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The SUF system: an ABC ATPase-dependent protein complex with a role in Fe–S cluster biogenesis

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ABSTRACT

Iron-sulfur (Fe–S) clusters are considered one of the most ancient and versatile inorganic cofactors present in the three domains of life. Fe–S clusters can act as redox sensors or catalysts and are found to be used by a large number of functional and structurally diverse proteins. Here, we cover current knowledge of the SUF multiprotein machinery that synthesizes and inserts Fe–S clusters into proteins. Specific focus is put on the ABC ATPase SufC, which contributes to building Fe–S clusters, and appeared early on during evolution.

1. Introduction

P-loop ATPases are one of the most prevalent protein families in the proteomes of organisms from all three domains of life. Phylogenetic analyses have suggested that the last universal common ancestor (LUCA) of all modern organisms already possessed multiple P-loop NTPases [1]. In the present-day organisms, P-loop NTPases are involved in a wide range of biological processes. P-loop ATPases are chemo-mechanical engines that use both the binding and the hydrolysis energy stored in adenosine triphosphate (ATP) to overcome energetic barriers. Among the P-loop superfamily one can distinguish the ATP-Binding Cassette (ABC) containing proteins. Initially the ABC proteins were described as energizing the transport across membranes of a wide array of solutes, from ions to macromolecules. Accordingly, ABC domains are part of, or associate with membrane-spanning proteins to form channels, whose opening and closing are regulated by cognates substrate binding and ABC proteins [2,3]. Subsequently, a subset of ABC proteins was found to be associated with a variety of non-transmembrane transport processes, such as structural maintenance of chromosomes, which is essential for chromosome segregation/condensation, and DNA repair [4]. Here, we present and discuss current information on the SUF system in which, SufC, an ABC ATPase has been associated early during evolution to ensure biogenesis of iron-sulfur (Fe–S) clusters [5–8].

2. Fe–S clusters and their biogenesis

Fe–S clusters are considered as ranking among the most ancient and versatile inorganic cofactors used in the three domains of life [6–9]. They can act as redox sensors or catalysts and are found to be used by a great number of proteins (over 150 in *Escherichia coli* [10]). Likewise, Fe–S proteins participate to diverse biological processes such as respiration, photosynthesis, metabolite biosynthesis, central metabolism, gene regulation or DNA repair [7,8,10,11].

Many types of Fe–S clusters are found in nature but the most common ones are the rhombic [2Fe–2S] and cubic [4Fe–4S] types. They are constituted by ferrous (Fe^{2+}) or ferric (Fe^{3+}) iron and sulfide (S^{2-}). In most cases, the thiolate from cysteine residues side chain coordinates iron ions of the cluster but examples have been reported wherein histidine (His), arginine (Arg), aspartate (Asp) or tyrosine (Tyr) residues can help to stabilize the cluster [7].

Fe–S clusters can be synthesized spontaneously *in vitro* with inorganic iron and sulfur sources but *in vivo*, cells require large multiprotein machineries such as SUF, NIF or ISC [12–14]. In this

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review we will focus on the SUF system (Fig. 1) which is composed of (i) the ABC ATPase-containing complex constituted by the SufBCD proteins, which represents the scaffold onto which Fe–S clusters are transiently assembled, (ii) the two-component cysteine desulfurase complex, SufSE, which mobilizes sulfur from L-cysteine, and (iii) the carrier SufA that delivers Fe–S clusters to target apo-proteins [5,15,16]. Recently, two additional Suf proteins were uncovered in the *Firmicutes*: SufT, a Fe–S cluster carrier protein, and SufU that can replace SufE as partner of the SufS cysteine desulfurase [17–19]. Strikingly, in most prokaryotes, genes encoding the Suf components are found to be located in a genomic cluster and variation in the number of genes and their relative order can arise among species (Fig. 2).

The SUF system was initially discovered in *E. coli* via a mutation in the *sufD* gene that prevents ferric siderophore utilization under iron limiting conditions [20]. Subsequently, the *suf* operon was discovered as contributing to virulence of the plant pathogen *Erwinia chrysanthemi* [21]. Spontaneous prototroph pseudo-revertant of *E. coli isc* strain were obtained, which contained mutation upregulating *suf* operon expression [22]. Since these early studies, the SUF system has been described in a wide array of organisms including chloroplasts in plants [23,24]. Initially, identification of an ABC ATPase in a Fe–S biogenesis system was interpreted as an indication that this system delivered clusters to membrane embedded and/or periplasmic apo-proteins. This hypothesis was however ruled out after subcellular fractionation and immunoblotting experiments, which showed that all three SufB, SufC and SufD proteins are cytosolic [5].

3. Physiological role of the SUF system

The SUF system was first described in *E. coli* and most of our knowledge on it derives from studies using this model organism [14]. Besides SUF, *E. coli* possesses the ISC system, another additional Fe–S cluster biogenesis system. Although they share some paralogous components, the two systems are not mere duplications of one another and appear to be examples of convergent evolution. First, the scaffold proteins differ from one system to another, SufB (see below) and IscU being structurally and phylogenetically unrelated. Secondly, the ATP-hydrolysing scaffold partners are different. Indeed, the SUF system uses an ABC ATPase (i.e. SufC), while the ISC system depends on ATP-using DnaK/J-like chaperones (i.e. HscA and HscB) [13,25]. In *E. coli*, SUF acts as a back-up system when the ISC system is unable to sustain cellular Fe–S demand. This occurs when Fe–S clusters biogenesis is difficult to achieve such as under iron limitation, and in conditions that damage Fe–S clusters, such as in the presence of oxygen reactive species (ROS). Consistently, *suf* mutants are hypersensitive to oxidative stress and iron limitation [5,20,26–28]. In *E. coli*, the SUF system is also required to cope with metallic stress such as cobalt exposure, which poisons the Fe–S cluster assembly process [29]. How the SUF proteins succeed to function in conditions where the ISC does not is not fully understood. The hypothesis that the SUF system is functional under oxidative stress or iron limitation has received support from biochemical and structural investigations [30,31]. Biochemical studies in particular showed that SufBCD-bound Fe–S clusters are more stable in the presence of hydrogen peroxide as compared to the IscU-bound cluster [30].

In *E. coli*, switching from ISC to SUF bears drastic physiological consequences (see [11] for a review on regulatory aspects). For example, an *E. coli* strain using only SUF to make Fe–S clusters exhibits a lower aminoglycoside uptake capacity, hence enhanced resistance to this class of antibiotics [32]. Indeed, aminoglycoside uptake is reduced because proton motive force-producing respiratory complexes that contains Fe–S are much less efficiently matured by the SUF machinery than the ISC machinery. Similarly, a recent study reported a reduced maturation of the major oxidoreductases in anaerobic growing *isc* mutants of *E. coli* [33].

The SUF machinery has been proven to be essential for viability in organisms that do not contain ISC or NIF systems, such as *Mycobacterium tuberculosis*, *Bacillus subtilis, Synechocystis* and *Staphylococcus aureus* [17,34–36]. Accordingly the *suf* genes are part of the *B. subtilis* strain engineered to contain a minimal genome [37].

4. Biochemistry of the SufBCD scaffold complex and its components

4.1. The SufB scaffold

SufB is able to assemble transiently a Fe–S cluster. The type of cluster however remains uncertain as *in vitro* reconstitution experiments reported the production of both a [2Fe–2S] and a [4Fe–4S] containing SufB [30,38]. *In vivo,* both [4Fe–4S] and [3Fe–4S] clusters-containing SufB have been identified [39]. As described below, SufB interacts with SufCD to yield a complex, which in its isolated form at an early stage of the purification exhibits a blackish-green colour and a typical [2Fe–2S] UV–visible spectrum [40]. However, the colour vanishes gradually during purification, because the Fe–S cluster is fragile in presence of oxygen [40]. Importantly, *in vitro*, both the [2Fe–2S] and [4Fe–4S] holoforms of SufB are able to transfer their cluster to [2Fe–2S] and

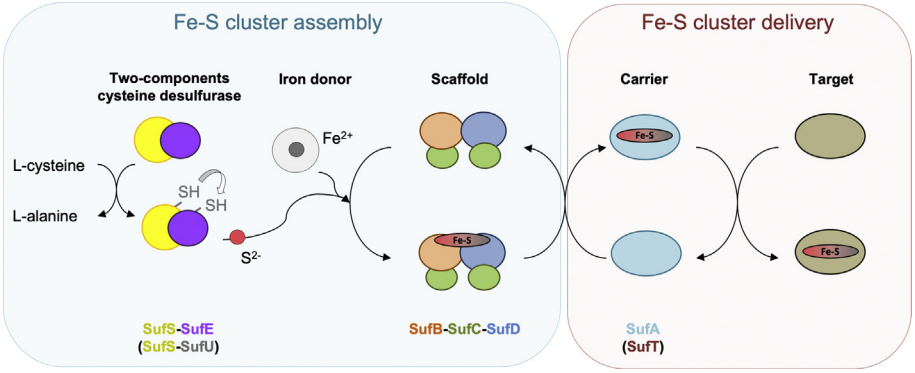

Fig. 1. General principles of the SUF Fe–S cluster biogenesis. The Fe–S cluster assembles on a scaffold protein ($SufBC_2D$ complex), which receives sulfur from a two-component cysteine desulfurase (SufSE or SufSU) and iron from an as yet non-identified source. Then, the pre-formed Fe–S cluster is transferred to a carrier protein (SufA and/or SufT), which delivers it to the final apotarget.

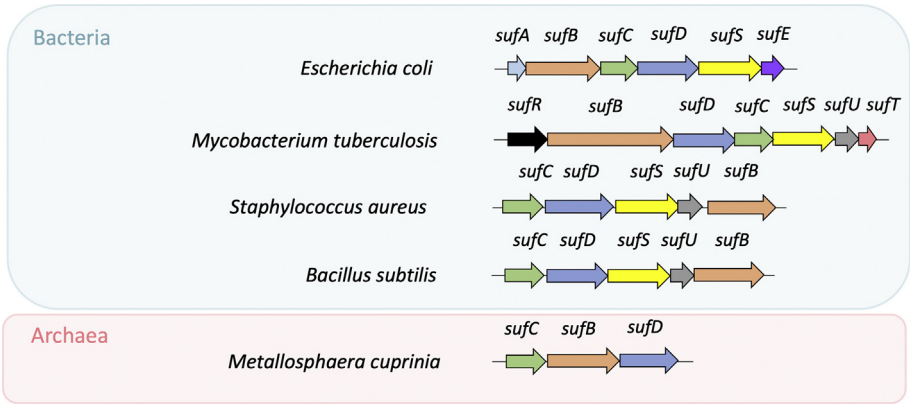

Fig. 2. Schematic representation of the *suf* locus in bacterial and archaeal species.

[4Fe–4S] client proteins [41–43]. The N-terminal region of SufB contains a putative Fe–S cluster motif (CxxCxxxC) that was proposed to provide the ligands of the Fe–S cluster [38], but such a prediction was ruled out by *in vivo* mutagenesis analysis [44]. Moreover both *in vivo* and structural studies pointed to the invariant Cys405 residue and Glu434, His433 and/or Glu432 residues as proposed Fe–S ligands (Fig. 3A, B) [40,44]. It is worth noting that the only available structure of SufB (PDB: 5AWF) corresponds to the form complexed with SufC and SufD (see below).

4.2. The SufC ABC ATPase

SufC exhibits signatures expected of ABC ATPases such as Walker A and B motifs, D- and Q-loops and was found to possess ATPase activity [5,21,45]. X-ray-solved crystal structures of *Thermus thermophilus* and *E. coli* SufC are available (PDB accession numbers: 2D2F; 2D3W) (Fig. 3C) [46,47]. A catalytic α/β domain containing the nucleotide-binding Walker A and B motifs, and a helical domain containing an ABC signature motif are connected by a Q-loop that contains a strictly conserved Gln residue (Gln85 in *E. coli* SufC). SufC presents differences with other ABC ATPase structures including a displacement of the Q-loop, and a difference in the solvent exposed surface, in particular the end of the Walker B motif [46,47]. The ATPase activity of SufC is significantly enhanced upon interaction with SufB or SufD (180-fold with SufB and 5-fold with SufD) [48,49].

4.3. The SufD component

In many organisms, SufB possesses a paralogue named SufD. In *E. coli*, SufB and SufD proteins share 17% identity and 37% similarity in protein primary sequence. However, SufD differs markedly from SufB as no Fe–S cluster was found associated with it. Early studies reported a link between SufD and iron metabolism [5,20,39,50]. However, *in vitro* evidence for a SufD/$Fe^{2+/3+}$ interaction is still lacking.

The structure of *E. coli* SufD has been resolved (PDB number: 1VH4) [51] and displays an atypical fold. It is a flattened right-handed β-helix of nine turns with two strands per turn. SufD can form homodimers, which yields to a doubling of the β-helix length (to 80 Å). Two highly conserved residues, Pro347 and His360, interact at the dimer interface (Fig. 3B) [51]. Interestingly, highly conserved residues are also found in SufB sequence such as Tyr374, Arg378, Gly379, Ala385 or Phe393, but their role remains unknown.

4.4. The $SufC_2D_2$ complex

SufC and SufD interact forming a $SufC_2D_2$ complex whose stoichiometry was determined by mass spectrometry and light scattering experiments [48,52]. Although the physiological significance of this complex is still unclear, as SufB is required for Fe–S cluster biogenesis, the information derived from the $SufC_2D_2$ complex might be of interest to understand how the SUF machinery works. The structure of the complex notably revealed that SufC and SufD interact through extensive hydrophobic interactions as well as by eight hydrogen bonds and one salt-bridge (PDB: 2ZU0) [52]. Interaction involves, on the SufD side, a series of hydrophobic residues that are conserved in SufD, but also in SufB, and, on the SufC side, the β6 strand, the α2 and α3 helices and the Q-loop. No structural changes were found when comparing SufD alone and SufD in the $SufC_2D_2$ complex. In contrast, significant structural changes occurred in SufC, especially near the catalytic site, which undergoes conformational modifications such as breaking-off of the salt bridge connecting Lys152 and the Glu171 residue of the Walker B motif whose side chain now faces the ATP-binding pocket [52] (Fig. 3D). Also, His203, another key residue for the activity of ABC ATPases, is shifted of approximately 5 Å toward Glu171. These conformational changes ensue a better ATP binding and hydrolysis and provide a rationale as to why SufC ATPase activity is boosted upon interaction with SufD [48,49].

4.5. The $SufBC_2D$ complex

In *E. coli*, the physiologically relevant scaffolding complex is the ternary $SufBC_2D$ complex with a 1:2:1 stoichiometry as indicated by mass spectrometry analysis and biochemical analyses [41]. Interestingly, the $SufBC_2D$ complex can be obtained *in vivo* in strains expressing the entire *suf* operon, but cannot be reconstituted *in vitro* starting from the purified components. Nevertheless, it remains to be investigated whether this reflects some sort of cotranslational folding/assembly constraint. The structure of the *E. coli* $SufBC_2D$ complex was solved at 2.95 Å resolution (PDB: 5AWF) (Fig. 3A) [40]. One SufC subunit binds SufB and the other binds SufD, while the ATP-binding motifs of each subunit face each other. Contrary to most ABC ATPases, the SufC subunits are widely separated (>40 Å). SufB and SufD share a similar structural organization, with an N-terminal helical domain, a core domain consisting of a right-handed parallel β-helix, and a C-terminal helical domain that contains the SufC binding site. SufC binds both of its partners and same structural changes are observed for SufC as in $SufC_2D_2$ complex (Fig. 3D). The association between SufC and SufB/SufD is made via the so-called “transmission interface” mode observed in ABC transporters [53]. This mode was proposed to allow transmission of the motion of the ABC ATPase to the transmembrane domain during ATP binding and hydrolysis [53]. Overall, this structural analysis indicates that the ATPase activity of SufC

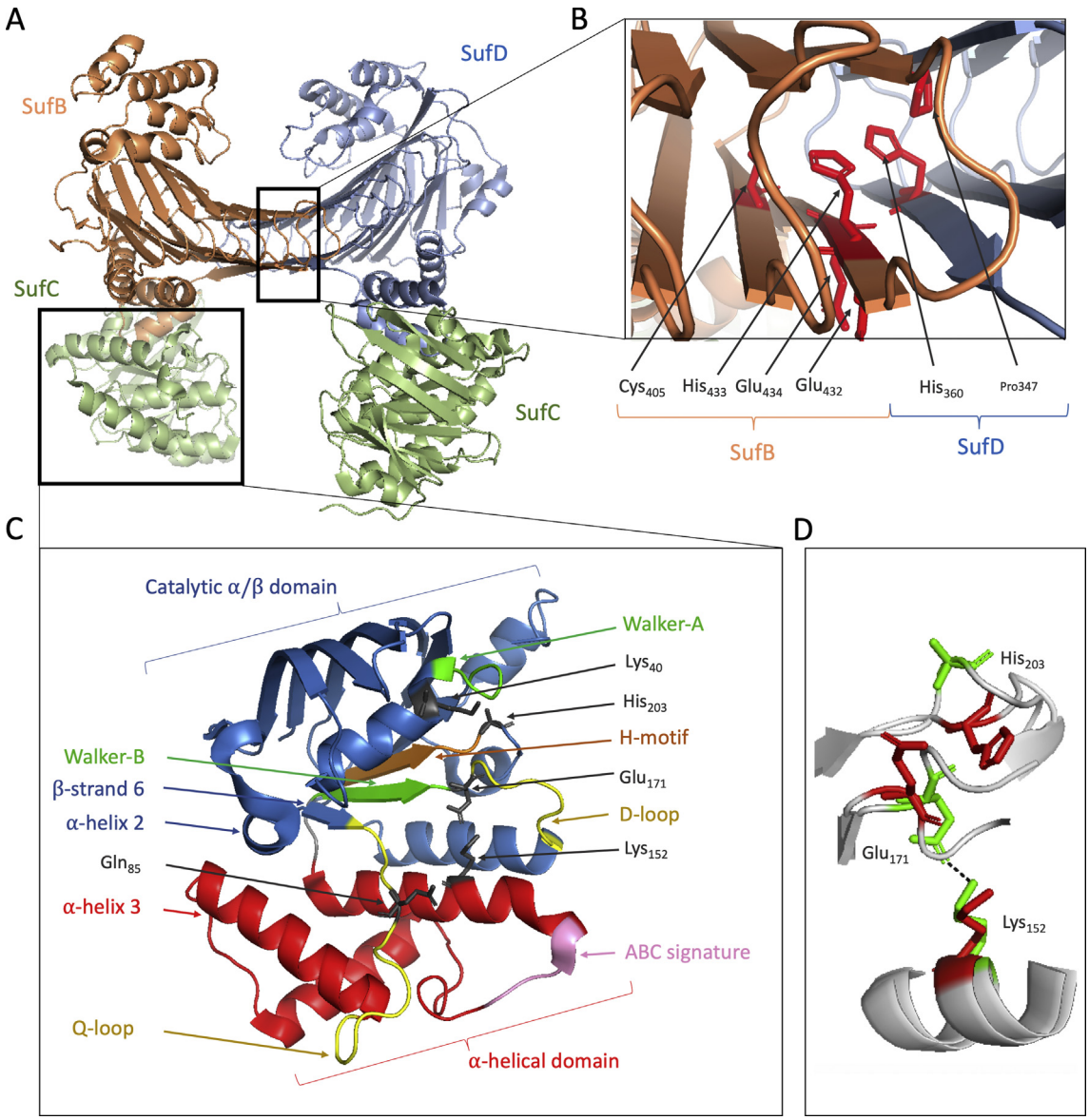

Fig. 3. Structure of the *E. coli* SufBCD components. A) Crystal structure of the *E. coli* SufBC$_2$D complex (SufB, orange; SufC, light green; SufD, light blue) (PDB: 5AWF) [40]. B) Close up of the SufBD interface with residues proposed to be involved in Fe–S cluster fixation and discussed in the text (represented in red). C) Details of the SufC ABC ATPase (PDB: 2D3W, [47]) that is composed of two domains: the catalytic alpha/beta domain (blue) and the alpha-helical domain (red). Important residues are represented in grey. D) Structural changes of major residues in SufC between the form complexed with SufD (PDB: 2ZU0, chain C, in red) and SufC alone (PDB: 2D3W, chain A, in green).

drives the conformational change of its SufB-SufD partners (Fig. 4) [40]. Moreover, these structural studies show that, despite not participating in a trans-membrane transport function, the ATP-dependent conformational changes of the SufBCD complex are similar to those arising within other ABC transporters.

Several important insights specific to Fe–S biogenesis were obtained by this structural study. The core domains of the SufB and SufD subunits are arranged in a right-handed parallel β-helix which upon interactions forms an atypical anti-parallel β-sheet heterodimeric structure (Fig. 3A). This is different from the transmembrane-constituting 12 helices found in classic ABC transporters or the long coiled-coil arm that forms a V-shaped dimeric molecule able to interact with DNA in ABC ATPases of the SMC family. The β-helix architecture of the SufB-SufD protomers was proposed to be specific of the Fe–S cluster biogenesis family of proteins [40].

Fluorescence labelling and cross-linking experiments, both used to follow conformational changes, revealed that SufC forms a transient head-to-tail dimer within the complex during the catalytic step of ATP binding and hydrolysis [40]. Moreover, SufC dimerization induces conformational changes in its two partners, SufB and SufD. Notably in SufB, the Cys405 residue thought to be a ligand for the Fe–S cluster becomes exposed at the surface [40]. It is likely that the conformational changes also allow His360 of SufD, another candidate for the cluster coordination residue [52], to locate close to Cys405 of SufB (Fig. 3B) [40]. Hence, ATP hydrolysis is required to drive conformational changes in order to make scaffold ligands accessible to build the Fe–S cluster.

5. Critical residues for the function of the SufBC$_2$D scaffold complex

Three strictly conserved amino acid residues of SufC, namely Lys40 residue in the Walker A box, Glu171 in the Walker B box, and His203 in the H-motif are considered essential for the SUF complex

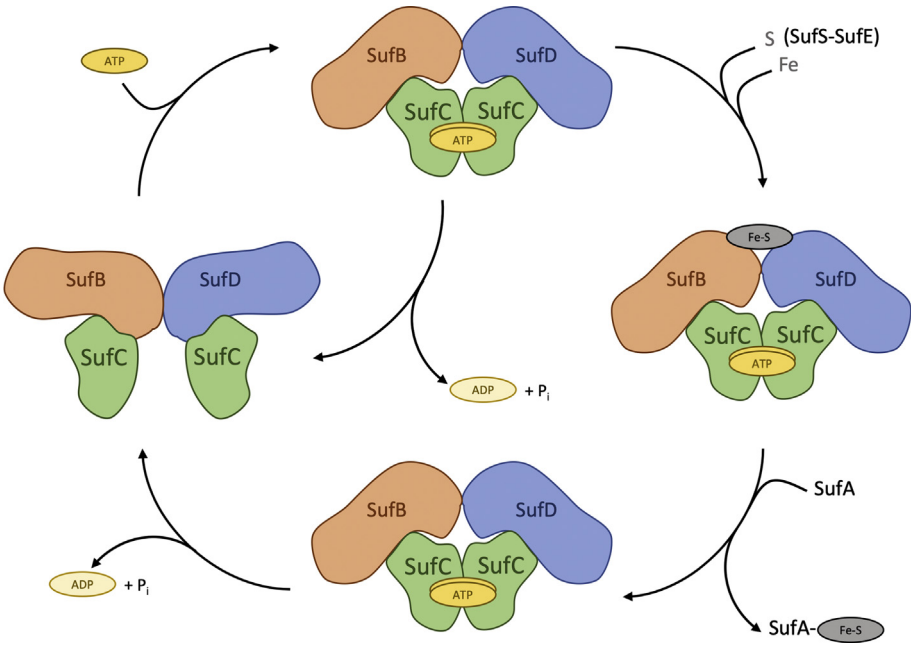

Fig. 4. Proposed mechanism of Fe–S cluster synthesis by the $SufBC_2D$ complex. This model is adapted from Hirabayashi et al., 2015 [40]. First, SufC binds ATP which induces the conformational changes of SufB and SufD (transmission interface). Then, Fe–S cluster is built at the interface of SufB and SufD using sulfur obtained from SufS/SufE. The Fe–S cluster is released to SufA and finally, the ATP is hydrolysed, leading to the initial state of the complex.

functioning. *In vitro* complexes containing mutated variants at any of these positions almost completely lack ATPase activity [40]. Based upon the blackish-green colour (assumed to reveal Fe–S assembly on the SufBCD complex) of the harvested host cells overproducing the SufBCD complex, *in vivo* analysis indicated that SufB C405A and SufD H360A mutants were unable to bind clusters whereas SufD C358A variant was. This supports the notion that both SufB Cys405 and SufD His360 serve as liganding sites for cluster binding but not SufD Cys358 [40]. Interestingly, SufBCD complex containing SufC variants lacking ATPase activity (Lys40, Glu171, His203 variants) failed to bind cluster supporting the notion that SufBD conformational changes induced by the SufC ATPase are indispensable for Fe–S cluster formation [40].

6. Integration of the SufBCD scaffold in the Fe–S biogenesis pathway line

The scaffold SufBCD sub-complex receives sulfur from the SufSE heterodimeric cysteine desulfurase (Fig. 4). The sulfur atom is mobilized from the L-cysteine by the SufS cysteine desulfurase. SufS is a pyridoxal 5′-phosphate (PLP)-containing enzyme exhibiting a dimeric aminotransferase V fold of the type II [54,55]. Briefly, once L-cysteine is bound, an external aldimine is formed with the lysine-bound PLP (Lys226 in *E. coli* SufS). Then, after formation of a ketamine intermediate, a conserved active site cysteine (Cys364 in *E. coli* SufS) attacks the cysteine sulfur, leading to C–S bond breakage and generation of the SufS persulfide ($SufS_{per}$) covalent intermediate and L-alanine. A specific feature of type II enzymes is a 19-residue insertion that forms a β-hairpin motif [55–57]. The hairpin from one SufS monomer reaches across the dimer interface to interact with the active site on the adjacent monomer and is thought to play a role in mediating interactions with the sulfur acceptor SufE [58]. The SufE protein binds to SufS, activates SufS activity and accepts the persulfide ($SufE_{per}$) on its conserved cysteine residue (Cys51 in *E. coli* SufE) [15,59]. From the SufSE complex the sulfur is then transferred to the SufBCD complex [38]. The interaction between SufSE and the SufB scaffold complex occurs only if SufC is present [38].

SufU is present in many Gram-positive bacteria such as *B. subtilis*, *S. aureus*, and in some *Mycobacteria* (*M. tuberculosis*) (Fig. 2). Interestingly, the SUF pathway of the SufU-containing organisms often lacks SufE. SufU of *B. subtilis* interacts with SufS, stimulates SufS activity and accepts the persulfide on the conserved cysteine (Cys41 in *B. subtilis* SufU) [17,19,56,60]. Despite the lack of homology between SufU and SufE, their tertiary structure exhibited analogous folding [17]. Interestingly, SufU exhibits additional cysteine residues that form a zinc binding site with the sulfur acceptor site and an additional aspartate residue (Cys66, Cys128, Asp43 in *B. subtilis* SufU) [19,61]. The exact role of this site remains to be determined. The interaction of SufU with the SufBCD complex has not been documented.

SufBCD was found to deliver its bound cluster to SufA, a member of the A-type Fe–S cluster carrier (ATC) family, including in *E. coli* two other paralogs, ErpA and IscA [42,62]. SufA of *E. coli* when co-expressed *in vivo* together with all Suf proteins, contains a [2Fe–2S] cluster after anaerobic purification [16]. SufA can transfer its cluster to a wide set of apo-proteins, including ferredoxin, which is a [2Fe–2S] protein, and biotin synthase or aconitase, which are [4Fe–4S] enzymes [16,42,43]. In contrast, SufA cannot transfer its cluster to SufBCD, suggesting an unidirectional pathway for cluster synthesis [42].

7. Taxonomic distribution of the SufBCD system

The SUF system is considered as the most widely distributed Fe–S biosynthesis system among prokaryotes [63]. We then explored the taxonomic distribution of homologues of the ABC ATPase SufC and of the two paralogues SufB and SufD in Bacteria and Archaea. We used a combination of BLASTP and HMM profiles search [64,65] on a database of 1990 complete proteomes of prokaryotes (March 2019, ftp://ftp.ncbi.nlm.nih.gov/genomes/all/), using the sequences of *E. coli* as a query (Fig. 5). Preliminary

phylogenetic trees were inferred to separate properly the paralogous SufB and SufD families, as well as the SufC family from other ABC transporters (MAFFT and FastTree, [66,67]). The number of copies per genome, the presence or absence in all proteomes of the database, the relative position of the corresponding genes in the genomes, and the size of sequences were taken into consideration to identify the most likely orthologues of each family. In agreement with previous analysis [63], the mapping of presence/absence ratio of SufBCD homologues in different prokaryotic phyla/classes indicates that the SUF system is widespread in both Archaea and

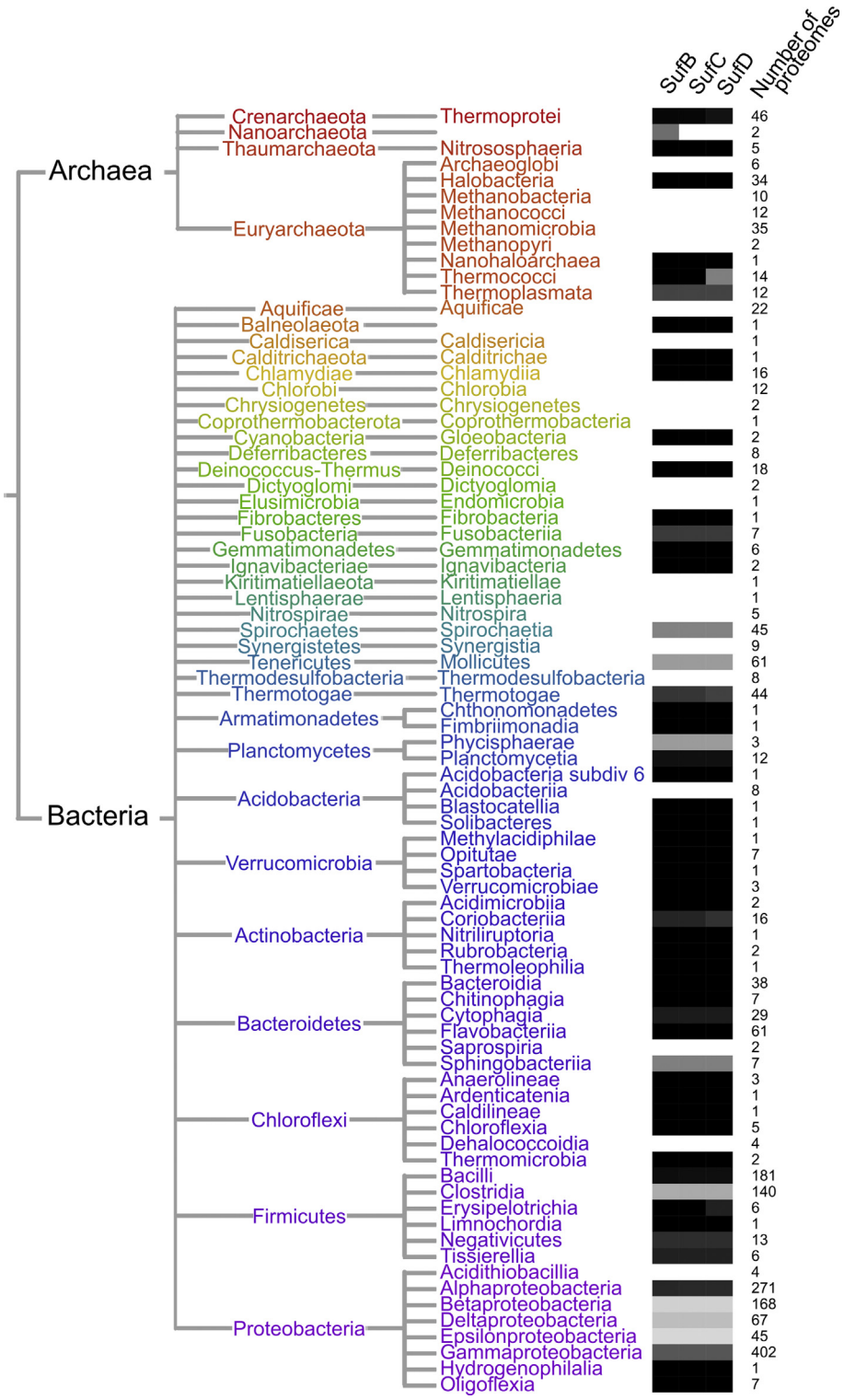

Fig. 5. Taxonomic distribution of SufBCD in major phyla/classes of Bacteria and Archaea. The tree corresponds to the NCBI taxonomy (https://www.ncbi.nlm.nih.gov/taxonomy). The first rank corresponds to the domain, the second to the phylum and the third one to the class. The number of proteomes possessing at least one copy of SufB, SufC and SufD in each taxa is represented by a shade of grey (black: 100% present, white: 100% absent). The number of proteomes by taxa is presented on the right.

Bacteria, which may suggest an ancient evolutionary origin (Fig. 5). SufB and SufD being paralogues, they also probably emerged by an ancient gene duplication. The majority of taxa possess Suf proteins homologues at the exception of some of them such as *Chlorobi*, *Chrysiogenetes, Dictyoglomi* or half of the *Euryarchaeota.* Under a parsimonious hypothesis, this most likely indicates that SUF system is ancient and that absences in these taxa are the result of several independent gene losses. Considering the Fe–S synthesis as crucial for most of living organisms, it is probable that these taxa present other Fe–S biosynthesis systems such as ISC. The general co-occurrence of SufB, SufC and SufD suggests the presence of a functional SUF system, and strengthens the link between its three main components. Interestingly, SufD seems to be less present than SufBC and SufC, which is in agreement with a previous analysis [63].

The Fe–S clusters biogenesis systems found in eukaryotes are diverse in terms of nature, evolutionary origin and DNA coding the corresponding genes. Contrary to mitochondria, which contain the ISC machinery, some plastids and especially from red algae possess a SUF system which derives from the cyanobacterial ancestor of chloroplasts [22]. Some plants such as *Arabidopsis thaliana* possess a SUF system encoded in the nuclear genome. Interestingly, the SUF machinery has been recently found to be present in the nuclear genome of some amitochondriate eukaryotes such as *Monocercomonoides exilis* [68,69]. The authors suggest that the acquisition of the SUF system could be a prerequisite for the loss of mitochondria [68]. The SUF system in such protists seems to originate from a horizontal gene transfer from prokaryotes but the precise donor taxon remains elusive. Finally, the human protozoan parasite *Blastocystis* has likely acquired its SUF system through lateral gene transfer from an archaeon related to the *Methanomicrobiales*, a lineage present in the human gastrointestinal tract [70].

8. The SUF system as a putative anti-pathogen target

The SUF system has a strong potential as an antipathogen target. Indeed, the SUF system is not present in humans and is the only Fe–S biogenesis pathway in some bacterial pathogens such as *S. aureus* or *M. tuberculosis* [34,71]. Research of potential inhibitors led to the identification of a polycyclic molecule that targets directly *S. aureus* SufC with good affinity [72]. Moreover, *in vivo* tests lend credence to the potential use of this compound as an antibacterial drug.

Some parasites such as *Toxoplasma gondii*, the protozoan parasite responsible for toxoplasmosis, and *Plasmodium falciparum*, the infectious agent of malaria rely exclusively on the SUF pathway [73]. Both belong to the *Apicomplexa*, whose members contain a membrane-bound organelle called apicoplast. The apicoplast is derived from a former plastid and contains four major prokaryote-like metabolic pathways, including the SUF pathway, the non-mevalonate (MEP) isoprenoid, the II fatty-acid (FAS II), and the heme biosynthesis pathways [74]. Interestingly, only the SufB/ORF470/Ycf24 gene has been retained in the apicoplast genome and most of the enzymes involved in the SUF pathway as well as the target proteins are encoded by the parasite's nuclear genome and transported to the apicoplast. In *P. falciparum*, the substitution of a conserved Lys by Ala (K140A) in *sufC* sequence has been shown to be toxic for the cell and led to the loss of apicoplast [75]. Hence the SUF pathway appears essential for apicoplast maintenance and parasite survival [75]. Similar observations were reported for *Plasmodium berghei*, for which SufC, SufD, SufE and SufS have been proven to be essential for cell survival but also during blood infection [76]. The research of inhibitors of SUF system of *P. falciparum* led to the identification of the D-cycloserine that inhibits, *in vitro,* the activity of cysteine desulfurase SufSE [77]. Furthermore, D-cycloserine was shown to inhibit the blood stage growth of *P. falciparum,* but whether it is caused by SufS inhibition was not demonstrated [77]. Altogether, these studies indicated that targeting the Plasmodium SUF machinery might be a promising way to fight against malaria.

9. Conclusion

The emergence of protein complexes to make use of Fe–S clusters in biological processes was likely an early event during evolution. As a matter of fact, at least three such systems arose, NIF, ISC and SUF, which, despite the fact that they share some related components, are more than mere duplications of one another. It is reasonable to hypothesize that these machineries possibly evolved from a common pool of components several times independently to meet with the same goal, *i.e.* the synthesis of Fe–S clusters. Interestingly, both ISC and SUF uses general ATP-using devices, Hsp70/Hsp40 and ABC ATPase, which subsequently specialized toward collaborating exclusively with their respective components of the Fe–S biogenesis machineries.

The basic mechanism of Fe–S cluster biogenesis is conserved between SUF and ISC. It can be described as a two-step process in which cluster transiently assembles on a scaffolding protein, and is eventually delivered by dedicated carriers to apo-protein clients. The ABC ATPase SufC, which is the main focus of the present review, participates in the scaffolding activity by allowing binding and formation of the cluster on SufB. Although much has been learned from genetics, biophysics and structural studies, key questions about the functioning of the SUF system remain. For instance, how is the cluster built and where is it located? Available evidences suggest it would position at the SufB/D interface, but this has to be fully demonstrated. Although the source of sulfur is well documented, that of iron, if there is a dedicated one, remains unknown. Evidences were provided that SufD could act as an entry point for iron while early studies had pointed potential connection between SUF and incoming ferric siderophore reductase [50], but much remains to be done to establish the role of SufD in iron harvesting. Evidently, a central question is the role of ATP in Fe–S cluster building and/or in its release from the SufBCD complex. Among other pending questions, an important one will be to decipher the molecular determinants that make SUF seemingly efficient under adverse conditions (iron limitation and potentially damaging oxidative stress). Finally, a reduced flavin (FADH2) was found associated to the as-isolated $SufBC_2D$ complex when purified under anaerobic conditions [41]. Studies on the role, if any, of this flavin in Fe–S cluster biogenesis is eagerly awaited.

It is clear that both its absence in humans and its exclusive presence in many pathogens make the SUF system an attractive target for anti-pathogen compounds. In the context of increasing paucity of efficient antibiotics, targeting the SUF machineries of *Mycobacterium*, *S. aureus* or *P. falciparum* might bear interesting alternatives.

Conflict of interest

The authors declare that no conflict of interest exists.

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