



Regulation of sulfur metabolism, homeostasis and adaptive responses to sulfur limitation in cyanobacteria

Surbhi Kharwar¹ · Samujjal Bhattacharjee¹ · Sindhunath Chakraborty¹ · Arun Kumar Mishra¹

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Abstract

Understanding of sulfur metabolism has reached a new dimension with comprehension of its molecular regulation; however, knowledge underlining the evolutionary perspective and dynamics of this metabolism in cyanobacteria is still limited. In this review, we provided a comprehensive overview of sulfur metabolism with special emphasis on cyanobacteria and discussed the biosynthesis of cysteine and its downstream sulfur containing metabolites. Here, we invested efforts to understand the possible regulatory mechanisms of cyanobacterial sulfur assimilation process by comparing with that of other bacteria and plants. The impact of sulfur limitation on the morpho-physiological, biochemical, and molecular responses in cyanobacteria was also elucidated. The present work reflected that the cyanobacterial sulfur assimilatory pathway is identical to that of bacteria and plants since all of them employ similar enzymes in the process; however, the regulatory mechanism may vary in cyanobacteria. This communication comprehends the recent progresses made in the field of cyanobacterial sulfur metabolism research along with a comparative account of sulfur metabolism in some related organisms. Therefore, this review not only gives a broad overview on cyanobacterial sulfur metabolism but also increases our understanding of the importance and evolutionary perspective of this crucial metabolism.

Keywords Assimilation · Cyanobacteria · Nutrient · Regulation · Sulfur · Uptake

Introduction

The versatility of sulfur in the life processes is unequivocal because it is a crucial component of cellular metabolites and exhibits redox-dependent activity (Giordano et al. 2005). With the evolution of the Earth, the concentration of sulfur, particularly in oceans, has changed (Table 1); however, in recent times, the concentration of sulfate in marine ecosystem is approximately 28 mM, which indicates it is the prime reservoir of sulfate (Ksionzek et al. 2016). In contrast, majority of the freshwater systems exhibit very low concentration of sulfate ranging from 10 to 50 μ M (Bochenek et al. 2013). Thus, organisms thriving in this low sulfate containing freshwater systems essentially require to compensate and maintain their

cellular homeostasis. Some algae and cyanobacteria can respond to sulfate deficiency (Prioretti et al. 2014). According to the sulfate facilitation hypothesis, red algae is more susceptible to low sulfate availability than green algae and cyanobacteria (Ratti et al. 2011, 2013), suggesting that changes in the oceanic sulfate concentration affect the phytoplankton dominance.

Despite its bioavailability in different habitats, the organic form of sulfur is predominant, with the total concentration being 95% in the environment, whereas the remaining 5% is present in the inorganic forms. Although organic sulfur accounts for a major proportion in the environment, it does not provide any significant advantage to living biota because they preferably utilize sulfate as the sole source of sulfur along with thiosulfates, cysteine, methionine, and glutathione to satisfy cellular requirements (Fernandez-Gonzalez et al. 2019). Some microalgae can release arylsulfatase and alkylsulfatase, which generate sulfate from organic compounds after catalysis through cleavage, making them available for growth.

Sulfate acquisition, its subsequent reduction into cysteine, and further catabolism into secondary sulfur metabolites are

✉ Arun Kumar Mishra
akmishraau@rediffmail.com

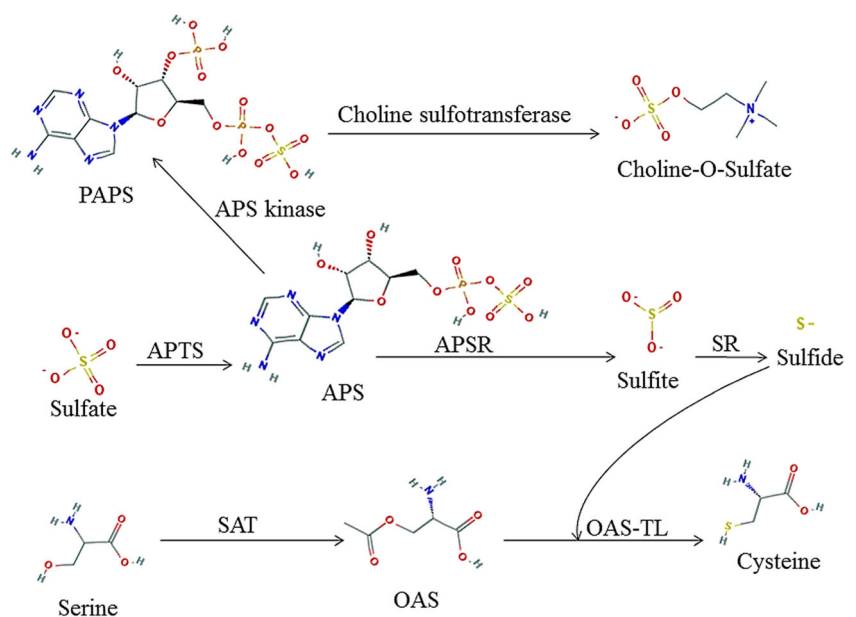
¹ Laboratory of Microbial Genetics, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi 221005, India

Table 1 Variation in sulfur availability with the evolutionary time scale

Habitat	Eon	Period	Sulfate concentration	References
Marine	Archean oceans	Precambrian	> 200 μM	Habicht et al. 2002
	Proterozoic oceans		1–5 mM	Kah et al. 2004
	Phanerozoic oceans	Late Ediacaran oceans	15 mM	Petrychenko et al. 2005
		Cambrian oceans	3–12 mM	Gill et al. 2011
		Carboniferous	> 15 mM	Gill et al. 2007
		Permian and Triassic ocean	20 mM	Lowenstein et al. 2003
		Late Paleozoic to Mesozoic oceans	13–27 mM	Gill et al. 2007
		Cenozoic (Today's) oceans	28–30 mM	Takahashi et al. 2011
Freshwater	Freshwater		0.01–1 mM	Giordano et al. 2008
	Oligotrophic lakes		< 300 μM	Holmer and Storkholm 2001
	Meso and eutropic lakes		700–800 μM	
	Acidic lakes		< 100 μM	

sequential multistep processes catalyzed by specific enzymes (Fig. 1). Sulfur assimilation, which involves the conversion of sulfate to cysteine, is an eight-electron reduction process that commences with the uptake of sulfate through specific membrane transporters (i.e., energy-dependent proton/sulfate [presumably $3\text{H}^+/\text{SO}_4^{2-}$] co-transporters) (Green and Grossman 1988; Laudenbach and Grossman 1991). To understand the crucial role of sulfur in the cell metabolism, it is vital to identify enzymes involved in the metabolism. Sulfate is first activated to form adenosine-5'-phosphosulfate (APS) and then phosphorylated to generate 3'-phosphoadenosine-5'-phosphosulfate (PAPS) by ATP sulfurylase (ATPS) and APS kinase (APSK), respectively. PAPS is successively reduced to sulfite (SO_3^{2-}) followed by sulfide (S^{2-}) by PAPS reductase and sulfite reductase (SR), respectively. The

produced SO_3^{2-} is required for sulfolipid biosynthesis, whereas S^{2-} is involved in the protein modification and redox buffering. The incorporation of sulfide formed in the reductive process into cysteine is mediated by serine acetyltransferase (SAT) that is involved in the acetylation of serine to O-acetylserine (OAS), while O-acetylserine (thiol) lyase (OAS-TL) is involved in the condensation of OAS and sulfide to form cysteine (Fig. 2). The first product of primary sulfate assimilation, namely cysteine, is further used in protein synthesis or as a reduced sulfur donor for the biosynthesis of downstream metabolites, such as methionine, SAM, and glutathione, as well as in coenzymes and cofactors. Cysteine not only serves as a central hub for the synthesis of different sulfur containing metabolites but also merges assimilatory sulfate, nitrogen, and carbon pathways (Fig. 3).

Fig. 1 Schematic representation of steps involve in sulfur metabolism

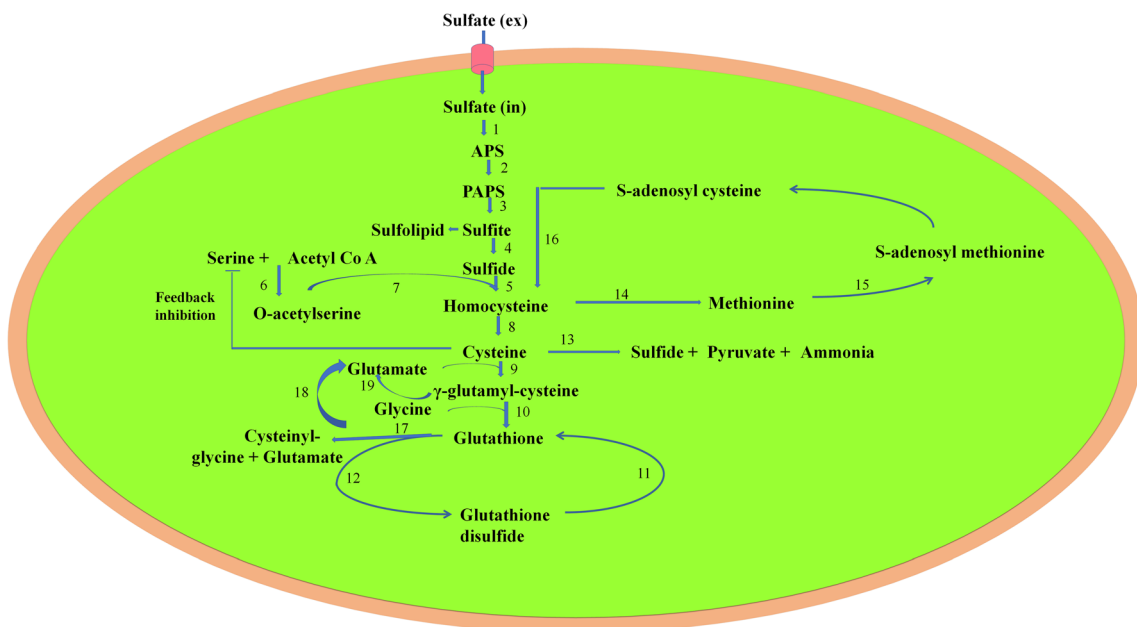


Fig. 2 Overview of sulfur assimilatory pathway and the key enzymes involved as follows: 1. ATP sulfurylase; 2. APS kinase; 3. APS reductase; 4. PAPS reductase; 5. Sulfite reductase; 6. Serine transacetylase; 7. Cysteine synthase; 8. Cystathionine β-synthase; 9. γ-Glutamylcysteine synthase; 10. Glutathione synthetase; 11. Glutathione

reductase; 12. Glutathione peroxidase; 13. Cysteine desulfurylase; 14. Homocysteine methyltransferase; 15. S-adenosylmethionine synthase; 16. S-adenosylcysteine hydrolase; 17. γ-Glutamyl transpeptidase; 18. γ-Glutamyl transpeptidase; 19. γ-Glutamyl transpeptidase (adapted from Hesse et al. 2004)

The regulation of sulfur metabolism and its homeostasis at the cellular level are achieved at multiple levels that include the regulation of transporters and enzymes involved in the assimilatory process and their transcriptional regulation through regulatory proteins. CysB, CysM, and CysR are the LysR-type

transcription regulators that control the expression of the *cys* operon in *Escherichia coli*, *Salmonella typhimurium*, and *Synechococcus*, respectively, whereas SAC1/SAC3 and SLIM1 are involved in green alga *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*, respectively.

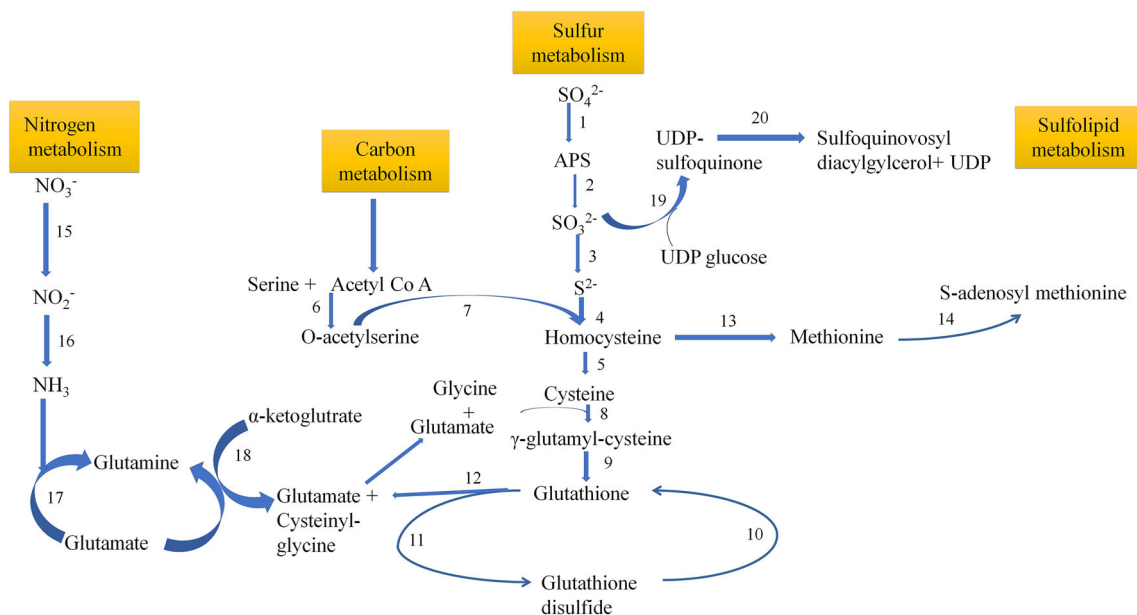


Fig. 3 The putative connection between carbon, nitrogen, and sulfate assimilation: 1. ATP sulfurylase; 2. APS reductase; 3. Sulfite reductase; 4.; 5. Cysteine synthase; 6. Serine acetyltransferase; 7. O-acetylserine thiol lyase; 8. Gamma-glutamylcysteine synthetase; 9. Glutathione synthetase; 10. Glutathione reductase; 11. Glutathione peroxidase; 12.

Gamma-glutamyl synthetase; 13. Homocysteine methyltransferase; 14. S-adenosyl methionine synthase; 15. Nitrate reductase; 16. Nitrite reductase; 17. Glutamate synthase; 18. Glutamine:2-oxoglutarate amidotransferase; 19. UDP-sulfoquinovose synthase; 20. SQDG synthase (adapted from Ravilious et al. 2012; Kumaresan et al., 2017)

Studies have reviewed the assimilation and regulation of sulfur and their roles as well as how this process affect other cellular processes either directly or indirectly in cyanobacteria. In cyanobacteria, cysteine synthesis is a major regulator of different reactions. So far, numerous studies on sulfur metabolism of photoautotrophs have been performed of which maximum are focused on physiology, biochemistry, and transcript analysis. Schmidt (1988) and Schmidt and Jäger (1992) published an in-depth review on cyanobacterial sulfur metabolism. However, the review particularly focusing on the sulfur starvation and its regulation in the cyanobacteria is still lacking.

Cyanobacterial gene expression databases and physiological studies have indicated that genes and enzymes involved in sulfate assimilation are expressed not only in photosynthetic organisms, but also in heterotrophic organisms (Zhang et al. 2008; Ludwig and Bryant 2012; Kolesinski et al. 2017; Kumaresan et al. 2017; Hughes et al. 2018; Kharwar et al. 2021). Transcriptomic analysis of *Synechocystis* by using a DNA array technique have been performed by Zhang et al. in 2008. Similar studies have also been done in case of *Arthrospira platensis* (Kumaresan et al. 2017). Recently, Kharwar and Mishra (2020) performed the expression analysis of some of the crucial genes involved in the photosynthetic and fatty acid metabolic pathway in *Anabaena* sp. PCC 7120 (hereafter referred to as *Anabaena* 7120). Ultrastructures; cell growth, differentiation, and survival; photopigments; protein profile; and nitrogen, carbon, and sulfolipid metabolism are affected by sulfur limitation. These changes can result from sulfur stress-mediated events such as photosynthetic damage, pigment destruction/degradation, protein/enzyme inactivation, reduced protein synthesis, reduced nutrient uptake, changes in redox homeostasis, and signal transduction. Although in-depth reviews are available for sulfur metabolism in bacteria, algae, and plants, particularly those based on model organisms, very few reviews have focused on cyanobacteria.

The present review summarizes sulfur metabolism and its regulation with particular emphasis on cyanobacteria. Here, we provide a comprehensive view of sulfur metabolism and its regulation by gathering patchy information and briefly appraising current updates in sulfur metabolism from physiology to complex processes by integrating morpho-physiological, biochemical, and molecular findings.

Sulfur uptake and assimilation: an overview

Uptake of sulfur

Assimilation of sulfur commences with the uptake of sulfate and then, sulfate is reduced in a sequential multistep process

(Fig. 2). Although sulfate is the most preferable source, alternative sources such as sulfonate, cysteine, methionine, glutathione, and taurine are also utilized by organisms. The model bacterium *E. coli* utilizes cysteine, glutathione, and various alkane sulfonates including taurine. In addition, some cyanobacteria can derive sulfur from sulfate ester (*Plectonema*), ethane sulfonate (*Anabaena variabilis*), methionine (*Synechococcus* 6301 and *A. variabilis*), taurine (*A. variabilis*) (Müller and Schmidt 1986; Biedlingmaier and Schmidt 1987), cysteine (*Synechococcus* 6301), cystine (*Synechococcus* sp.), thiosulfate (*Synechococcus* sp.), thiocyanate (*Synechococcus* sp.), and reduced glutathione (*Synechococcus* 6301) (Schmidt et al. 1982; Lawry and Jensen 1986; Laudenbach and Grossman 1991).

The uptake process of sulfate is a highly regulated as it involves different proteins and function jointly to acquire sulfur from the external environment; these proteins are encoded by a specific set of genes. Uptake of sulfur in case of cyanobacteria is an energy-requiring, light-dependent process and varies with both temperature and pH (Utkilen et al. 1976; Jeanjean and Broda 1977; Utkilen 1982). Metabolic poisons and structural analogs of sulfate such as chromate, selenate, molybdate, and sulfur compounds inhibit sulfate transport (Jeanjean and Broda 1977). The capacity for sulfate uptake increases in the deprived cyanobacterial cells. Many studies have observed that sulfate uptake increased within an hour after the removal of sulfate from growth medium and its sequential decrease after the resupply of sulfur, indicating that a low sulfur concentration induces the expression and activity of sulfate transporters, whereas high sulfur content suppresses them. Low sulfur content is determined by a decrease in the intracellular sulfur pools that cause an increase in nutrient-dependent sulfate uptake. Initial studies on *Synechococcus* PCC 7942 (hereafter referred to as PCC 7942) have demonstrated that V_{\max} value for sulfate transport increases by approximately 10–20 folds during sulfur deprivation, whereas the value of $K_{1/2}$ remains 1 μM in both sulfur-sufficient and -deficient medium, indicating that sulfur deficiency causes increased accumulation of sulfate transporters (Green and Grossman 1988). Utkilen and his colleagues reported a $K_{1/2}$ and V_{\max} of 0.75×10^{-6} M and 0.7 pmol (10^6 cells \times min $^{-1}$), respectively, at 42 °C for sulfate transport in sulfur-starved cells of *Synechococcus* (Utkilen et al. 1976). Once sulfate is transported into the cell, it is reduced through the PAPS sulfotransferase pathway (i.e., bacterial type of sulfate reduction system) in case of *Synechococcus* (Schmidt and Christen 1978), whereas other cyanobacteria reduce sulfate through the APS sulfotransferase pathway (i.e., plant type of sulfate reduction system) (Tsang and Schiff 1975). The specificity of enzymatic activities toward these sulfate donors for further reduction process (APS and PAPS) serve as a useful taxonomic marker for studying the relationship between different groups of cyanobacteria and between cyanobacteria and other

bacteria as well as for examining the phylogenetic problems of plant origin (Schmidt 1977a, 1977b).

Pioneering work identified that the genes such as *cysP*, *cysT*, *cysW*, *cysA*, and *cysM* harbored present on the *cys* operon encode transporter proteins for sulfate uptake in *E. coli* K-12. Activation of these genes requires CysB, an LysR-type autoregulatory protein (Hryniewicz and Kredich 1991). Moreover, the *cys* operon consists of many genes required for sulfur assimilation including enzymes catalyzing the conversion of sulfate to sulfide, OAS synthesis, and its further conversion to L-cysteine. The *cys* operon is regulated through the feedback inhibition of OAS by cysteine along with some activators (Ostrowski et al. 1989).

In cyanobacteria, genes encoding sulfate permease were first identified in PCC 7942 in an analogous region of *S. typhimurium* that contains genes crucial for their growth. A map of this region shown in Fig. 4a indicated that the transcripts of many genes were either undetectable or detected at a very low level in sulfur-deficient cyanobacteria. An increase in the accumulation of mRNA transcribed from these genes is apparent only when the cells are deprived of sulfur. Moreover, in the *S. typhimurium* DNA probe approach was utilized to determine genes involved in transportation; the following genes were determined to be involved: *cysA*, *cys T*, *cysW*, and *sbpA*. Furthermore, to prove the system involved in the sulfate transport, each of these genes was inactivated through mutagenesis, and the results suggested that if genes encoding integral membrane components and nucleotide-binding protein were inactivated, then the mutated cells could no longer transport sulfate; in this case, cells would require an alternate

sulfur source for their growth. By contrast, if *sbpA* was inactivated, then mutated cells grew if sulfate was supplied as the sole source and did not show a marked increase in the transcript of permease genes exhibited by sulfur-deprived wild type cells, suggesting that Sbp is not required for growth (Green et al. 1989; Laudenbach et al. 1991; Laudenbach and Grossman 1991).

The first gene present in the restriction map of a region characterized which encodes a nucleotide-binding protein i.e., CysA of a periplasmic permease system. The transporter subunits namely *cysA*, *cysT*, *cysW*, and *sbpA*, are clustered on the pANL plasmid along with *cysR* (Table 2). This sulfate permease is likely to be the only sulfate transporter in PCC 7942 because the deletion of *cysA*, *cysT*, and *cysW* resulted in no observable growth when sulfate was supplied as the sole sulfur source. Schmidt and Jäger (1992) described the order of genes in PCC 7942: *rhda cysV cysU sbpB cysW cysR orfδ1 cysT sbpA cysA*. The transcription of *cysA* and *sbpA* occurs in the opposite direction. Genes encoding sulfate permease have different arrangements among cyanobacteria (Aguilar-Barajas et al. 2011). Sulfate uptake is a light- and pH-dependent process (Utkilen et al. 1976; Jeanjean and Broda 1977). Ritchie (1996) suggested the presence of ATP-dependent active sulfate transport system rather than symport or antiport (Fig. 4b).

The influx of sulfate in the prokaryotes is carried out by specific ABC-type transporters (Fig. 4b) consisting of a periplasmic substrate-binding protein, a pair of intrinsic channel proteins, and an ATP-binding cytoplasmic protein (Laudenbach and Grossman 1991; Laudenbach et al. 1991). These proteins have conserved domain(s) among

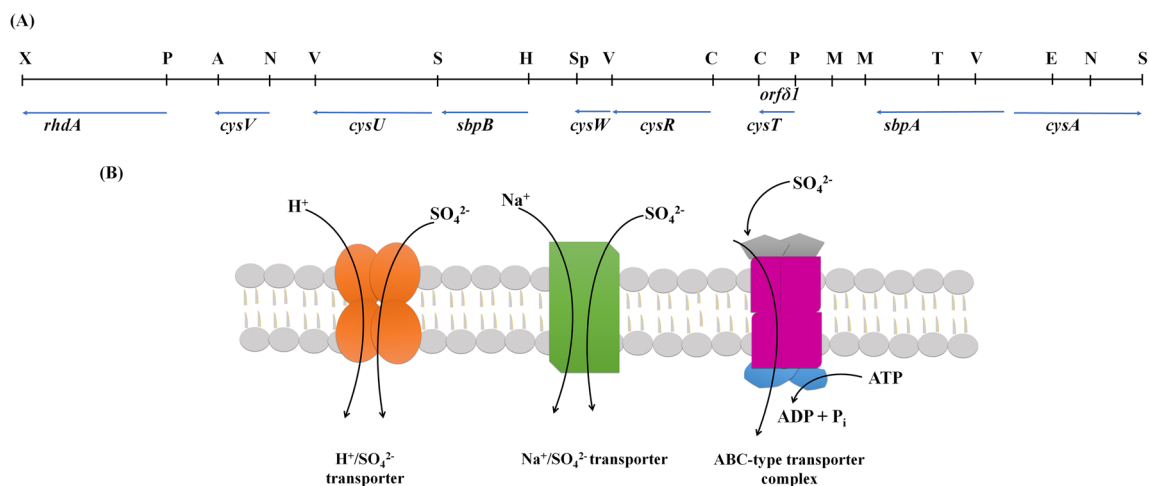


Fig. 4 a Restriction map of sulfate operon of *Synechococcus* PCC 7942 genome. The restriction sites on the map are abbreviated as follows: X, *XbaI*; P *PstI*; A, *ScaI*; N, *NaeI*; V, *EcoRV*; S, *SalI*; H, *HindIII*; Sp, *SphI*; C, *ClaI*; M, *SmaI*; T, *StuI*; B, *BglII*. The positions of open reading frames, their gene designations, and the direction in which they are transcribed (indicated with arrows) are given below in the map. The operon encodes for two periplasmic sulfate binding proteins namely SbpA and SbpB, and four cytoplasmic membrane proteins (CysT, CysU, CysV, and CysW),

and membrane associated ATP-binding proteins CysA. In addition, the *cysR* gene encodes a protein belonging to crp family of prokaryotic the transcriptional regulators; *rhda* encodes a protein that exhibits similarity rohdanese; and *orfδ1* encodes for unknown protein (adapted from Laudenbach et al. 1991). b Transporters involved in sulfur metabolism. The name of the transporters indicated below the suggested sulfate transport mechanisms (adapted from Laudenbach et al. 1991)

Table 2 Comparative accounts of genes involved in sulfur metabolism in *E. coli*, *Bacillus subtilis*, and *Synechococcus* PCC 7942 (adopted from Laudenbach et al. 1991; Sekowska et al. 2000)

<i>E. coli</i>		<i>Bacillus subtilis</i>		<i>Synechococcus</i> PCC 7942	
Genes	Functions	Genes	Functions	Genes	Functions
<i>cysP</i>	Periplasmic thiosulfate binding protein	<i>yvgK</i>	Transport sulfate, thiosulfate; or part of membrane sulfatases transport	<i>cysV</i> , <i>cysU</i>	Thiosulfate binding protein
<i>cysT</i>	Transmembrane protein of sulfate/thiosulfate permease	<i>cysT</i>	Sulfate ABC transporter permease	<i>cysT</i>	Transmembrane periplasmic binding protein
<i>cysW</i>	Transmembrane protein of sulfate/thiosulfate permease			<i>cysW</i>	Transmembrane periplasmic binding protein
<i>cysA</i>	ATP-binding protein of sulfate/thiosulfate permease			<i>cysA</i>	ATP-binding protein sulfate permease
<i>sbp</i>	Periplasmic sulfate binding protein	<i>yugK</i> <i>ytmJ</i>	Sulfate binding Sulfonate binding	<i>sbpA</i>	Sulfate binding protein
<i>cysU</i>	Thiosulfate permease				
<i>cysC</i>	APS kinase	<i>yisZ</i> , <i>ylnC</i>	Adenyl sulfate kinase		
<i>cysH</i>	PAPS sulfotransferase	<i>cysH</i> , <i>yitB</i> <i>ylnA</i> <i>ylnB</i> <i>ylnC</i>	Adenylsulfate reductase, of <i>cysD</i> ; member of “S-box” regulon Sulfate permease, similar to <i>pitA</i> and <i>pitB</i> in <i>E. coli</i> , tetradral anion permease Adenyl transferase (archaeobacteria, eukaryotes, cyanobacteria) Adenylsulfate kinase (archaeobacteria, eukaryotes, cyanobacteria); could be dephospho CoA kinase		
<i>cysE</i>	Serine transacetylase	<i>cysE</i>	Serine acetyltransferase	<i>srpH</i>	Serine acetyltransferase
<i>cysK</i>	O-acetylserine (thiol)-lyase A	<i>cysK</i> <i>ytkP</i> , <i>yrhA</i> , <i>cysK</i> , <i>ytkP</i> , <i>yrhA</i> <i>ygrR</i>	O-acetylserine (thiol)-lyase O-acetylhomoserine (thiol)-lyase O-acetylserine (thiol)-lyase Sulfite reductase (α subunit)	<i>srpG</i>	O-acetylserine (thiol)-lyase
<i>cysM</i>	O-acetylserine (thiol)-lyase B				
<i>cysJ</i>	α subunit of sulfite reductase (flavoprotein)				
<i>cysI</i>	β subunit of sulfite reductase (heme protein)	<i>yvgQ</i>	Sulfite reductase (β subunit)		
<i>cysD</i>	Smaller subunit of ATP sulfurlyase	<i>cysH</i>	Sulfate adenyltransferase		
<i>cysN</i>	Larger subunit of ATP sulfurlyase; GTPase	<i>tufA</i> , <i>lepA</i> , <i>ylaG</i> , <i>infB</i> , <i>fus</i>	Similar to GTP binding proteins, couple with GTP hydrolysis		
<i>metA</i>	β -cystathionase	<i>metB</i>	Homoserine O-acetyltransferase		
<i>metB</i>	γ -cystathionine synthase	<i>yrhB</i> , <i>yicI</i>	Cystathionine synthase or lyases		
<i>metC</i>	Cystathionine γ -synthase	<i>yicI</i> , <i>yrhB</i> , <i>yicI</i>	Several operons of <i>metB</i> in <i>E. coli</i>		
<i>metE</i>	Tetrahydropteroyltryptoliglutamate dehydrogenase	<i>metC</i>	Methionine synthase (cobalamine-independent)		
<i>metH</i>	Homocysteine methyltransferase	<i>yilJ</i> <i>ybgG</i>	Methylene tetrahydrofolate reductase Methionine methyltransferase Not known Not known		
<i>gshA</i>	γ -glutamylcysteine synthase			<i>gshA</i>	γ -glutamylcysteine synthase
<i>gshB</i>	Glutathionine synthase			<i>gshB</i>	Glutathionine synthase
<i>ggt</i>	γ -glutamyltranspeptidase	<i>ggt</i> , <i>yvrD</i>	γ -glutamyltranspeptidase	<i>ggt</i>	γ -glutamyltranspeptidase
<i>gst</i>	Glutathione S-transferase	No similarity		<i>gst</i>	Glutathione S-transferase

Table 2 (continued)

<i>E. coli</i>		<i>Bacillus subtilis</i>		<i>Synechococcus</i> PCC 7942	
Genes	Functions	Genes	Functions	Genes	Functions
<i>tauA</i>	Taurine periplasmic binding protein	<i>ssuB</i>	Arylsulfonate ABC permease (ATP-binding site)		
<i>tauB</i>	Taurine ABC permease (ATP binding site)	<i>ssuC</i>	Arylsulfonate ABC permease (membrane protein)		
<i>tauC</i>	Taurine permease (membrane protein)	<i>ssuA</i>	Arylsulfonate binding lipoprotein		
<i>asl</i>	Arylsulfatase		No similarity		

cyanobacteria and other bacteria (Table 3). One of the components localized in the periplasmic space is a polypeptide that is involved in the binding of the substrate called as substrate-binding protein (Sbp) that has two globular domains separated by a flexible hinge. When sulfate binds to globular domains, the conformation of Sbp changes leading to the bending of the hinge, thus resulting in the trapping of sulfate. Subsequently, a protein–ligand complex interacts with hydrophobic proteins such as CysT and CysW, which span over the cytoplasmic membrane and form a pore. Furthermore, this interaction causes the release of sulfate, which traverses pores and enters into a cell against a concentration gradient through ATP hydrolysis. However, preliminary analysis suggested that CysA constitute sulfate permease may also transport thiosulfate. A mutational analysis of *cysA* was performed to confirm proposed the gene function, and the finding suggested that *cysA* is associated with thiosulfate transport. The thiosulfate transport system can be augmented by binding of thiosulfate to a specific protein such as Sbp and CysP in *E. coli* (Hryniewicz and Kredich 1991). The sequence homology analysis of *E. coli* genes to *cysP* in PCC 7942 suggested its position as immediate downstream of *cysW*. The downstream position of thiosulfate transport genes, designated *rhdA*, encodes a protein that shows similarity to rhodanese, which cleaves the sulfane bond and transfers the thiol group to a thiophilic acceptor molecule (Laudenbach et al. 1991). These findings indicated that the function of *rhdA* is crucial for the survival of cyanobacteria during sulfur deficiency.

A sequence analysis of the sulfate/thiosulfate binding protein of *E. coli* K-12 showed similarities with the Sbp of *S. typhimurium* (Pardee et al. 1966). In addition, in *E. coli*, *cysZ* was identified as a crucial factor for sulfate influx (Parra et al. 1983; Byrne et al. 1988). Sulfate uptake in green alga *C. reinhardtii* commences through transporters present on the membrane and then actively translocated in the chloroplast. *Chlamydomonas* harbors two distinct sulfate transport systems, namely H^+/SO_4^{2-} co-transporters (SULTR), Na^+/SO_4^{2-} transporters (SLT) (Fig. 4b) which are localized and function at plasma membrane and chloroplast envelope (Melis and Chen 2005), respectively. By contrast, sulfate influx in plants occurs through proton/sulfate (H^+/SO_4^{2-}) co-transporters which utilize proton motive force (Lass and Ullrich-Eberius 1984; Smith et al. 1995a) suggested that *Chlamydomonas* diverge from the plant lineage before the loss of Na^+/SO_4^{2-} transporters (Pootakham et al. 2010). Structural analysis of co-transporters revealed that they belong to the intrinsic solute transporter family that contains 12 membrane-spanning domains (Smith et al. 1995b). However, in *Arabidopsis*, sulfate transporters encoded by 12–16 genes are classified into five distinct subfamilies (SULTR1 to SULTR5) based on protein sequence similarities (Takahashi et al. 1997; Takahashi 2010, Takahashi et al. 2011). In *A. thaliana*, three high-affinity sulfate transporters, namely AtSULTR1;1, AtSULTR1;2, and

Table 3 Description of domain configuration and domain superfamily of the transporters involved in sulfur metabolism using Conserve Domain Database of NCBI (<https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>)

S. No.	Species	Protein Domain details			MW (kDa)	Protein length
		Domain	Domain superfamily	Descriptions/features		
1	<i>Syechococcus</i> PCC 7942	CysA ATP binding domain TOBE domain	AAA TOBE2	ATP binding domain Transporter associated OB domain occur as dimer at C terminal end of each domain. Probably involve in the recognition of small ligands such as Mo and SO_4^{2-} . The domains occur after immediately ATPase domain in ABC transporter.	37.8	344
2	<i>Syechococcus</i> PCC 7942	CysT Transmembrane periplasmic binding protein	TM-PBP2	Transmembrane perimease i.e., Periplasmic Binding Protein (PBP-2) dependent ATP-Binding Cassette (ABC) involve in solute uptake like SO_4^{2-} in this case.	30.4	278
3	<i>Syechococcus</i> PCC 7942	CysW Transmembrane periplasmic binding protein	TM-PBP2	Paralogous to CysT in the ABC transporter encoded by CysA involve in SO_4^{2-} uptake.	30.6	286
4	<i>E. coli</i> O25b:H4	CysP Thiosulfate transporter subunit	PBP-LTR substrate	Substrate binding domain, component of ABC transporter complex involves in the uptake of SO_4^{2-} /thiosulfate.	37.6	338
5	<i>E. coli</i>	CysT Sulfate/ thiosulfate transporter subunit	TM-PBP2	Substrate binding domain, component of ABC transporter complex. The protein involves in specific binding and transmembrane transport of thiosulfate.	30.2	277
6	<i>E. coli</i> FVEC1302	CysW Sulfate/ thiosulfate transporter subunit	TM-PBP2	Substrate binding domain, component of ABC transporter complex, involve in specific binding and transmembrane transport of thiosulfate.	32.5	291
7	<i>E. coli</i>	CysA Sulfate/ thiosulfate transporter subunit	AAA	SO_4^{2-} ABC transporter, ATP –binding protein (CysA) is a part of transporter complex CysAWTP and is responsible for energy coupling.	41.0	365

AtSULTR1;3, have been identified through knockout analysis; the functions of proteins are listed in the Table 4. These high-affinity sulfate transporters that have a low K_m value are induced under sulfur limitation. Among them, AtSULTR1;1 and AtSULTR1;2 facilitates sulfate uptake in the root system, whereas AtSULTR1;3 is expressed only in phloem, establishing a source-sink relationship (Yoshimoto et al. 2007; Berberon et al. 2008). Despite the high-affinity of sulfate transporters, AtSULTR1;1 is expressed less in the roots compared with AtSULTR1;2 which is expressed in the root epidermis and plasma membrane of the root cortical cells. AtSULTR1;2 serves as a major sulfate permease because its mutant shows a considerable decrease in the uptake of sulfate (El Kassi et al. 2007). Apart from these sulfate transporters, several low-affinity sulfate transporters (AtSULTR2;1 and AtSULTR2;2) are expressed in vascular cylinders (Takahashi et al. 2012) and facilitate the vertical translocation of sulfate from the root to shoot. AtSULTR2;1 mediates sulfate influx into the xylem parenchyma along with AtSULTR3;5, which acts synergistically with AtSULTR2;1, resulting in the enhanced translocation of sulfate to a larger distance (Kataoka et al. 2004a, 2004b). The low-affinity sulfate transporter i.e., AtSULTR2;1 is expressed in the developing seeds (Awazuhara et al. 2005), whereas AtSULTR2;2 is expressed and controls sulfate influx into the companion cells (Takahashi et al. 2000). In additionally, two sub-families of the SULTR4 transporter i.e., AtSULTR4;1 and AtSULTR4;2 are responsible for the remobilization of vacuolar sulfate to the cytosol (Kataoka et al. 2004b). Moreover, SULTR5 lacks both N- and C-terminal hydrophilic domains common to other transporter sub-families that have the STAS region and perhaps perform regulatory function. This transporter subfamily also contains a molybdenum transporter, namely MOT1 (SULTR5;2) (Tomatsu et al. 2007). However, knowledge regarding plastidic sulfate transporters in plants is limited, although studies have reported that plastidic sulfate transporters in green algae *C. reinhardtii* belong to ABC-type transporters (Lindberg and Melis 2008), reflecting the theory of endosymbiotic association.

Reduction of sulfate

Sulfate reduction commences with its uptake and ultimately leads to the biosynthesis of sulfur-containing metabolites (Fig. 5). Although the sulfate assimilation process is similar, the cellular localization process differs between prokaryotes and eukaryotes. Prokaryotes assimilate sulfate in the cytosol, whereas eukaryotic phototrophs assimilate sulfate in the membrane-bound organelles (plastid and mitochondria) and cytosol. Moreover, sulfate assimilation in *C. reinhardtii* is performed in the chloroplast. Similar to other bacteria and cyanobacteria, higher plants also possess all enzymes involved in sulfate acquisition, namely ATPS, APSK, APS

reductase (APR), PAPS sulfotransferase, SR, and OAS-TL indicating that assimilation is likely to be conserved among these life forms (Table 4).

Genes involved in sulfur assimilation are well described in other bacteria (Table 2). These genes are harbored on the chromosomes in the order *cysCNDHIIJ* in *E. coli* K-12, whereas they are arranged in a different order i.e., *cysJIHDC* in *S. typhimurium*. Expression of these genes is controlled by CysB in *E. coli*, which is in contrast to cyanobacterial sulfur assimilation where genes involved in sulfur metabolism are controlled by the regulatory protein, CysR that belongs to the Crp superfamily of transcriptional regulator (Nicholson et al. 1995; Nicholson and Laudenbach 1995).

The standard reduction potential for the conversion of sulfate to sulfide is -454 mV, which is higher and beyond the range of biological reduction potential (Thauer et al. 1977). In the first step, sulfate must be activated before it can be reduced. In the bacterial system, ATPS acquires a tetrameric form, which consists of two regulatory and two catalytic domains. The regulatory domain, *cysN* (62 kDa) exhibits GTPase activity and hydrolyzes one GTP molecule for each APS formed, whereas the catalytic domain, *cysD* (27 kDa) is involved in a thermodynamically unfavorable reaction. The activation of sulfate catalyzed by ATPS was found to be inhibited by AMP, ADP, and inorganic phosphate in *A. cylindrica*, *A. platensis*, and *Synechococcus* 6301 (Schmidt 1977b, 1979; Menon and Varma 1978). Patron et al. (2008) performed a phylogenetic analysis of ATPS and observed that cyanobacteria and viridiplantae are not monophyletic. The tree depicted that the lateral gene transfer of ATPS among cyanobacteria rather than the cyanobacterial origin of enzymes in plastids plays a key role in the redox regulation (Prioretti et al. 2014).

APS, a product formed by ATPS, serves as a critical metabolic branch point channeling sulfur to cysteine (primary pathway) or PAPS (secondary pathway), where PAPS further serves as a substrate for the sulfation of metabolites (Giordano and Raven 2014). Assimilatory reduction of sulfur occurs either through the APS or PAPS pathway depending on the organisms. The findings of phylogenetic analysis indicated that in *Plectonema* strain 73,110, APS reductase lies in the same branch as plant APS reductases (implying that plants obtained genes for APS reductase from a chloroplast ancestor; cyanobacterial genes were then allocated to the plant nuclear genome through endosymbiotic gene transfer), but not as PAPS reductases from other cyanobacteria (*Synechococcus* and *Synechocystis*). Horizontal gene transfer plays a crucial role in the distribution of APS and PAPS. Furthermore, the APS-dependent pathway is an ancestral pathway because APS reductase was present in the ancient sulfate-reducing microbes which require one less ATP in the APS-dependent pathway than in the PAPS-dependent pathway (Kopriva et al. 2002). The primary pathway mainly occurs in PCC 7942, whereas

Table 4 Comparative accounts of proteins involved in sulfur metabolism in *Synechococcus* PCC 7942, *C. reinhardtii*, and *A. thaliana* (adopted from Saito 2004; Melis and Chen 2005)

<i>Synechococcus</i> PCC 7942	<i>C. reinhardtii</i>	<i>A. thaliana</i>	Descriptions
CysT, CysW, CysA, sbp	SulP, SulP2, Sabc, Sbp, Sultr1, Sultr2, Sultr3, SAC-1-like	SULTR1;1, SULTR1;2, SULTR1;3, SULTR2;1, SULTR2;2, SULTR3;1, SULTR3;2, SULTR3;3, SULTR3;4, SULTR3;5, SULTR4;1, SULTR4;2, SULTR5;1, SULTR5;2	ABC transporter type-sulfate transporter
ATPS	ATSI, ATS2	APSI, APS2, APS3, APS4	ATP sulfurylase (ATPS)
APSK	AKN2	AKN1, AKN2, APS kinase	Adenylylsulfate kinase2 (APSK)
APR	APR1	APR1, APR2, APR3	APS reductase (APR)
SR	SIR1, SIR2, SIR3	SIR	Sulfite reductase (SIR)
SAT	SAT1, SAT2, SAT3	SAT1, SAT2, SAT3, SAT4, SAT5	Serine acetyl transferase (SAT)
OAS-TL	OASTL1, OASTL2, OASTL3, OASTL4	OASA1, OASB, OAS-TL	O-acetyl serine (thiol) lyase (OAS-TL)
MS	METH, METE	ATMS1, ATMS2, ATMS3	Methionine synthase (MS)
GshA	GSH1	GSH1, RML1, CAD2, PAD2	Glutamino-cysteine lyase (GCL)/ γ -glutamylcysteine synthetase (γ ESCS)
GshB	GSH2	GSH2	Glutathione synthetase
PCS	PCS	AtPCS1	Phytochelatins synthase
Not known	SUOX1	SOX	Sulfite oxidase
CGS	METC	CGS, CGS1, MTO1	Cystathionine β -lyase (CGS)
CBL	Not known	CBL	Cystathionine β -lyase (CBL)

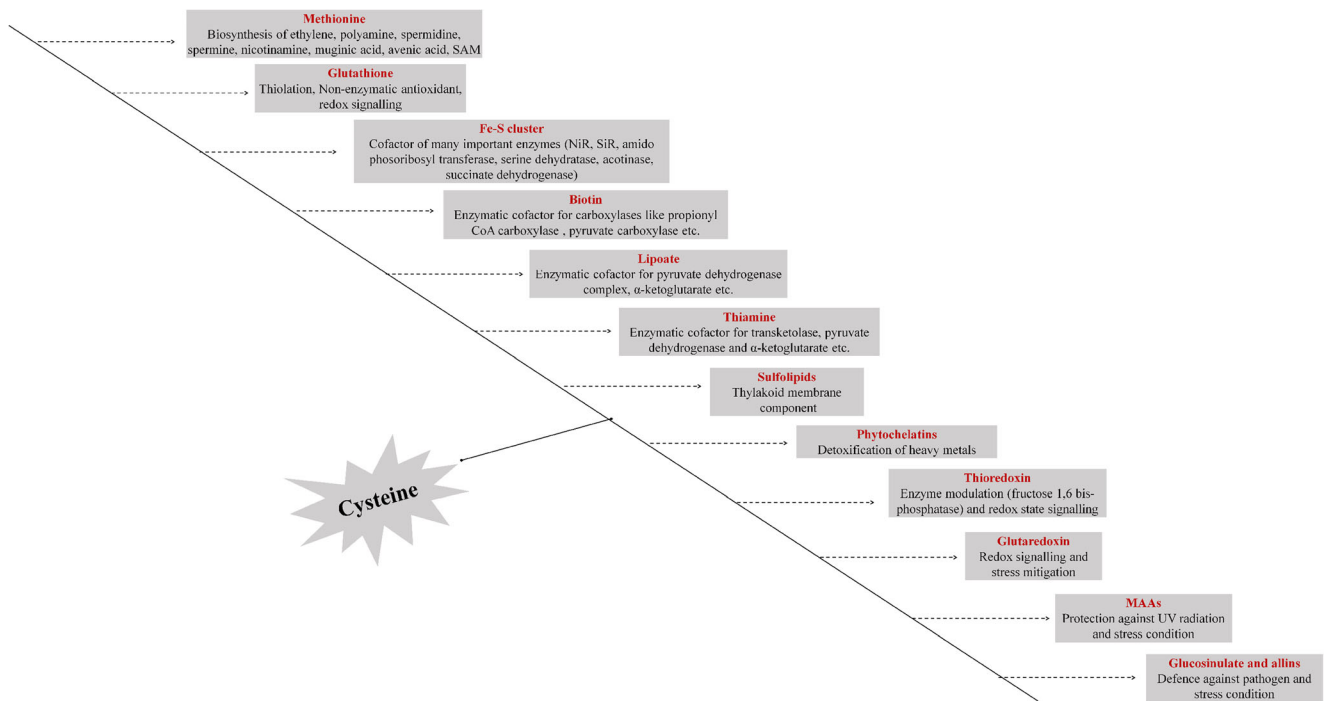


Fig. 5 A schematic representation of the involvement of sulfur in the biosynthesis of sulfur-containing metabolites. Sulfur-containing metabolites are directly involved as constituents or indirectly as cofactors, prosthetic groups, methyl group donor, or generally as integral part of proteins and enzymes

the PAPS pathway occurs in all cyanobacteria (Tsang and Schiff 1975). Patron et al. (2008) performed a chemical analysis and observed APS reduction in *Symploca*, *Nodularia*, *Leptolyngbya*, and *Chroococcus*. And also suggested that APR phylogeny supports the theory of endosymbiotic origin.

APS is reduced to sulfite and subsequently to sulfide by APSR and SR, respectively. Conversion of sulfite to sulfide requires six electrons from NADPH in non-photosynthetic bacteria. However, in cyanobacteria, SR activity was observed in the presence of reduced ferredoxin, suggesting similar coupling of SiR and reducing power with green algae and plants (Schmidt 1979). SR consists of α - and β -subunits encoded by *cysJ* and *cysI* with the $\alpha_8\beta_4$ structure, respectively (Siegel and Davis 1974). The α -subunit contains four FAD, four FMN, and NADPH-cytochrome c reductase activity, whereas the β -subunit consists of four Fe_4S_4 clusters and one siroheme group (Siegel and Davis 1974; Siegel et al. 1973; Siegel et al. 1974). Within the process, electron flow from NADPH to sulfite in the following sequential manner: $NADPH \rightarrow FAD \rightarrow FMN \rightarrow Fe_4S_4 \rightarrow$ siroheme \rightarrow sulfite (Siegel et al. 1974).

Cyanobacteria shares probable homology in cysteine biosynthesis with other life forms

The assimilatory pathway proceeds toward cysteine biosynthesis from OAS and sulfide, catalyzed by OAS-TL. The production of OAS from serine and acetyl-CoA is catalyzed by SAT and regulated by cysteine through feedback inhibition. In

E. coli, SAT is encoded by *cysE*, whereas in PCC 7942, SAT is encoded by *srpH* harbored on the pANL plasmid (Nicholson et al. 1995). Furthermore, Kredich and his co-workers reported that *cysK* and *cysM* encoded OAS-TL A and OAS-TL B, respectively. By contrast, *srpG* harbored on the plasmid of PCC 7942 encodes a single form of OAS-TL (Nicholson and Laudenbach 1995). In 1981, Diessner and Schmidt (1981) proposed the presence of two distinct cysteine synthases, cysteine synthase I and II, in *Synechococcus* 6301 that are involved in the formation of cysteine, like cysteine synthase complex (CSC) in *E. coli*.

Pathways downstream of cysteine: sulfur-containing metabolites

Cysteine is the first molecule involved in the synthesis of sulfur-containing metabolites such as methionine (Met), glutathione (GSH), thioredoxin (Trx), and glutaredoxin (Grx) (Fig. 5). The thiol group of cysteine forms a disulfide bridge between two cysteine residues and play a role in maintaining the tertiary structure of the proteins. Similar to cysteine, Met is involved in translational processes. In addition, Met acts as a universal methylating agent for nucleic acids, proteins, and several other metabolites through SAM formation. GSH, a tripeptide composed of cysteine, glycine, and glutamic acid that has a thiol group of cysteine residue as an active group, is synthesized through the sequential action of two ATP-requiring enzymes. First, γ -glutamylcysteine synthetase encoded by *gshA* forms γ -

glutamylcysteine to which glutathione synthase (encoded by *gshB*) add glycine and produce GSH. Furthermore, GSH is catabolized by γ -glutamyl transpeptidase (*ggt*), releasing cysteinyl-glycine dipeptide and transferring the glutamyl part to either water, thereby regenerating glutamate or amino acids producing glutamate-containing dipeptides (Narainsamy et al. 2016). GSH serves as a redox buffer and stabilizes cellular homeostasis based on the interconversion between reduced and oxidized forms.

Trx comprise a crucial antioxidant protein family. Trx was recently identified in cyanobacteria (Gleason 1994) and exhibits two distinct types: T1, thioredoxins similar to bacterial thioredoxins (in terms of structure and redox activity). The other protein (T2) is relatively unstable and appears to be unique among cyanobacteria. Several metabolic pathways such as carbon dioxide fixation, carbon catabolism, and nitrogen metabolism are redox regulated through Trx. Similar to Trx, another redox-regulated protein, Grx, also has two different types: class 1 and class 2. Class 1 forms a classical dithiol group and class 2 contains monothiols based on the number of cysteine residues present in their active site (Couturier et al. 2009, 2011). Grxs play crucial role in the various cellular processes such as photosynthesis, respiration, iron homeostasis, sulfur and nitrogen assimilation, chlorophyll catabolism, apoptosis, and the survival, growth, and fitness of the organism under fluctuating environment (Rouhier et al. 2010; Li and Outten 2012).

Phytochelatin abbreviated as PC (a cysteine-rich peptide), a polymer of γ -glutamylcysteine with the terminal glycine residue found in all the organisms, is synthesized non-ribosomally by PC synthase (PCS). Synthesis of PC is a two-step process. The first step involves the cleavage of glycine from GSH and the generation of γ -Glu-Cys. In the second step, the formed γ -Glu-Cys unit is transferred to an acceptor molecule that is either GSH or an oligomeric PC peptide to generate PC_{n+1} (Grill et al. 1989). PC has been ascribed as a chelator compound that detoxifies heavy metals (Howe and Merchant 1992) and is involved in metal tolerance and xenobiotic metabolism (Gekeler et al. 1988).

Sulfur is needed for the production of redox active Fe–S centres. The Fe–S cluster is the most ancient and functionally versatile prosthetic group. In brief, S and Fe in metabolism meet at the scaffold protein, which provides a molecular platform to form the Fe–S cluster. Three biogenesis machineries for Fe–S biosynthesis have been proposed in prokaryotes: NIF system specialized in the assembly of enzyme complexes such as nitrogenase; ISC system responsible for the generation of the majority of cellular Fe–S proteins, and the SUF system, an independent assemblage used preferentially under oxidative and Fe stress (Zheng et al. 1998; Patzer and Hantke 1999). Fe–S clusters have a wide range of redox potential (–500 to +300 mV) and are thus involved in transfer of electron (Meyer 2008) in the biological processes.

Despite conventional phospholipid, sulfur-containing lipid (sulfolipid) is found in the most of photosynthetic and few non-photosynthetic organisms (Harwood and Okanenko 2003). Sulfolipid i.e., sulfoquinovosyldiacylglycerol (SQDG) is a glycolipid characterized by 6-deoxy-6-sulfoglucose head group at the *sn*-3 position of the glycerol backbone of diacylglycerol (Sato 2004). Synthesis of SQDG requires two enzymes: (i) UDP-SQ synthase responsible for the biosynthesis of the donor head group and (ii) SQDG synthase that catalyzes the final assembly of sulfolipid. Sulfolipid found in the thylakoid membrane have been reported in cyanobacteria except *Gloeobacter violaceus* which lack thylakoid and perform “thylakoid reactions” in its cell membrane (Selstam and Campbell 1996; Sato et al. 2017). In addition, SQDG helps in maintaining the structural and functional integrity of the membrane by stabilizing the pigment complex (Barber and Gounaris 1986; Sato et al. 1995; Sato et al. 2003a, b; Sato et al. 2004).

Regulation of sulfur metabolism

Regulation of sulfur metabolism is based on its availability and demand, which are regulated at multiple levels to ensure intracellular homeostasis (Saito 2004). In prokaryotes, sulfur uptake is commenced by ABC-type transporters encoded by set of genes that are localized on *cys* operon (Table 4). However, gene encoding transporters, namely H⁺/SO₄²⁻ co-transporters and ABC-type transporters, have been identified in *Synechocystis* 6803 (Kohn and Schumann 1993; Kaneko et al. 1996), thus raising questions regarding the coordination of functions between these two transporters. Moreover, the plants utilize H⁺/SO₄²⁻ co-transporter to acquire sulfate.

Green et al. (1989) laid down the foundation to understand the regulation of sulfur metabolism in cyanobacteria; in addition, they identified ABC-type transporters that act as sulfate permease in *Synechococcus* 6301 and anticipated an elevated level of sulfate transport upon sulfur starvation. Permease activity increases with an increase in the V_{max} value, whereas the value of K_m remains constant. Furthermore, Laudenbach et al. (1991) described that the accumulation of transcripts encoding sulfate permease is responsible for increased V_{max} value. A 33 kDa polypeptide, rhodanese, was found to be a crucial factor and accumulate under sulfur deficiency. This protein encoded by *rdhA* harbored on the *cys* regulon shows thiosulfate reductase activity and is involved in thiol transfer from thiosulfate to an acceptor molecule. Moreover, further characterization of genes encoding sulfate permease in PCC 7942 showed that *cysA*, *cysT*, and *cysW* mutants failed to uptake sulfate under sulfur starvation, thus conferring their role. Cyanobacteria possess strong potential orthologs of *cysT* in viridiplantae and are considered as an ancestor (Lyubetsky et al. 2013). Laudenbach and Grossman (1991) mutated *sbpA* and *cysR* genes and indicated that *sbpA*

and *cysR* function as a sulfate binding protein and regulator, respectively. CysR shows structural similarities to NtcA and BifA, allows its DNA binding, and further regulates the transcription of 36 kDa periplasmic protein (i.e., sulfate permease) (Nicholson and Laudenbach 1995). CysR contains SPXX motif located at its N-terminal domain (Churchill and Suzuki 1989; Suzuki et al. 1993) that is involved in DNA binding at the AT-rich regions and further guide sulfur metabolism. Insertional inactivation of *cysR* prevents an increment in the V_{\max} value of sulfate permease upon sulfur deprivation compared with its wild type (Laudenbach and Grossman 1991).

Similar to cyanobacteria, sulfate uptake increases in other bacteria because of the accumulation of transporters transcript under sulfur limitation. Transporters in *E. coli* are encoded by *cysPUWAM* operon and are homologous to cyanobacterial *cysATW* operon (Table 4). Regulation of sulfur metabolism in *E. coli* is achieved through two levels: the first level involves, inhibition of SAT encoded by *cysE*, whereas the second level involves the allosteric inhibition of CysB through sulfide and thiosulfate (Hryniewicz and Kredich 1991; Kredich 1966). CysB function as an analog to CysR for the derepression of *cys* regulon upon sulfur starvation. CysB acts either positively or negatively depending on presence or absence of N-acetyl serine (NAS) (Kredich 1966; Parry and Clark 2002). Transcriptional studies have revealed that the DNA binding site of CysB lies upstream of promoter –35 region, and the interaction of CysB to major activator sites allows stable conformation and recruitment of RNA polymerase, leading to the transcription of genes (Guédon and Martin-Verstraete 2006). Although CysB acts as a positive regulator, it represses its own transcription by blocking the binding site of RNA polymerase due to the overlapping of CysB with the promoter of its own gene (Kredich 1996). Such autoregulation of CysR is not yet reported in cyanobacteria. The three-dimensional structures of regulators generated using I-TASSER server (<https://zhanglab.ccmb.med.umich.edu/I-TASSER/>) are shown in Fig. 6, and the quality of models were evaluated using the RAMPAGE plot (<http://mordred.bioc.cam.ac.uk/rapper/rampage.php>). Furthermore, String (<http://string-db.org/>) network analysis was performed to understand the spectrum of protein–protein interactions for CysB and CysR that act as query protein (Fig. 7). CysB interacts with different proteins such as RpoA, CysE, and TopA, possibly exhibiting DNA-dependent RNA polymerase activity; catalyzes the transcription of DNA into RNA; participates in cysteine biosynthesis, and exhibits DNA topoisomerase activity. Several other proteins, namely CysD, CysH, CysJ, TauA, CysP, and CysI, involved in sulfur assimilation also interact with CysB, indicating that all possible interactive partners are regulated by CysB. However, CysR interacts with CysW and SbpA, which are involved in the sulfate transport system. In addition, CysR interacts with other proteins such as

CysA, SrpH, CysE, SrpG, and CysT, suggesting the regulatory role played by CysR.

Similar to inorganic sulfur compounds, the assimilation of aliphatic sulfur (i.e., sulfonate and taurine) is regulated by CysB interacting with *ssu* and *tau* operon, respectively. The *tauABCD* and *ssuEADCB* operons of *E. coli* are induced in the absence of preferred sulfur sources such as inorganic sulfate, thiosulfate, and cysteine, whereas their expression is suppressed in the presence of these sources. Such phenomenon is termed sulfate starvation response, which are controlled by two transcriptional regulators, namely CysB and Cbl. No direct evidence is related to the binding of CysB to the *ssu* promoter, although it exerts a direct effect on *tau* operon in vivo (Van Der Ploeg et al. 2001). Furthermore, Iwanicka-Nowicka and Hryniewicz (1995) reported a second regulator, Cbl, regulated by CysB. In 2002, Bykowski and his colleagues reported that the direct interaction of Cbl with the –35 promoter region of both operons and activate gene transcription (Bykowski et al. 2002). By contrast, negative regulation of APS by Cbl is reported by the same research group. However, such a descriptive illustration for the regulation of sulfonate and taurine assimilation are not known in cyanobacteria. Moreover, knowledge regarding sulfonate and taurine assimilation in cyanobacteria is majorly confined to the work of Biedlingmaier and Schmidt (1987), who depicted that the growth of cyanobacteria depends on ethane sulfonate and taurine as the source of sulfur. Under sulfur limitation, *A. variabilis* uses ethane sulfonate and taurine, whereas no such apparent uptake was detected in *Synechococcus* 6301, indicating that some cyanobacteria possess sulfonic acid permease and thus utilize sulfonate and taurine as sulfur source. Recently, genome analysis of *Vulcanococcus limneticus* indicated that cyanobacteria utilize alkane sulfonate through two alkanesulfonate monooxygenases and six sulfonate ABC transporters (TauABCD and ssuEADCB) (Di Cesare et al. 2018).

Symbiotic cyanobacteria such as *Nostoc*, showed enriched functions for the transport and metabolism of organic sulfur compounds. Recently, Warshan et al. (2018) performed transcript profiling to identify gene families present in *Nostoc* and observed that domains involved in sulfur metabolism include putative aliphatic sulfonate transporters, substrate binding proteins (alkanesulfonate monooxygenase), and a putative aliphatic sulfonate monooxygenase. Up-regulation of these proteins during physical contact in the symbiont-component strains indicated that aliphatic sulfonate act as “currency” and are involved in symbiosis, reflecting that feathermoss probably transfers aliphatic sulfonate to the cyanobiont in exchange for fixed nitrogen (Warshan et al. 2017; Warshan et al. 2018).

The regulatory mechanism of *C. reinhardtii* is distinct from that of cyanobacteria because three genes, namely *sac1*, *sac2*, and *sac3*, are involved in the sulfur assimilation. Mutational analysis of these genes showed aberrant arylsulfatase activity

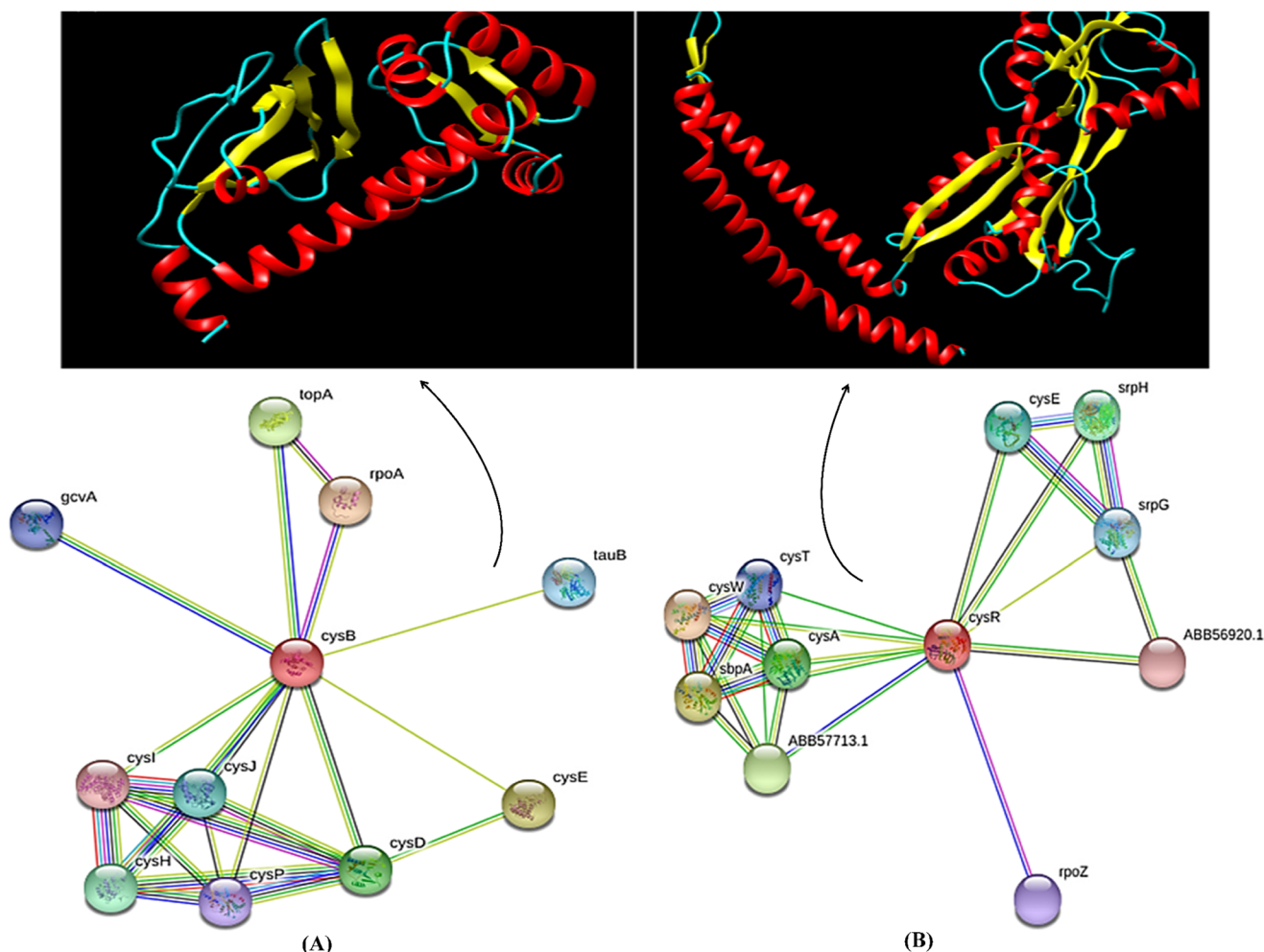


Fig. 6 3D structure of the regulatory proteins: **a** CysR (*Synechococcus* PCC 7942) and **b** CysB (*E. coli*) generated by I-TASSER server. The models were further refined by two-step atomic-level energy minimization based on C α traces using ModRefiner (<http://zhanglab.ccmb.med.umich.edu/ModRefiner/>). These proteins consist of α helix (red), β -strand (yellow), and random coil/loops (cyan). The secondary structure analysis revealed that CysR and CysB have C-score value of -0.08 and -0.04 , respectively. Ramachandran plot was used for the validation of the predicted models by measuring the backbone dihedral phi (ϕ) and psi (Ψ) angles. Quality of the generated models were evaluated by RAMPAGE

server (<http://mordred.bioc.cam.ac.uk/rapper/rampage.php>) showed 97.1% (198 amino acid residues) lies in favored region, 2% (4 amino acid residues) in allowed region, and 1% (2 amino acid residues) lies in the outlier region for CysR protein; whereas 93.2% (300 amino acid residues) lies in favored region, 5.6% (18 amino acid residues) in allowed region, and 1.2% (4 amino acid residues) lies in the outlier region for CysB protein indicating stability of the models. Functional interactive networks of CysB (A) and CysR (B) with other proteins using STRING database

in the mutant compared with its wild type. SAC1 has been characterized and appeared to play a crucial role in the regulation of sulfur assimilation because its mutation blocks APSR transcription (Davies et al. 1994, 1996; Wykoff et al. 1998). Nevertheless, SAC1 controls the distribution of resources under sulfur limitation by sensing the intracellular sulfur status. Moreover, *sac1* controls cell wall reorganization by inducing the expression of proteins upon sulfur deficiency. Furthermore, SAC2, a second regulator, acted at translational and post-translational levels (Shibagaki and Grossman 2008) in a SAC2 mutant and showed the accumulation of APSR transcript with no significant increase in its protein level (Ravina et al. 2002). However, SAC3 is considered a putative SFN1-like serine–threonine kinase; however, it does not exert

any significant effect on the transcription of genes involved in sulfur assimilation (Davies et al. 1999).

In recent years, increasing attention has been focused on plants for examining molecular mechanisms underlying sulfur metabolism. Metabolic products of sulfur metabolism, such as GSH and OAS, are considered which play central role in the signaling pathway and elicit several regulatory responses including the expression of most of the genes encoding sulfate transporters and sulfur assimilation enzymes. OAS does not perform a parallel role as NAS in prokaryotes (Hopkins et al. 2005; Davidian and Kopriva 2010). Moreover, no correlation has been demonstrated between the transcript levels of high-affinity sulfate transporters (*AtSULTR1;1* and *AtSULTR1;2*) with intracellular OAS concentration in *A. thaliana* (Rouhier

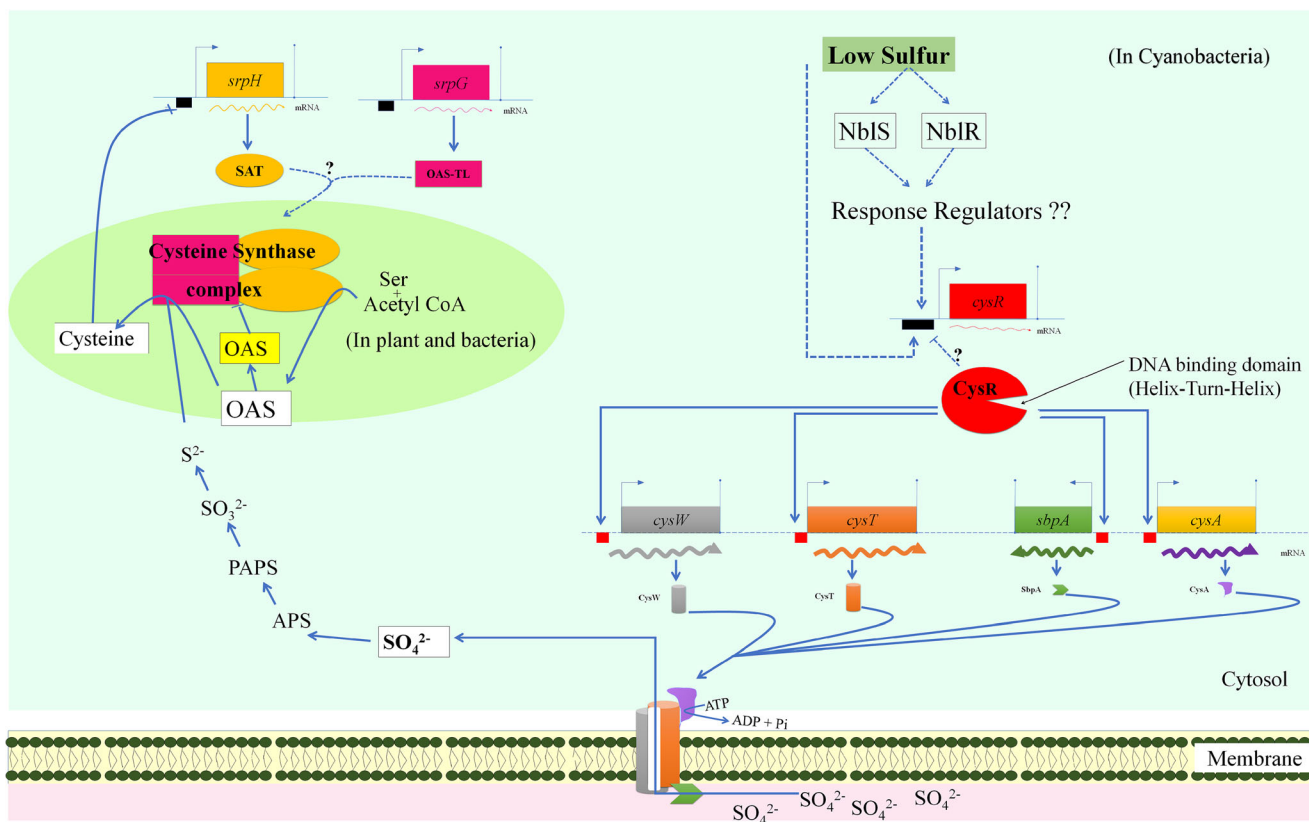


Fig. 7 An integrated view of probable regulatory mechanism of sulfur assimilation in cyanobacteria, showing activation of CysR under sulfur limited condition; CysR induced accumulation of transporter proteins transcripts under low intracellular sulfur concentration. The thickness of transcript shows its accumulation (shown in bold). Solid arrow indicates the known mechanism prevails in the cyanobacteria whereas dotted arrow indicated possible mechanisms that may occur. The green zone highlights the regulation of sulfur metabolism which is known in plant and bacteria but not reported in cyanobacteria, though, OAS-TL and SAT perform similar catalysis in cyanobacteria. Regulation of sulfur metabolism in

cyanobacteria triggered by deprivation of sulfur inside the cell and sense by NblS-NblR. NblS-NblR is homologues of histidine kinase sensors and response regulators of two component signal transduction pathway. Dotted arrow indicates as yet unknown response regulatory pathway, whereas yellow and green colors indicated the pool of OAS, regulatory process inside the plant and bacteria. NblS, sensor kinase; NblR, response regulator; *cysA*, *cysT*, *cysW*, and *shpA* encodes ATP binding domain; transmembrane periplasmic binding protein, transmembrane periplasmic binding protein; sulfite binding protein, respectively, ** shows increased accumulation

et al. 2010). Despite showing a similar response under sulfur limitation, regulatory mechanism of plants is more complex, consisting of several regulators coordinating together at multiple levels, unlike *cys* regulon-mediated regulation as observed in other bacteria and cyanobacteria. Maruyama-Nakashita et al. (2005) described SURE as a *cis*-acting element with 16 bp sequences in *A. thaliana*. Maruyama-Nakashita et al. found that the gene of AtSULTR1;1 harbored SURE element in its promoter. However, Howarth et al. (2009) depicted another *cis*-acting element (i.e., *sdi-1*) occupying a similar position in the promoter as the SURE element. Moreover, Maruyama-Nakashita et al. (2005) reported that SLIM, another transcriptional regulator, induces the genes of AtSULTR1;1, AtSULTR1;2, and AtSULTR4;2 upon sulfur limitation. The inducibility of AtSULTR1;1 is higher than that of AtSULTR1;2 because AtSULTR1;2 lacks the SURE element in its promoter, suggesting that SURE as an element induces high-affinity sulfate transporters under sulfur stress condition.

In plants, sulfur assimilation is dependent on APSR expression, which acts as a link between sulfur and nitrogen metabolism. Reduced sulfur compounds such as hydrogen sulfide, cysteine, and GSH inhibit APSR, whereas sulfur limitation restores APSR. In addition, Brunold and Suter (1984) observed that nitrogen starvation represses APSR, whereas ammonium and amino acid induces it. Kopriva and Koprivova (2004) described similar inducibility of APSR under sulfur and nitrogen stress condition. Other genes such as SAT and OAS-TL are strongly regulated by protein–protein interactions forming CSC which are feedback inhibited by OAS through the dissociating enzyme complex (Riemenschneider et al. 2005). Sulfide strengthens the association of both SAT and OAS-TL, thus suggesting that cysteine biosynthesis occurs only in the presence of adequate sulfide. This substrate-level regulation of CSC was observed in *E. coli* (Kredich et al. 1969). However, in cyanobacteria, CSC was not reported, despite the presence of SAT and OAS-TL, indicating that substrate-level regulation or protein–protein

interactions between these two proteins suggest the presence of a unique regulatory pathway. Figure 7 shows an integrated view of sulfur assimilation in cyanobacteria. Although we have summarized the role of CysR, there are still unanswered questions concerning their elaborative mechanism of action that need further investigations. Little is known regarding the functional role of CysR. Because CysR acts as a key regulator of sulfur homeostasis by controlling sulfur acquisition, elucidation of other targets should provide novel insights into sulfur-dependent biochemical processes.

Metabolic adjustments upon sulfur stress

Schwarz and Forchhammer (2005) proposed two forms of state response upon nutrient limitation: general and specific. Rapid anabolism and arrested catabolism are considered as a general response, whereas specific response include modulation of metabolic circuits. These responses are either short- or long-term depending on the nutritional status (Hirai and Saito 2004). Likewise, sulfur deficiency elicits both short- and long-term as well as general and specific responses based on the magnitude of deficiency. Transcriptome profiling in *A. platensis* upon sulfur starvation showed that genes involved in the Fe–S cluster, amino acid biosynthesis, protein folding, translational machineries, and ribosomal assembly were down-regulated, whereas genes related to carbohydrate metabolism, phosphor-relay sensor kinase, membrane proteins, and DNA repair mechanisms were up-regulated (Kumaresan et al. 2017). In addition, the corresponding down-regulation of chaperon molecules, adenylylsulfate kinase, tyrosinephenol lyase, aspartate aminotransferase, adenosylhomocysteinase, and phosphate acetyl transferase were observed, suggesting changes in different cellular metabolism upon sulfur limitation. Some of the pronounced effects of sulfur deprivation on the various metabolic processes are as follows:

Effect of sulfur deprivation on the growth, ultrastructures, and cellular inclusions

A reduction in sulfur not only reduces cyanobacterial growth (Kharwar et al. 2021) but also alters the cellular ultrastructure (Allen 1984; Ariño et al. 1995). Specific responses upon sulfur deficiency in cyanobacteria include the accumulation of glycogen and polyphosphate bodies mimicking the responses of nitrogen limitation (Lawry and Simon 1982). Likewise, deposition of phosphate granules was observed in few bacteria and red alga (Callow and Evans 1979). Moreover, *C. reinhardtii* synthesizes polyphosphate granules under sulfur limitation (Ruiz et al. 2001). Deposition of condensed phosphate in *Synechococcus* prevent nutrient imbalance, and the concurrent loss of orthophosphate from growth media reflects phosphorus uptake by cyanobacteria (Lawry and Jensen 1979). Additionally, polyphosphate was significantly

increased in *Synechococcus leopoliensis* upon sulfur limitation (Jensen and Rachlin 1984). A similar response shown by *Klebsiella aerogenes* (*Enterobacter aerogenes*) indicated the reciprocity of uptake and assimilation which exist between orthophosphate and sulfate.

Similar to many photosynthetic organisms, cyanobacteria produce polyhydroxybutyrate (PHB), a stored product (Hai et al. 2001). Granules of polyphosphate and lipid bodies containing β -hydroxybutyrate have been reported in cyanobacteria with photoautotrophic PHB production reaching less than 10% of dry cell weight (Bhati et al. 2010). In *Aulosira fertilissima*, the PHB content increased by 8.7% of dry cell weight upon sulfur deprivation (Samantaray and Mallick 2015), whereas *Gloeothece* PCC 6909 accumulated sulfate in its sheath during sulfur deficiency (Günel et al. 2019). A study reported concomitant repression of total cellular proteins and up-regulation of transcript levels and/or translated proteins involved in the biosynthesis of PHB, namely *phaA–C* and *phaE* for class III PHB synthase upon sulfur starvation (Hirai et al. 2019). An ultra-structural assessment of sulfur-deficient and -replete cells of cyanobacteria revealed morphological alterations such as structure-less sheath; cell wall thickening; and accumulation of cyanophycin, PHB, polyphosphate, and glycogen granules alongwith prominent reduction and/or disintegration of the thylakoid membrane. When PCC 7942 was grown in the presence of sulfate, 3–4 prominent concentric rings of the thylakoid membrane were produced, whereas the membrane was reduced to a single layer in sulfur-starved cells.

Under prolonged sulfur limitation, all vegetative cells eventually differentiated into akinetes in *Nostoc ANTH* (Kyndiah and Rai 2006), demonstrating sulfur limitation as a better circumstance to trigger akinete formation. In addition, reduction in cell size and subsequent programmed cell death of *Anabaena* 7120 were observed upon sulfur limitation (Kharwar and Mishra 2020).

Sulfur limitation alters photosynthesis and fluxes of central carbon metabolism

The optimum availability of sulfur is a pre-requisite for photosynthesis because the process displays a high demand for sulfur compounds; therefore, sulfur limitation exerts inhibitory effects on the process. Sulfur deprivation leads to the degradation of pigment displaying chlorosis/bleaching of the cells (Collier and Grossman 1992, 1994; Davies and Grossman 1998). Changes in the color of cyanobacteria from blue–green to yellow occur as a consequence of pigment degradation and cessation of new pigment biosynthesis (Bryant and Liu 2013). Plasticity and restructuring of phycobilisome are evident upon sulfur limitation (Schwarz and Grossman 1998; Richaud et al. 2001) largely due to phycobiliproteins (PBP) degradation and

repressed transcript synthesis. A reduction in PBPs possibly reduces light harvesting capacity by narrowing PAR absorption (Schmidt et al. 1982). However, an alternative adaptive mechanism was observed in *Fremeyella diplosiphon*, where low sulfur-containing PBPs synthesized under sulfur limitation (Gutu et al. 2011).

Sulfur starvation damage the photosynthetic reaction center. The biosynthesis of D1 protein and PSII repair process was found to be ceased not only in *Anabaena* 7120 and PCC 7942 but also in green alga *C. reinhardtii* (Vasilikiotis and Melis 1994; Wykoff et al. 1998; Melis 1999; Zhang et al. 2002; Zhang et al. 2004; Kharwar and Mishra 2020). Moreover, *rbcL* encoding the Rubisco large subunit was down-regulated (Kharwar and Mishra 2020). Furthermore, decreased transcription of cytochrome b6/f and ATP synthase accounts for decreased photosynthetic activity (Zhang et al. 2008). Thus, sulfur deficiency reduces the maximum photosynthetic efficiency (P_{max}), but in *Gloeothece*, the photosynthetic efficiency (α) was less affected (Ortega-Calvo and Stal 1994). The decreased rate of photosynthetic electron transport chain resulted in the reduction of ATP and NADPH, thus affecting carbon dioxide fixation (Giordano et al. 2000, Giordano et al. 2005, 2008, 2013). However, no significant effect on the glycogen content was observed in *Synechocystis* PCC 6803 (Monshupanee and Incharoensakdi 2016). Moreover, long-term sulfur deficiency causes significant perturbation in the gene expressions and metabolites of the Calvin cycle, leading to lower photosynthate assimilation (Lindblad 1999; Nikiforova et al. 2003; Hoefgen and Nikiforova 2008).

Similar to photosynthesis, the effect of sulfur limitation on the respiration was evident because of alterations in gene expressions involved in the TCA cycle and glycolysis. In the marine microalgae, *Emiliania huxleyi*, sulfur deficiency resulted in the up- or down-regulation of the transcripts of succinyl CoA synthetase, succinate dehydrogenase, and citrate synthase (Bochenek et al. 2013). Moreover, Zhang et al. (2008) reported the down-regulation of *ccbA*, which encodes fructose-1,6-bisphosphate aldolase, causes glycolysis impairment in *Synechocystis* 6803 upon sulfur deprivation. Sulfur deficiency affects the acetyl-CoA content and thiamine pyrophosphatase affecting respiration, thus causing lower energy assimilation. Genes encoding transketolase (*tklA*) and pentose-5-phosphate-3-epimerase (*cfxE*) were also altered, leading to the activation of sugar accumulation in cyanobacteria (Zhang et al. 2008). Recently, Klanchui and his colleagues demonstrated that *A. platensis* C1 (iAK888) increased their glycogen fluxes upon sulfate starvation. Thus, sulfur limitation affects carbon metabolism by modulating the activities of phosphoglycerate mutase, enolase, pyruvate kinase, and malate dehydrogenase (Klanchui et al. 2018).

Regulation of nitrogen and amino acid metabolism upon sulfur stress

It is imperative to maintain the harmony between sulfur and nitrogen metabolism for the optimal cell metabolism; therefore, the level of nitrogen assimilatory proteins was perceived as an indicator to balance the S:N ratio. At a low sulfur concentration, a lower expression of genes involved in nitrogen metabolism was observed to maintain the S:N ratio. In *Gloeothece* and *Anabaena* 7120, sulfur deprivation represses nitrogenase, whereas sulfur replenishment reactivates it (Ortega-Calvo and Stal 1994). Furthermore, Nishihara et al. (2018) reported that the loss of nitrogenase activity in thermophilic microbes was partially recovered by the addition of hydrogen sulfate and carbon dioxide, anticipating their roles. In addition, impairment in the nitrate uptake and assimilation has been observed in plants under sulfur limitation (Prosser et al. 1997; Migge et al. 2000), suggesting the involvement of sulfur in nitrogen assimilation. A reduction in the nitrate uptake was probably due to decreased cysteine production upon sulfur deficiency, leading to OAS accumulation that acted as a feedback inhibitor (Prosser et al. 2001). Kaur et al. (2011) suggested that nitrate reductase (NR) and glutamine synthetase activity was reduced, whereas nitrite reductase (NiR) and glutamate synthase (GS) were not significantly affected. However, transcriptome analysis indicated a significant reduction in the mRNA expression of NR and GS, although the reduction in the transcript level of NR was more prominent than latter, indicating reduction in NR activity and its mRNA level appear to be an adaptive response. Furthermore, nitrite excretion occurred at a saturating carbon dioxide concentration during sulfur starvation in *Synechococcus* 6301 (Krämer and Schmidt 1989).

In *A. variabilis*, assimilatory sulfate reduction was restricted in the vegetative cells only, whereas heterocysts were restricted at a reduced level of sulfur metabolism, suggesting that heterocysts must be provided with reduced sulfur compounds (Giddings Jr et al. 1981). Moreover, reduced sulfur and their subsequent reduction into cysteine alter their translation, leading to the accumulation of organic nitrogen compounds such as asparagine and arginine in cyanobacteria (Kiyota et al. 2012). By contrast, decreased NR and nitrite accumulation upon sulfur starvation was observed by Krämer and Schmidt (1989), suggesting perturbation in the amino acid metabolism and changes in the physiology. Sulfur deficiency exerts different effects on the individual amino acids based on their composition and properties. Sulfur deficiency in plants resulted in a decreased rate of protein synthesis and increased contents of free amino acids, amides, and inorganic nitrogen (Thomas 1958). An increased content of basic amino acids such as glutamine, asparagine, and arginine has been reported in plants and cyanobacteria, whereas no significant change was reported in aspartate,

glutamate, serine, and alanine contents upon sulfur deprivation (Kiyota et al. 2014). Similarly, the total amino acid content in sulfur-deficient and starved cells increased significantly when compared with control cells in *Anabaena* 7120; however, reduction in methionine and cysteine was evident in *Anabaena* 7120 (Kharwar and Mishra 2020).

Nitrogen and sulfur act as an integral component of protein; thus, the nitrogen content was correlated with cellular protein. A decrease in the nitrogen content upon sulfur stress condition has been recently reported in cyanobacteria, suggesting the nitrogen content depends on the availability of sulfur (Kharwar and Mishra 2020; Zhang et al. 2020).

Effect of sulfur stress on lipid metabolism

Sulfur deficiency causes down-regulation of the fatty acid metabolism and exerts an adverse effect on both phospholipid and sulfolipid. Studies on *E. huxleyi* concluded that genes involved in fatty acid biosynthesis, including acetyl-CoA carboxylase, β -ketoacyl-ACP reductase, and acetyl-CoA dehydrogenase were affected when the sulfur content was low (Bochenek et al. 2013). Furthermore, increased *desC* transcript and fatty acid unsaturation were observed upon sulfur limitation in *Anabaena* 7120 (Kharwar and Mishra 2020). In addition, Zhang et al. (2020) found an increased accumulation of C16:0 and C18:1 in sulfur-limited groups that could be further used in the application of biodiesel production. However, no significant effect on the lipids was observed in *Synechocystis* 6803 (Monshupanee and Incharoensakdi 2016).

In *C. reinhardtii*, sulfolipid degrade within 6–12 h upon sulfur starvation, suggesting that sulfolipid act as a major source of sulfur for the protein synthesis (Sato et al. 2017). By contrast, high stability of SQDG is maintained in the cyanobacterium, *Synechococcus* and *Synechocystis* under sulfur-limiting condition (Sato 2004; Sato et al. 2017). However, in *A. thaliana*, genes involved in sulfolipid biosynthesis were down-regulated, thus resulting in the reduced sulfolipid biosynthesis upon sulfur limitation.

Increased hydrogen production upon sulfur stress

Nutrient starvation has been recently used for enhancing hydrogen (H_2) production in the microorganisms by altering their cellular metabolism. Sulfur limitation triggers H_2 production because the degradation of D1 protein results in a more rapid decline of photosynthesis than respiration enabling anaerobic condition at equilibrium, which is ideal for H_2 production. In addition, sulfur starvation inhibits carbon dioxide fixation and thus removes another important electron sink. Furthermore, this process likely leads to decreased sugar catabolism and culminated carbohydrate accumulation that are crucial for sustained H_2 production (Antal and Lindblad 2005). Similar to nitrogen starvation, mechanism underlining increased H_2 production upon sulfur limitation involve a higher expression of the *hox* gene encoding cyanobacterial Hox-hydrogenase (Antal and Lindblad 2005; Baebprasert et al. 2010). The large-scale H_2 production involves treating cells with sulfur limitation through inhibiting oxygenic photosynthesis in *Gloeocapsa alpicola*, *Synechocystis* 6803, and

Fig. 8 Proposed generalized scheme of MC and MAA biosynthetic pathway and their interaction with sulfur metabolism. The intermediate products formed and the steps involve in the process are not indicated (adapted from Long 2010)

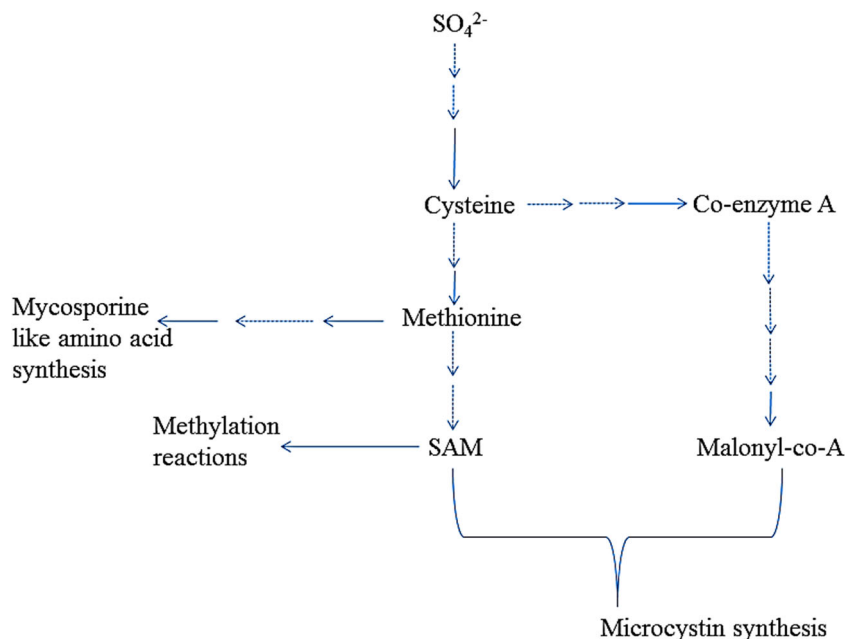
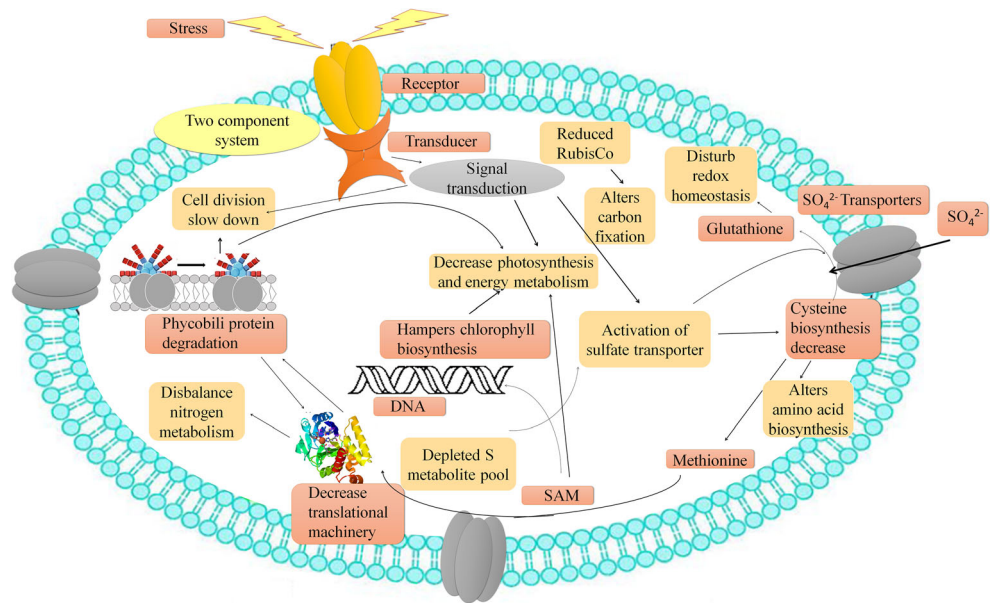


Fig. 9 Scheme of sulfur stress induced responses and modulations in the metabolic processes of cyanobacteria



Anabaena 7120 (Antal and Lindblad 2005; Zhang et al. 2008; Hifney and Abdel-Basset 2014). Yodsang and his colleagues showed 17-fold increased H₂ production under combined nitrogen and sulfur deprivation in *Fischerella muscicola* (Yodsang et al. 2018).

Modulations in the secondary metabolism in response to sulfur stress

Sulfur-containing compounds are known for their protective mechanism and enhance overall fitness. Cyindrospermopsin, a sulfur-containing hepatotoxin produced by *Aphanizomenon ovalisporum*, is affected by sulfate availability. Bácsi and his coworkers reported a reduction in the cyindrospermopsin pool, indicating the role of sulfur and its interaction with the biosynthesis of cyindrospermopsin (Bácsi et al. 2006). Furthermore, microcystin (MC), a hexapeptide, was affected after limiting sulfur supply, suggesting limitation of SAM production may outcompete for MC biosynthesis, thus requiring sulfur (methionine as metabolic demand) in the biosynthetic process of MC (Fig. 8) (Long 2010).

Mycosporine-like amino acids (MAAs) contain sulfate esters or glycosidic linkages through imine substituents, indicating the role of sulfur in the biosynthesis of MAAs. MAAs play a crucial role in the recycling of sulfur (in the form of methionine) under sulfur-deficient condition, in addition to their well-known photoprotective role (Singh et al. 2010). Under sulfur deficiency, methionine and its derivative SAM become limiting while increase in the homocysteine concentration. Furthermore, Singh et al. reported that the bioconversion of primary MAAs (MAA-shinorine) into secondary MAA (MAA palythine-serine) is regulated by sulfur in *A. variabilis*. This cyanobacterium synthesizes MAA-

shinorine under control condition, whereas MAA palythine-serine is produced upon sulfur limiting condition for the recycling of sulfur (Fig. 8).

Future prospects and goals of sulfur research

With the increase in research on sulfur, we are moving toward deciphering the canonical regulation of sulfur metabolism in cyanobacteria. Although CysR regulation has been described in unicellular cyanobacteria, in-depth investigation of regulation in filamentous cyanobacteria has not yet been performed. Thus, future research should examine the effects of sulfur limitation and its crosstalk with other metabolic processes at genetic and proteomic levels, evaluate the perception of sulfur stress-induced signals, explore how sulfur regulates nutrient uptake, and investigate the ameliorative role of sulfur under stress condition. Possibly integrated omic approaches can be used to elucidate the role of sulfur in cyanobacteria. Figure 9 provide an integrated view of sulfur stress-mediated responses in cyanobacteria. Studies on this versatile macronutrient are crucial to broaden our understanding and help us further elucidate the response mechanisms and adaptive physiology of cyanobacteria.

Abbreviations ABC, ATP- binding cassette; APS , Adenosine-5'-phosphosulfate; ATPS, ATP sulfurylase; CSC, Cysteine synthase complex; SAM, S-adenosylmethionine; STAS , Sulphate Transporter and Anti-Sigma factor antagonist.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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