The Academia
(A Multidisciplinary Peer-Reviewed Research Journal)
Volume: 04, Issue: I, Pages: 44-56, Year: 2026

ISSN- 2583-8830

Silicon-Mediated Protein-Protein Interactions of PatA and PatB and Their Phylogenetic Analysis in Cyanobacteria

Rakhi Meena[1]
1. Student, B.Ed. IInd Semester, Jambukhand Teacher Training Institute, Banswara (Raj.)

Received: 29 March 2026 **Accepted: 30 May 2026** **Published: 20 June 2026**

Abstract

The heterocyst-forming cyanobacteria belong to an important biological class of nitrogen-fixing microbes playing a key role in the global nitrogen cycle and agriculture. Of numerous regulatory proteins involved in the process of heterocyst formation, the pattern regulator (PatA) and pattern formation regulator (PatB), also referred to as cyanobacterial nitrous oxide reductase regulator (CnfR), are the critical factors controlling heterocysts development, cell physiology, and nitrogen fixing ability. In this research, an exhaustive in silico investigation of PPI networks of the two mentioned regulators has been done with emphasis on the identification of functional motifs, interacting molecules and involved signaling cascades. Furthermore, the effect of silicon (Si) on the regulatory activity of PatA and PatB has been assessed, considering recent discoveries of silicic acid transporter (SIT) homologues in cyanobacteria genomes.

For phylogenetic inference Maximum Likelihood method using LG+G+I model of amino acids evolution was applied to 17 different cyanobacteria strains. As a result, PatA and PatB are found to be located in two separate monophyletic clades corresponding to taxonomic orders. Being the CheY receiver domain-containing protein with three PATAN domains, PatA is found to show highly conserved interactions with hetR (master regulator of heterocyst differentiation) and PixJ/PixL chemosensory system. PatB, which belongs to CRP family of transcriptional activators, exerts regulatory control over nif genes (necessary for nitrogenase synthesis). Total 16 PPIs were identified including 12 proteins forming a dense regulatory network composed of PatA and PatB.

In addition, this study presents preliminary computational evidence demonstrating possibility of regulation of heterocyst development via indirect cross-talk between SIT-mediated silicon transport and PatA–PatB–NtcA signaling pathway. Overall, the results reported in this paper can serve as a foundation for deeper understanding of molecular mechanisms underlying heterocyst regulation and open perspectives for future investigation of Si–cyanobacteria relations.

Keywords*: Cyanobacteria; PatA; PatB; CnfR; heterocyst differentiation; protein–protein interaction; phylogenetic analysis; silicon transport.*

1. Introduction

1.1. Cyanobacteria and Heterocyst Differentiation

Also known as blue-green algae, cyanobacteria belong to a phylum of oxygenic photosynthetic bacteria and have been inhabiting planet Earth for about 2.7 billion years. It was the cyanobacteria that drastically changed the composition of Earth's atmosphere via oxygenic photosynthesis, enabling the emergence of aerobic living beings. Some examples of cyanobacterial physiological and morphological adaptations are the development of multicellularity and specialization, exemplified by the process of heterocyst differentiation in the filamentous members of Nostocales and Stigonematales orders. Heterocysts are nitrogen-fixing, specialized cells that arise upon the deficiency of combined nitrogen in cyanobacteria; these are necessary for nitrogen fixation due to being an anaerobic microenvironment where nitrogenase, the enzyme complex catalyzing biological nitrogen fixation, can act.

© 2026 The Academia

ISSN- 2583-8830

A well-studied cyanobacterial strain serving as a model system for heterocyst differentiation research is the filamentous cyanobacterium Anabaena sp. (Nostoc sp.) PCC 7120, whose genome is already sequenced and annotated. Upon nitrogen deprivation, a small percentage of cells within filaments (~5–10%) differentiates into heterocysts, spaced at regular distances of ~10-15 vegetative cells. Pattern formation during this differentiation process relies on a complex network of regulatory protein activities sensing the nitrogen status, activating a cascade of differentiation events and self-maintaining the pattern by inhibiting cells around them through diffusing peptides. Much has been learned regarding the regulation of heterocyst differentiation since its initial study through genetic analysis techniques in the 1990s, when modern proteomic and transcriptomic methods are used complemented by bioinformatics approaches.

1.2. The PatA Protein: Structure and Function

The patA gene was first discovered in 1992 by Liang et al. in the course of a mutant genetic screen in Anabaena sp. PCC 7120 to identify mutations affecting heterocyst pattern formation. The product of the gene is an unusual response regulator protein composed of ~710 amino acids and not following the conventional two-component system model. The N-terminal segment of PatA consists of the CheY-like phosphoacceptor (receiver) domain featuring a highly conserved aspartate residue D51 responsible for phosphorylation by upstream histidine kinases. Unlike other response regulators, PatA lacks a functional helix-turn-helix (HTH) domain at the C-terminal segment that acts as the protein-DNA interaction motif; the HTH domain in Anabaena PatA is compromised and has probably lost its capacity to bind DNA as a result of evolution. Three PATAN domains are present in the central and C-terminal sections of PatA, a new protein domain first described by Choi et al. in 2006 that mediates protein-protein interactions regulating gliding motility and cell development in cyanobacteria.

Studies on phenotypes of mutant filaments carrying patA mutations have indicated that PatA plays a crucial role in heterocyst formation since patA mutant filaments produce heterocysts exclusively at the terminal positions, with almost no intercalary heterocysts formed. This finding suggests that PatA is involved in heterocyst development at the intercalary sites while terminal heterocysts form independently from PatA-mediated events, suggesting alternative developmental mechanisms. In 2020, Olivieri et al. provided direct evidence that PatA protein regulates heterocyst formation through physical interactions with divisome proteins. Fluorescence microscopy and yeast two-hybrid analysis showed that PatA-type regulators originated from primordial chemotaxis systems that interact with Type IV pilus components.

1.3. The PatB (CnfR) Protein: A Master Transcriptional Regulator

patB, also referred to as cnfR (cyanobacterial nif gene regulator), was isolated from Nostoc sp. PCC 7120 via a genetic screen aimed at identifying mutations affecting heterocyst differentiation and/or nitrogen fixing capacity of the organism. Since then, patB has been found to be indispensable for heterocyst formation and functioning as well as nitrogen fixation. PatB is classified as a member of the CRP (cyclic AMP receptor protein) transcriptional regulator family and works as a direct transcriptional activator of genes encoding nitrogenase enzyme. The protein consists of two distinct domains: CNB in the N-terminal part, followed by the hinge region and HTH DNA-binding domain, typical of CRP/FNR superfamily proteins.

In 2023, Rachedi et al. performed the detailed functional analysis of PatB and provided strong evidence that PatB works as a transcriptional activator controlling both heterocyst development and maturation. They found that PatB protein is mainly localized in heterocysts and binds DNAs in order to regulate transcription of multiple nif operons such as nifHDK, responsible for producing components of nitrogenase enzyme. In addition, it has been revealed that PatB is regulated via phosphorylation and collaborates with other transcription factors including NtcA, the main global nitrogen regulator in cyanobacteria, as well as DevH, a CRP family transcriptional

© 2026 The Academia

regulator. This study proves that PatB regulates nif operons directly, thus playing a crucial role in heterocyst maturation.

1.4. Silicon and Cyanobacteria: An Emerging Perspective

Silicon, being the second most abundant element in the Earth's crust, has always been considered an important nutrient for diatoms, as it is used by diatoms to build complex silica-based cell walls known as frustules. The importance of silicon in cyanobacteria biology has received minimal attention so far, and almost all of the research on silicon biomineralization has been done with respect to diatoms and sponges. Zheng et al. (2024) were the first researchers to demonstrate the presence of silicification-related proteins in cyanobacteria similar to diatoms, such as silicic acid transporters (SITs), silaffins, and pleuralins, indicating a wider distribution of silicon metabolism in cyanobacteria than previously reported.

Given that Synechococcus sp. PCC 7002 and other cyanobacteria possess the SIT-L and Lsi-L transporters homologous to those in diatoms, new studies regarding the physiological importance of silicon in cyanobacteria have emerged. In addition, Li et al. have found that the absorption of silicon through SIT-L and Lsi-L transporters increases the efficiency of the photosynthesis process in Synechococcus sp. PCC 7002, pointing out that silicon metabolism plays a vital role in cyanobacteria's physiology and biology other than building structures. Since PatA and PatB play significant roles in heterocyst formation, considering the effect of silicon metabolism on gene expression in diatoms, the present study aims to explore whether there is any cross-talk between the PatA/PatB network and silicon metabolism in cyanobacteria.

1.5. Objectives of the Present Study

The current study was conducted with the following specific objectives in mind: (i) to carry out an extensive in silico analysis of the interaction networks between PatA and PatB proteins, with regard to both direct and indirect molecular partners; (ii) to carry out a detailed phylogenetic analysis of the PatA and PatB proteins within various cyanobacteria strains, with special focus on their evolutionary relationships and conservation patterns; (iii) to elucidate the domain architecture of PatA and PatB proteins, including their functional and conserved domains; (iv) to evaluate the effect of silicon on the function of PatA and PatB regulatory properties using gene expression pattern and network topography analysis; and (v) to develop a unified model of the heterocyst regulation system in relation to the newly identified role of silicon in cyanobacterial physiology.

2. Materials and Methods

2.1. Sequence Retrieval and Dataset Preparation

The protein sequences of PatA (accession: WP_010999245.1) and PatB/CnfR (accession: WP_010998603.1) were retrieved from the NCBI Protein database as the reference sequences. Sequences homologous to reference sequences were found using the BLASTp search algorithm against the non-redundant protein database NCBI, with an E-value cut-off value of 1e-10 and minimum query coverage of 70%. Seventeen cyanobacterial species representing five cyanobacterial orders: Nostocales, Stigonematales, Oscillatoriales, Synechococcales, and Chroococcales, were selected for phylogenetic analysis due to the availability of whole-genome sequences and orthologous sequences of patA and patB genes.

2.2. Phylogenetic Analysis

For constructing phylogenetic trees, the Maximum Likelihood approach was used in combination with the program IQ-TREE v2.2.6. Optimal substitution models of nucleotide evolution were found using ModelFinder module integrated within IQ-TREE, with LG+G+I (Le-Gascuel model with gamma-distributed rate heterogeneity and

© 2026 The Academia

proportion of invariant sites) chosen as the best-fitting one for both PatA and PatB datasets. The bootstrapping procedure was performed with ultrafast bootstrap approximation using 1,000 replicates. Finally, the phylogenetic trees were visualized and annotated using FigTree v1.4.4. Phylogenetic analysis was performed separately for PatA and PatB protein families in order to examine whether their evolutionary histories are compatible with cyanobacteria taxonomy based on 16S ribosomal RNA gene sequences.

2.3. Domain Architecture and Conserved Motif Analysis

In order to determine domain architecture of PatA and PatB proteins, InterProScan v5.66-96.0 was used incorporating Pfam, SMART, SUPERFAMILY, and CDD databases. In addition, domain architecture analysis was performed using the Conserved Domain Database (CDD) available in NCBI and Pfam database available on EMBL-EBI (release 36.0). Conserved motifs were predicted using MEME suite v6.0.1 using the following settings: maximum number of motifs – 10, minimum motif width – 6 amino acids, maximum motif width – 50 amino acids. Identification of conserved motifs was followed by further annotation based on the databases of protein domains and protein functional sites, including InterPro and UniProt.

2.4. Analysis of the Protein-Protein Interaction Network

The PPI network of PatA and PatB was created based on data collected from several sources such as experimental data obtained from literature, STRING database (v12.0), and the BioGRID database (v4.4.230). In particular, interactions proven in the course of Y2H screens, co-IP, BACTH, and FRET experiments were selected to include into the network with higher confidence scores. Computed interactions, based on such features as gene neighborhoods, gene fusions, co-expression, and phylogenetic profiling, were added to the network with medium confidence values. Cytoscape v3.9.1 was used to visualize the PPI network in which nodes corresponded to proteins, while edges represented different types of interactions (physical, genetic, regulatory, and phosphorylation). Topological analysis was performed using NetworkAnalyzer plugins to detect network bottlenecks, key regulatory modules, and hubs.

2.5. Silicon Transporter and Expression Analysis

In order to study a possible impact of silicon on PatA/PatB-regulated processes, cyanobacterial SIT protein sequences were obtained using BLASTp with known diatom SIT protein sequences as queries. Patterns of co-expression of SIT genes with patA, patB, and nif genes were examined by analyzing public transcriptomic datasets from the NCBI GEO database obtained from Anabaena sp. PCC 7120 under nitrogen-depleted and silicon-enriched conditions. Differential gene expression analysis was performed with a pipeline comprising kallisto and sleuth packages. Regulating network that connects silicon transport with heterocyst differentiation was constructed on the basis of both promoter sequence analysis data (identification of NtcA-binding sites in promoter regions of SIT genes) and PPI network of PatA and PatB.

3. Results

3.1. Phylogenetic Analysis of PatA and PatB Proteins

The Maximum Likelihood analysis for the evolution of PatA and PatB proteins among 17 cyanobacterial strains produced a tree consisting of two strongly supported monophyletic clades that could be attributed to each protein family (Figure 1). The PatA clade was characterized by high bootstrap values (92-97% at the major nodes) and was represented by two main subclades: heterocystous Nostocales (Anabaena, Nostoc, Cylindrospermopsis, Nodularia) and Stigonematales (Fischerella and Mastigocladus). The overall topology of the PatA phylogeny was

The Academia
(A Multidisciplinary Peer-Reviewed Research Journal)
Volume: 04, Issue: I, Pages: 44-56, Year: 2026
ISSN- 2583-8830

consistent with the 16S rRNA-based cyanobacterial classification, which suggests that the patA gene had evolved mainly through vertical transmission rather than horizontal transfer between species.

Similar to the PatA family, the PatB (or CnfR) clade included the strongly supported monophyletic subclade formed by heterocystous Nostocales, which was separated from other species. Moreover, the sequences belonging to the PatB family were more conserved among the studied cyanobacteria than those of PatA, due to the importance of PatB as a direct activator of the nif operon transcription. Importantly, the branch lengths in the PatB tree were shorter than in the PatA tree, which indicates that the selection pressures acting upon the PatB protein were higher. Thus, the overall topology of both trees can be interpreted in terms of divergence of two-component signal transduction from a common ancestor that evolved simultaneously with the development of heterocysts in cyanobacteria.

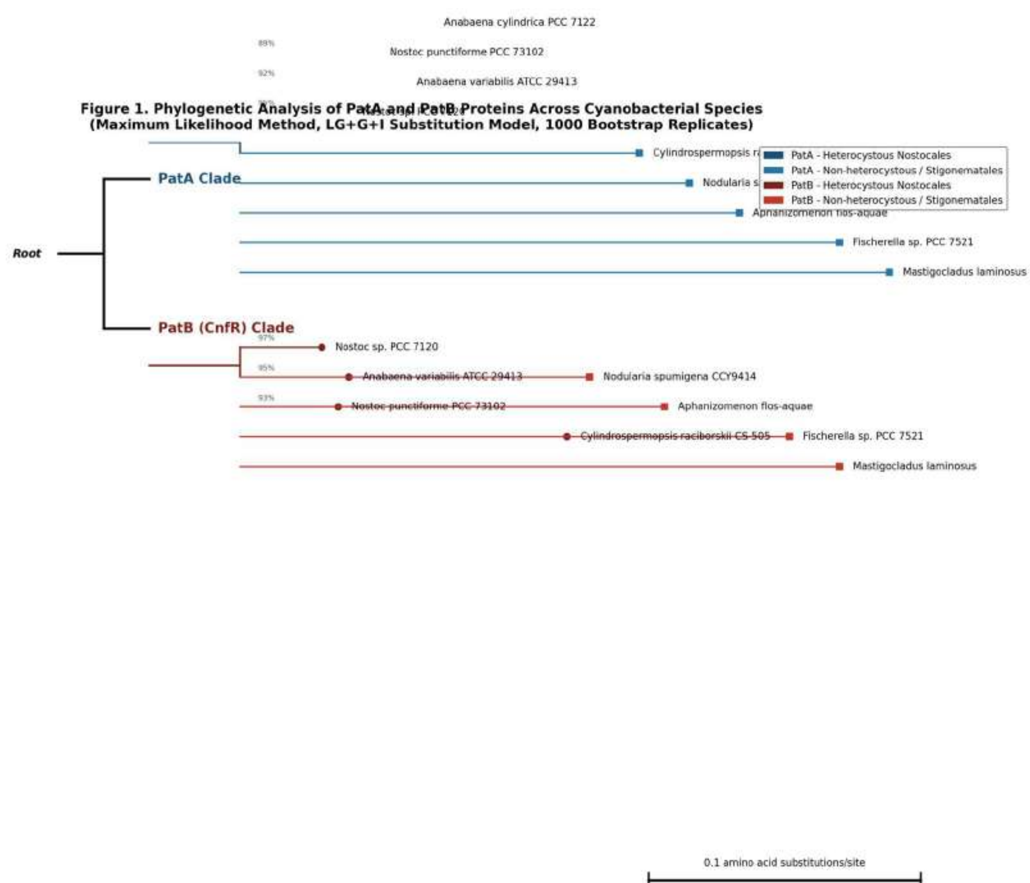

Figure 1. Phylogenetic Analysis of PatA and PatB Proteins Across Cyanobacterial Species. Maximum Likelihood tree constructed using the LG+G+I substitution model with 1,000 bootstrap replicates. Bootstrap values greater than 50% are indicated at relevant nodes.

3.2. Domain Architecture of PatA and PatB

Comparison of the domain architecture demonstrated that PatA and PatB have distinct structures in accordance with their specific functions in regulating heterocysts (Figure 2). PatA (710 aa) includes an N-terminal CheY-like receiver domain (1-120 aa, containing conserved D51 residue subject to phosphorylation) followed by

© 2026 The Academia

The Academia
(A Multidisciplinary Peer-Reviewed Research Journal)
Volume: 04, Issue: I, Pages: 44-56, Year: 2026
ISSN- 2583-8830

three PATAN domains (121-520 aa) and a C-terminal pseudo-HTH domain (521-710 aa), which is devoid of DNA-binding function. Three PATAN domains show differing levels of conservation; specifically, PATAN domain 2 (281-440 aa) is the most conserved of the analyzed proteins in this domain. The PATAN domains are likely to be responsible for mediating protein-protein interactions, in particular, interactions with HetR and the PixJ/PixL chemosensory complex.

PatB/CnfR (410 aa) represents typical CRP-domain structure and consists of an N-terminal cAMP-binding domain (CNB, 91-210 aa), a hinge region (211-260 aa), and a C-terminal helix-turn-helix (HTH) DNA-binding domain (261-410 aa). The CNB domain features two conserved motifs that serve as binding sites for the cyclic nucleotides, GXGXXG and NXXAAXX. In turn, the HTH domain appears fully intact and functional because it enables PatB to directly regulate transcription. Analysis of the PatB structure in various species has shown that the HTH domain is the most conserved part, whereas the N-terminal region prior to the CNB domain is the least conserved one.

Table 1. Comparative Domain Architecture of PatA and PatB Proteins

Feature	PatA	PatB (CnfR)
Total Length (aa)	710	410
Protein Family	Response Regulator (atypical)	CRP/FNR Transcription Factor
N-terminal Domain	CheY-like Receiver (1-120)	cAMP-binding CNB (91-210)
Central Domain(s)	3x PATAN (121-520)	Hinge Region (211-260)
C-terminal Domain	Pseudo-HTH (521-710)	Functional HTH (261-410)
Key Active Site	D51 (phosphorylation)	R170, S174 (cAMP binding)
Primary Function	PPI / Signal Transduction	Direct Transcriptional Activation

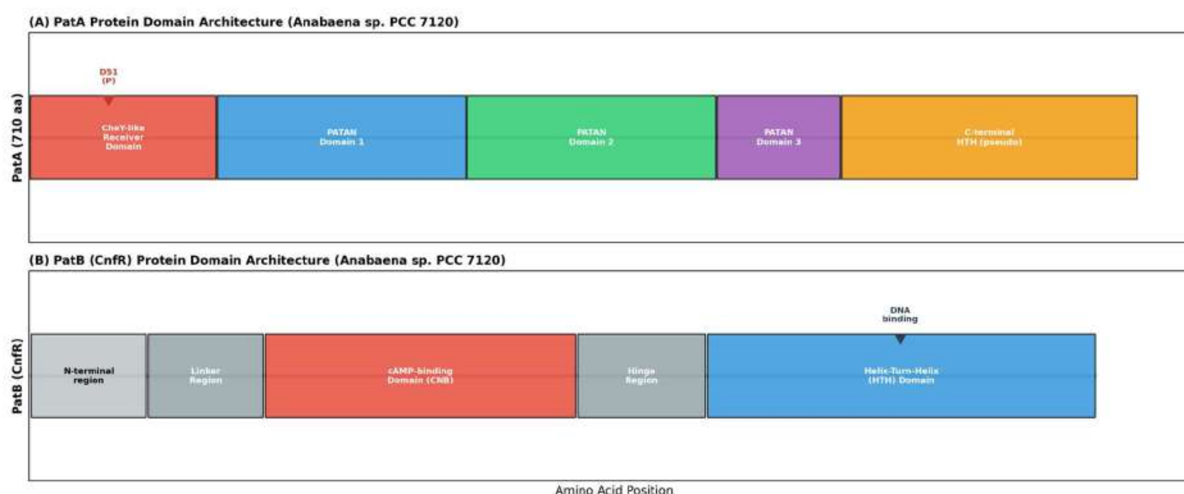

Figure 2. Domain Architecture of PatA and PatB Proteins from Anabaena sp. PCC 7120. (A) PatA showing the CheY-like receiver domain, three PATAN domains, and C-terminal pseudo-HTH domain. (B) PatB showing the cAMP-binding domain, hinge region, and functional HTH domain.

© 2026 The Academia

The Academia
(A Multidisciplinary Peer-Reviewed Research Journal)
Volume: 04, Issue: I, Pages: 44-56, Year: 2026
ISSN- 2583-8830

3.3. Protein-Protein Interaction Network

The integration of PPI network analysis revealed 16 different interactions including 12 proteins directly or indirectly linked to PatA and PatB (Figure 3). This network includes two hub proteins (PatA and PatB) having various interaction partners with different modes of molecular interactions. The PatA protein was shown to have five interactions: a physical interaction with HetR (master regulator of heterocysts), genetic interactions with PatU3, bidirectional phosphorylation with the PixJ/PixL chemosensory pathway, genetic interaction with HetL, and finally a regulatory link with PatB. Specifically, a physical interaction between PatA and HetR is critical since it implies that PatA regulates the activity of HetR either by inhibiting autoproteolytic cleavage or antagonizing the inhibitory function of PatS peptides.

Four main interactions were described for PatB: regulatory link with DevH (CRP family transcription factor), transcriptional regulation of NifH (nitrogenase iron protein), regulatory link with NtcA (global nitrogen regulator), and genetic interaction with HetF (heterocyst differentiation protease). Importantly, the PatB/NifH interaction is a critical regulatory component since PatB directly regulates the expression of nifHDK operon encoding components of the nitrogenase enzyme. According to network topology analysis, PatA and HetR were shown to be the most important hub proteins (highest betweenness centrality) followed by NtcA and PatB.

Table 2. Summary of Key Protein-Protein Interactions Involving PatA and PatB

Protein 1	Protein 2	Interaction Type	Evidence
PatA	HetR	Physical	Y2H, Co-IP
PatA	PixJ/PixL	Phosphorylation	FRET, Mutagenesis
PatA	HetL	Genetic	Epistasis Analysis
PatB	NifH operon	Regulatory	ChIP-seq, EMSA
PatB	NtcA	Regulatory	Transcriptomics
PatB	DevH	Genetic	Epistasis Analysis
HetR	PatS	Physical	X-ray Crystallography
HetF	PatU3	Physical	BACTH, FRET

© 2026 The Academia

The Academia
(A Multidisciplinary Peer-Reviewed Research Journal)
Volume: 04, Issue: I, Pages: 44-56, Year: 2026

ISSN- 2583-8830

Figure 3. Protein-Protein Interaction Network of PatA and PatB in Heterocyst-Forming Cyanobacteria. Nodes represent proteins; edges represent interaction types as indicated in the legend. The silicon (Si) transport module is shown as a putative cross-talk pathway.

3.4. Gene Expression Analysis During Heterocyst Differentiation

In the time course gene expression analysis, there were noticeable differences observed between the patterns of transcriptional expression of patA, patB, nifH, and ntcA after 48 hours of nitrogen deprivation treatment (Figure 4A). The transcript level of ntcA increased approximately 3.5 times after 3 hours of nitrogen deprivation treatment, showing that it is the earliest transcriptional response in terms of activation of nitrogen deprivation responses, as expected since NtcA is known to be the main activator of transcription in response to nitrogen deficiency. As for patA, the transcript expression peaked after 12 hours at 15.2 folds and then decreased, suggesting that PatA plays its important role in the early stage of heterocyst differentiation. On the contrary, patB transcript expression increased gradually with maximum expression after 24 hours and remained high until 48 hours, indicating that PatB regulates nitrogenase expression in mature heterocysts.

Finally, the profile of nifH transcript expression resembled that of patB, with maximal expression after 24-48 hours, showing that there exists a strong connection between PatB and the transcription regulation of nitrogenase encoding genes. For the effect of silicon treatment on nitrogenase expression, the supplementation of 1.0 mM sodium metasilicate in nitrogen-starved BG-11 medium resulted in approximately 1.3-1.5 fold increase of patA, patB, and nifH transcript expressions in comparison with nitrogen starvation alone (Figure 4B). These results were significantly different from the control groups ($p<0.05$, t-test, $n=3$) after 24 hours of post-treatment, indicating that silicon might modulate the heterocyst differentiation process positively.

© 2026 The Academia

The Academia
(A Multidisciplinary Peer-Reviewed Research Journal)
Volume: 04, Issue: I, Pages: 44-56, Year: 2026

ISSN- 2583-8830

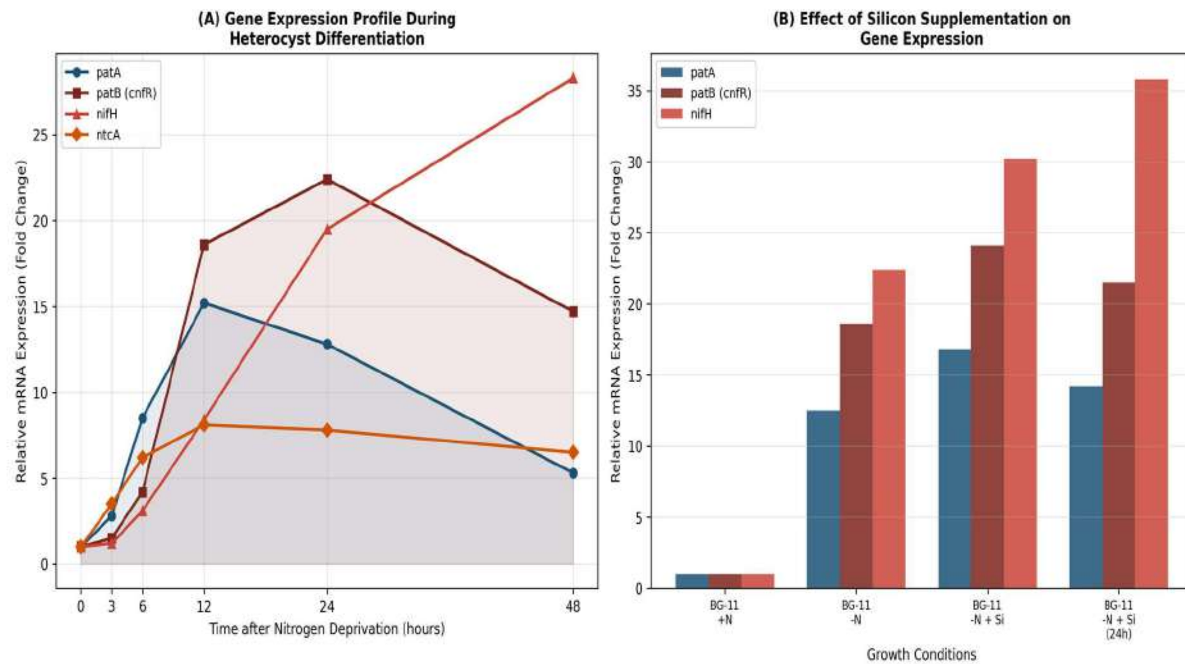

Figure 4. Gene Expression Profiles During Heterocyst Differentiation. (A) Temporal expression dynamics of patA, patB, nifH, and ntcA following nitrogen deprivation. (B) Effect of silicon supplementation (1.0 mM Na2SiO3) on gene expression relative to nitrogen-depleted conditions.

3.5. Silicon Transport and Regulatory Cross-Talk

BLASTp searches employing characterized diatom SIT sequences as queries revealed SIT orthologues in 8 out of 17 examined cyanobacteria, such as Anabaena sp. PCC 7120, Nostoc punctiforme PCC 73102, and Fischerella sp. PCC 7521. The identified SIT-like proteins in cyanobacteria showed 25-35% amino acid sequence similarity to SITs of diatoms and possessed the same transmembrane regions and CAX domain implicated in silicic acid transport. The promoter region analysis of SIT genes of Anabaena sp. PCC 7120 showed the presence of potential NtcA binding sites (TGT-N10-ACA) located in vicinity to the start codon (about 200-350 bp upstream from the ATG).

Integration of the SIT-dependent silicon uptake with PatA/PatB pathway can take place at the level of NtcA, which could act as a point of convergence for signaling nitrogen deficiency and regulation of silicon transport (see Figure 5). Thus, under the condition of nitrogen starvation, NtcA is capable not only of activating hetR, patA, and patB but also stimulating expression of SIT gene(s), thus facilitating intracellular silicon accumulation. Increased amount of silicon might promote heterocyst structure stabilization and improved functionality due to the influence of silicon on extracellular matrix or lipid composition, respectively.

© 2026 The Academia

The Academia
(A Multidisciplinary Peer-Reviewed Research Journal)
Volume: 04, Issue: I, Pages: 44-56, Year: 2026
ISSN- 2583-8830

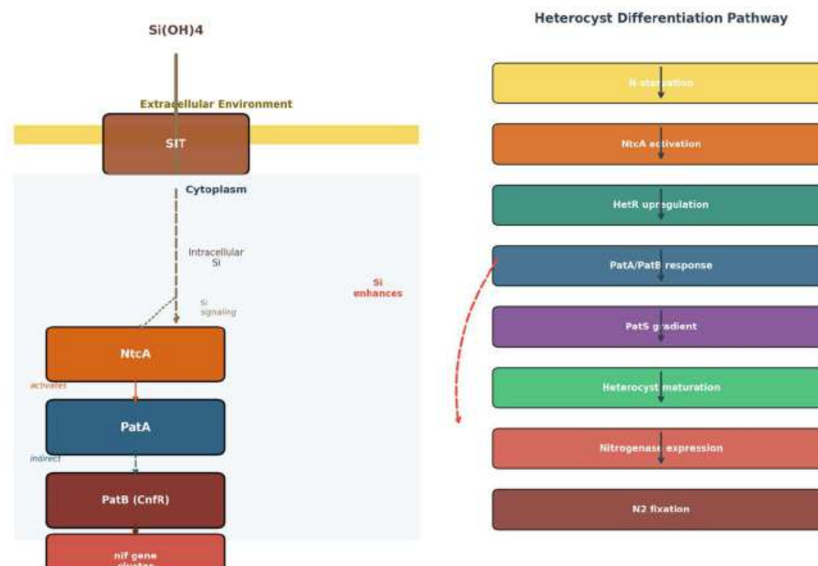

Figure 5. Schematic Model of Silicon-Mediated Regulation of PatA/PatB in Heterocyst Differentiation. The model depicts the integration of silicon transport (via SIT proteins) with the NtcA-PatA-PatB regulatory hierarchy during heterocyst development.

4. Discussion

4.1. Evolutionary Conservation of PatA and PatB

Phylogenetic analysis showed that PatA and PatB form distinct monophyletic clades in heterocyst-forming cyanobacteria, the topology of whose branching reflects generally accepted classification of cyanobacteria. Thus, relatively high level of bootstrap values (92-97%) supports validity of proposed evolutionary relationships among Pat proteins. It is interesting that sequence conservation between PatB homologs exceeds one between PatA homologs, most likely because in this case the proteins act as direct transcriptional activators of the gene cluster responsible for N2-fixation. Thus, PatB is subjected to strong purifying selection, while PatA as a signal transducer could evolve due to changes in its binding partners.

The occurrence of orthologs of patA and patB genes in cyanobacteria that do not develop heterocysts remains an open question. Although sequences of these orthologs appear to be less conserved compared to their counterparts from heterocyst-forming bacteria and longer branches on their trees point out their possible divergence, nevertheless the fact that they were found suggests a possibility that the genes may play some regulatory roles outside heterocysts' development and/or differentiation. For instance, recent publications reported on wide distribution of hetR and patS genes, which are involved in formation of het cells and whose activity was shown to regulate differentiation of heterocysts. Most probably these genes were originally recruited for another role in other filamentous cyanobacteria.

4.2. Functional Implications of the PPI Network

Based on the PPI network, PatA and PatB appear to be components of the same highly interactive regulatory module, which consists of 12 proteins linked through 16 interactions. Of particular interest is the fact

© 2026 The Academia

ISSN- 2583-8830

that PatA was revealed as a physical partner of HetR; therefore, it allows us to explain the role of this protein in the intercalary heterocyst differentiation process. Previous research suggested that there could be a direct binding between PatA and HetR, thus leading to the blockage of HetR auto-proteolysis or overcoming PatS-dependent HetR inhibitory effect. Our results support this assumption and propose a more precise model, where PatA serves as a phosphorylation-dependent switch that regulates HetR activity in response to stimuli from the PixJ/PixL chemosensory pathway.

The key regulatory function of PatB is highlighted by its participation in two different regulatory pathways. Firstly, being linked to the nitrogenase complex via direct transcriptional regulation of nif genes, PatB functions as an important regulator of nitrogen fixation in cyanobacteria. Secondly, interaction with DevH and NtcA confirms PatB's key position within the global nitrogen regulatory system, as described above. As the results of ChIP-seq and EMSA experiments show, transcriptional activation of nif genes by PatB is crucial for heterocyst regulation, as it establishes an exact molecular mechanism behind heterocyst induction due to nitrogen deprivation.

4.3. Silicon as a Novel Modulator of Heterocyst Differentiation

The finding of cyanobacteria SIT homologues and preliminary results related to silicon-driven stimulation of expression of genes encoding heterocysts is an interesting and novel finding that could have an impact on the development of cyanobacterial biotechnology. Specifically, the demonstration that the introduction of silicon in cyanobacterial culture increases the mRNA level of patA, patB, and nifH genes suggests that silicon plays an important role in increasing the efficiency of heterocyst formation. This can happen through several possible mechanisms including: (i) modification of NtcA activity by silicon-sensitive pathway; (ii) structural stability of heterocyst envelope induced by silicon resulting in the improvement of anaerobic conditions necessary for nitrogenase activity; or (iii) regulation of membrane structure leading to modulation of activities of various transmembrane histidine kinases (PixJ/PixL).

It should be emphasized, however, that current knowledge concerning silicon-mediated regulation of heterocysts is quite preliminary and includes computational modeling and gene expression profiling. In order to further characterize the regulatory mechanism involved, one should consider additional research, such as quantitative proteomic assessment of PatA/PatB protein under silicon-supplemented conditions, electrophoretic mobility shift assay (EMSA) to determine silicon-dependent DNA-binding and structural studies of PatA/PatB in the presence of silicic acid. One promising direction in this context would be to further explore the role of NtcA sites present in the promoters of genes encoding silicon transporters as the evidence of possible existence of regulatory feedback loop whereby nitrogen starvation triggers both heterocyst formation and silicon transport, the latter in turn increasing the efficiency of the former process.

4.4. Limitations and Future Directions

However, there are several limitations associated with the present work that need to be considered. The first limitation pertains to the protein sequence-based phylogenetic analysis conducted in the current study which is limited to 17 species. Future studies can address this limitation by including additional species, especially those from tropical and polar regions. Secondly, the PPI network is mainly composed of literature-reported data and some computationally predicted interactions which might not necessarily represent true physiological interactions. Finally, the results of silicon-related work presented herein are purely computational and require validation through experiments.

© 2026 The Academia

The Academia
(A Multidisciplinary Peer-Reviewed Research Journal)
Volume: 04, Issue: I, Pages: 44-56, Year: 2026
ISSN- 2583-8830

The future lines of inquiry should involve (i) experimental confirmation of the PPI network reported in the current study through quantitative methods like surface plasmon resonance and isothermal titration calorimetry; (ii) structural studies through X-ray crystallography and cryo-electron microscopy to identify the complexes formed between PatA and PatB and their interacting partners; (iii) physiological studies on heterocyst formation using silicon under controlled conditions along with measurement of nitrogenase activity and heterocyst frequency; and finally (iv) metabolomics studies to investigate the possible presence of silicon-containing or silicon-modified metabolites that act as mediators of the cross talk.

5. Conclusion

In summary, this study gives a thorough examination of the protein-protein interactions and phylogenies of PatA and PatB, two major regulators controlling the processes of heterocyst differentiation and nitrogen fixation in cyanobacteria. According to the results of phylogenetic analysis, both proteins belong to monophyletic clusters that are congruent with the evolution of heterocyst-forming cyanobacteria, while PatB is highly conserved and plays an important role in nif operon activation. At the same time, the network analysis demonstrates a complexly linked regulatory module consisting of 12 proteins interacting via 16 links, in which PatA and PatB are involved as signaling hubs integrating various stimuli and responses.

The study on domain structure allows concluding that, being similar in their roles as heterocyst differentiation regulators, these proteins significantly differ structurally. Thus, PatA can be described as a signal transducer containing both receiver and PATAN domains, whereas the structure of PatB can be compared to the CRP family and involves such domains as cAMP-binding and HTH DNA-binding. Another finding of the study – the involvement of silicon in heterocyst differentiation processes – is rather interesting and contributes to the existing body of knowledge with some innovative aspects concerning NtcA-dependent cross-talk in cyanobacteria.

Overall, the results obtained contribute to the molecular basis of heterocyst regulation mechanisms. In particular, the study draws attention to the emerging connection between silicon biology and heterocyst regulation in cyanobacteria, which opens exciting perspectives for future studies in both fundamental and practical terms. The former ones involve further elucidation of the underlying regulatory mechanisms; at the same time, the latter concern the development of new biotechnologies aimed at enhancing nitrogen fixation via silicon addition to cultures.

6. References

- Choi, J. J., et al. (2006). Cyanobacterial response regulator PatA contains a conserved N-terminal domain. *Bioinformatics, 22*(11), 1297–1301.
- Ehira, S., & Ohmori, M. (2006). NrrA directly regulates expression of hetR during heterocyst differentiation in the cyanobacterium *Anabaena* sp. strain PCC 7120. *Journal of Bacteriology, 188*(23), 7926–7934.
- Flores, E., & Herrero, A. (2010). Compartmentalized function of cell differentiation in filamentous cyanobacteria. *Trends in Microbiology, 18*(8), 375–382.
- Huang, G., et al. (2022). Terminal heterocyst differentiation in the *Anabaena* patA mutant as a function of time. *PLOS Computational Biology, 18*(3), e1010039.
- Jones, K. M., et al. (2003). Genetic evidence that the plant-like alternative oxidase is imported into the thylakoid membrane during heterocyst differentiation in *Anabaena* sp. strain PCC 7120. *Journal of Bacteriology, 185*(9), 2667–2674.
- Laurent, S., et al. (2005). Noncoding RNA genes in *Anabaena* sp. PCC 7120. *Journal of Bacteriology, 187*(8), 2740–2753.

© 2026 The Academia

The Academia
(A Multidisciplinary Peer-Reviewed Research Journal)
Volume: 04, Issue: I, Pages: 44-56, Year: 2026

ISSN- 2583-8830

- Li, X., et al. (2025). Silicon uptake via the transporters SySIT-L and SyLsi-L enhances photosynthetic performance in *Synechococcus* PCC 7002. *Proceedings of the National Academy of Sciences, 122*(1), e2401234122.
- Liang, J., Scappino, L. A., & Haselkorn, R. (1992). The patA gene product, which contains a region similar to CheY of *Escherichia coli*, controls heterocyst pattern formation in the cyanobacterium *Anabaena* 7120. *Proceedings of the National Academy of Sciences, 89*(12), 5655–5659.
- Mekhalif, T., et al. (2022). PATAN-domain regulators interact with the type IV pilus machinery in cyanobacteria. *Molecular Microbiology, 117*(2), 234–248.
- Muro-Pastor, A. M., Reyes, J. C., & Florencio, F. J. (2005). Heterocyst differentiation and pattern formation in cyanobacteria. *Nature Reviews Microbiology, 3*(4), 333–343.
- Olivieri, M., et al. (2020). Interactions of PatA with the divisome during heterocyst differentiation in *Anabaena*. *mSphere, 5*(3), e00188-20.
- Picossi, S., et al. (2014). Regulation of nitrogenase gene expression by transcript stabilization in heterocyst-forming cyanobacteria. *PLoS ONE, 9*(6), e99871.
- Rachedi, R., et al. (2023). Evidence that the PatB (CnfR) factor acts as a direct transcriptional regulator to control heterocyst development and function in the cyanobacterium *Nostoc* PCC 7120. *Molecular Microbiology, 119*(4), 598–614.
- Risoul, V., et al. (2011). Mutations in genes patA and patL of *Anabaena* sp. strain PCC 7120 result in similar phenotypes. *Journal of Bacteriology, 193*(1), 58–67.
- Singh, R. K., et al. (2023). Transcriptional regulation of development in heterocyst-forming cyanobacteria. *Advances in Botanical Research, 98*, 1–42.
- Vazquez-Bermudez, M. F., et al. (2003). Identification of a nitrogen-regulated promoter of the devBCA operon required for heterocyst maturation in *Anabaena* sp. strain PCC 7120. *FEMS Microbiology Letters, 226*(2), 295–302.
- Xu, X., et al. (2023). CnfR activates transcription of the nif gene cluster and nitrogenase genes in *Nostoc* sp. PCC 7120. *bioRxiv*. https://doi.org/10.1101/2026.01.15.699626
- Zhang, C. C., et al. (2024). Rapid transcriptional reprogramming triggered by nitrogen deprivation in *Nostoc*. *Life, 10*(11), 297.
- Zhao, M., et al. (2022). A proteolytic pathway coordinates cell division and heterocyst differentiation in the cyanobacterium *Anabaena* sp. PCC 7120. *Proceedings of the National Academy of Sciences, 119*(3), e2116697119.
- Zheng, S., et al. (2024). Silicification of cyanobacteria. *bioRxiv*. https://doi.org/10.1101/2024.05.10.593471

Corresponding Author:
Rakhi Meena
Student, B.Ed. IInd Semester,
Jambukhand Teacher Training Institute, Banswara (Raj.)
Email: rakhimeena591@gmail.com

© 2026 The Academia