Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

# Recent advances in the Suf Fe–S cluster biogenesis pathway: Beyond the Proteobacteria ☆

F. Wayne Outten *

*University of South Carolina, Department of Chemistry and Biochemistry, 631 Sumter Street, Columbia, SC 29208, USA*

ARTICLE INFO

*Article history:*
Received 17 September 2014
Received in revised form 31 October 2014
Accepted 3 November 2014
Available online xxxx

*Keywords:*
Suf
Isc
Iron–Sulfur
Fe–S cluster
Plasmodium
*B. subtilis*

ABSTRACT

Fe–S clusters play critical roles in cellular function throughout all three kingdoms of life. Consequently, Fe–S cluster biogenesis systems are present in most organisms. The Suf (sulfur formation) system is the most ancient of the three characterized Fe–S cluster biogenesis pathways, which also include the Isc and Nif systems. Much of the first work on the Suf system took place in Gram-negative Proteobacteria used as model organisms. These early studies led to a wealth of biochemical, genetic, and physiological information on Suf function. From those studies we have learned that SufB functions as an Fe–S scaffold in conjunction with SufC (and in some cases SufD). SufS and SufE together mobilize sulfur for cluster assembly and SufA traffics the complete Fe–S cluster from SufB to target apo-proteins. However, recent progress on the Suf system in other organisms has opened up new avenues of research and new hypotheses about Suf function. This review focuses primarily on the most recent discoveries about the Suf pathway and where those new models may lead the field. This article is part of a Special Issue entitled: Fe/S proteins: Analysis, structure, function, biogenesis and diseases.



## 1. Introduction

Iron–sulfur (Fe–S) cluster metalloproteins play myriad roles in cell function, ranging from amino acid biosynthesis to transcriptional regulation. These diverse functions arise from the multiple types of Fe–S clusters assembled in vivo, ranging from relatively simple [2Fe–2S] clusters, found in some classes of ferredoxin, to complex, mixed-metal clusters, such as the [Mo–7Fe–9S] cluster (or FeMo cofactor) of nitrogenase [23,56]. Fe–S cluster function is also intimately associated with the protein frameworks to which they are bound. Protein environment can greatly alter Fe–S cluster reduction potentials and overall sensitivity to oxidation, providing a means to subtly tailor Fe–S cluster function to fit specific biochemical functions. Due to their versatility and the ready availability of ferrous iron and sulfide in the early Earth environment, Fe–S cluster metalloproteins have become intimately associated with key metabolic and regulatory pathways in most organisms.

The addition of oxygen to the atmosphere exposed Fe–S clusters as a potential Achilles heel of cellular metabolism [58]. Solvent exposed Fe–S clusters can readily react with oxygen and its derivatives ($H_2O_2$ and $O_2^{\bullet-}$) [16,27,30,34]. Such oxidation events often lead to cluster disassembly and release of $Fe^{2+}$, which in the cellular milieu can undergo further Fenton chemistry with $H_2O_2$ to produce highly toxic hydroxyl radicals. Under this selective pressure, some Fe–S cluster proteins were replaced with less sensitive, often iron-free alternative enzymes.

Despite their sensitivity to oxidation, the essential roles of many Fe–S cluster proteins have been maintained throughout evolution. As a supplemental strategy to replacing these key metalloproteins with other, less sensitive alternatives, many aerobic organisms have evolved with complex antioxidant systems that minimize the accumulation of reactive oxygen species in the cell [26,28]. In addition, the in vivo biogenesis systems for Fe–S clusters themselves have become a complex and interlocking network of highly regulated pathways that maintain adequate levels of Fe–S cluster assembly even under adverse conditions such as oxidative stress or iron starvation. The Fe–S cluster biogenesis systems of aerobic and facultative aerobic organisms have been selected for their robust activity and careful handling of assembly intermediates that allow them to keep pace with the increased Fe–S cluster demand that comes with an aerobic lifestyle.

One such Fe–S cluster system, the Suf pathway, was likely present in a simple form in the earliest progenitors of modern species [7,72]. While Suf is still maintained in a simple form in many anaerobes, the pathway has progressively grown more complex throughout evolution [7,62,72,76,77]. Over the past 15 years the Suf system has been the subject of in depth biochemical, genetic, and regulatory studies. Much of this accumulated knowledge of Suf function has been well reviewed in other publications, to which I point the reader for more in depth coverage of the Suf system [5,7,29,63,82]. Here I will focus primarily on recent progress on Suf in more diverse organisms beyond the Gram-negative model organisms, *Erwinia chrysanthemi* (recently renamed *Dickeya dadantii*) and *Escherichia coli*, in which it was first characterized [49,53,

☆ This article is part of a Special Issue entitled: Fe/S proteins: Analysis, structure, function, biogenesis and diseases.

* Tel.: +1 803 777 8151.
*E-mail address:* outtenf@mailbox.sc.edu.

http://dx.doi.org/10.1016/j.bbamcr.2014.11.001
0167-4889/

55,72]. Where necessary I will allude back to results from *E. chrysanthemi* and *E. coli* in order to compare and contrast that work to the newest discoveries in other organisms.

## 2. The Suf Fe–S cluster biogenesis system: An overview

The core Suf system is composed of the SufB Fe–S cluster scaffold and the SufC ATPase (Fig. 1) [7,72]. SufB seems distinct from the IscU scaffold found in the well-characterized Isc pathway. SufB is unstable and prone to heterogeneous oligomer formation when expressed alone. Fe–S cluster assembly on SufB results in formation of a [4Fe–4S] cluster but, unlike IscU, no stable [2Fe–2S] intermediate is observed during SufB cluster assembly [9,38,79]. SufC is homologous to the nucleotide hydrolysis domains of the ATP-binding cassette (ABC) family of transporters that use ATP hydrolysis to drive transport across cellular membranes. Together the two proteins can form a $SufB_2C_2$ complex that is capable of forming and transferring Fe–S clusters to apo-acceptors [8,38,57,60]. The exact role of the SufC ATPase activity in this process is unknown but deletion of *sufC* or mutations in the ATP binding site abolish in vivo function of the Suf pathway [18,48,64,80].

The core Suf system can be augmented by a number of accessory proteins. SufD is a paralogue of SufB but does not appear to function as an Fe–S scaffold. SufD interacts with SufC and SufB to form a $SufBC_2D$ complex (Fig. 1) [48,54,60]. The $SufBC_2D$ complex may replace $SufB_2C_2$ as the scaffold complex or it may be used for a distinct step in cluster assembly prior to formation of the final cluster on $SufB_2C_2$ (see next paragraph) [8,64]. SufA is a member of the A-type carrier (ATC) family of Fe–S cluster carrier proteins, which includes IscA and ErpA [77]. SufA can accept Fe–S clusters from the SufB scaffold and transfer them to downstream apo-proteins [9,20]. The ATCs are just one class of Fe–S cluster carrier proteins. Monothiol glutaredoxins and Nfu-like proteins have also been implicated in trafficking Fe–S clusters from scaffolds to target proteins [4,6,21,32,46,59,90]. In some systems the *suf* operon has its own dedicated transcriptional regulator while in other cases the *suf* pathway is controlled by multiple regulators that respond to stresses that disrupt Fe–S cluster metabolism [39,50,53,55,70,78,86,89].

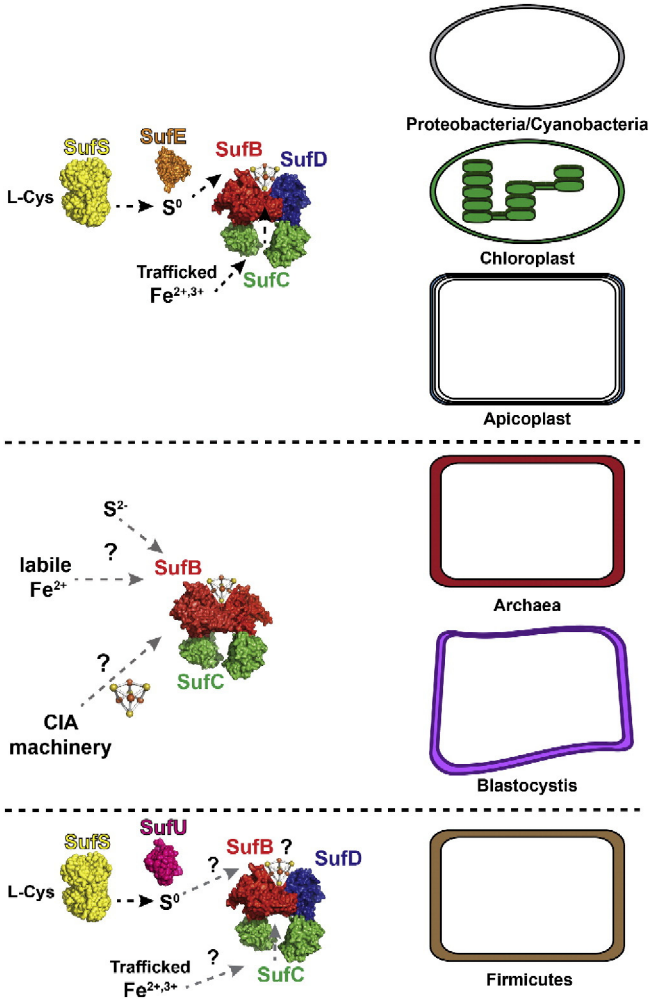


**Fig. 1.** Models of Suf function in multiple organisms and organelles. Solvent accessible structures are used for SufB (red), SufC (green), SufD (blue), SufE (orange), SufS (yellow), and SufU (magenta). Gray arrows with question marks indicate possible Fe–S cluster assembly/trafficking steps that have not been verified experimentally.

Stepwise Fe–S cluster assembly requires the donation of sulfide and iron. While sodium sulfide can be used for in vitro Fe–S cluster reconstitution, most Fe–S cluster biogenesis systems that have been studied at the biochemical level contain a cysteine desulfurase enzyme (SufS in the Suf pathway) that uses a pyridoxal 5′-phosphate (PLP) cofactor to mobilize sulfur from L-cysteine (Fig. 1) [1,47,65,87,88]. This sulfur, bound to the cysteine desulfurase as a persulfide (R–S–SH) intermediate, is ultimately reduced and incorporated into the Fe–S cluster as sulfide ($S^{2-}$). This mechanism limits the release of toxic sulfide in the cytoplasm, mitochondria, or chloropolast. In vivo iron donation for Fe–S cluster assembly is quite murky but the Suf system seems to require both SufC ATPase activity as well as the presence of SufD in order for efficient iron acquisition in vivo (Fig. 1) [64]. These results suggest $SufBC_2D$ formation is required for iron incorporation into the SufB scaffold. Whether this effect is due to a direct role for SufC and SufD in iron mobilization or indirectly due to disruption of some other step of cluster assembly is not clearly understood. Clearly some organisms lack SufD but are still able to use SufBC for cluster assembly, suggesting there may be more than one route for iron acquisition or that organisms in certain ecological niches do not require specialized iron trafficking pathways (Fig. 1) [7].

One clear theme of the Suf system in Proteobacteria is that the stepwise Fe–S cluster assembly pathway is highly regulated by protein–protein interactions. The interaction of SufB with SufC is required to enhance the low basal ATPase activity of SufC in *Thermotoga maritima* [14,57] and similar effects have been observed when comparing *E. coli* SufC to $SufB_2C_2$ and $SufBC_2D$ (F.W. Outten and K.S. Thomas, unpublished data). In Proteobacteria and the chloroplast, SufS has low intrinsic cysteine desulfurase activity unless it is stimulated by the SufE partner protein that accepts persulfide from SufS via a conserved Cys residue [12,41,54,68,81,84]. Transfer of persulfide cycles the SufS desulfurase enzyme back to its resting state to allow initiation of another round of catalysis [52,54,68,81,84]. Recent studies have also suggested that SufE in *E. coli* may be able to allosterically enhance SufS activity by stimulating L-cysteine binding when the two proteins interact [71]. The concerted SufS–SufE cysteine desulfurase activity is further enhanced by the addition of the $SufBC_2D$ complex in *E. coli* [54]. The enhancement of SufS–SufE by the $SufBC_2D$ complex occurs because SufE physically interacts with SufB to transfer persulfide to the scaffold protein for cluster assembly [38]. The efficient transfer of persulfide to SufB allows SufE to accept another persulfide from SufS, thereby stimulating the overall production of persulfide from L-cysteine [38,68].

Since two key enzymes of the Suf pathway (SufC and SufS) are essentially inactive unless they interact with the correct partner proteins, this tight regulation controls the stepwise assembly of Fe–S clusters in vivo. One reason for the tight regulation of SufS activity could be to protect the reactive persulfide intermediate from perturbation by oxygen or reactive oxygen species. It was recently shown that *E. coli* SufS is more resistant to $H_2O_2$ mediated thiol oxidation than the *E. coli* IscS cysteine desulfurase [12]. The resistance of SufS is likely tied to the conformation of its active site Cys residue, which is more shielded when compared to the relatively exposed active site of IscS [11,17,40]. The

Please cite this article as: F. Wayne Outten, Recent advances in the Suf Fe–S cluster biogenesis pathway: Beyond the Proteobacteria, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbamcr.2014.11.001