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Interactions with sulfur acceptors modulate the reactivity of cysteine desulfurases and define their physiological functions

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ABSTRACT

Sulfur-containing biomolecules such as [Fe—S] clusters, thiamin, biotin, molybdenum cofactor, and sulfur-containing tRNA nucleosides are essential for various biochemical reactions. The amino acid L-cysteine serves as the major sulfur source for the biosynthetic pathways of these sulfur-containing cofactors in prokaryotic and eukaryotic systems. The first reaction in the sulfur mobilization involves a class of pyridoxal-5′-phosphate (PLP) dependent enzymes catalyzing a Cys:sulfur acceptor sulfurtransferase reaction. The first half of the catalytic reaction involves a PLP-dependent C—S bond cleavage, resulting in a persulfide enzyme intermediate. The second half of the reaction involves the subsequent transfer of the thiol group to a specific acceptor molecule, which is responsible for the physiological role of the enzyme. Structural and biochemical analysis of these Cys sulfurtransferase enzymes shows that specific protein-protein interactions with sulfur acceptors modulate their catalytic reactivity and restrict their biochemical functions.

1. Introduction to thio-cofactors

Sulfur-containing biomolecules are involved in essential biochemical reactions in all three domains of life. Besides the universal and essential presence of sulfur amino acids cysteine and methionine, all known living organisms use additional thio-biomolecules in various aspects of metabolism [1–9] (Fig. 1). It is well-established that inactivation of pathways leading to the synthesis of these sulfur-containing molecules leads to pleiotropic cellular defects associated with the loss of function of their protein partners, compromising cellular viability and, in some cases, leading to lethal phenotypes.

The diverse functions of organic and inorganic thio-molecules are attributed to versatile chemistries that these molecules perform in biological systems. Their broad range of reactivities is a result of their vastly distinct chemical structures, the wide range of oxidation states of sulfur, and the protein/nucleic acid environments where these molecules function in nature [10]. For instance, low-molecular-weight (LMW) thiols glutathione (GSH), mycothiol (MSH), bacillithiol (BSH), ergothioneine (EGT), and trypanothione (TSH) serve as intracellular redox buffers mediating reactions with electrophilic chemicals, antibiotics, reactive oxygen and nitrogen species (ROS and RNS) as well as participating in important roles in metal homeostasis. Sulfur-containing coenzymes A and M (CoA and CoM) and S-adenosylmethionine (SAM) are involved in a variety of chemical reactions by partnering with enzymes displaying diverse folds and functions. Organic sulfur-containing protein cofactors biotin, thiamine, lipoic acid, and molybdenum cofactor assist a range of chemical transformations, including carboxylation, decarboxylation, ketoacid oxidation, and oxidative hydroxylation, respectively [11,12]. Proteins coordinating inorganic iron-sulfur complexes (Fe—S clusters) participate in non-catalytic structural and metabolic sensing roles as well as in a variety of catalytic functions, including electron transfer, substrate activation and reduction, molybdenum and sulfur donation, and complex substrate structural arrangements [13–15]. In tRNA, sulfur addition to nucleoside bases occurs at a post-transcriptional level. These modifications expand the functionality of tRNA to improve folding and control the formation of non-canonical base pairing, which guarantee fidelity of the degenerate genetic code and allows tRNA to act as a sensor for environmental and nutritional status [8,16–17]. In DNA, phosphorothioate, an oxygen-sulfur switch onto non-bridging oxygen of DNA, provides resistance to nuclease action and confers an advantage in growth and survival rates during various environmental stresses [18,19]. Thus, the ubiquitous and diverse involvement of thio-cofactors in various aspects of metabolism elevates the importance of sulfur in biology. As a result, biological systems have

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developed dedicated and tightly regulated mechanisms for sulfur acquisition, activation, and incorporation into biomolecules.

2. Cysteine as the source in thio-cofactor biosynthesis

The amino acid cysteine serves as the sulfur source for most, if not all, thio-cofactors in bacteria, eukaryotes, and some species of archaea [20,21]. The biosynthesis of LMW thiols BSH, TSH, GSH, MSH, and CoA involves the incorporation of cysteine via a reaction with its amino group. Whereas the synthesis of other cofactors occurs through direct reaction of the Cys thiol moiety. A specific class of enzymes, denoted cysteine desulfurases, are involved in the initial step of sulfur activation on the biosynthesis of thio-cofactors, in which only the sulfur atom is retained from the amino acid cysteine in a reaction that involves the cleavage of the C—S bond [22]. This review explores specific features of bacterial cysteine desulfurases in the biosynthesis of thio-cofactors that direct the physiological functions of these enzymes with a focus on the importance of interactions with sulfur acceptors, reactivity towards reductants, and their associated pathways. Fundamental principles of reactions promoted by bacterial enzymes are expected to be replicated in eukaryotic ortholog enzymes despite the occurrence of more complex biosynthetic schemes involving additional components and reaction steps confined to cellular organelles [23–25].

3. Discovery of cysteine desulfurases

The first cysteine desulfurase was discovered in the diazotrophic organism, *Azotobacter vinelandii*, thanks to pioneering work established by the Dennis Dean laboratory [22]. The enzyme, denoted NifS, was first identified due to its involvement in the activation of the two Mo-nitrogenase components. Although the function of NifS was elusive at first, the initial hypothesis was that the enzyme was involved in the maturation of metalloclusters, a shared feature in both components required for nitrogen reduction. The nitrogenase component 1, MoFe protein (NifDK), coordinates two types of metal centers, the [8Fe7S] P cluster and the [Mo7Fe9SC-homocitrate] FeMo-cofactor, whereas component 2, Fe-protein (NifH), coordinates a [4Fe—4S] cluster [26]. Biochemical characterization of NifS showed the presence of a pyridoxal-5′-phosphate attributed to the yellow coloration of the purified enzyme dimer. Reaction with L-cysteine, but not the other 19 amino acids or D-cysteine, resulted in an absorption spectra shift, indicating the interaction of the cofactor with the substrate Cys [22]. Subsequent analysis of reaction products established that alanine and sulfide were formed under reducing reaction conditions and that Cys325 thiol was involved in the reaction mechanism by forming a persulfide covalent intermediate [27]. Interestingly, the *nifS* gene is co-transcribed with *nafG* (cysE1) coding for an *O*-acetyl serine transferase, which catalyzes the rate-limiting step for de novo cysteine synthesis [28]. Additionally, the inactivation of *nifU*, a gene located adjacent to *nifS*, exhibited an equivalent phenotype

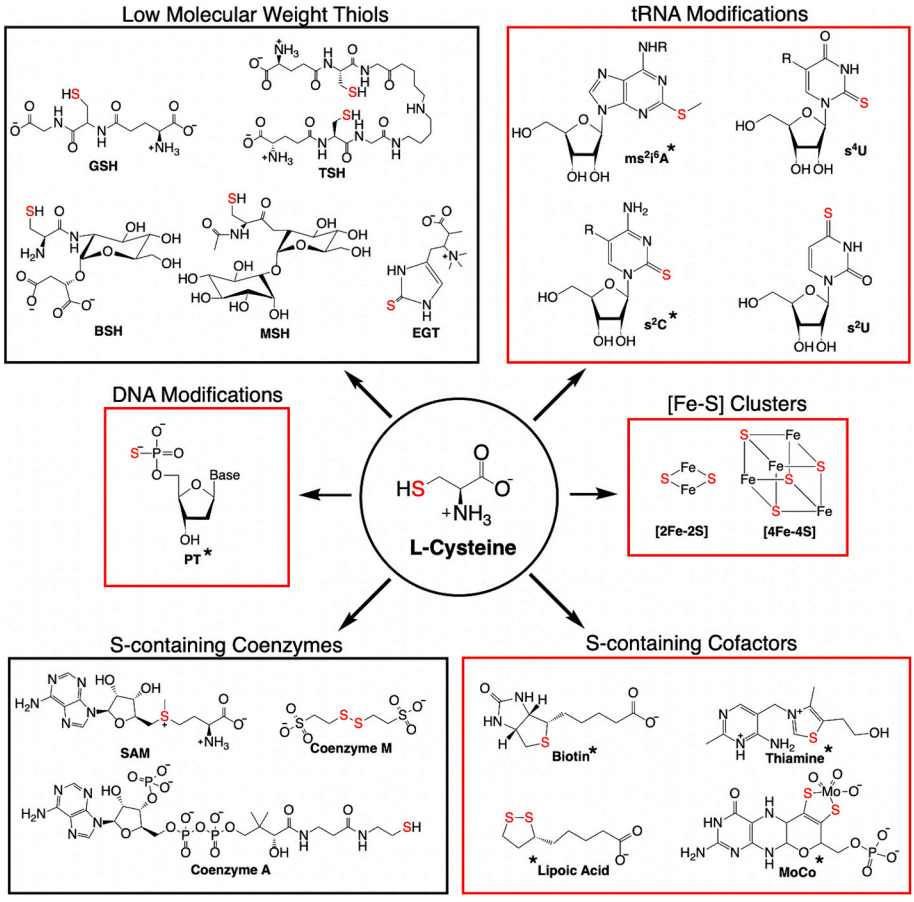

Fig. 1. Cysteine serves as a source of sulfur in the biosynthesis of thiol-containing biomolecules. The figure shows: (top left) low molecular weight thiols glutathione (GSH), trypanothione (TSH), bacillithiol (BSH), mycothiol (MSH), and ergothioneine (EGT), (top right) post transcriptional tRNA modifications 2-methylthio-N6-isopentenyl-adenonsine (ms2 i 6A), 2-thiocytidine (s2C), 4-thiouridine (s4U), and 2-thiouridine (s2U), (middle left) post replication DNA modification phosphorothioate (PT), (middle right) cubic and rhombic forms of basic [Fe—S] clusters, (bottom right) sulfur-containing coenzymes S-adenosylmethionine (SAM), coenzyme M, and coenzyme A, (bottom left) sulfur-containing cofactors biotin, lipoic acid, thiamine, and molybdenum cofactor (MoCo). The biosynthesis of molecules contained in red boxes is dependent on the activity of cysteine desulfurases and synthesis of cofactors marked with a star also depend on the activity of [Fe—S] cluster-containing enzymes

impacting the activity of both NifH and NifDK, suggesting a potential partnership between NifU and NifS. Experimental validation of this proposal later demonstrated that NifS provides sulfur for the transient assembly of Fe—S clusters onto NifU. These pre-assembled clusters then serve as the building blocks for the maturation of three metallocofactors P cluster, FeMo-cofactor and [4Fe—4S] cluster. Together NifU-NifS has been proposed to serve as the minimal toolbox for the biological assembly of Fe—S clusters [29].

The identification of a specialized system for the assembly of nitrogenase metalloclusters prompted the investigation of equivalent systems with general functions in the synthesis of Fe—S clusters and associated sulfur mobilization of sulfur for a wider range of enzymes with roles not restricted to nitrogen fixation. Purification of a cysteine desulfurase protein from a strain of *Azotobacter vinelandii* having *nifS* deleted led to the discovery of IscS [30]. IscS, a paralog of NifS, also displays a dimer quaternary conformation coordinating a PLP cofactor in each active site monomer. The *iscS* gene was found to be adjacent to *iscU* whose product displayed amino acid sequence similarity to the N-terminal domain of NifU. While inactivation of *iscS* led to a lethal phenotype in *A. vinelandii*, parallel studies in *E. coli* established that *iscS*, along with other genes within the *isc* operon (*iscRSUA-hscAB-fdx*) were involved in the housekeeping formation of Fe—S clusters [3]. This proposal was later validated in *S. enterica* [31], *A. vinelandii* [32], and many other organisms carrying the Isc system. Furthermore, functional analysis of *iscS* demonstrated that its role is not limited to the sulfur mobilization reactions for the synthesis of Fe—S clusters but also involved in supplying sulfur for the synthesis of other thio-cofactors including thiamin, molybdenum cofactor, thionucleosides [4,5,9,31,33]. In these additional roles, IscS interacts with a variety of proteins, displaying distinct structural folds and participating in pathways for the biosynthesis of these cofactors.

Genomic analysis of *E. coli iscS* suppressor mutants led to the discovery of the Suf system (named after sulfur mobilization factor) [34]. Since the inactivation of *E. coli* iscS was not lethal, as in the case of *A. vinelandii*, it hinted at the existence of perhaps a backup system supporting the synthesis of essential Fe—S enzymes in this organism. Takahashi and collaborators identified the *suf* operon (*sufABCDSE*) to be upregulated in isolates that reverted the slow-growth phenotype of *iscS* null genetic background. The cysteine desulfurase SufS, along with its sulfur acceptor SufE and other components SufA and SufBCD, were then characterized as a secondary Fe—S cluster biosynthetic pathway under conditions of iron starvation and oxidative stress [35,36]. The Suf system is absent in *A. vinelandii*, explaining the essentiality of the Isc system in this organism [37].

Interestingly, the Suf system is widely distributed in microbial species and plays an essential role in Firmicutes, which lacks the Isc pathway. It is now known that most organisms contain at least one ortholog of NifS/IscS/SufS [38–40]. Notable exceptions to the nearly essential utilization of cysteine desulfurases as the main sulfur donor for thiocofactor synthesis are species of archaea and selected thermophilic bacteria that directly utilize sulfide [7,41–42]; the mechanisms of sulfur incorporation have not been fully explored but could involve persulfide intermediates or direct sulfide addition to these biomolecules. In either case, alternate systems bypassing the enzyme-bound persulfide chemistry afforded by cysteine desulfurases represent an exciting development area in biological sulfur chemistry.

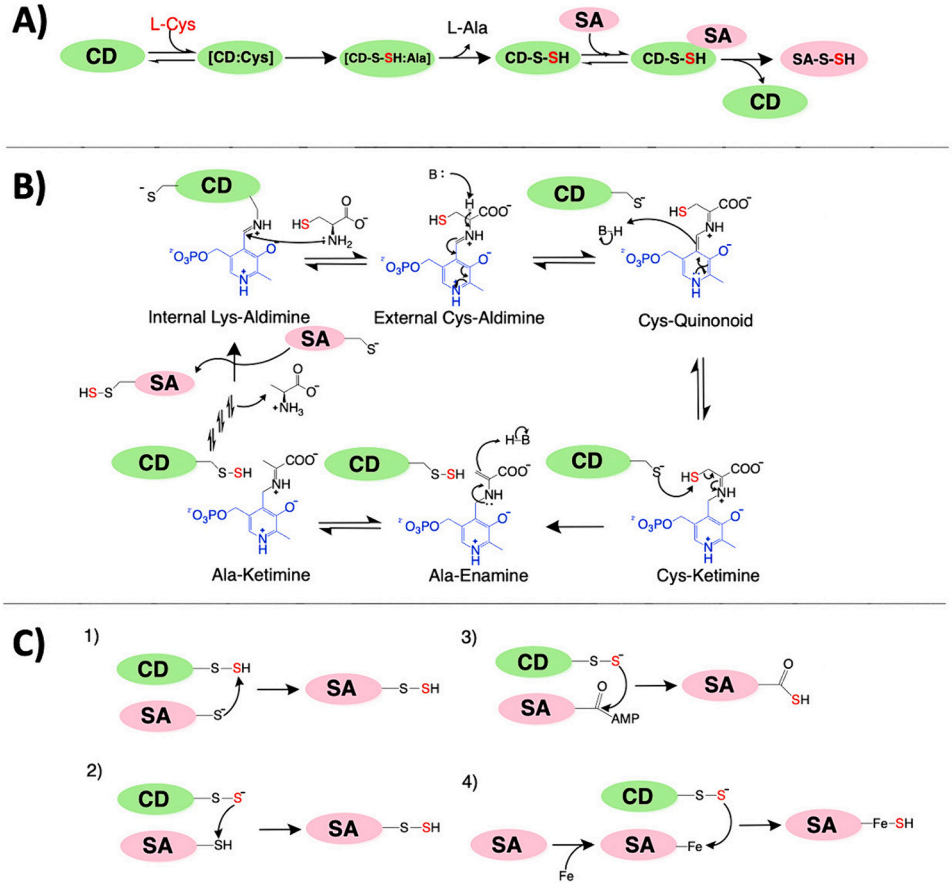

Fig. 2. Cysteine desulfurase (CD):sulfur acceptor (SA) sulfur transferase reaction. (A) Double-displacement mechanism shows the sulfur abstracted from cysteine in the first half of the reaction, resulting in a persulfide intermediate, and subsequently transferred to a sulfur-accepting partner in the second half of the reaction. (B) PLP-dependent (*shown in blue*) mechanism for the cysteine desulfurase abstraction of sulfur from cysteine resulting in the release of alanine in the first half of the reaction. (C) Proposed mechanisms of sulfur transfer in the second half of the reaction.

3.1. Cys:sulfur acceptor sulfurtransferase reaction

In a physiological context, cysteine desulfurases are, in fact, Cys: sulfur acceptor sulfurtransferases (E.C.2.8.1.7) following the proposed double displacement mechanism (ping-pong) with a characteristic reaction scheme (Fig. 2A). All cysteine desulfurases described to date utilize pyridoxal-5′-phosphate (PLP) to activate the Cys substrate via the formation of an external Schiff base displacing the internal Lys-PLP Schiff base observed in the resting state of the enzyme [27,43–45] (Fig. 2B). The formation of a Cys-PLP aldimine Schiff base intermediate leads to conjugation of the π orbital on the pyridine ring, enabling abstraction of the alpha proton of the Cys substrate by a general base residue at the active site, leading to a transient Cys-PLP quinonoid intermediate. Protonation at the C4′ position of the Cys-PLP quinonoid intermediate leads to formation of a Cys-PLP ketimine intermediate, directly proceeding C—S bond cleavage. For Class I cysteine desulfurases, the active site cysteine contained on the flexible loop has been proposed to serve as the proton donor residue involved in the Cys-PLP ketimine intermediate formation [27,44]. While for Class II cysteine desulfurases, the active site cysteine has been ruled out as the proton donor in this step due to the distance between this residue and C4′ position of the Cys-PLP quinonoid intermediate. In this case, the Schiff base lysine has been proposed to be involved in protonation of the quinonoid intermediate to form the ketimine intermediate [43]. A committed step in this reaction mechanism is the nucleophilic attack of the deprotonated active site Cys thiol onto the substrate thiol, which results in a persulfide covalent enzyme intermediate (R-SSH, perthiol, sulfane sulfur) and an Ala-PLP aldimine. The release of Ala product follows the reverse order of Cys-PLP activation through regeneration of the internal Lys-PLP Schiff base. In some members of this class of enzymes, the slow dissociation of Ala leads to its further decomposition into pyruvate and ammonia following a subsequent deamination reaction [46]. The formation of the enzyme persulfide intermediate marks the first half of the catalytic cycle and provides an activated sulfur form for the synthesis of thio-cofactors.

In the second half of the sulfurtransferase reaction, the persulfide intermediate is transferred to sulfur acceptor proteins participating in downstream biosynthetic steps. As discussed later, it is anticipated that protein-protein interactions are required for an effective sulfur transfer reaction to the acceptor protein. Although not fully established, several modes of sulfur transfer have been proposed (Fig. 2C): 1) a deprotonated thiol on the sulfur acceptor may promote the nucleophilic attack on the enzyme terminal persulfide, 2) the persulfide sulfur acts as a nucleophile attacking a thiol on the sulfur acceptor, 3) the persulfide sulfur attacks an adenylated carbonyl to form a thiocarboxylate, and 4) the persulfide sulfur is directly transfered to a nascent Fe—S cluster directly binding to the metal. The R-SSH intermediate can also be directly reduced by liberating sulfide (S^{2-}). These distinct mechanisms are not mutually exclusive and may vary with the nature of the sulfur acceptor, the protonation, and the metal-bound state of interacting molecules.

It has been demonstrated through in vitro studies that artificial reducing agents compete with sulfur acceptor substrates [47–48]. In some cases, reductants bypass the sulfurtransferase reaction directly reducing the enzyme persulfide liberating sulfide. This type of reaction masks the involvement of the second substrate (sulfur acceptor), which is critical for its vivo function. In the absence of sulfur acceptors and reductants, the enzyme RSSH intermediate can also engage in subsequent reaction cycles, forming a polysulfide enzyme intermediate (R-SSnH, n = 2–7). The kinetic profile of each of these three reaction paths differs and impacts the enzyme catalytic turnover rate. Furthermore, the availability of sulfur acceptors and their sulfuration state, the presence of modulator factors, the pH of the reaction, and the nature and concentration of reducing agents drastically affect the activity levels of these enzymes in vitro and in vivo.

3.2. Probing the cysteine desulfurase/sulfurtransferase reaction

The cysteine desulfurase reaction can be probed at various stages of the catalytic cycle (Fig. 3). The PLP cofactor displays distinct visible absorption features at different intermediate steps and shows absorbance shifts from the internal PLP-Lys aldimine upon substrate binding. Pre-steady state kinetic analysis using stop-flow measurement allowed characterization of the aldimine (385–420 nm), quinonoid (500–506 nm), and ketimine (340–350 nm) PLP-substrate adducts [22,43–44,49], suggesting that the binding of the substrate is a fast reaction step and likely the transfer or reduction of the persulfide bond constitutes a rate-limiting step in the overall reaction (Fig. 2B). In *E. coli* SufS, the Cys-aldimine is proposed to adopt tautomeric enolimine (343 nm) and ketoenamine (424 nm) conformation in the presence of Cys substrate and in the absence of a sulfur acceptor SufE, suggesting that the persulfurated form of the enzyme may engage another molecule of the substrate Cys [43]. Mutagenesis studies also show the involvement of the PLP-ligating Lys, general base His, and persulfide-forming Cys. The first half of the reaction is marked by the formation of a covalent persulfide enzyme intermediate and one equivalent of Ala. In vitro, the persulfide intermediate has been detected through mass spectrometry [50,51], crystallography [52–54], selective alkylation [55,56], and ^{35}S labeling studies [44,57–59].

Kinetic analysis of reaction products can be measured through the rate of alanine formation and, in reactions performed under reducing conditions, through the rate of sulfide production (Fig. 3). Reducing agents, both artificial and physiological, have demonstrated reactivity towards the persulfide intermediates of the cysteine desulfurase, leading to the direct release of free sulfide (S^{2-}) [47]. The artificial reducing agent dithiothreitol (DTT) has been routinely used in cysteine desulfurase activity assays, probing the rate of sulfide production. While those measurements provide a general readout of enzyme activity, DTT is known to interact with the PLP active site, perturbing the absorption spectrum and also competing with physiological sulfur acceptors. Thus, artificial reductants make it challenging to determine the sulfurtransferase reaction of these enzymes as they compete for the enzyme persulfide sulfur.

Sulfide released upon direct reduction of persulfides formed at cysteine desulfurases or indirectly through reduction of persulfides accumulated at sulfur acceptors can be monitored through a colorimetric methylene blue assay. In cysteine desulfurase assays under reducing conditions, sulfide can be quantified by reacting time point aliquots with *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) and $FeCl_3$ to form methylene blue (Fig. 3). Formation of methylene blue (Abs650nm) is limited by the concentration of sulfide present in the reaction, reporting the amount of sulfide generated in each assay time point. Additionally, the formation of methylene blue occurs under acidic conditions. Under these conditions, sulfide is found as hydrogen sulfide gas (H2Sg). Therefore, reaction vessels must be sealed after stopping the reaction and before methylene blue synthesis to avoid H2Sg loss before color development. It is worth noting that some reducing agents like tris (2-carboxyethyl)phosphine (TCEP) can reduce methylene blue, and while it is an effective reductant of persulfide, it is not a suitable reagent in this assay. The sulfide/methylene blue assay can also be adapted to examine the Cys:sulfur acceptor sulfurtransferase activity under non-reducing conditions. In this case, the reaction time point aliquots are quenched and reduced prior to the development of methylene blue.

Alanine, a byproduct of the first half-reaction, can be quantified either in enzymatic-coupled assays or through fluorescence derivatization. Indirect quantification of Ala is achieved in reactions containing alanine dehydrogenase in the presence of a ketoacid that leads to the formation of pyruvate that can react with lactate dehydrogenase to form lactate at the expense of NADH oxidation (Abs340nm) [60]. Alternatively, alanine can undergo a naphthalene dialdehyde (NDA)-derivatization reaction, resulting in a fluorescent a fluorescent 1-cyano-2-alkylbenz[*f*]isoindole adduct (Fig. 3). This latter method has higher

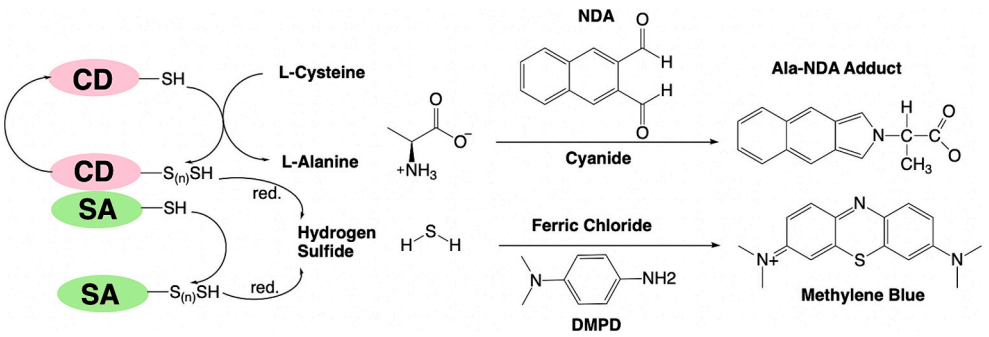

Fig. 3. Methods to probing the cysteine desulfurase/sulfur transferase activity. (Top) The enzyme activity can be measured by quantifying alanine via an NDA derivatization reaction resulting in a fluorescent adduct. (Bottom) The enzyme activity can be quantified through the reduction of persulfides on both cysteine desulfurase and/or sulfur acceptor and quantified via methylene blue formation.

sensitivity (0.1 pmol) when coupled to HPLC separation, providing a major advantage over other methods including the methylene blue method. Additionally, alanine formation is not dependent or affected by the presence of a reducing agent, allowing kinetic studies on reactions performed under non-reducing conditions. For instance, reaction assays performed in the presence of TCEP display the highest catalytic turnover rates [57,61–62], supporting the model that the cleavage of the persulfide is the rate-limiting step in reactions performed by cysteine desulfurases.

4. Impact of sulfur acceptors

The interactions between cysteine desulfurases and physiological sulfur acceptors dictate their biological functions in vivo. The sulfurtransferase activity of cysteine desulfurases involves interactions with specific partners in the second half of the reaction of the catalytic cycle (Fig. 2A). Thus, the binding interface shared between donor and acceptor enables productive sulfur transfer, where the persulfide intermediate is directly passed onto the acceptor protein participating in downstream biosynthetic pathways. In fact, some enzymes have their activity modulated by the presence of the sulfur acceptor and other modulators that are proposed to induce conformational changes to the enzyme to either allow or restrict catalytic steps preceding the transfer of the persulfide. It is assumed that all cysteine desulfurases characterized so far perform the first half of the reaction following the same chemical steps, leading to the persulfide intermediate in the cysteine desulfurase active site. However, the mechanism of persulfide transfer from the cysteine desulfurase has been found to be unique and dependent on the sulfur-accepting partner.

Sequence and structural elements within cysteine desulfurases allow their classification into class I, IscS-like and class II, SufS-like [63]. Members of class I have a characteristic flexible loop containing the active site cysteine involved in persulfide formation. Whereas for members of class II enzymes, the active site cysteine is located in a shorter, well-defined loop where the Cys active residue is protected from reductants, partially explaining the dependency of the sulfur acceptor partner to complete the catalytic cycle (Fig. 4). The importance of the active site loop has been acknowledged as a determinant in controlling the reactivity of these enzymes, especially towards direct action of reducing agents. However, this feature alone does not explain the lack of cross-reactivity of these enzymes in vivo, indicating that additional protein interactions control the reactivity of these enzymes and restrict their physiological functions.

4.1. Class II — SufS-like cysteine desulfurases

The activity of SufS and SufS-like enzymes is marked by the dependency on a single dedicated acceptor (Fig. 5). In *E. coli*, SufS and SufE are components of the Suf system (*sufABCDSE*) and serve as the sulfur donor for the [Fe—S] cluster biogenesis under conditions of oxidative stress and iron starvation [36]. In this system, SufS is the cysteine desulfurase and SufE serves as the direct sulfur acceptor (Fig. 5C). The *sufE* gene is located immediately downstream of *sufS*, as has been found to be the case for most cysteine desulfurases and sulfur acceptor-specific pairs [57]. SufE accepts sulfur from SufS and subsequently transfers it to SufB. The SufBC2D has been proposed to serve as the [Fe—S] cluster scaffold in this system. Kinetic analysis of SufS alone revealed low basal cysteine desulfurase activity under reducing conditions, however, in the presence of SufE, SufS activity was enhanced up to 70-fold [57]. Under these conditions, the reaction follows a biphasic profile. The first phase involves rapid turnover of SufS as sulfur is transferred to SufE Cys51, followed by a slower second phase as polysulfides build up on SufE. Additionally, in the presence of $SufBC_2D$ complex, the activity is further enhanced, providing strong evidence that the physiological activity of SufS is dependent on its dedicated sulfur acceptor partner serving as an intermediate sulfurtransferase. In this linked catalytic scheme, the availability of the scaffold regulates the turnover rate of sulfur mobilization by SufS [36,57].

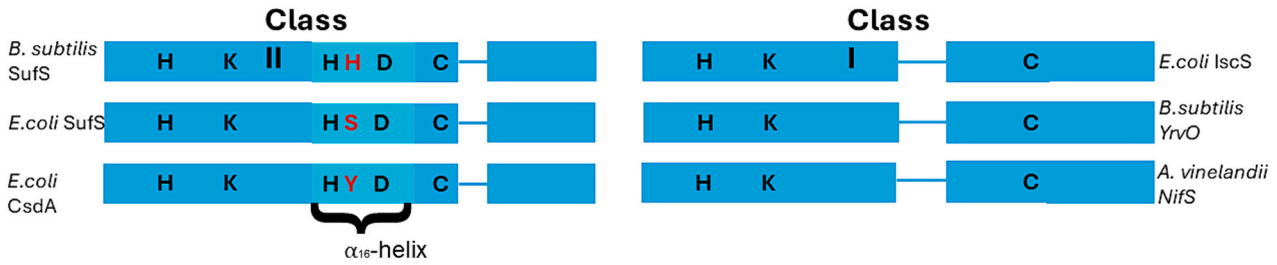

Fig. 4. Diagram representation of important primary sequence elements in Class I and Class II cysteine desulfurases. Residues shown for both classes include conserved histidine proposed to serve as a general acid/base, lysine involved in the internal PLP coordination, and the active site cysteines. Important residues of the α16-helix controlling reactivity with sulfur acceptors in Class II enzymes are shown. Shown in red, is the Zn-coordinating H342 residue of *B. subtilis* SufS, and the equivalent residues in *E. coli* SufS and *E. coli* CsdA.

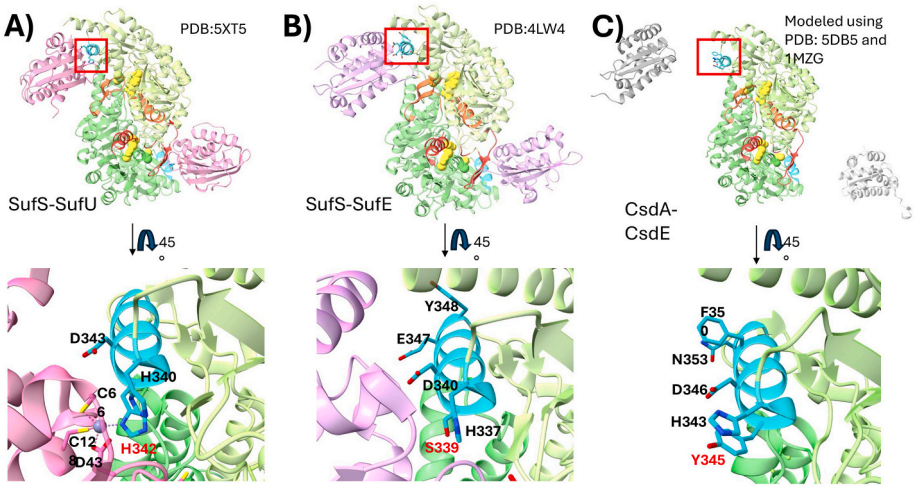

Fig. 5. Structures of Suf and Suf-like cysteine desulfurases in complex with sulfur acceptor partners. For all structures, the cysteine desulfurase monomers are shown in dark and light green, and the sulfur acceptors are shown in pink or purple. PLP cofactors (yellow) and active site cysteines are displayed in space-filling form. β-Latch structural elements (β-hairpin, Gly-rich loop, proline pivot, and α6-helix) for each active site are highlighted in orange and red. The β-latch shown in orange protects the active site of the light green monomer, while the β-latch shown in red protects the active site of the dark green monomer. The zoomed-in images highlight the conserved α16-helix structural element in Type II cysteine desulfurases. A) *B. subtilis* SufS–SufU showing the residues involved in Zn-coordination. Additionally, Asp343 of SufS, which is conserved across all Type II enzymes regardless of the acceptor, is also shown [54]. B) *E. coli* CsdA–CsdE complex. Residues highlighted on the α16-helix include residues involved in driving complex formation [66]. C) A model of *E. coli* SufS–SufE complex positioned afar but expected to bind at the same orientation as other structures. Residues highlighted on the α16-helix include residues identified to drive complex formation [65]. The figures show at the start of the helix structure, the conserved His residue (His340 in B. s. SufS, His337 in E.c. CsdA, and His343 in E. c. SufS) and the residues Tyr345 of E. c. SufS and Ser339 at the equivalent position of His342 in *B.s.* SufS.

In *E. coli* SufS and other members of the Class II cysteine desulfurases, the enzyme reactivity is controlled by the accessibility to the catalytic cysteine Cys364. This residue is buried into a solvent-excluded hydrophobic pocket formed at the dimer interface of SufS, protected from the effects of reductants and likely non-physiological sulfur acceptors [64]. A beta-hairpin from one subunit of the dimer shields the Cys364 persulfide of the active site within the other subunit (Fig. 5C). A conformational change promoted by a conserved β-latch structure is proposed to direct the SufS-Cys364 persulfide to the SufE-Cys51 catalytic residue, allowing sulfur transfer [65]. This β-latch is suggested to be involved in a conformational switch modulating the reactivity of the enzyme towards its physiological sulfur acceptor. In fact, disruption of the β-latch at a site remote from the active site partially decouples the dependency on SufE for enzyme reactivity [65]. Thus, the protected sulfur transfer reactions involving SufS and SufE have physiological significance as the Suf system operates under oxidative stress. Trafficking sulfur through coordinated mechanisms prevents undesirable oxidation of persulfide intermediates and guarantees the transfer of the persulfide cargo to its final acceptor SufBC2D, for the synthesis of Fe—S clusters under these conditions [64].

The interaction between SufS-SufE and its effect on SufS's reactivity is not matched by other sulfur acceptors of cysteine desulfurases. SufS-SufE pair has roles dedicated to the cluster assembly, presumably through a commitment to the other components of the Suf system, which limits its role in Fe—S cluster biogenesis. Therefore, the SufS and SufE do not cross-react with components of the Isc system [67]. Overexpression of the *sufABCDSE*, but not *sufABCDS*, recovers Fe—S cluster defects caused by the inactivation of the Isc system [34]. That is, the functionality of this system relies on the presence of SufE, which indicates that the sulfur transfer of SufS to SufE and SufE to SufBC2D complex is specific and not provided by other *E. coli* proteins.

The *B. subtilis* SufS has its activity dependent on the zinc-dependent sulfurtransferase SufU (Fig. 5A) [68]. Different from the *E. coli* Suf system, the *B. subtilis* Suf, encoded by the *sufCDSUB* operon, has a more general function as opposed to a specialized role during stressed conditions [69–70]. Another major difference between these two systems is the absence of a gene encoding SufE and the presence of a gene encoding SufU, which is proposed to operate in an equivalent role as SufE in mediating the trafficking of sulfur from SufS to the SufBC2D scaffold. Interestingly, SufE and SufU tend not to co-occur within the same species, indicating their mutually exclusive roles [41]. The initial proposed function of SufU was as a [Fe—S] scaffold protein due to the primary sequence similarity to IscU, a known scaffold protein, including two conserved cysteine residues near the active site cysteine involved in persulfide formation. However, Mossbauer and EPR spectroscopy experiments, along with cluster reconstitution assays, were unsuccessful in characterizing purified forms of SufU coordinating Fe—S clusters [69].

The specific partnership of SufS-SufU performing analogous roles to that of SufS-SufE was also evidenced through in vivo complementation studies. First, cross-complementation of individual components in *E. coli* and *B. subtilis* knockout strains failed to recover defects associated with the inactivation of individual components. Second, co-expression of *B. subtilis* SufS and SufU rescued defects associated with *E. coli* Δ*sufS* and Δ*sufE* strains and co-expression of *E. coli* SufS and SufE rescued the lethal phenotype of *B. subtilis* Δ*sufS* and Δ*sufU* strains [71]. Third, these genetic complementation studies are supported by in vitro biochemical data demonstrating that sulfur acceptors *E. coli* SufE and *B. subtilis* SufU can only enhance the turnover rate of their species-specific cysteine desulfurase counterparts (PDS personal communication).

Despite the equivalent roles of SufSE and SufSU, the mechanisms of sulfur transfer differ between these two systems. In the presence of SufU, SufS activity is enhanced up to 200-fold, and the sulfur transfer mechanism is dependent on the presence of zinc coordinated by conserved Cys41, Cys66, Cys128, and Asp43 [68]. The tightly bound Zn (Ka = 10^{17}) likely restricts the role of SufU as sulfurtransferase as opposed to Fe—S cluster as initially proposed. Interactions between SufS and SufU lead to significant conformational changes, driven by a Zn-ligand swapping event in which SufU Cys41 is replaced by SufS His342, freeing Cys41 to engage in sulfur transfer [54]. Additionally, the active site persulfide (Cys361) of SufS is brought into proximity of SufU Cys41. The pH-activity profile of the SufS–SufU reaction is compatible with a model that the deprotonated form of SufU Cys41 is the active participant in the second half of the reaction, promoting the nucleophilic attack onto the SufS Cys361 persulfide (Fig. 2A).

Following sulfur transfer, the interacting cysteine residues move apart. Mutagenesis experiments on residues involved in Zn coordination impair Zn binding and consequently disrupt the ability of SufU to enhance the turnover rate of SufS [68]. Pull-down assays also revealed that substituting His342 of SufS to a non-Zn-coordinating residue eliminates complex formation between SufS and SufU and impairs activity [72]. The Zn dependency in SufU sulfurtransferase reaction expanded the role of this metal in biology and makes a distinct mechanism from that between *E. coli* SufS and SufE. Ligand swapping provides a unique mechanistic strategy to activate the enzyme for sulfur transfer and illustrates the impact of specific protein-protein interactions between cysteine desulfurase and sulfur acceptors in regulating sulfur transfer.

CsdA, another SufS-like enzyme, partners with CsdE to perform similar roles in sulfur mobilization reactions (Fig. 5B). The function of this sulfur donor-acceptor pair is still not fully understood. Earlier studies identified that overexpression of CsdAE could suppress the lethal phenotype of *E. coli* Δ*iscS* strain grown in LB medium supplemented with thiamine and nicotinic acid, indicating that they could partially restore defects associated with sulfur trafficking [72–73]. Overexpression of CsdA but not CsdAE complements lack of SufS [73], indicating that the presence of the sulfur acceptor CsdE restricts the reactivity of the cysteine desulfurase towards non-physiological acceptors. Pull-down experiments showed the interaction and reactivity with TcdA (CsdL) as a sulfur acceptor of CsdAE reaction [73]. The function of TcdA was subsequently assigned as ATP-dependent dehydratase catalyzing the conversion of N^6-threonylcarbamoyladenosine (t^6A) to a cyclized hydantoin ring (ct^6A) at position 37 tRNA [74]. Interestingly, even though ct^6A is not a sulfur modification, the inactivation of *csdA* or *csdE* leads to a drastic reduction of ct^6A levels, revealing the unprecedented involvement of a cysteine desulfurase and sulfur acceptor in the synthesis of a non-thiolated metabolite [75].

While the function of CsdAE in ct^6A formation remains to be characterized, the sulfur mobilization reaction catalyzed by this sulfurtransferase displays features distinct from other studied SufS enzymes. First, the cysteine desulfurase reaction of CsdA shows high levels of alanine and sulfide formation under reducing conditions and in the absence of its sulfur acceptor CsdE [72]. Second, the addition of the CsdE to the in vitro reaction only enhanced the turnover rate of the enzyme two-fold as opposed to 50–100 fold, as observed for SufS [48,64,72]. Third, binding experiments show a monophasic 1:1 tight complex formation between CsdA and CsdE ($Kd = 0.117$ μM) as opposed to the biphasic behavior of SufS–SufE complex formation ($Kd1 = 3.59$ μM and $Kd2 = 312$ μM) [64,76]. These differences can partially be explained by comparing the individual structures of CsdA and SufS. In contrast to SufS, the a-helix 4 is bent 50 degrees, resulting in a much more solvent-exposed active site, which reductants can reach more readily [77]. The binding profile of CsdAE resembles the binding profile observed for SufS with the alkylated form of SufE ($Kd = 0.263$ μM), hinting that CsdA adopts a conformation ready for interaction with sulfur acceptor and transfer. In fact, a comparison between CsdA and CsdE individual structures and the CsdAE complex structure shows that the structure of CsdA is largely unchanged with the active site Cys358 solvent-exposed [66]. Meanwhile, CsdE undergoes a conformational change, reorienting the sulfur-accepting Cys61 to be positioned in close proximity to Cys358 of CsdA. Combined, these analyses provide a structural justification for the reactivity of CsdA and the limited activity enhancement caused by CsdE.

4.2. Structural and mechanistic features of Class II cysteine desulfurases

Structures of class II cysteine desulfurases and sulfur acceptors give insight into areas of interactions and structural elements controlling the reactivity of these enzymes. The monophasic binding of CsdAE suggests that binding one monomer of CsdE to the dimer of CsdA does not affect the binding of the second molecule of CsdE. While the binding profile of CsdAE in the absence of cysteine does not support the idea of half-site reactivity (flip-flop) of CsdA dimer, structural differences on the two CsdE monomers of the complex along with the conservation of residues participating in CsdA dimer interaction provides support for flip-flop mechanism characterized for SufS enzymes [48,66]. In *B. subtilis* SufS-SufU reaction, the flip-flop mechanism is supported by a kinetic burst profile of 1 equivalent of alanine per SufS dimer in reactions in the absence of SufU and by cooperative inhibition profile of iodoacetamide treated SufU (SufUalk) (Hill coef = 2), indicating that binding of one monomer of SufUalk is sufficient to inactivate both active sites of SufS dimer [48]. The structure of the SufSU complex points to areas of specific protein-protein interaction promoting complex formation. The distance between sulfur-donating Cys361 of SufS and sulfur-accepting Cys41 of SufU varies with sulfuration status and across subunits of the dimer. In the structure of the SufSU resting state complex (PDB5XT5), the distance between the sulfur donating on Cys361 of SufS and sulfur accepting on Cys41 of SufU is 10 Å and 6.6 Å at each catalytic pair [54]. Incubation with cysteine leads to trapping catalytic intermediates containing a persulfide at Cys41 and/or a second persulfide with Cys361. Again, structure analysis of the intermediate complex reveals differences across the catalytic pair of the dimer with varying distances of 11 Å and 5.7 Å between Cys thiols pairs (PDB5XT6) [54].

The residues remotely located from the PLP active site but involved in protein-protein interactions with sulfur acceptor likely control the specific function of cysteine desulfurases. Despite differences in both SufSU complex structures, the coordination of His342 of *B. subtilis* SufS to the Zn within SufU indicates that the kinetic double displacement mechanism observed for this enzyme (Ping-Pong) is not attributed to the formation of the complex, but rather the sequence of chemical steps afforded by repositioning of the Cys thiols involved in the second step of the catalytic cycle. The His342 of *B. subtilis* SufS, the residue involved in Zn-coordination with SufU, is largely conserved among SufS sequences from systems containing a SufU partner (over 50 bacterial species containing SufCDSUB). This residue is located in a conserved helical structure (α-helix 16) that serves as a point for contact between the enzyme and sulfur acceptors in other studied enzymes (Fig. 5) [54]. In the CsdAE complex structure, this helix also provides contact points with CsdE, in which Ser339 is equivalent to His342 [66]. Although no structural information is yet available for a SufSE complex, sequence and structural alignments show that Tyr345 occupies an equivalent position to Ser339 of CsdA and His342 of *B. subtilis* SufS.

The lack of conservation of this Histidine residue across Class II cysteine desulfurases provides a mechanistic rationale for the lack of cross-reactivity among these enzymes and corresponding sulfur acceptors [77,78]. Adjacent to Tyr345, Asp346 of *E. coli* SufS is known to be involved in interaction with SufE. Substituting Asp346 to Arg decreases the affinity for SufE [65], and substituting SufE Asp74 to Arg also affects complex formation [79]. Mutagenesis combined with HD-exchange analyses indicates that SufE binds to an equivalent surface of SufS involving residues within α-helix 16. Although class II cysteine desulfurases display similar structural folds, these enzymes have evolved to select their physiological sulfur acceptor counterparts through specific protein-protein interactions that promote a coordinated conformational movement of the complex and positioning of catalytic residues in the proper orientation for catalysis.

4.3. Class I — IscS-like cysteine desulfurases

Members of class I cysteine desulfurases display architecture similar to that of Class II enzymes but have structurally distinct elements that impact the mechanism of sulfur transfer. A conserved sequence surrounding the active site cysteine can be used to distinguish Class I (-SSGSA**C**TS) from Class II (-RXGHH**C**A-) [80]. Importantly, class I enzymes do not contain the β-latch structural elements conserved in Class II enzymes, leaving the active site cysteine more solvent exposed [65]. Additionally, the catalytic loop in class I enzymes is usually about ten

amino acids longer, making it more extended and flexible as opposed to class II enzymes where the catalytic cysteine is found structured between two alpha-helices [63]. These structural features explain the enhanced reactivity of the enzyme towards reducing agents, which leads to a high turnover rate when compared to class II enzyme reactions in the absence of their physiological S-acceptor substrates. Furthermore, the accessibility of the persulfide was initially used to justify the reactivity of IscS towards more than one physiological acceptor, however subsequent studies show that the IscS offers distinct binding sites to sulfur acceptor proteins. Therefore, a simplistic model does not explain the lack of cross-complementation of other Class I cysteine desulfurases, indicating that additional structural elements serve as recognition points between the enzyme and S-acceptors.

The *E. coli* IscS is perhaps the best-studied Class I cysteine desulfurase and serves as the hub of sulfur mobilization and delivery for various pathways (Fig. 6) [45]. IscS interacts with multiple sulfur acceptors, including IscU, TusA, and ThiI. These acceptor proteins display diverse structural folds, demonstrating the versatile role of IscS in metabolism. For *Azotobacter vinelandii*, IscS is an essential enzyme, meaning deletion of the gene encoding IscS leads to a lethal phenotype, explained by the lack of a general backup [Fe—S] cluster biogenesis pathway in *A. vinelandii*. In *E. coli*, the deletion of IscS leads to severe biochemical growth defects, including lack of thionucleosides, impaired activity of Fe—S enzymes, and auxotrophy for thiamine, nicotinic acid, and branched-chain amino acid, and a lethal phenotype when grown in glucose minimal medium conditions. However, the viability of *E. coli* ΔIscS cells grown in minimal media can be restored with the addition of thiamine and nicotinic acid under aerobic conditions. [3–5,32,81–82].

Structural and mutagenesis analyses attempting to map the protein-protein interactions of IscS with TusA, IscU, ThiI, CyaY, and IscX showed that interacting proteins bind to the cysteine desulfurase through distinctive modes, in some cases, allow ternary complexes to form [82–84]. For example, CyaY and IscX binding to IscS do not prevent interaction with the sulfur acceptor IscU [85–86], suggesting that both proteins play regulatory roles. In contrast, ThiI and TusA display overlapping binding sites, indicating that sulfur transfer to their respective pathways are mutually exclusive events. In vivo analysis showed that a strain of *E. coli* carrying a deletion of *tusA* had more Fe—S clusters [87]. This observation provides experimental support for the model that despite distinct binding sites, TusA and IscU are competing sulfur donors of IscS. Overall, the general involvement of IscS in several biochemical pathways, not limited to Fe—S cluster synthesis, stems from its ability to interact with a suite of sulfur acceptors with diverse folds and functions. Although the identity of many sulfur partners has been determined [1], it is possible that IscS interacts with other, as yet unidentified proteins.

Other characterized members of class I cysteine desulfurases partner with specific sulfur acceptors. In *B. subtilis*, for example, YrvO, NifZ, and NifS are class I cysteine desulfurases performing specialized roles in sulfur metabolism directly interacting with single sulfur acceptors MnmA, ThiI, and NadA, respectively. Inactivation of these enzymes led to specific phenotypes, each characterized by the deficiency of s2U, s4U, or nicotinic acid auxotrophy [8,20,88]. Interestingly, despite their shared sequence similarities, these enzymes do not display cross-reactivity in vivo, indicating that interactions with sulfur acceptors restrict their physiological functions. *B. subtilis* cysteine desulfurases also do not restore defects associated with *iscS* inactivation in *E. coli*. In fact, abundant expression of *B. subtilis* YrvO exacerbates the slow growth phenotype of *E. coli* Δ*iscS* strain [8]. The lack of interspecies cross-complementation in these systems shows that despite the presence of orthologous sulfur acceptors, Bacillus enzymes utilize species-specific elements that control sulfur flow to their associated pathways and provide alternate mechanisms for regulating individual pathways.

YrvO is the closest ortholog to IscS in *B. subtilis*, and its function is restricted to the synthesis of 2-thiouridine (s^2U) at the wobble position 34 of Glu, Gln, and Lys tRNA [19]. This modification stabilizes the overall anticodon structure and improves ribosomal binding [89–90]. In *E.coli*, the initial sulfur-accepting partner of IscS for the biosynthesis of s^2U is TusA, which then further transfers the sulfur to the TusBCD complex and subsequently relayed to TusE, mediating the transfer of sulfur onto the MnmA tRNA modifying enzyme [5,91]. MnmA serves a bifunctional role in promoting ATP-dependent activation of the C2 of the uridine, which enables the final sulfur transfer reaction to form s^2U. In *B. subtilis*, TusA, TusBCD, and TusE are absent, and the cysteine desulfurase YrvO partners directly with MnmA in an abbreviated pathway [8]. Genes coding *yrvO* and *mnmA* are located within the same transcriptional unit as *cymR*, a gene coding for the master regulator of cysteine metabolism. Levels of both YrvO and MnmA proteins, but not of other *B. subtilis* cysteine desulfurases, respond to sulfur nutrient availability and consequently impact the accumulation of s^2U tRNA in a dose-response manner [89]. Attempts to construct *B. subtilis* strains with *yrvO* and/or *mnmA* deleted were unsuccessful, leading to the assumption that these genes are essential [8,92].

Inactivation of the Bsu *yrvO* ortholog, *iscS1*, in *Mycobacterium tuberculosis* leads to a slow growth phenotype associated with a myriad of metabolic defects with yet not fully established biochemical function. Characterization of *M. turberculosis* Δ*iscS1* strain showed the accumulation of reactive oxygen species, reduction in the activity of certain metabolic enzymes, including those containing Fe—S clusters, altered respiratory capacity, accumulation of several amino acids, and up-regulation of the SUF pathway involved in Fe—S cluster biogenesis [93–94]. Contrary to expectations, this complex phenotype led to a hypervirulence of Mtb *iscS1* knockout strain, a phenotype that was attributed to up-regulation of the *suf* operon [93]. While Mtb IscS1 has been proposed to be involved in Fe—S metabolism due to its similarity to Eco IscS, *M. tuberculosis* does not encode additional components of the ISC system within the iscS1 transcriptional unit or anywhere else in the chromosome. Putative ISC components that could promote the assembly and transfer of Fe—S clusters have not been identified either. Notably, this organism is known to utilize the essential SUF system for the housekeeping synthesis of Fe—S clusters, and the genomic location of *iscS1* gene is adjacent to *mnmA*, similar to that of *B. subtilis* and several other Gram-positive species. Given the high degree of sequence

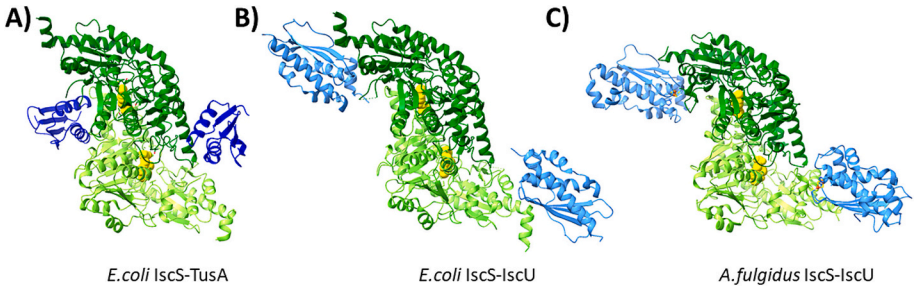

Fig. 6. IscS complexed with sulfur acceptors, highlighting different binding regions of different sulfur acceptors. IscS monomers are shown in green. Sulfur acceptors are shown in blue. PLP cofactors are shown in yellow. A) *E. coli* IscS-TusA complex (PDB: 3LVJ). B) *E. coli* IscS-IscU complex (PDB: 3LVL) C) *A. fulgidus* IscS-IscU complex (PDB: 4EB5).

similarity to the studied *B. subtilis* YrvO-MnmA s^2U biosynthetic pair, it is reasonable to propose that Mtb IscS1-MnmA functions in an equivalent capacity. It is not fully understood, however, why this presumed function has not yet been experimentally investigated.

Kinetic and labeling studies demonstrate the specific and direct partnership of *B. subtilis* YrvO and MnmA in the abbreviated s^2U pathway. The ATP-bound form of MnmA is the active form of the sulfur acceptor, as demonstrated in ^{35}S-Cys labeling experiments. The Cys: MnmA (ATP) sulfur transfer reaction indicates the mandatory requirement of Cys325 of YvrO in persulfide formation and the essentiality of conserved cysteine residues in MnmA (Cys104, Cys200, Cys51). Both Cys104 and Cys200 are found in the active site of the enzyme, while Cys51 is presumed to be located on the enzyme surface, leaving unanswered questions about its involvement in tRNA thiolation [8]. In vitro kinetic analysis shows that the presence of ATP-MnmA stimulates the catalytic turnover rate of YrvO two-fold in reactions performed in the presence of artificial reductant DTT [8]. Interestingly, reactions including the thioredoxin and thioredoxin reductase as the reductant system led to 24-fold activity enhancement of YrvO activity by MnmA, demonstrating that physiological persulfide reductase system is effective in reducing persulfides within the sulfur acceptor but not at the cysteine desulfurase. Steady-state kinetic analysis of YrvO-MnmA reactions shows a similar reaction profile as proposed for other cysteine desulfurases where the availability of the sulfur acceptor and, in turn, its regeneration limit the overall reaction rate when considering physiological reaction components.

Similarly, the biosynthesis of 4-thiouridine (s^4U) at position 8 of several tRNA also involves the direct sulfur transfer reaction from a cysteine desulfurase to a tRNA modifying enzyme. The reaction mechanism of the 4-thiouridylase ThiI follows similar chemical steps as the ones described for MnmA, including adenylation followed by sulfur transfer. In *E. coli*, *Salmonella enterica* and several other species, ThiI contains an additional rhodanese (Rhd) domain located at the C-terminal end that mediates sulfur transfer from IscS to s^4U and thiamin biosynthesis [95–97]. In species of Bacillus and most Gram-positive bacteria, ThiI lacks the Rhd domain and consequently is not involved in thiamin biosynthesis while retaining its role in s^4U synthesis [20,98]. Shared domains in all ThiI sequences include a THUMP domain involved in tRNA binding and a PP-loop pyrophosphatase domain responsible for adenylation reaction. The absence of the Rhd domain in most ThiI sequences suggests that ThiI sequences likely contain an alternate site for sulfur transfer. The *B. subtilis thiI* gene is co-transcribed with the *nifZ* cysteine desulfurase, suggesting a specific partnership between sulfur donor-acceptor equivalent to that observed for YrvO-MnmA pair. Indeed, ThiI-NifZ pair is sufficient to complete s^4U synthesis both in vitro and in vivo and individual components do not crosstalk with *E. coli* orthologs [20]. That is, neither Bsu NifZ nor Bsu ThiI can complement the functions of Eco IscS or Eco ThiI in vivo, however co-expression of both Bsu NifZ-ThiI pair can restore s^4U tRNA levels in *E. coli* Δ*iscS* or Δ*thiI* strains. Kinetic analysis shows that the presence of ThiI enhances the rate of desulfurase of NifZ in reactions in the absence of an artificial reductant and that the requirement of the specific cysteine desulfurase is bypassed in reactions using sulfide as the sulfur source. However, the presence of artificial reductants masks the involvement of dedicated desulfurase S-donors in s^4U as well as in the synthesis of other thiomolecules known to be critical for their in vivo biochemical functions.

Specific partnership of cysteine desulfurases is also observed in pathways involving the synthesis of Fe—S clusters for other class I enzymes. As discussed, IscS partners with IscU for the synthesis of Fe—S clusters. The structure of IscS and IscU complex shows the binding regions of both S-donor and S-acceptor proteins, which is distinct from the IscS-TusA binding interface [98,99] (Fig. 6). The Fe—S cluster formation promoted by the IscS-IscU pair also includes ferredoxin serving as the electron source and additional components involved in the delivery of clusters of these cofactors to final target proteins [100]. In *A. vinelandii* the IscS system serves as the general system for the maturation of Fe—S clusters, while the desulfurase NifS specifically partners with sulfur acceptor NifU for assembling Fe—S clusters destined to nitrogenase [30,101–102]. The three-domain NifU serves as the scaffold site for the preconstruction of Fe—S clusters where the N-terminal domain is similar to IscU and is believed to operate in an equivalent capacity. The central domain harbors a ferredoxin-like 2Fe—2S cluster proposed to function in an equivalent capacity as the Isc ferredoxin connected through a linker to the C-terminal domain similar to Nfu cluster carriers [103]. Despite their shared sequence motifs, limited functional replacement between NIF and ISC systems is observed in *A. vinelandii*. IscU and NifU sulfur acceptors, when expressed at their native levels, do not exhibit cross-reactivity, indicating the specificities with their respective pathway components. Over-expression of these components shows that NifU has some capacity to engage in reactions promoted by IscS, but NifS does not provide productive interaction with IscU even at abundant levels [101,104]. NifU-NifS biosynthetic pair is well-described in nitrogen fixation, and the occurrence of this system has been associated with the capacity to sustain nitrogen fixation in aerobes [105]. In fact, the requirement of this system is eliminated under low oxygen conditions in *A. vinelandii* [104]. Fe—S cluster synthesis by NifUS system is also utilized by non-nitrogen fixing species for the general housing-keeping synthesis of Fe—S clusters [106]. The occurrence of a two-component system harboring sulfur mobilization, cluster assembly, reduction, and transfer functions may be seen as an optimized model for activating and trafficking S- and Fe—S species under conditions of high demand and offers a simplified model to study discrete steps in Fe—S cluster formation.

5. Concluding remarks

Cysteine desulfurases participate in the biosynthesis of several sulfur-containing cofactors. The reaction performed by these enzymes requires the partnership with specific sulfur acceptors at the intersection of several metabolic pathways where the activated sulfur in the form of a covalent persulfide intermediate is directly transferred to acceptor partners. While these enzymes have been studied for three decades, most studies describing the characterization of these enzymes have been performed in the presence of artificial reductants. These conditions liberate sulfide and, in some cases, bypass the involvement of the sulfur acceptor protein that is critical for the biochemical function of these enzymes. More recent structural and function analyses show sequence motifs promoting communication between subunits of the functional dimer and unique areas of interactions with sulfur acceptors that likely limit the reactivity towards non-physiological targets. These recognition points of interaction offer a unique strategy to selectively disrupt pathways involving cysteine desulfurases at species-specific and pathway-specific levels.

While the chemistry promoted by members of this family is identical — PLP-dependent cleavage of the C—S of cysteine and transfer of the persulfide sulfur- diversification of their biological functions is achieved through distinct strategies. Thus, the utilization of multiple cysteine desulfurases matched with dedicated acceptors or alternate contact points of a single enzyme partnering with multiple acceptors provides diverse solutions to regulate sulfur activation and trafficking in microbial systems. The distribution of these enzymes is nearly universal, and their functions are essential in the vast majority of species due to the essentiality of cofactors products resulting from their participating biosynthetic pathways. However, selected species of archaea and thermophiles lack cysteine desulfurases or encode non-active copies of these enzymes [7,107]. In these cases, sulfide is provided as the source of sulfur, and the mechanisms of incorporation into those pathways are not fully understood. However, it is an exciting area that remains to be explored from a mechanistic and evolutionary perspective.

CRediT authorship contribution statement

Jimmy Swindell: Writing – review & editing, Writing – original draft, Conceptualization. **Patricia C. Dos Santos:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

Declaration of competing interest

Patricia C. Dos Santos reports financial support was provided by National Science Foundation. 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

No data was used for the research described in the article.

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