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Review

Mrp and SufT, Two Bacterial Homologs of Eukaryotic CIA Factors Involved in Fe-S Clusters Biogenesis

Corinne Aubert, Pierre Mandin and Béatrice Py *

Laboratoire de Chimie Bactérienne (UMR7283), Institut de Microbiologie de la Méditerranée, Institut Microbiologie Bioénergies et Biotechnologie, Centre National de la Recherche Scientifique, Aix-Marseille Université, 13009 Marseille, France; aubert@imm.cnrs.fr (C.A.); pmandin@imm.cnrs.fr (P.M.)
* Correspondence: py@imm.cnrs.fr

Abstract: Fe-S clusters are essential cofactors for the activity of a large variety of metalloproteins that play important roles in respiration, photosynthesis, nitrogen fixation, regulation of gene expression, and numerous metabolic pathways, including biosynthesis of other protein cofactors. Assembly of iron and sulfur atoms into a cluster, followed by its insertion into the polypeptide chain, is a complex process ensured by multiproteic systems. Through evolution, eukaryotes have acquired two Fe-S protein biogenesis systems by endosymbiosis from bacteria. These systems, ISC and SUF, are compartmentalized in mitochondria and plastids, respectively. The eukaryotic Fe-S protein biogenesis system (CIA) is dedicated to the biogenesis of cytosolic and nuclear Fe-S proteins. While the CIA system is absent in bacteria, at least two of its components share homologies with bacterial Fe-S protein biogenesis factors, Mrp and SufT. Here, we provide an overview of the role of Mrp and SufT in Fe-S protein biogenesis in bacteria, aiming to put forward specific but also common features with their eukaryotic CIA counterparts.

Keywords: iron-sulfur clusters; Mrp/NBP35/CFD1/NUBP1-2; DUF59 domain; SufT/Cia2/CIAO2A-B/FAM96A-B

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1. Introduction

Iron-sulfur (Fe-S) clusters are prevalent and multifariously employed cofactors that were already used by proteins at an early stage during evolution. Amongst contemporary organisms, only very few exceptions are thought to be devoid of Fe-S cluster proteins. A rare example is that of *Borrelia burgdorferi*, the causative agent of Lyme disease, one of the few pathogenic bacteria that can survive without iron [1]. Thanks to their chemical versatility, Fe-S clusters are crucial for the activity of a myriad of proteins [2,3]. To cite only a few of their properties, Fe-S clusters in proteins transfer electrons and help to establish a proton gradient that powers ATP production during cellular respiration and photosynthesis, catalytically act in numerous enzymatic reactions, or participate in perceiving environmental conditions such as oxygen and iron concentration. In addition, a special class of Fe-S proteins can self-sacrifice their cluster to provide sulfur in the biosynthetic pathway of other protein cofactors such as lipoic acid and biotin.

A bioinformatic survey has revealed that Fe-S proteins represent between 1.8 and 4.8% of the proteome of prokaryotes [4]. In *Escherichia coli*, a recent search has updated the number of Fe-S proteins to 181 [5]. The global Fe-S proteins repertoire has been increased, with Fe-S proteins taking a key role in genome maintenance in eukaryotes and also in viruses with Nsp12 and Nsp13, the very recently demonstrated RNA-dependent RNA polymerase and helicase of the SARS-CoV-2 virus, respectively [6–8]. Thus, Fe-S clusters have indisputably played a key role in the evolution of life and are still central in contemporary organisms.

Fe-S clusters are most frequently found in proteins as cubic [4Fe-4S] and rhombic [2Fe-2S] types. Other types of clusters, albeit less frequent, are cubic [3Fe-4S] clusters that

for instance arise from the degradation of a [4Fe-4S] cluster, linear [3Fe-4S] clusters, or even more complex clusters, such as the H-cluster in hydrogenase and the P-cluster and FeMo-cofactor in nitrogenase [3,9]. Generally, the cluster is bound to the polypeptide chain by four ligands. However, in particular cases the cluster is coordinated by fewer ligand residues, either to allow one Fe to interact with the enzyme substrate, such as in aconitase, or with the use of external ligand molecules, such as S-adenosylmethionine (SAM) in radical SAM enzymes or glutathione in monothiol glutaredoxin. In the vast majority of cases, the thiolate from cysteine residues coordinates the iron ions of the Fe-S cluster. Histidine and arginine are other examples of residues able to anchor a Fe-S cluster via a nitrogen coordination, but also aspartate, or tyrosine and serine via an oxygen coordination.

During more than 30 years, Fe-S cluster formation was thought to be a spontaneous process because in vitro Fe-S clusters can spontaneously assemble into proteins when providing inorganic iron and sulfur under reductive and anaerobic conditions. However, to ensure the activity of Fe-S proteins, cells possess multiproteic systems that assemble iron and sulfur atoms into Fe-S clusters and deliver them to client proteins (Figure 1). Pioneering work from the Dean's lab unveiled that in vivo maturation of the *Azotobacter vinelandii* nitrogenase required the concerted action of multiple proteins that define what is now termed the NIF Fe-S cluster biogenesis system [10]. Since then, two other bacterial systems for de novo Fe-S cluster biogenesis have also been extensively documented, ISC and SUF (Figure 1) [11–13]. While the NIF system is specialized for nitrogenase maturation, ISC and SUF are generalist systems that are able to maturate many, if not all, client Fe-S proteins. The *nif*, *isc* and *suf* genes are generally organized in an operonic structure facilitating the identification of the components of each of these systems. These three Fe-S protein biogenesis systems obey the same general principle (Figure 1). The central piece is the scaffold protein, which confers the appropriate receptacle to receive and assemble sulfur and iron atoms into a Fe-S cluster. Sulfur is provided by a cysteine desulfurase that abstracts the sulfur atom of L-cysteine. Whether these systems require an as yet unidentified dedicated factor for iron acquisition is still an open question. Then, the preformed cluster is delivered to the client apo-protein. Recently, by combining exhaustive homology searches with genomic context analysis and phylogeny, the Fe-S cluster biogenesis systems of over 10,000 archaeal and bacterial genomes have been precisely identified [14]. In addition to ISC, SUF and NIF, two clearly distinct 'minimal' systems for Fe-S cluster assembly, MIS (minimal iron-sulfur system) and SMS (SUF-like minimal system) have been brought to light [14]. From this study, a scenario for the origin and evolution of Fe-S cluster synthesis machineries was proposed in which the ISC, SUF and NIF systems have evolved from MIS and SMS by duplication/speciation and recruitment of additional components to secure the use of a chaperone, a source of electrons and of Fe-S cluster carriers [14].

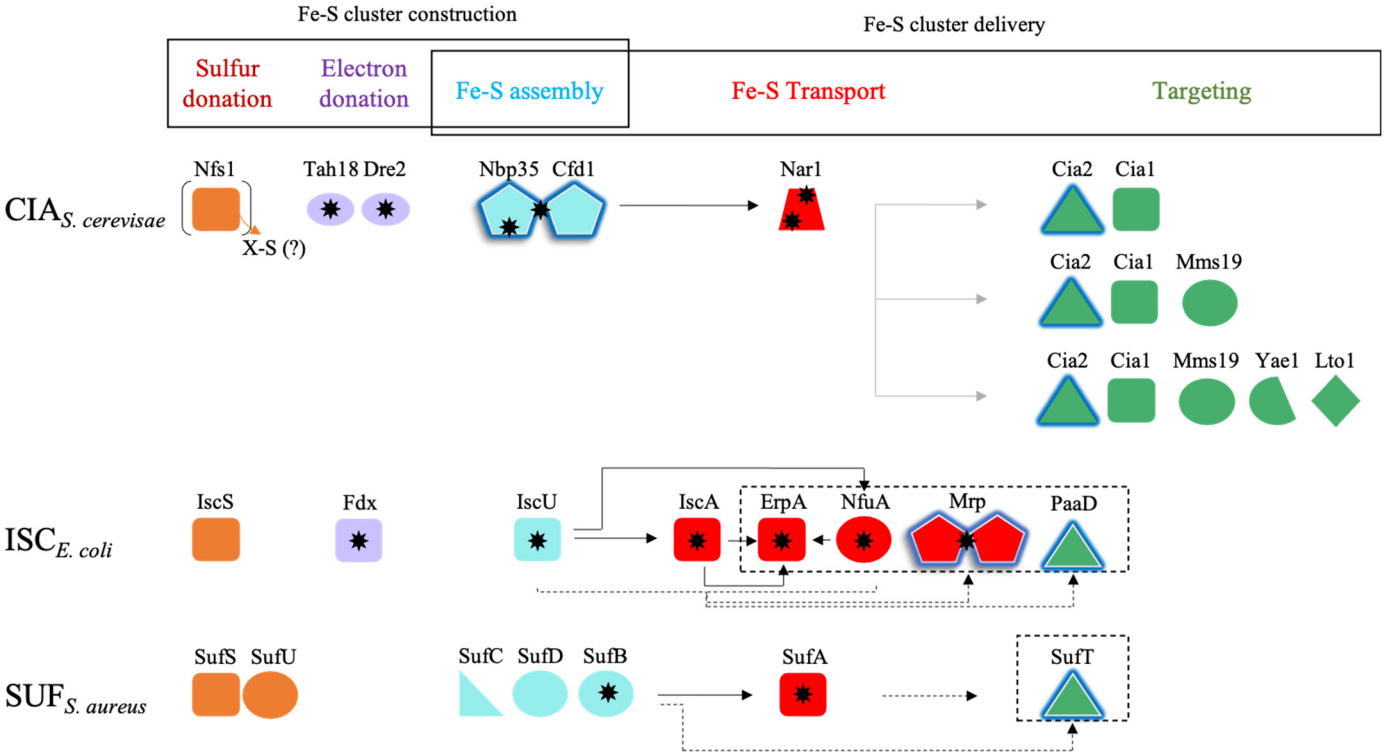

Figure 1. General principle of the Fe-S cluster biogenesis assembly line. De novo Fe-S proteins biogenesis proceeds by assembly of a cluster on a scaffold protein by acquisition of sulfur and iron atoms (early step), followed by delivery of the cluster to the final apo-target (late step). Main biogenesis factors of three Fe-S proteins biogenesis systems are represented: the CIA system of *Saccharomyces cerevisiae*, the ISC system of *E. coli* and the SUF system of *Staphylococcus aureus*. Fe-S clusters are represented by black stars. Proteins that are the focus of this review, Mrp/Nbp35 and DUF59 domain-containing proteins, are highlighted in blue. For the sake of clarity, the formation of dimers is only indicated for Mrp/Nbp35 proteins. In order to have a simplified view of the Fe-S protein biogenesis process, members of the cellular thiol redox systems, such as glutathione and glutaredoxins, are not represented. On this latter topic the reader can refer to the following reviews [15,16]. Fe-S cluster biogenesis components carrying out similar function are shown with the same color. Sulfur donation (orange) (orange arrow); electron donation (purple); Fe-S cluster assembly by different type of scaffold proteins (turquoise). Fe-S cluster transporters (red) receive Fe-S cluster, assembled by scaffold proteins, and redistribute it. Targeting factors (green) participate to Fe-S cluster delivery by interacting directly with apo-proteins. Transporter proteins and multiple targeting factors of the CIA system interact in different combination to form specific delivery branches pathways for dedicated client apo-proteins (grey arrows) [17]. In bacteria, a dynamic network is proposed between the different Fe-S cluster delivery/targeting factors, which depends on the client protein and the environmental conditions encountered [11]. Black solid arrows indicate the flows of Fe-S clusters at the delivery step that have been experimentally shown. Black dashed arrows indicate the putative flows of Fe-S clusters. The Fe-S cluster delivery pathways involving *E. coli* SufA are not represented since in this scheme only the $ISC_{E.\ coli}$ machinery is represented. No preferred source of iron atoms has yet been identified. Components inside the dotted boxes are those encoded by genes that are not included in the *E. coli isc* or *suf* operon nor in the *S. aureus suf* operon.

Eukaryotes have acquired the ISC and SUF systems by endosymbiosis, presumably with ancestors of α-proteobacteria and photosynthetic cyanobacteria, respectively. While the first endosymbiosis event is thought to be the origin of mitochondria, the second event gave rise to plastids. This evolutive scenario explains why in eukaryotes the ISC system locates in the mitochondria, and the SUF system in plastids [17,18]. In the anerobic parasitic protist *Entamoeba histolytica* that possesses a reduced mitochondrion-related or-

ganelle (MRO) called mitosome, the ISC system is missing but a minimal NIF system is present, possibly acquired by horizontal gene transfer from ε-proteobacteria [19,20]. In all eukaryotes, cytoplasmic and nuclear Fe-S proteins acquire their cluster by a dedicated Fe-S cluster biogenesis system CIA (cytosolic iron-sulfur assembly) that functions downstream of the ISC system [17,21,22]. Detailed description of how the CIA pathway is connected to the ISC system is outside the scope of this review, and more information can be found in reviews from Braymer et al., 2021 and Maio et al., 2020 [17,22]. Briefly, the prevalent model is that the CIA system uses as the source of sulfur a sulfur-containing molecule (X-S) that is synthetized inside the mitochondria thanks to the core components of the ISC system, and which is exported to the cytoplasm by the ABC transporter Atm1 [17]. Alternatively, another model postulates the existence, at least in mammalian cells, of cytosolic ISC components that would supply sulfur to the CIA system [22].

Following the symbiotic origin of eukaryotic Fe-S cluster biogenesis machinery in organelles, and according to the latest hypothesis on the endosymbiotic host during eukaryogenesis, one could have expected that the system for the maturation of cytoplasmic and nuclear Fe-S proteins would be related to Archaea [23–25]. However, CIA components do not exhibit a specific affiliation to archaeal proteins [25]. Interestingly, CIA components are homologous to bacterial Fe-S cluster biogenesis proteins, as illustrated by the *Saccharomyces cerevisiae* scaffold complex Nbp35/Cfd1 and the targeting factor Cia2, which are related to Mrp and SufT proteins, respectively (Figure 1) [21,25,26]. Here, we will review the contribution of Mrp and SufT for maintaining Fe-S cluster biogenesis in bacteria, two factors whose precise role in Fe-S protein biogenesis is still not fully understood, in an attempt to highlight resemblances and differences with their counterparts in the CIA system.

2. Mrp Proteins: A Universal Fe-S Cluster Biogenesis Factor

The Mrp proteins are part of the P-loop NTPase family, one of the largest class of proteins, whose members are involved in a wide variety of functions [26,27]. The *mrp* gene was named regarding its proximity to *metG* in *E. coli* (*mrp* stands for "*metG*-related protein"), but no other relationship between the two genes has ever been reported [28]. Based on a unique set of sequences and structural signatures, Mrp proteins form the Mrp/Nbp35 subfamily of the SIMIBI class of P-loop GTPase, designated after representative members: the Signal recognition particle GTPase, the septum site-determining protein MinD, and the ATP-dependent dethiobiotin synthetase BioD [27]. The Mrp/Nbp35 subfamily shows a universal phyletic distribution compatible with its presence in the last universal common ancestor [21,25–27].

The link between bacterial Mrp and Fe-S cluster metabolism in vivo was first obtained in *Salmonella enterica* Serovar Typhimurium, where ApbC (that we will refer to as Mrp_{Se} for the sake of clarity) is required for Fe-S cluster dependent enzymatic activities (aconitase, succinate dehydrogenase) and Fe-S cluster dependent pathways (alternative pyrimidine biosynthetic, tricarballylate catabolism pathways) [29–31]. This explains the thiamine auxotrophy and the inability to grow on tricarballylate as carbon source of the *S. enterica* Δ*mrp* mutant [29,31]. In *E. coli*, Mrp is required for stability and full assembly of the NADH:ubiquinone oxidoreductase (respiratory complex I) [32]. In contrast to *S. enterica*, no major phenotype has been reported for the *E. coli* Δ*mrp* mutant [32]. The *mrp* homolog of *Haemophilus influenzae* was shown to play an important role in regulating LPS biosynthesis by impacting incorporation of Galα(1-4)βGal [33]. However, whether this phenotype is directly linked to Fe-S metabolism has not yet been elucidated.

That eukaryotic P-loop NTPase proteins are responsible for maturation of cytoplasmic and nuclear Fe-S proteins was first demonstrated for the eukaryotic homologs of Mrp in *S. cerevisiae*, Cfd1 and Nbp35 [34,35]. In yeast, deficiency of either Cfd1 or Nbp35 is lethal, a situation clearly different to *E. coli* and *S. enterica* where *mrp* mutants are viable. This difference might illustrate some degree of specificity of bacterial Mrp towards Fe-S protein targets that are required in particular growth conditions.

Biochemical characterization of Mrp proteins from *S. enterica* and *Desulfovibrio vulgaris* Hildenborough (Mrp_{ORP}, here referred Mrp_{Dv}) showed that, after anaerobic purification or in vitro reconstitution, they both bind a [4Fe-4S] cluster [36,37]. In vitro, holo-Mrp_{Se} can rapidly and effectively transfer its cluster to an apo-protein, such as Leu1 [36]. Mrp_{Dv} has an additional C-terminal dinitrogenase domain that binds a [3Fe-4S] cluster (Figure 2), but only the [4Fe-4S] cluster of the P-loop NTPase domain can be transferred to apo-proteins, such as the aconitase, and to physiological partners of Mrp_{Dv}, the Fe-S Orp proteins of the orange protein complex [37]. In vivo, direct interactions between Fe-S targets and Mrp_{Ec}, Mrp_{Dv} and Mrp of *Helicobacter pylori* (Mrp_{Hp}) have been shown by various methods, such as co-purification and two-hybrid approaches, pointing out a late role of these proteins in Fe-S proteins biogenesis [32,37,38].

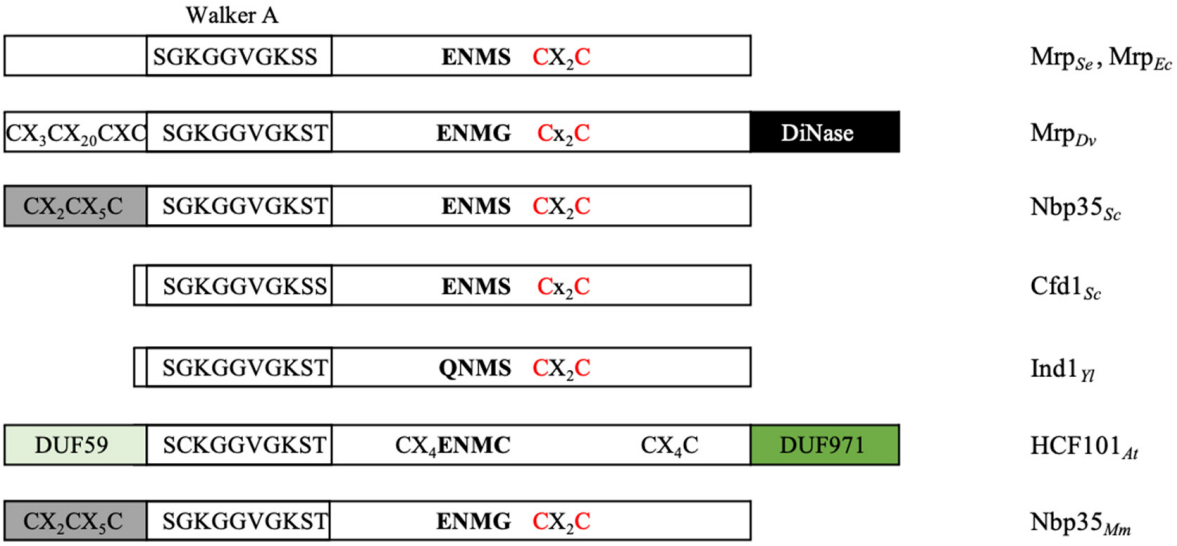

Figure 2. Schematic representation of members of Mrp/Nbp35 subfamily. Bacterial, archaeal and eukaryotic members of the Mrp/Nbp35 subfamily with distinct specificity in their primary sequence are represented (*E. coli* Mrp, *S. enterica* Mrp, *S. cerevisiae* Nbp35 and Cfd1, *Arabidopsis thaliana* HCF101, *Yarrowia lipolytica* Ind1, *Methanococcus maripaludis* Nbp35). Coordinating cysteines of the transferable Fe-S cluster are in red. The conserved Walker A motif that interacts with the α- and β-phosphate moieties of the nucleoside and the magnesium ion is indicated. The sequence replacing the NKXD motif, which leads to a loss of specificity for GTP is indicated in bold. An amino-terminal domain (grey) with typical [4Fe-4S] cluster binding motif is found in archaeal homologs of Mrp and in eukaryotic Nbp35. Mrp from *Desulfovibrio vulgaris* Hildenborough (Mrp_{Dv}), possesses a C-terminal dinitrogenase iron-molybdenum cofactor biosynthesis domain (Di-Nase) (black), and a non-canonical motif containing cysteines residues in its N-terminal domain (white). HCF101 possesses at its N- and C-terminus two additional domains: DUF59 that composes the monodomain Fe-S cluster biogenesis factor SufT from *S. aureus*, and DUF971 a domain of unknown function. The mitochondrial transit peptide of $Ind1_{Yl}$ and the chloroplast transit peptide of $HCF101_{At}$ are not represented.

The transferable Fe-S cluster is very likely bridged at an Mrp homodimer interface by two conserved and functionally essential Cys residues, in the CXXC motif of each monomer. Thanks to the crystal structure of the holo-form of CIA component Cfd1 from the thermophilic fungus *Chaetomium thermophilum* ($Cft1_{Ct}$), the presence of a subunit-bridging a [4Fe-4S] cluster at the surface of the homodimer was confirmed [39]. The $Cfd1_{Ct}$-dimeric architecture of the nucleotide binding regions is similar to other P-loop NTPases, and to other SIMIBI proteins [39,40]. Like Mrp_{Dv}, Nbp35 possesses a second Fe-S cluster in its additional N-terminal domain, which is not transferred to apo-client proteins (Figure 2). The role of this second cluster in Mrp_{Dv} and Nbp35 proteins remains unknown.

The Mrp/Nbp35 proteins exhibit a deviant Walker A box that is essential in vivo for their function, as shown for the bacterial Mrp_{Se} [41]. They also lack the classical NKXD motif that usually provides specificity for GTP [27]. Hence, as observed for Mrp_{Se}, P_i is released at a higher rate with ATP than with GTP or CTP [41]. As for Mrp_{Se}, genetic studies in yeast have early shown the functional importance of conserved residues of the NTPase domains of the CIA components Nbp35 and Cfd1, in vivo [41–43]. In contrast, demonstrating their capacity to bind and hydrolyze ATP in vitro has been quite challenging [44,45]. Ultimately, the Nbp35 homodimer and the Nbp35-Cfd1 heterodimer were shown to exhibit ATPase activity, whereas the Cfd1 homodimer had no or very low ATPase activity [44].

Thanks to complementary in vivo genetic dissection and biochemical characterization of the bacterial Mrp_{Se} protein, ATP binding/hydrolysis was proposed to be required for Fe-S cluster formation on Mrp_{Se}. Hence, a variant in the Walker A motif (S122A) was nonfunctional in vivo, had no detectable ATPase activity, but could still bind and rapidly transfer the Fe-S cluster when chemically pre-loaded with a Fe-S cluster in vitro [41]. This hypothesis is further supported by in vivo data in yeast showing that Fe-S cluster incorporation into Walker A-mutated versions of Cfd1 or Nbp35 was severely decreased [43,46].

Altogether, a working model for the bacterial Mrp reaction cycle can be proposed (Figure 3). First, dimerization of Mrp forms the ATPase site at the dimer interface. So far, in contrast to the dimeric SIMIBI proteins, SRP-SR and MinD, involved in protein targeting and localization, no trigger molecule has been shown to intervene at this step in order to prevent futile Mrp dimerization [40]. Second, dimerization creates new interfaces for the binding of proteins, allowing the loading of a preformed cluster. Whether and how Mrp cooperates with the Fe-S cluster biogenesis systems ISC and SUF remains to be investigated. Third, Mrp transfers its Fe-S cluster to client proteins. It is still formally unknown whether ATP hydrolysis is linked to cluster acquisition or cluster transfer. By analogies with the other SIMIBI dimeric proteins, ATP hydrolysis could switch Mrp to the OFF state, and therefore might occur at a late step in the Mrp reaction cycle [40]. It would now be interesting to investigate whether cellular factors are controlling the activity of the bacterial Mrp/Nbp35 proteins, a common characteristic of the dimeric SIMIBI-type NTPases [40].

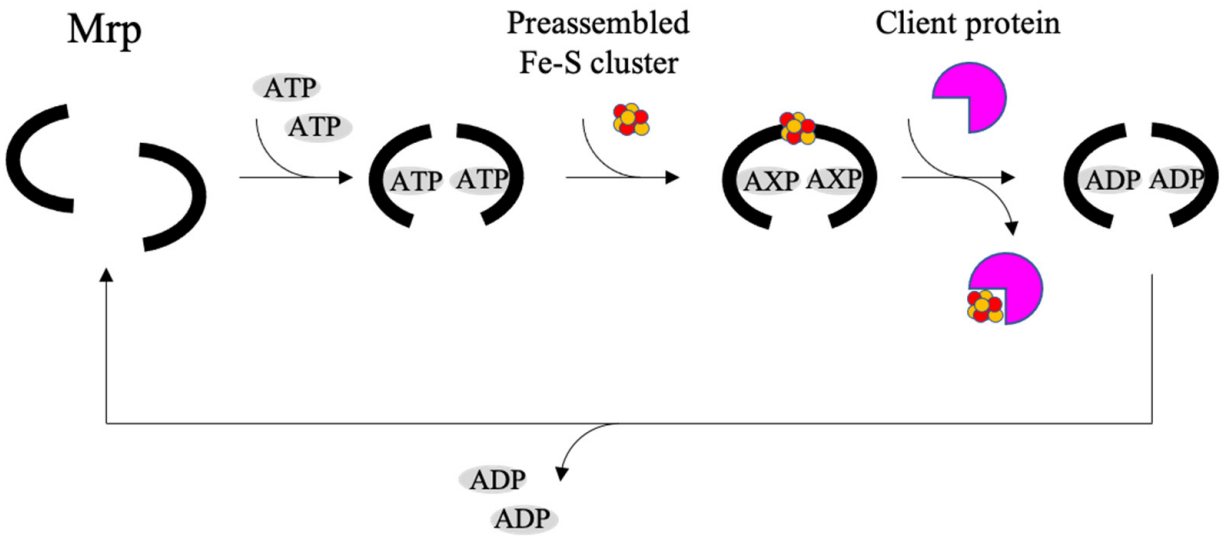

Figure 3. Working model for the reaction cycle of bacterial Mrp. (i) Dimerization of Mrp forms the ATPase site at the dimer interface, (ii) Mrp acquires its cluster, and (iii) the Fe-S cluster is transferred to apo-targets. Whether ATP hydrolysis occurs before or after Fe-S cluster transfer is not known, therefore the type nucleotide bound to holo-Mrp is indicated by AXP which stands for ATP or ADP, and release of inorganic phosphate is not indicated.

Altogether these studies have undisputedly established that bacterial and eukaryotic CIA Mrp/Nbp35 proteins participate in Fe-S protein biogenesis and that they share extensive biochemical properties. These studies have also provided a compelling example of how during evolution a Fe-S cluster biogenesis cofactor has been exploited in different manners. Indeed, while the bacterial Mrp/Nbp35 proteins seem to act at a late step in Fe-S protein maturation by delivering clusters to client proteins, the eukaryotic CIA Mrp/Nbp35 proteins, in contrast, appear to act at an early step. Indeed, in eukaryotes, data suggest that CIA Mrp/Nbp35 proteins act as a scaffold and, like the IscU scaffold of the ISC machinery, they are connected to electron donors required for Fe-S cluster biogenesis (Figure 1). However, the possibility that bacterial Mrp/Nbp35 proteins could also be scaffold proteins seems to be ruled out. Indeed, while *iscU* provided in trans in the *S. enterica apbC* mutant restored growth on tricarballylate, such a suppressor effect also required undetermined components of the ISC system (the Fe-S cluster carrier IscA, the chaperone/cochaperone HscAB, the electron donor Fdx and/or IscX) [31]. In addition, Mrp_{Ec} is not able to compensate for the loss of the ISC and SUF scaffolds since an *E. coli* mutant lacking both ISC and SUF Fe-S cluster biogenesis system is unviable [47–49]. Thus, the body of current data rather supports that the role of bacterial Mrp proteins is to be a delivery factors, although a role in assembling a [4Fe-4S] cluster by a reductive coupling of two [2Fe-2S] clusters with an electron source cannot be excluded. Remarkably, this delivery role is also exploited in eukaryotes by Mrp/Npbp35 proteins located in organelles. Indeed, plastidic and mitochondrial proteins of the Mrp/Nbp35 family are proposed to act late for the maturation of a subset of Fe-S protein. The plastidic HCF101 protein in *Arabidopsis thaliana* and the mitochondrial Ind1 protein of *Yarrrowia lipolytica* and human are required for the maturation of Fe-S containing complexes, such as photosystem I and Complex I, respectively [50–54].

Formation of the clusters on eukaryotic Mrp/Npbp35 proteins fully requires the ISC function, a cytosolic electron transfer chain, the multidomain monothiol glutaredoxin Grx3/GLRX3 that is complexed with BolA2 in human [17,55,56]. How bacterial Mrp/Nbp35 proteins acquire their cluster remains a key question in the field for at least two reasons. First, the bacterial Mrp is probably the sole Fe-S cluster biogenesis factor present with any type of de novo Fe-S cluster biogenesis machinery. Indeed, Mrp is found in bacteria possessing ISC, SUF, MIS, and/or SMS pathways [14,32,37,38]. Such association with different Fe-S cluster biogenesis machineries has also recently been further illustrated for eukaryotic Mrp/Nbp35 homologs by a bioinformatic study. Indeed, so far HCF101 was only described in plastids (SUF containing compartment), but a HCF101 paralog containing a targeting signal for the mitochondria (ISC containing compartment) was recently predicted in unicellular eukaryotes from the Stramenopila, Alveolata, Rhizaria, Haptista and Cryptista supergroups [57]. Such adaptation of Mrp/Nbp35 proteins to cooperate with different Fe-S cluster biogenesis pathways during evolution might be a hallmark of proteins acting at the delivery step. Second, a further understanding of bacterial Mrp will provide important information (e.g., regions of interaction, key residues, role of ATP binding/hydrolysis) to tackle the puzzling and long standing question of what is the sulfur source for Fe-S cluster assembly in eukaryotic CIA Mrp/Nbp35. Deciphering how these universal proteins which, despite extensive homologies, seem to acquire their cluster in a different manner is of great evolutionary interest.

Knowing whether and how archaeal Mrp/Nbp35 proteins intervene in Fe-S cluster metabolism will be informative to better apprehend this class of proteins. Archaeal Mrp/Nbp35 proteins are still insufficiently documented. To our knowledge MMP0704, from *Methanococcus maripaludis*, (Mrp_{Mm}) is the sole archaeal Mrp/Nbp35 protein characterized both in vivo and in vitro [58,59]. Data suggest that, like bacterial and eukaryotic Mrp/Nbp35 proteins, Mrp_{Mm} is able to bind a labile [4Fe-4S] cluster and to transfer it to target proteins, such as apo-aconitase [58,59]. No phenotype has been shown for the *M. maripaludis* mutant lacking Mrp_{Mm}, suggesting that either a potential compensation mechanism exists or that Mrp_{Mm} is acting under a specific condition that remains to be found [58].

Finally, ectopic expression of Mrp_{Mm} could restore growth of the *S. enterica* Δ*mrp* mutant on tricarballylate [59]. Additional genetic studies showed that, whereas the conserved Walker box serine and the CXXC motif of Mrp_{Mm} were both required for function in vivo, its additional N-terminal domain was dispensable [59]. Altogether, these results suggest that archaeal Mrp/Nbp35 proteins might act late in the Fe-S cluster biogenesis process as a Fe-S cluster carrier, like their bacterial counterparts. This raises the possibility that turning a late actor into an early factor in the Fe-S protein biogenesis process constitutes a eukaryotic innovation at the heart of the emergence of the CIA system.

3. SufT Proteins: A Fe-S Cluster Delivery Factor

The implication of SufT in maturation of multiple Fe-S proteins has been shown in bacteria such as *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and *Sinorhizobium meliloti* [60–63]. The SufT proteins contain a domain of unknown function, DUF59 (also referred to as MIP18/IPR002744), and its corresponding gene is often located in the gene cluster encoding the SUF Fe-S cluster biogenesis machinery (Figure 4) [21,61,63]. Phylogenetic analysis indicated that *sufT* recruitment in the *suf* operon is recent in its evolutionary history, and that it was acquired and lost multiple times during evolution [14,61]. By a similar approach, it was concluded that virtually all eukaryotes possess at least one homologue of SufT [21]. The SufT proteins are predominantly composed of the unique DUF59 domain but in some cases alternate protein architectures have been described with additional N- and/or C- terminal domains [21,61,64]. Amongst these additional modules many are related to Fe-S protein biogenesis factors, such as a the Mrp/Nb35 P-loop NTPase described above (Figure 2), or to Fe-S target proteins themselves (Rieske protein, ferredoxin) [61,64].

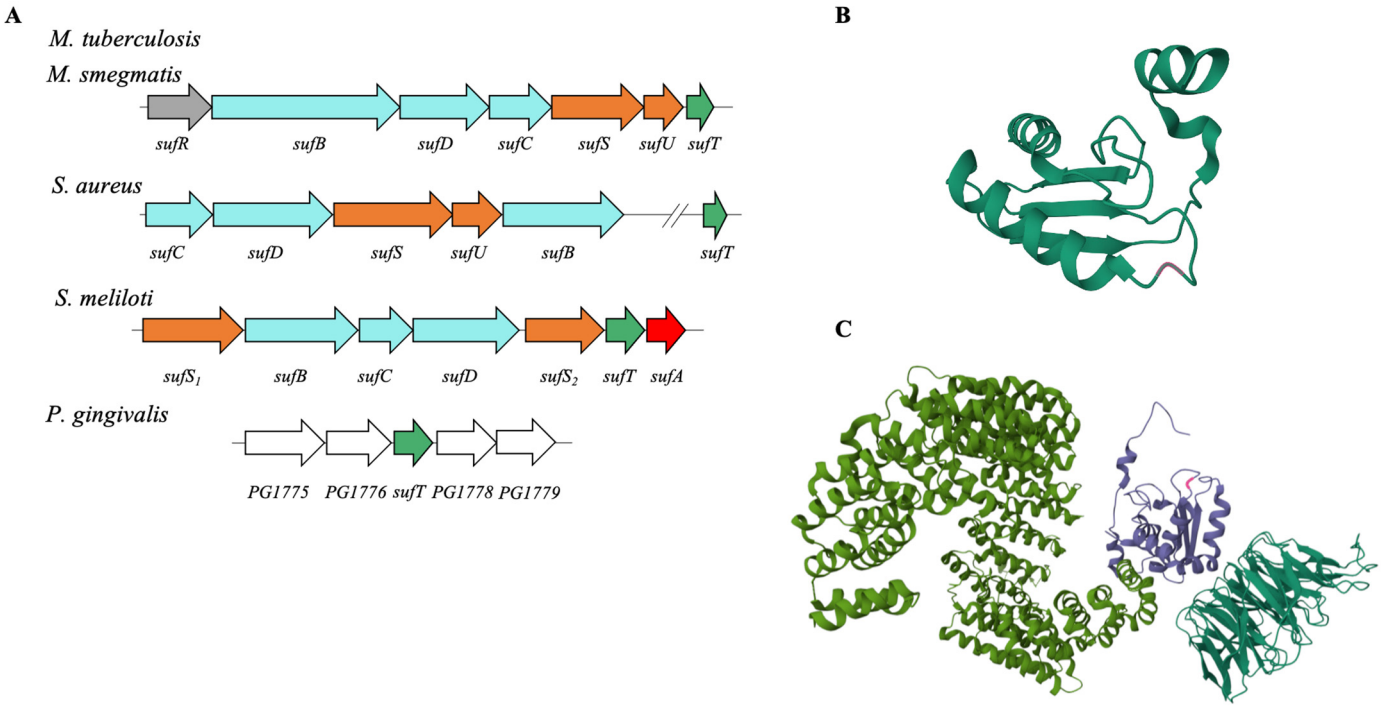

Figure 4. Schematic representation of the chromosomal location of the *sufT* genes and architecture of SufT proteins. (**A**) The chromosomal region containing *sufT* in *M. tuberculosis* H37Rv, *Mycobacterium smegmatis*, community-acquired methicillin-resistant *S. aureus* subsp. *aureus* USA300_FPR3757, *S. meliloti* 1021, and *Porphyromonas gingivalis* W83 strains is shown. The intein element inserted in *M. tuberculosis sufB* gene is not represented [65]. The *PG1775*, *PG1776*, *PG1778* and *PG1779* genes are predicted to putatively code for the nucleotide exchange factor GrpE, the chaperone protein DnaJ, a putative UDP-2,3-diacylglucosamine hydrolase and an O-acetyl-ADP-ribose deacetylase, respectively [66]. Genes are color-coded according to their role in Fe-S cluster biogenesis as in Figure 1.

The gene encoding the transcriptional regulator SufR is indicated in grey. Genes with no demonstrated experimental link with the SUF biogenesis machinery are depicted by white arrows. (**B**) Ribbon diagram of the structure of SufT (BA_5281) from *Bacillus anthracis* obtained by X-ray diffraction (PDB ID: 3LNO). The conserved Cys residue is highlighted in red. (**C**) Crystal structure of the *Drosophila melanogaster* CIA targeting complex containing the DUF59 protein CIAO2B (blue), MMS19 (light green), and CIAO1 (dark green) (PDB ID: 6TC0) [67]. The conserved Cys residue of CIAO2B is highlighted in red.

Deletion of *sufT* led to decreased activity of several Fe-S proteins, including aconitase (AcnA), isopropyl malate isomerase (LeuCD), dihydroxy-acid dehydratase (IlvD) and lipoyl synthase (LipA) in the human pathogen *S. aureus*, and AcnA, glutamate synthase (GltS), and 6-Phosphogluconate dehydratase (6-PGDH) in *S. meliloti*, a diazotrophic bacteria able to fix atmospheric nitrogen into ammonia for their legume symbionts [60,61,63]. In these bacteria and in *Mycobacterium smegmatis*, SufT is required when cells experience Fe-S cluster biogenesis stressful conditions, such as ROS exposure and/or iron limitation [60,61,63,68]. Also, in *S. aureus*, the physiological importance of SufT is maximal in conditions imposing a high demand for Fe-S assembly such as respiratory versus fermentative growth [61].

In *M. tuberculosis*, SufT is essential for viability under standard growth conditions but SufT could be depleted thanks to the CRISPR interference technology [62,69,70]. SufT depleted cells exhibit lowered activity of aconitase and of SufR, a Fe-S dependent transcriptional regulator. These cells are more sensitive to oxidative and NO stresses and showed decreased survival inside macrophages [62]. Moreover, SufT depletion results in a widespread metabolic change as determined by quantitative liquid chromatography-mass spectrometry-based approach. Hence, substrate and/or product of several Fe-S enzymes intervening in carbon, amino acid and nucleotide metabolism were increased and decreased, respectively [62]. The global importance of SufT likely explains its crucial role in bacteria/host interaction such as, the *S. meliloti* Fix^+ phenotype, *S. aureus* biofilm formation, and *M. tuberculosis* survival in macrophages and persistence in mice [61–63].

A gene coding for a SufT-like protein is located in a phenylacetate metabolic gene cluster conserved in *E. coli* and *Pseudomonas putida*, named *paa* and *pha*, respectively [71,72]. Interestingly, in this catabolic pathway, phenylacetate-CoA is hydroxylated by a multicomponent oxygenase complex (PaaABCE/PhaFGI) that contains a Fe-S reductase subunit (PaaE/PhaI). *P. putida* mutants lacking the SufT protein PhaH or components of the oxygenase complex are unable to use phenylacetate as a carbon source. The *E. coli* SufT protein (PaaD) is dispensable in vitro for the activity of the oxygenase complex. However, this complex was purified from the *E. coli* wt strain and already contained its cofactors. Thus, it is very likely that *E. coli* and *P. putida* SufT proteins, PaaD and PhaH, are specifically dedicated to the maturation of the Fe-S reductase subunit in the phenylacetate-CoA oxygenase complex. Other example of target specialization might also be found in lactobacilli in which *sufT* homologs are in putative operons with genes encoding for Fe-S proteins, such as the anaerobic ribonucleoside-triphosphate activating enzyme or the serine dehydratase [61].

The DUF59-containing proteins in human (CIAO2A and CIAO2B) and in *A. thaliana* (AE7) are required for maturation of cytoplasmic and nuclear Fe-S proteins [73,74]. They act downstream the intermediate carrier protein CIAO3 (Nar1 in yeast) and interact in different combination with additional factors to form various CIA targeting complexes (CTC) that each recognizes directly a subset of apo-proteins [17,73,75–79]. For example, CIAO2A interacts with CIAO1 for maturation of aconitase, CIAO2B and CIAO1 participate to Fe-S cluster insertion into viperin, while CIAO2B together with CIAO1 and MMS19 delivers Fe-S clusters to proteins involved in DNA metabolism and genome integrity [17,73,75–79]. The heteromeric species formed by two molecules of CIAO2A and one of CIAO1 can bind one [4Fe-4S] cluster that is able to be transferred to apo-aconitase [77]. No such [Fe-S]-containing stable targeting complexes have been identified yet for bacterial DUF59-containing proteins.

Interestingly, binding of the [4Fe-4S] cluster in the CIAO2A/CIAO1 complex involves the conserved cysteine residue of CIAO2A. This strictly conserved essential cysteine has been pointed out in a relatively conserved DPE-X_{26-31}-T-X_{2-3}-C motif and was found to

be hyper-reactive in DUF59-containing proteins of mammalian and yeast cells [80]. As observed in the crystal structure of the bacterial *Bacillus anthracis* DUF59 protein, and in the NMR structure of archaeal and human DUF59 proteins, the Cys, Asp, Glu and Thr residues are in close proximity, with most of them in solvent-exposed loops [64,81,82]. These residues form a small surface of conserved homology that has been proposed to bind small ligands, such as a metal ion or a Fe-S cluster [62,64,81]. While, there is no experimental evidence to support that bacterial SufT proteins bind a Fe-S cluster, the SufT protein PG1777 of *Porphyromonas gingivalis*, the etiological agent in the chronic adult periodontitis, was suggested to be an iron-binding proteins [66]. Binding of Fe-S cluster in DUF59 proteins would require the formation of homo- or hetero-multimers since Fe-S clusters are always liganded by more than one cysteine. Recently, the crystal structure of the CIAO2B-containing targeting complex showed a dimeric arrangement with an extensive interface between the two CIAO2B protomers [67]. Interestingly, the potential Fe-S coordinating Cys residue of the two CIAO2B protomers are in proximity to each other in the dimer [67]. Thus, CIAO2B was proposed to both transiently accept a Fe-S cluster and bridge together components of the CIA system to deliver Fe-S clusters to apo-proteins. By using the mycobacterial two-hybrid approach, a bridging role could also be envisioned for bacterial DUF59 proteins, since *M. tuberculosis* SufT is able to interact with proteins of the SUF system, SufS and SufU, and with Fe-S proteins such as the aconitase and SufR [62]. Remarkably, this study also showed that *M. tuberculosis* SufT forms a homodimer and that the substitution of the conserved cysteine residue by an alanine abolished SufT homodimerization and association with other SUF proteins [62]. A role for bacterial SufT proteins at the delivery step, like for eukaryotic proteins, is consistent with genetic interactions between *sufT* and the *nfu* gene that encodes a Fe-S cluster carrier. Indeed, *sufT* and *nfu* display a synergistic effect on growth and aconitase activity of *S. aureus* cells [61]. Furthermore, expression of *nfu* from a multicopy plasmid suppressed phenotypes of the *S. aureus* Δ*sufT* mutant [60,61].

Finally, the implication of SufT in repair of oxidatively damaged clusters was tested by monitoring over time the reactivation of aconitase activity in a cell-free lysates assay. The rate of reactivation was identical in the parental *S. aureus* strain and Δ*sufT* mutant, suggesting that SufT was dispensable for repair of damaged Fe-S clusters [61]. Intriguingly, using a similar assay, an opposite conclusion was reached for *M. tuberculosis* SufT. This discrepancy might be explained by an indirect effect due to the widespread and drastic metabolic changes that occur in *M. tuberculosis* SufT depleted cells [62].

Altogether, these studies indicate that DUF59 proteins are able to establish interactions with different Fe-S cluster biogenesis factors in eukaryotes and bacteria, which allow them to participate to the Fe-S cluster delivery step in both type of organisms.

4. Conclusions

The high conservation of the ISC and SUF Fe-S cluster biogenesis systems between prokaryotes and eukaryotes has offered a great opportunity to exploit the assets of very diverse model organisms to understand and to draw general mechanistic concepts. Given the common features shared by the bacterial Fe-S cluster biogenesis factors, Mrp and SufT, with their CIA counterpart, the continuation of this strategy will offer the opportunity to deepen our knowledge. For instance, further dissection of the Mrp/Nbp35 cycle will shed light on the role of ATP, and on novel actors that might control such a key step in Fe-S protein biogenesis. Unveiling biochemical parameters allowing the SufT/DUF59 proteins to participate and to guide Fe-S cluster delivery will lead to an even more detailed mechanistic view of the delivery step. Finally, such molecular understanding may be exploited either to improve Fe-S protein biogenesis, for example in cases of human diseases related to insufficient Fe-S cluster biogenesis, or in contrast to weaken Fe-S protein biogenesis in pathogenic bacteria.

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