heterodimer interface (and probably H_{360} of SufD). These proposed structural rearrangements have to be demonstrated in the future using biochemical or structural studies.

3.2.4. Genetic analysis of the $SufBC_2D$ complex

Genetic analysis identified important residues for $SufBC_2D$ function. The *E. coli* strain UT109, which is a Δ(*iscUA-hscBA*) Δ*sufABCDSE* MG1655 derivative was used [49]. This strain cannot grow because the isoprenoid biosynthetic pathway that depends upon the Fe—S proteins IspG and IspH, is not functional. However, bringing in the eukaryotic isoprenoid biosynthesis pathway that is independent of Fe—S proteins, and depends upon the sole presence of exogenously added mevalonate (MVA), can rescue the UT109 strain. A UT109 strain carrying *sufCDSE* genes on a plasmid and *sufAB* genes on a separate plasmid was used to screen for mutations in *sufB* or *sufD*. Loss of function of such mutations could be identified as giving rise to MVA-dependent growth. A series of residues in SufB were selected for mutation based on their conservation among SufB orthologs. Accordingly, variants SufB (triple Cys_{96}–Cys_{99}–Cys_{103} to Ala, Cys_{254} to Ala, Cys_{405} to Ala, Arg_{226} to Ala, Asn_{228} to Ala, Gln_{285} to Ala, Trp_{287} to Ala, Lys_{303} to Ala or Glu_{434} to Ala) as well as SufD (His_{360} to Ala) were unable to complement the *E. coli* strain UT109 for growth in the absence of MVA [49]. Interestingly, although the single substitution Asp_{406} to Ala, Glu_{432} to Ala, His_{433} to Ala (SufB) or Cys_{358} to Ser (SufD) had no significant effect on their own, combination of Asp_{406} to Ala, Glu_{432} to Ala and His_{433} to Ala of SufB elicited slow-growth phenotypes in the absence of MVA. The combination of Glu_{432} to Ala, His_{433} to Ala and Cys_{358} to Ser also prevented complementation. Altogether, these results highlight Cys_{254}, Cys_{405}, Glu_{432}, His_{433}, Glu_{434}, Arg_{226}, Asn_{228}, Gln_{285}, Trp_{287}, and Lys_{303} of SufB as well as His_{360} and Cys_{358} of SufD as functionally important (Fig. 5). These residues are conserved, excepted for Cys_{358} which is absent in *B. subtilis* [49].

From structural and genetic studies, the location of the cluster binding site in $SufBC_2D$ is currently proposed to be at the SufD-SufB interface [49], involving Cysteine, Histidine and/or Glutamate residues meaning sulfur, nitrogen and/or oxygen atoms from both SufD and SufB subunits. All spectroscopies used so far to characterize Fe—S containing $SufBC_2D$ did not provide a clear-cut answer regarding Fe—S cluster ligands. Therefore, for the moment the question of the cluster ligands is still opened.

3.2.5. Flavin is bound by the $SufBC_2D$ complex

A remarkable and still unexplained feature of the entire $SufBC_2D$ complex concerns its ability to bind flavin ($FADH_2$) during anaerobic purification [52,59]. The complex does not bind the oxidized form of flavin (FAD) and exhibits rapid loss of flavin when exposed to oxygen [59]. It is possible to reload the apo-$SufBC_2D$ with $FADH_2$ under anaerobic conditions with good yield (0.8–1 $FADH_2$/complex) and a dissociation constant for the binding of $FADH_2$ to $SufBC_2D$ could be determined (12 μM) [59]. SufB alone or SufB in the presence of SufC is able to bind $FADH_2$, albeit to a lesser extent (0.1–0.3 mol of $FADH_2$ per mol of protein, respectively). SufC and SufD alone are not capable to bind the Flavin [59]. The reduced flavin in the $SufBC_2D$ complex is sufficiently accessible and reactive to donate its electrons to iron-binding proteins (CyaY-Fe), suggesting that the $SufBC_2D$ complex may serve as a flavin-dependent system to reduce ferric iron from an unknown iron source [59]. A role of the $FADH_2$ for the reduction of the persulfide on SufB during Fe—S assembly is also possible. Concerning the $FADH_2$ binding site, Wollers et al. proposed three motifs which are present in the SufB amino- acid sequence: the GXXL motif, the $P(X)_6G(A)XN$ motif and the $R(X)_6EXXYXXXXXG(X)_8Y$ motif. These motifs are characteristic of the flavin-binding motif found in the p-cresol-methylhydroxylase family [61]. The crystal structure of the $SufBC_2D$ complex does not help to determine whether these motifs could be involved in $FADH_2$ binding, as the N-term (amino-acids 1 to 33 and 80 to 156) of SufB, that contain the $P(X)_6G(A)XN$ motif, is not properly folded. It is important to note that although the three motifs are present in some SufB proteins (*Salmonella* and *Yersinia* for example), they are not all well conserved among all SufB proteins. Moreover, substitution of some residues in the $R(X)_6EXXYXXXXXG(X)_8Y$ motif, (R237A or E244A) does not affect $SufBC_2D$ function *in vivo* [49]. Hence, the assignment of the $FADH_2$ binding site requires further studies and it is not excluded that $FADH_2$ is bound to $SufBC_2D$ through residues from different subunits. Yet, the exact role and function of flavin in the $SufBC_2D$ complex is not fully understood.

3.2.6. A model for $SufBC_2D$-mediated assembly of a Fe—S cluster

The two crystal structures of the $SufBC_2D$ complex, combined with the *in vivo* experiments led to a proposed mechanism for the Fe—S cluster assembly on $SufBC_2D$. After extraction of sulfur from the substrate L-cysteine by the action of SufS and its delivery *via* SufE to SufB Cys_{254}, the bound S^0 is reduced to S^{2-}, released from SufB Cys_{254}, and migrates through the hydrophilic tunnel that traverses the beta-helix core domain of SufB to reach SufB Cys_{405}. In the presence of iron, the cluster is assembled using Cys_{405}, Glu_{434} of SufB and His_{360} of SufD as essential ligands in combination with a fourth ligand that can be either Glu_{432}, His_{433} of SufB or Cys_{358} of SufD. Interestingly, in the $SufBC_2D$ structure with Hg^{2+} ions, as already mentioned, one Hg^{2+} is bound to Cys_{405} (SufB), and the other to Cys_{358} (SufD) that is adjacent to His_{360} (SufD). Therefore, residues Cys_{405}, Glu_{434} in SufB, His_{360} and Cys_{358} in SufD appear as good candidates for Fe—S cluster ligation [49]. However, the side chains of SufB Cys_{405} and SufD His_{360} are buried inside the beta-helix, whereas Glu_{434} protrudes outside making the binding of the cluster impossible [49]. Glu432 or His433 in SufB could be the fourth ligand, with a preference for the more conserved His433. Only the use of advanced spectroscopies such as XAS, Hyscore and ENDOR or crystallography carried out on the native Fe—S containing $SufBC_2D$ complex will enable the determination of the cluster ligands. We cannot exclude that the cluster at the SufB-SufD interface in $SufBC_2D$ is a transient binding site within $SufBC_2D$ complex and that the final cluster binding site is located somewhere in SufB subunit. Therefore, for the nature of the cluster ligands remains unknown. SufC dimerization and ATP could initiate important structural changes of the $SufBC_2D$ complex, allowing Fe—S delivery, probably initiated by the transmission interface created by the dimerization of the two SufC subunits. A schematized mechanism of Fe—S assembly on the $SufBC_2D$ scaffold is proposed Fig. 6A. Before to close, it might be worth reporting that another complex, a $SufB_2C_2$ complex, could be isolated after anaerobic purification from *E. coli* cells overexpressing SufBCD proteins [52]. This complex contains 3.2 iron and 4.2 sulfur/complex likely organized as a [4Fe—4S] and no flavin [52,62]. *In vitro* studies indicated that $SufB_2C_2$ is more efficient in *de novo* assembly of clusters in the ferredoxin target protein than the $SufBC_2D$ complex [62]. The location of the Fe—S cluster within this complex is not known [62]. Although it is still unclear whether such a sub-complex occurs *in vivo*, knowledge derived from its analysis might prove useful to decipher the functioning of minimal SmsCB complex (see

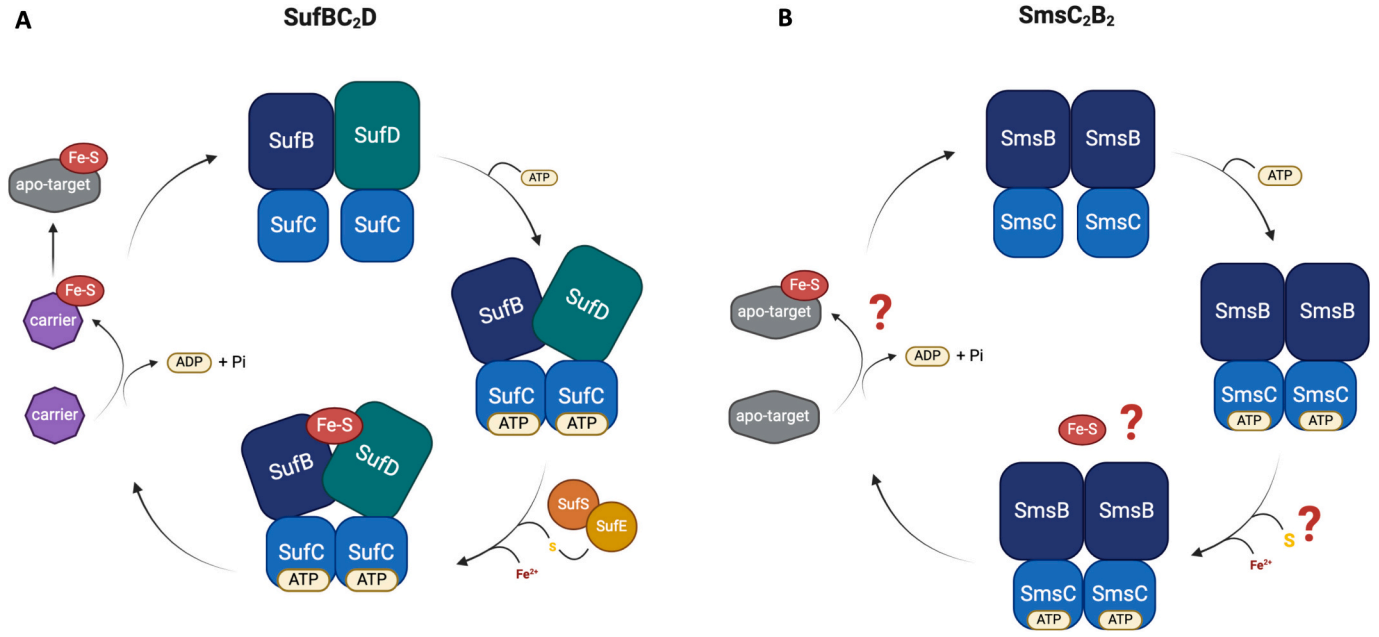

Fig. 6. Simplified proposed mechanisms of Fe—S cluster assembly by the $SufBC_2D$ complex and the ancestral minimal SMS system.

below).

(A) SufC binds ATP inducing a conformational change of SufB and SufD leading to a transmission interface. Then, using sulfur obtained from the two-component cysteine desulfurase SufS-SufE, and iron (unknown source) the Fe—S cluster is built at the interface of SufB and SufD. Finally, the Fe—S cluster is transferred to the carrier of the SUF system, the ATP is hydrolyzed, leading to the initial state of conformation of the complex. **(B)** For SMS system, there is no identified sulfur donor (X) and carrier. The ATPase activity of SmsC has not been described. For both systems, the iron source is unknown.

4. The gram-positive SUF system referred to SUF-like system

4.1. Physiology

Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*, *Mycobacterium tuberculosis* and *Enterococcus faecalis* possess only SUF Fe—S biogenesis systems (Fig. 1). These systems very much resemble the *E. coli* SUF, except SufE that is substituted by SufU, referring to them as SUF-like systems. *M. tuberculosis* (*Mtb*) is the causative agent of tuberculosis while *S. aureus* is a human commensal that causes morbidity and mortality worldwide. *E. faecalis* is responsible for 80–90 % of clinical isolates in nosocomial infections [63] including infections of the urinary tract, wounds, bloodstream and endocardium. The SUF system is essential for viability of *Mtb* [64–66] and *S. aureus* [67,68] and therefore constitute a potential target for therapeutic treatments, as humans have no SUF.

4.2. Sulfur acquisition: Structure-function of SufS and SufU

In SUF-like systems, sulfur acquisition is performed by the SufS and SufU proteins. Gram (+) SufS is, like Gram (−) SufS, a dimeric type II cysteine desulfurase enzyme displaying a low cysteine desulfurase activity dependent on the conserved cysteine residue (*B. subtilis* Cys_{361}, *Mtb* Cys_{373}, *S. aureus* Cys_{389}, *E. faecalis* Cys_{153}) [69–72]. Gram (+) SufS is characterized by a half-site reactivity (still not fully demonstrated for Gram(−) SufS), defined by one active site functional at a time [69,70,73], meaning that the active site of each monomer operate alternately.; In the case of SufS enzymes, there is no evidence for either case. Structural studies showed that the catalytic loop of SufS is adjacent to the PLP in the resting state and undergoes a small structural conformational change in orientation (by 3°) of Cα-Cβ-Sγ of the Cys_{361} during catalysis. Such a small rotation of the side chain is necessary for nucleophilic attack toward the PLP-L-cysteine [74]. This loop of *B. subtilis* SufS lacks structural flexibility and solvent accessibility (like Gram (−) SufS, which likely explains its low cysteine desulfurase activity. Several crystallographic studies, including X-ray crystallo-snapshot analysis, were carried out on *B. subtilis* SufS during catalysis with L-cysteine [74,75]. In particular, two intermediate external aldimines were trapped: a) PLP-L-cysteine intermediate, which was modeled as an Cys-aldimine intermediate like in *E. coli* SufS system [23], b) the PLP-L-alanine aldimine (ala-eneamine) with Cys361-SSH which was derived from the first catalytic conversion of L-cysteine to L-alanine.

Biochemical studies have unveiled that SufU, a monomeric protein of 16 kDa, interacts in solution with SufS forming a complex with a 2:2 stoichiometry for the SufS:SufU interaction [69,70,76]. Surprisingly, SufU shares more similarity with NifU and IscU scaffolds than with SufE and, in fact, *B. subtilis* SufU can accommodate Fe—S cluster after *in vitro* chemical reconstitution and transfer it [71,76]. Therefore, *B. subtilis* SufU was initially proposed to act as a scaffold. However, several arguments do not favor this view. (i) the LPPVK motif present on NifU/IscU scaffolds, which permits interaction with HscA, is absent in SufU proteins. (ii) a 19-amino acid insertion is present between the second and third conserved cysteine residues, which discriminates SufU from IscU. (iii) on the N-terminal side of the third conserved cysteine lies a conserved lysine in SufU, which is a conserved histidine in IscU. (iv) attempts to characterize the SufU-FeS cluster by Mössbauer and EPR spectroscopies were unsuccessful, strongly suggesting that binding of Fe—S in SufU is aspecific [73]. (v) heterologous complementation studies using *B. subtilis* and *E. coli* mutants showed that *B. subtilis* SufSU is interchangeable with *E. coli* SufSE but not with IscSU [77]. (vi) *Mtb* Zn-SufU was shown not to be an intermediate during Fe—S formation and apo-SufU does not bind an Fe—S cluster [70,73]. Altogether, these observations support the notion that SufU is involved in sulfur mobilization with SufS rather than acting as a Fe—S assembly scaffold.

In fact, SufU as-purified binds a zinc ion (affinity constant of 10^{16} M^{-1}-10^{17} M^{-1}), which is coordinated *via* conserved residues, three cysteines (Cys_{35}, Cys_{41} and Cys_{128} in *B. subtilis*) and an aspartate (Asp_{43} in *B. subtilis*) [69,72,73,78]. Enzymology experiments revealed that SufU is a co-substrate of SufS (with L-cysteine) explaining the ping-pong

mechanism [69,70]. SufU is a sulfurtransferase that enhances the activity of SufS (Table 1) [70,72,73], and the zinc ion is important for the sulfur transfer reaction between SufS and SufU [69,70]. Interestingly, *E. coli* SufE that does not contain any metal is not able to enhance the Gram(+) SufS activity [70]. Moreover, a strictly conserved His residue of SufS (His_{354} in Mtb SufS, His_{342} in *B. subtilis*) plays an essential role in the sulfur transfer reaction [70,79]. This residue is absent in Gram-negative SufS proteins associated with SufE. These observations show a specificity in the sulfur transfer between Gram(+) and Gram(−) bacteria [70].

The crystal structure of *Mtb* and *B. subtilis* Zn-SufU-SufS complexes were solved at 1.65 Å and at 2.3 Å resolution, respectively (PDB 8ODQ and PDB 5XT5) [70,79]. In these structures, the SufS-SufU complex is in the $(SufS)_2$-$(SufU)_2$ state. These crystal structures revealed the importance of Zn^{2+} for both the SufU-SufS binding and the sulfur transfer from SufS to SufU. These studies unveiled a Zn ligand exchange reaction upon SufS-SufU complexation; the first conserved Cys (*B. subtilis* Cys_{41} or *Mtb* Cys_{40}) is exchanged with the SufS conserved histidine residue (*B. subtilis* His_{342}, *Mtb* His_{354}). Consistently, changing this His to tyrosine resulted in a significant loss of desulfurase activity of the SufS-SufU complex [79]. The structural studies of the complex show also that the histidine (Mtb His_{354}), which is located at the extremity of an alpha-helix and points out of the surface of SufS, is inserted between the Zn ion and the second conserved Cys_{40} in SufU [70]. Therefore, Zn ligand swapping provides a free thiol from a Cys of SufU to act as a sulfur acceptor. The sulfur transfer mechanism between SufS and SufU was beautifully demonstrated *in cristallo* using SufS-SufU from *B. subtilis* with several trapped catalytic intermediates (persulfide on SufS and then transfer to SufU) [79]. The *Mtb* SufS-SufU structure has provided in addition new insight regarding the conformational changes that SufU undergoes upon docking to SufS, in particular regarding the loop containing the conserved Cys_{40} residue. A comparative analysis of the *Mtb* SufU unit (as it is in complex) extracted from *Mtb* SufS-SufU with SufU from *B. subtilis* (PDB 6JZV) revealed that the hairpin loop of SufU, including Cys_{40}, undergoes a major reorientation when docking to SufS. From this analysis, a sequential molecular mechanism was proposed (Fig. 7): (1) SufU docks onto SufS; (2) Zn swaps its ligand from Cys_{40} to His_{354}; (3) the released SufU hairpin loop undergoes a major movement from a closed to an open conformation; (4) His_{354} stabilizes the hairpin in the open state (through a hydrogen bond with the Cys_{40} carbonyl oxygen) and creates a projection inside SufS; (5) the Cys_{40} side-chain flips into the cavity of SufS to position Cys_{40} close to Cys_{373} (6 Å). In this position, Cys_{40} can accept sulfur atom(s) from SufS Cys_{373}. In the *Mtb* SufS-SufU structure, the electron density map at Cys_{40} revealed an extra density that could be modeled as Cys_{40} persulfide (Cys_{40}-SSH).

Differences in cysteine-oxidation patterns were noted between the *Mtb* SufSU and *E. coli* SufSE systems [78]. Indeed, in the presence of H_2O_2, SufE homodimers formed whether SufS was present or not. In contrast, in the same oxidizing conditions SufS-SufU heterodimers formed. These differences might explain the better cysteine desulfurase activity measured of *Mtb* SufSU in the presence of oxidative stress [70]. In this regard, it is tempting to propose that Zn ion protects the SufU sulfur shuttle conserved cysteine residue from oxidation. In comparing the sulfur mobilization mechanism by SufSU and SufSE, it seems that SufSU has a specificity which consists in making its sulfur shuttle cysteine free for sulfur transfer only upon interaction with SufS.

4.3. *Fe—S cluster assembly by the SufBCD scaffold*

The SufBCD scaffold of Gram-positive bacteria received specific focus in *Mtb* as it is essential and exhibits original features. Indeed *Mtb* SufB possesses an intein from residues 253–611 (over a total of 846 aa). SufB, SufC, and SufD have been shown to interact *in cellulo* [64] and SufB splicing appears to have a critical role in modulating interaction with SufC and SufD [80,81]. The SufB intein could act as a sensor for oxidative and nitrosative stress in *Mtb* [81]. After splicing, *Mtb* SufB is around 54 kDa in size, like other SufB proteins. *Mtb* SufB primary sequence contains residues identified as functionally important *in vivo* for *E. coli* SufB such as the conserved sulfur entry Cys_{253} (Cys_{254} in *E. coli*) and terminal sulfur acceptor Cys_{397} (Cys_{405} in *E. coli*) sites, the potential Fe—S ligands His_{425} (His_{433} in *E. coli*), Glu_{426} (Glu_{434} in *E. coli*), and the sulfur production and/or sulfur channeling residues Gln_{284} residue (Gln_{285} in *E. coli*), Lys_{296} (Lys_{303} in *E. coli*) and Trp_{286} (Trp_{287} in *E. coli*) [49]. *Mtb* SufC displays 50 % amino-acid sequence identity with *E. coli* SufC, including Walker A and Walker B domain, ABC signature as well as key amino-acids for ATPase activity such as the Lys residue in the Walker A motif (corresponding to the Lys_{40} in *E. coli* SufC), the Glu_{176} residue immediately following the Walker B motif (the Glu_{171} in *E. coli* SufC), and the His_{209} residue in the characteristic ABC ATPase H-motif (the His_{203} in *E. coli SufC*) [56]. Surprisingly, *Mtb* SufD displays only 23 % sequence identity with *E. coli* SufD. However, its sequence contains the His_{327} residue (His_{360} in *E. coli*), proposed to be functionally important [49,56,82]. The prediction is that SufB, SufD and SufC proteins interact to assemble the Fe—S cluster before transfer to targets. However, these three proteins as well as their interaction remain to be functionally and structurally characterized. SufBCD was also studied within the context of development of a new anti-bacterial compound. Likewise, a small inhibitory molecule, “882”, was identified as targeting *S. aureus* SufBCD [83]. Pull-down experiments from cellular lysate using biotin-conjugated 882, showed that "882″ molecule interacts with SufBCD proteins and subsequent biolayer interferometry (BLI) experiments revealed that the inhibitor interacts directly with SufC (Kd = 4 μM) [83].

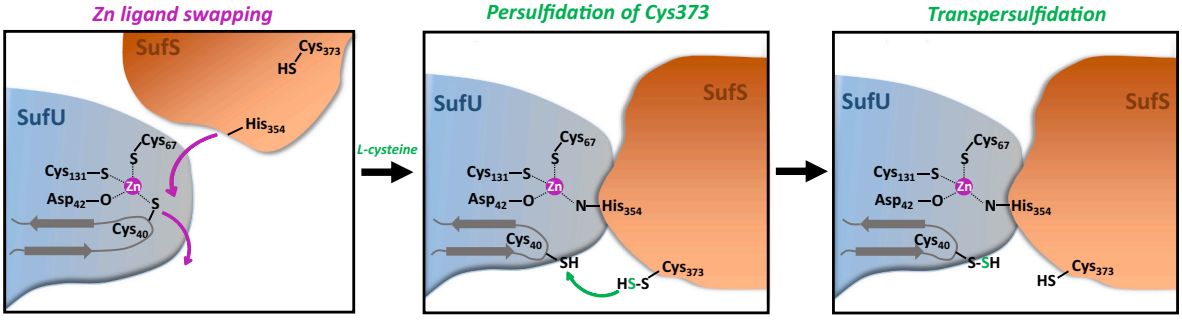

Fig. 7. Proposed mechanism of sulfur mobilization by Suf-like system (with Mtb system). SufU zinc (Zn) coordinated by Cys_{65}, Cys_{40}, Cys_{131} and Asp_{67} interacts with SufS. Upon interaction, Zn atom swaps its ligand from Cys_{40} to His_{354}. SufU hairpin loop (grey) undergoes a reorientation movement from a closed to an open conformation. Cys_{40} side-chain flips into the dedicated cavity of SufS close to Cys_{373}. Cys_{40} can accept sulfur atom from SufS Cys_{373}.

5. The SUF system in parasites: *Plasmodium falciparum*

5.1. Physiology

Malaria is responsible for around 500,000 deaths each year and is caused by the parasite *Plasmodium falciparum*. The plastid (apicoplast) of *P. falciparum*, essential for parasite survival [84], houses several important biochemical pathways such as type II fatty acid biosynthesis, non-mevalonate pathway of isoprenoid synthesis and biosynthesis of heme [85–87]. The existence of a SUF pathway (Fig. 1) in apicoplast has been initially suggested by bioinformatics studies [88,89]. One protein (SufB/YCF24) is predicted to be encoded by the apicoplast genome itself, while SufC, SufD, SufS and SufE proteins are nuclear encoded proteins with N-terminal bipartite apicoplast targeting elements [85]. A SufA-like protein has been predicted to be apicoplast-targeted. The SUF pathway was reported to be essential for apicoplast maintenance and parasite survival [58,90] and therefore constitutes a potential target for antimalarial drug discovery. Discovery of inhibitors requires biochemical and structural characterization of its components.

5.2. Sulfur acquisition: The structure-function of *P. falciparum SufS and SufE*

Although *P. falciparum* SufS and *P. falciparum* SufE proteins have low overall sequence identity with their *E. coli* counterparts, they carry conserved domains and residues that are critical for cofactor binding and function. *P. falciparum* SufS contains a PLP-binding motif (SGHK) with a conserved Lys residue that forms an internal aldimine with PLP. *P. falciparum* SufS has the conserved Cys_{497} residue (corresponding to *E. coli* Cys_{364}) in a typical consensus region, RXGHHCA, found in the group II cysteine desulfurases. *P. falciparum* SufE is predicted to have a 102-aa N-terminal apicoplast targeting leader sequence and carries a conserved Cys_{154} residue (corresponding to Cys_{51} in *E. coli* SufE) that is predicted to receive sulfur from SufS. Purified *P. falciparum* SufS and SufE proteins were studied *in vitro* [91]. SufS forms a complex with SufE and exhibits a low cysteine desulfurase activity, which is greatly enhanced by SufE in a concentration-dependent manner (up to 17-fold) (Table 1). Moreover, SufS Cys_{497} is the critical residue involved in sulfur mobilization from the cysteine substrate. Structural modelling of the *P. falciparum* SufS-SufE interaction revealed that Cys_{497} of SufS is only 8.4 Å from Cys_{154} of SufE, a distance which would be further reduced by persulfide on SufS, making a sulfur transfer possible between these two residues. *P. falciparum* SufE is able to activate also *E. coli* SufS and *P. falciparum* SufS can complement *in vivo* the loss of *E. coli* SufS [58]. *P. falciparum* SufS does not have the conserved Histidine residue (His_{354} in *Mtb*, His_{342} in *B. subtilis*) confirming that *P. falciparum* SufS works in tandem with a SufE protein. D-cycloserine, a PLP binding drug, was shown to inhibit *P. falciparum* SufS *in vitro* (IC_{50}: 29 μM) [91].

5.3. Fe—S cluster assembly by the *P. falciparum SufBCD scaffold*

P. falciparum SufC exhibits 44 % sequence identity with its *E. coli* counterpart. The unprocessed protein has a predicted mass of 40 kDa containing a 96 amino acid extension at the N-terminus that is predicted to contain the bipartite element for apicoplast targeting [85,92]. *P. falciparum* SufC carries Walker A and Walker B motifs together with a conserved ATP binding cassette (ABC) signature [93]. Accordingly, it is endowed with an ATPase activity [94].

P. falciparum SufB is a 54 kDa protein that exhibits 37 % primary sequence identity with *E. coli* SufB. Interaction between purified *P. falciparum* SufC ATPase and scaffold component *P. falciparum* SufB was observed *in vitro* and further confirmed by a pull-down assay [94]. Unlike *E. coli* SufB, but similar to *Arabidopsis* plastid SufB, *P. falciparum* SufB exhibits an ATPase activity, which is however 3-fold lower than SufC activity [94–96]. *In vitro* interaction experiments and *in vivo* crosslinking showed that apicoplast-targeted SufD (21 % identity with its *E. coli* homolog) interacts with apicoplast-encoded *P. falciparum* SufB and apicoplast-targeted *P. falciparum* SufC forming a complex with a 1:1:2 stoichiometry [96]. *P. falciparum* SufD, but not SufB, enhances the SufC ATPase activity by two-fold [96]. The $SufBC_2D$ complex can function as a scaffold to assemble [4Fe—4S] clusters *in vitro* [96] . In a structural model of $SufBC_2D$, Cys_{379} in SufB (Cys_{405} in SufB *E. coli*) and His_{1396} of SufD (His_{360} in *E. coli* SufD) are well oriented and positioned for cluster ligation [96]. The knowledge of other SUF proteins, particularly those constituting the Fe—S scaffold, will provide the opportunity for identifying drugs that would target SUF system and thus inhibit parasite growth [91].

6. The SMS system: Ancestor of the SUF system

6.1. The SMS in archaea

SMS is composed of two homologs of the SUF scaffold proteins SufBC, called SmsCB. Surprisingly no cysteine desulfurase, carrier or homolog of SufD encoding gene was found in the vicinity of the *smsCB* genes or even in the genome of multiple species carrying *smsCB* (Fig. 1). SMS was experimentally validated as a *bona fide* Fe—S cluster biogenesis scaffold [20]. Interactions between SmsC and SmsB proteins of *M. jannaschii* and *M. acetivorans* were shown by two-hybrid technique and copurification in anaerobiosis of SmsB with 6His-tagged SmsC, suggesting that SmsCB act as a complex. As purified SmsCB proteins of *M. jannaschii* and *M. acetivorans* were shown to bind Fe and sulfur by UV–visible spectroscopic analysis and iron and sulfur analyses [20]. *M. acetivorans* SmsCB proteins display great amount of iron and sulfur as purified (4 Fe and S per $SmsC_2B_2$) while *in vitro* reconstitution of the Fe—S cluster was necessary for SmsCB of *M. jannaschii* that contains substoichiometric amount of Fe and S (0.1 nd O.2/per complex). After reconstitution it binds around 4 Fe and 4 S per $SmsC_2B_2$. Moreover, both as purified and reconstituted SmsCB were able to transfer their Fe—S cluster to *E. coli* aconitase and reactivate it, showing that the SmsCB complex can promote both formation and transfer of a Fe—S of a cellular apo target [20].

Crystal structure of SmsB homodimers from *Methanosarcina mazei* Go1 is available (PDB 4DN7). Interestingly, the structure reveals the existence of a tunnel similar to that observed in SufB, ranging from Cys_{177} (corresponds to *E. coli* SufB Cys_{254}) to Cys_{319} (corresponds to *E. coli* SufB Cys_{405}). Recently, a first attempt to analyze the contribution of SMS to Fe—S biogenesis *in vivo* was reported in the methanogenic archaeon *Methanosarcina acetivorans* [98]. This methanogen has two predicted SMS systems, referred to as SmsC1B1 and SmsC2B2. Authors utilized genetic methods to repress or delete the *smsC1B1* and *smsC2B2* genes, and the results revealed that neither dual repression nor deletion of both operons affected the organism's growth under various conditions, including diazotrophy. Moreover, the deletion of *smsC1B1* and/or *smsC2B2* did not affect the total Fe—S cluster content in *M. acetivorans* cells. However, the deletion of only *smsC1B1* led to a delayed-growth phenotype. The study concluded that the SMS systems are not required for Fe—S cluster biogenesis in *M. acetivorans* and raised questions about the *in vivo* function of SmsCB in methanogens.

6.2. The SMS in blastocystis

Blastocystis (*Bh*) is a unicellular anaerobic parasite, part of the genus of unicellular protozoa of the stramenopile group, which includes algae, diatoms and oomycetes. It can be found in the intestinal tract of animals and humans, leading to infections. It harbors a ISC system and a SmsCB system that was previously misannotated as SufBC [99,100] (Fig. 1). Curiously, SmsC and B are actually fused, forming a single polypeptide, in which both domains interact. Immunofluorescence and immunoelectron microscopy have revealed that the *Blastocystis* SMS system is localized in the cytoplasm [99]. *Bh* SmsCB exhibits ATPase activity (V_{max} value: 0.049 μmol Pi/min/mg; Km ATP: 196 μM). These values are

comparable to those of the *E. coli* SufBC$_2$D complex [99]. *Bh* SmsCB enhances the cysteine desulfurase activity of the SufSE complex from *E. coli*, similar to the SufBC$_2$D complex [99]. Last, *Bh* SmsCB was found to contain an average of 4.2 Fe atoms and 4.5 sulfur atoms per dimer, upon reconstitution [99]. Thus, despite it underwent a fusion event, the *Bh* SmsCB protein exhibits biochemical features similar to the *E. coli* SufBC$_2$D complex. Interestingly, SmsBC was likely acquired through horizontal gene transfer from an archaea [99].

7. Perspectives

Despite progress on the SUF system, notably through structural studies, much remains to be learned. Obtaining the structure of the SufSE complex will be crucial for elucidating the mechanism of sulfur mobilization in Gram negative bacteria. Similarly, structural data on SufBC$_2$D with flavin and the Fe—S cluster are eagerly awaited, and combined with biochemical studies will enable us to determine the nature of the Fe—S cluster ligands and to understand the role of flavin in the complex. In particular, it would be interesting to determine whether flavin is a specific cofactor of the Gram-negative SUF machinery, or whether it also occurs in Gram-positive SUF systems and certain parasites. The study of Gram-positive SUF systems has made great strides in recent years and should soon make it possible to find inhibitors of this machinery as antibacterials.

The ancestral and minimal SMS system, composed of only simple SmsCB scaffold raises different questions. The first one is the source of sulfur, as this minimal system has no cysteine desulfurase (Fig. 3B**)**. One possibility is that environmental exogenous sulfide is used as sulfur source instead of L-cysteine [101,102]. Another possibility, proposed by Boyd and collaborators, is that pyrite is the sulfur source of methanogens. Indeed, anaerobic methanogens have the ability to reduce FeS2 and release Fe and S for their nutritional needs [103]. A third possibility for organisms living in non-sulfide-rich environments, is that MIS, the minimal machinery of Fe—S clusters, ancestral of the ISC system could provide sulfur to SMS through its cysteine desulfurase MisS (as it is the case between *csdAE* and *sufBCD* in *E. coli* [104]. Indeed, MIS and SMS largely co-occur (even if they are never side-by-side in the genomes) and this co-existence was largely retained in the archaea, possibly linked to their more limited range of lifestyles (strictly anaerobic) with respect to bacteria.

The SMS system has no homolog of SufD. We discussed above that the existence of sub-complex SufB$_2$C$_2$. If it occurs at all *in vivo*, a possibility put forward by Outten et al. is that it would be the terminal scaffold complex once SufD has been released from it [19,62]. In that sense, SmsC$_2$B$_2$ could simply act as the principal assembly and donor platform of Fe—S clusters in an environment rich in iron (Fig. 3B) [62]. The other possibility is that the SmsB protein could play both the role of SufB and SufD.

CRediT authorship contribution statement

Macha Dussouchaud: Writing – review & editing, Data curation. **Frédéric Barras:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Conceptualization. **Sandrine Ollagnier de Choudens:** Writing – review & editing, Writing – original draft, Validation, Supervision, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Ollagnier Sandrine reports was provided by National Centre for Scientific Research. Ollagnier Sandrine reports a relationship with National Centre for Scientific Research that includes: employment, funding grants, and non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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