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A model for cell type-specific differential gene expression during heterocyst development and the constitution of aerobic nitrogen fixation ability in *Anabaena* sp. strain PCC 7120

SHREE KUMAR APTE† and G NARESHKUMAR*
Cell Biology Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India
*Department of Biochemistry, M S University of Baroda, Vadodara 390002, India

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Abstract. When deprived of combined nitrogen, aerobically-grown filaments of *Anabaena* sp. strain PCC7120 differentiate specialized cells called the heterocysts. The differentiation process is an elaborate and well orchestrated programme involving sensing of environmental and developmental signals, commitment of cells to development, gene rearrangements, intricate DNA-protein interactions, and differential expression of several genes. It culminates in a physiological division of labour between heterocysts, which become the sole sites of aerobic nitrogen fixation, and vegetative cells, that provide photosynthate to the heterocysts in return for nitrogen supplies. We propose a model, to describe the chronology of the important events and to explain how cell type-specific differential gene expression is facilitated by DNA-protein interactions leading to the development of heterocysts and constitution of nitrogen-fixing apparatus in *Anabaena.*

Keywords. ***Anabaena;*** **heterocyst development; nitrogen fixation; differential gene expression.**

1. Introduction

Heterocystous cyanobacteria, such as *Anabaena, Nostoc,* have the unique ability to simultaneously carry out mutually exclusive processes of the O_2-evolving photosynthesis and the highly oxygen-sensitive nitrogen fixation, during aerobic growth (Stewart 1980). They do so by separating these phenomena in space, i.e., under aerobic conditions photosynthesis occurs in the vegetative cells (90-95% cells of the filament), while nitrogen fixation is restricted to 5-10% specialized cells called the heterocysts (Haselkorn 1978). The vegetative cells provide heterocysts with photosynthetically generated carbohydrate which acts as a source of reductant and of ATP needed for nitrogen fixation. In turn, heterocysts fix and assimilate nitrogen as glutamine which is then released to the neighbouring vegetative cells down the filament (Wolk 1982). At the level of prokaryotes this probably forms the best instance of a "physiological division of labour" (Apte 1992, 1993).

Heterocysts differentiate from vegetative cells when the filaments are subjected to nitrogen deficiency (hereafter referred to as nitrogen stepdown) during aerobic growth. Elegant structural and biochemical modifications aimed at optimising conditions for N_2 fixation occur in heterocysts during differentiation (Haselkorn *et al* 1990; Wolk 1991; Buikema and Haselkorn 1993). Photosynthetic machinery is broken down in differentiating cells, resulting in (i) loss of phycocyanin responsible for photolytic O_2

†Corresponding author (Fax 022-5560750)

evolution from water (Thomas 1972), (ii) degradation of carboxysomes which house the prime CO_2 assimilating enzyme ribulose 1,5-bisphosphate carboxylase (Rubisco), and (iii) repression of *rbcLS* operon (encoding Rubisco) and Rubisco synthesis. Heterocysts gain a new envelope consisting of additional wall layers of novel glycolipids and polysaccha- rides, impervious to most gases and solutes. As a consequence heterocysts are adequately protected from oxygen but become totally dependent on vegetative cells for photosynthate. Other important gains in heterocysts, which support efficient nitrogen fixation, include (i) synthesis of nitrogenase proteins, (ii) enhanced synthesis of oxidative pentose phosphate pathway enzymes and heterocyst-specific ferredoxins to provide reductant to nitrogenase, (iii) enhanced levels of glutamine synthatase for rapid assimilation of NH_4^+, and (iv) additional enzyme systems (such as uptake hydrogenase, superoxide dismutase etc.,) to scavenge O_2 (see Apte 1992, 1993, for a review).

An important episode in hetereocyst development is the occurrence of three developmentally regulated gene rearrangements late during heterocyst differentiation (> 18 h) which result in creation of three functional Operons. Two of these belong to nitrogen fixation (*nif*) genes (Golden *et al* 1985,1988) and the third occurs in a gene that encodes an uptake hydrogenase (Carrasco *et al* 1995). These Operons do not express in vegetative cells but only express subsequent to their rearrangement in heterocysts. Several genes that express during heterocyst development or in the mature heterocysts and certain regulatory proteins which appear to bring about cell-specific differential gene expression have been identified in the last 5 years by very elegant techniques and novel experimental approaches. Although these have revealed some exciting facets of the phenomena involved, they have also raised several interesting questions. Prominent among these refer to the perception of environmental/developmental signals, nature of commitment of cells to differentiation, chronology of the various molecular events, nature and regulation of DNA-protein interactions and the overall co-ordination of the structural and functional differentiation. This article proposes a model to explain the molecular basis of various events that occur subsequent to nitrogen stepdown and lead to the establishment of aerobic nitrogen fixation in *Anabaena.*

2. Genes encoding structural and biochemical components associated with nitrogen fixation in heterocysts

The prominent genes/operons associated with nitrogen fixation in heterocysts are (*nif H, D, K*), (*nif B, J dxN, nif S, U*), *f dxH,* (*hupSL*), *devA, hepA, hglK* and *gln A* (table 1). The genes in the *nifH, D, K* operon encode structural proteins, the dinitrogenase and dinitrogenase reductase, that together constitute the nitrogenase complex. The *nif B* product contributes to the synthesis of FeMoco—the active site of dinitrogenase, the *f dxN* encodes a heterocyst-specific ferredoxin of unknown function (Mulligan *et al* 1988), while *nifS, U* gene products probably aid the maturation of dinitrogenase (Haselkorn *et al* 1990, 1993). The *f dxH* gene encodes a heterocyst specific ferredoxin (Bohme and Haselkorn 1988) that acts as the terminal electron donor to nitrogenase complex during catalysis (Scrautemeier and Bohme 1985).

The *hupL* gene encodes a heterocyst-specific uptake hydrogenase (Carrasco *et al 1995*) that recycles H_2, evolved by nitrogenase as a byproduct, and helps to scavenge oxygen inside heterocysts (Houchins 1984). A barrier against oxygen from the environment is provided in heterocysts by products of the *hep A, hglK* and *devA* genes. The

Table 1. Genes expressed in different cell types of *Anabaena* PCC 7120 following nitrogen stepdown.

Gene/ protein	Probable function	Expression time (h)	Mutant phenotype	Reference
hetR/HetR	Counteracts N repressor, expresses selectively in heterocysts	2 (12–24)	No heterocysts formed	Buikema and Haselkorn 1991a, b; Black *et al* 1993
patA/PatA	NtrC-like transcriptional regulator, affects pattern of heterocysts	6 (6–12)	Terminal heterocyst, unaffected by the HetR	Liang *et al* 1982
hetP/HetP	Unknown	6 (6–24)	No heterocysts formed	Fernandez-Pinas *et al* 1994
sigA/SigA	Principle σ-factor	6 (12–24)	Not attempted	Brahamsha and Haselkorn 1991
sigB/SigB	Alternate σ-factor during nitrogen stepdown	12 (12–18)	Altered morphology of colonies, Het^+, Nif^+	Brahamsha and Haselkorn 1992
sigC/SigC	Alternate σ-factor during nitrogen stepdown	6	Altered morphology of colonies, Het^+, Nif^+	Brahamsha and Haselkorn 1992
devA/DevA	Periplasmic permease (like traffic ATPases) of heterocysts	6 (6–24)	Heterocysts lack envelope polysaccharides and glycolipids Nif^- in air	Maldener *et al* 1994
hepA/HepA	Deposits heterocyst envelope polysaccharides	7–8 (12–24)	No mature heterocysts	Holland and Wolk 1990; Wolk *et al* 1993
hglK/HglK	Heterocyst glycolipid	—	Defective heterocysts	Buikema and Haselkorn 1993
patB/PatB	Fnr-like transcriptional regulator, affects pattern of heterocysts	12 (12–24)	Slow development of heterocysts, HetR byepasses phenotype	Liang *et al* 1993
ntcA/NtcA also BifA	Transcription regulator of *glnA* > *xisA* > *rbcLS* > *nifH*, and *hetR*, *ORF1*, *devA* etc.	12 (12–36)	Het^-, Nif^-, lack the 11 kb and 55 kb element rearrangements	Wei *et al* 1993, 1994; Ramasubramanian *et al* 1994
Factor 2	Positive regulator of *rbcLS* operon, found only in the vegetative cells	—	—	Ramasubramanian *et al* 1994
glnA/GS 1·7 kb mRNA	Glutamine synthetase, prime ammonia assimilating enzyme, expresses from "*nif*-like" promoter in heterocysts	18 (18–36)	Excretes NH_4^+ in the growth medium	Tumer *et al* 1983; Wei *et al* 1994
xisA/XisA	Site-specific recombinase, rearranges the 11 kb element	24 (24–36)	Het^+, Nif^-, lacks 11 kb rearrangement	Lammers *et al* 1986 Golden and Wiest 1988
xisF/XisF	Site-specific recombinase, rearranges the 55 kb element	24 (24–36)	Het^+, Nif^-, lacks 55 kb rearrangement	Carrasco *et al* 1994

(*Continued*)

Table 1. (*Continued*)

Gene/ protein	Probable function	Expression time (h)	Mutant phenotype	Reference
xisC/XisC	Site-specific recombinase, rearranges 10·5 kb element	24 (24–42)	—	Carrasco *et al* 1995
hupL/HupL	Uptake hydrogenase of heterocysts, recycles H_2	24 (24–30)	Expected to decrease N_2 fixation	Carrasco *et al* 1995
nifHDK/ NifHDK	Nitrogenase in heterocysts, facilitates N_2 fixation	18 (18–42)	Nif$^-$, unable to grow in N-free media	Golden *et al* 1991 Wei *et al* 1994
nifB, fdxN nifS, U	Involved in synthesis of FeMoco and processing of nitrogenase in heterocysts	18 (18–36)	—	Golden *et al* 1988 Golden *et al* 1991
ORF1	Contributes to nitrogen fixation in an unknown way	18 (18–30)	Grow poorly in combined nitrogen-free media	Borthakur *et al* 1990
fdxH/FdxH	Heterocyst ferredoxin, acts as terminal electron donor to nitrogenase	18	—	Schrautemeier and Bohme 1985; Bohme and Haselkorn 1988
rbcLS/ Rubisco	Prime CO_2 fixation enzyme of vegetative cells, absent in heterocysts	Always present	—	Madan *et al* 1993

hepA gene codes for a heterocyst-specific polysaccharide that forms the outermost layer of heterocyst envelope (Holland and Wolk 1990; Wolk *et al* 1993), while the *hglK* codes for a heterocyst-specific unique glycolipid which forms a layer internal to the polysaccharide layer (Buikema and Haselkorn 1993). A heterocyst-specific periplasmic permease, the *devA* protein, facilitates the transport of the *hglK* and *hepA* products to create heterocyst envelope (Maldener *et al* 1994).

The first and foremost ammonia assimilating enzyme in *Anabaena* is glutamine synthetase or GS (Thomas *et al* 1975). The *glnA* gene which codes for GS, expresses both in the vegetative cells as also in the heterocysts but a novel 1·7 kb *glnA* mRNA transcript is found only during nitrogen stepdown (Wei *et al* 1994). The *glnA* gene has several promoters (Tumer *et al* 1983) and the 1·7 kb transcript emanates from a "*nif*-like" promoter which is reportedly expressed only in the heterocysts (Wei *et al* 1994).

3. Genes responsible for the developmental gene rearrangements

Three genes involved in N_2 fixation in *Anabaena* are interrupted by large intervening DNA elements. Thus, an 11 kb element is found in the *nifD* gene (Golden *et al* 1985), a 55 kb element in the *fdxN* gene (Golden *et al* 1988) and a 10·5 kb element in the *hupL* gene (Carracso *et al* 1995). The (*nif HDK*) and (*nif B, fdxN, nif S, U*) operons are located next to each other while the *hupSL* operon is located > 700 kb away on *Anabaena*

chromosome (Kuritz *et al* 1993). The interrupting sequences are precisely removed late during heterocyst differentiation (see Apte and Prabhavathi 1994, for a review) by means of three independent site specific recombination events, resulting in gene rearrangements and creation of three functional Operons. Thus, during development of heterocysts three large circular DNAs of 11 kb, 55 kb and 10·5 kb are excised from the chromosome to generate three functional Operons. The excised circles persist in the heterocysts with no discernible function (Haselkorn 1992). The mechanisms underlying these rearrangements have been reviewed extensively (Haselkorn 1992; Apte and Prabhavathi 1994).

The aforesaid gene rearrangements in *Anabaena* PCC 7120 are brought about by three different excisases, the XisA, XisF and XisC, encoded by three independent genes, namely the *xisA* (Lammers *et al* 1986), *xisF* (Carrasco *et al* 1994) and *xisC* (Carrasco *et al* 1995), respectively (table 1). Interestingly each of these genes resides near the left border of the same DNA element which it excises during development, i.e., the 11 kb element harbours *xisA,* the 55 kb element harbours the *xisF* and the 10·5 kb element contains the *xisC.* The 11 kb element is flanked by a 11 bp direct repeat (GCCTCAT-TAGG) and the 55 kb by a 5 bp direct repeat (TATTC) at each end (Golden *et al* 1987) while the 10·5 kb element has a 16 bp direct repeat (CACAGCAGTTATATGG) at the left and right borders (Carrasco *et al* 1995). Each excisase carries out a site-specific recombination event involving the respective direct repeats resulting in the excision of the DNA contained within the direct repeats (Haselkorn 1992). The *xisA* and *xisF* are essential genes for nitrogen fixation since mutating them results in Nif⁻ phenotype and inhibits diazotrophic growth (Golden and Wiest 1988; Carrasco *et al* 1994) (table 1). Mutation in *xisC* have not been obtained yet, but such mutations are expected to only decrease the efficiency of N_2 fixation (Carrasco *et al* 1995).

All the three excisase genes are developmentally regulated, i.e., they do not express in the vegetative cells. Neither the transcripts nor the protein products of these genes have been visualized in *Anabaena,* so far. The exact transcription start sites are not known and the functional promoters have not been mapped (Brusca *et al* 1990; Carrasco *et al* 1994). The only way to measure their activity has been to screen for the respective gene rearrangements. Based on such studies, the *xisA, xisF* and *xisC* seem to express at low levels and only transiently, late during heterocyst differentiation (nearly 18 h after nitrogen stepdown). (Haselkorn 1992; Carrasco *et al* 1994). The putative promoter region of *xisA* (— 100 bp to — 170 bp upstream of the second ATG) contains two divergent promoters P_1 and P_2 (Lammers *et al* 1986). The P_1 promoter is stronger than P_2 and can cause transcription away from the ORF. As a consequence, it can create a strong interference for *xisA* transcription. A negative regulatory element (NRE) located between — 65 bp to — 192 bp strongly controls expression of *xisA* (Brusca *et al* 1990). At least two DNA-binding proteins, BifA (now NtcA) and factor 2, associate with the NRE (between — 152bp to — 223 bp) and regulate its expression (Ramasubramanian *et al* 1994). When the NRE was deleted completely, the *xisA* could be expressed from a strong *tac* promoter even in vegetative cells (Brusca *et al* 1990). Deletions up to — 123 bp (which remove binding sites for both BifA and factor 2) caused only low level expression while deletions extending to — 65 bp (which also removes the P_1 promoter) caused high level expression (Brusca *et al* 1990). Thus, *xisA* expression in vegetative cells appears to be blocked both by transcriptional interference as well as by binding of regulatory proteins at NRE. Additionally, the *xisA* gene also contains a "*nif*-like" promoter (TCTAC at - 57 bp and CAAATAT at - 97 bp). As we shall describe later

(see §5 below), in the heterocysts an expression from this "*nif*-like" promoter may overcome the repression of transcription of this gene. The features likely to be responsible for the developmental regulation of *xisF* and *xisC* genes are completely unknown, at this point.

4. Genes encoding heterocyst-specific regulatory proteins

Prominent genes in this group include the *hetR, patA, patB, sigB, sigC* and *ntcA* (table 1). The *hetR* gene controls heterocyst development and is also autoregulatory. The *hetR* mutants do not form heterocysts while the *hetR* overexpression produces multiple heterocysts even during nitrate-supplemented growth (Buikema and Haselkorn 1991b). Expression of *hetR* commences within 2h after nitrogen stepdown (Buikema and Haselkorn 1991b; Black *et al* 1993) at low levels in all the cells and by 3·5h is localized to differentiating cells only. During the peak expression period (6-24h) the gene expresses abundantly, mainly in the heterocysts (Black *et al* 1993). A repressor of heterocyst development is believed to prevent heterocyst differentiation in the presence of combined nitrogen as also prevent cells adjacent to a heterocyst from differentiating in *Anabaena* (Wolk 1991). The nature of such repressor is unknown but some recently characterized genes *hetN, hetM* and *hetI* appear to be involved in production of a regulatory secondary metabolite (cyclic peptide?) that inhibits heterocyst formation or their spacing (Black and Wolk 1994). In some way, the HetR protein appears to interfere and titrate out such a hypothetical repressor. The expression of at least one heterocyst specific gene *hepA* has been shown to depend on *hetR* (Wolk *et al* 1993). The predicted HetR protein possesses no structural motifs typical of transcription factors (Buikema and Haselkorn 1991b), and its precise function is not known.

Two independent genes *patA* and *patB* have been implicated in pattern formation in *Anabaena* PCC 7120 (table 1). The *patA* mutant develops only terminal heterocysts (Liang *et al* 1992) while in the *patB* mutant heterocysts develop very slowly but eventually there are many more closely spaced heterocysts compared to the wild type (Liang *et al* 1993). The *patA* sequence resembles that of *cheY* and *ntrC* (Liang *et al* 1992), which belong to the well known two component signal sensing/response regulating systems of bacteria (Stock *et al* 1989). It has been suggested that *patA* product may be the response regulator (transcriptional activator) of a pair of environment-sensing (nitrogen levels ?) system that controls some aspect of heterocyst differentiation (Buikema and Haselkorn 1993). The PatB protein is equally interesting in that it has a 4Fe-4S bacterial-type ferredoxin domain near the N-terminus and a helix-turn-helix DNA-binding motif at the C-terminus. This structure resembles transcriptional regulators like Fnr in bacteria or the LIM domain in eukaryotes (Liang *et al* 1993). The PatB may, therefore, function as a sensor of redox state or of iron levels in the cells and may regulate transcription of certain heterocyst-specific genes. Recently, iron levels have been shown to be important for certain heterocyst-specific events such as gene rearrangements and nitrogen fixation (Razquin *et al* 1994). Interestingly, *hetR* overexpression has no effect on the phenotype of *patA* mutation but byepasses that of *patB* mutation (Buikema and Haselkorn 1993). While the intricacies of their interactions remain to be elucidated, the *hetR, patA* and *patB* offer themselves as possible candidates for triggering heterocyst differentiation in response to appropriate environment.

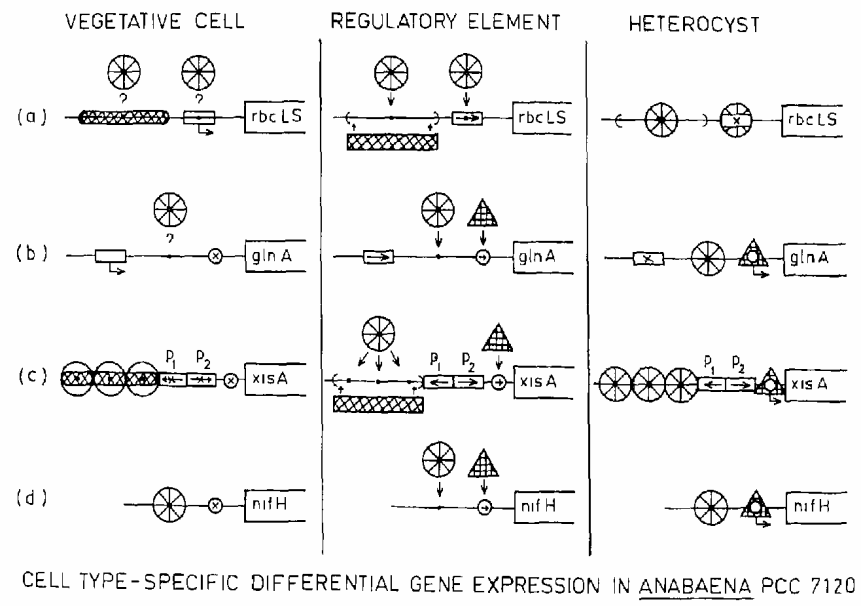

Figure 1. A scheme to explain cell type-specific differential gene expression in *Anabaena* PCC 7120. Important differentially expressed genes are shown in the middle column along with their upstream regulatory elements denoted by (▭), a constitutive "*E. coli*-type" promoter; (O), a "*nif*-like" promoter; (●), a NtcA-binding site; and , a factor 2-binding site. Of the three DNA-binding proteins, NtcA/BifA (⊛) is present in both cell types, the factor 2 (▩) only in the vegetative cells, and the σ_{nif} (▲) is proposed to be present only in the heterocysts. The downward arrows (↓) point to the binding sites, the horizontal arrows (←, →) indicate the direction of transcription, while the arrow (↳) signifies induction of transcription. The *rbcLS* expression in vegetative cells is activated by factor 2 by outcompeting the NtcA repressor; in heterocysts NtcA represses *rbcLS* in the absence of factor 2. The *glnA* expresses in vegetative cells from "*E. coli*-type" promoter; in heterocysts NtcA represses expression from "*E. coli*-type" promoter and induces expression from "*nif*-like" promoter in conjunction with σ_{nif} . The *xisA* does not express in vegetative cells due to divergent P_1/P_2 promoters and repression by binding of NtcA and factor 2; in heterocysts the NtcA and σ_{nif} induce *xisA* expression from the "*nif*-like" promoter. The *nifH* does not express in the vegetative cells due to non-availability of σ_{nif} which induces its expression in the heterocysts in combination with the NtcA.

It has been estimated that nearly 15-25% of the genome (> 1000 genes) may selectively express in the *Anabaena* heterocysts (Lynn *et al* 1986). This prompted efforts aimed at identifying heterocyst specific σ-factors among other transcriptional factors (Buikema and Haselkorn 1993). Expression of at least two alternate σ-factor genes, the *sigB* and *sigC,* commences within 6 h of nitrogen stepdown and is maximal between 12 to 18 h—the peak period for heterocyst differentiation, gene rearrangements etc (Brahamsha and Haselkorn 1992). Although, these alternate σ-factors express only under nitrogen deficiency, individual mutants in *sigB/sigC* or a double mutant lacking both SigB and SigC, do not affect nitrogen fixation in *Anabaena* sp. strain PCC 7120 (Brahamsha and Haselkorn 1992). The target genes of these σ-factors and their possible role remains unknown at present.

A global nitrogen control gene *ntcA* was initially identified in a unicellular adiazotroph, *Synechococcus* sp. strain PCC 7942 (Vega-Palas *et al* 1990,1992). In parallel the gene was also cloned and identified as *bifA* (DNA-binding factor A, earlier called the

vegetative cell factor or VF1) in *Anabaena* sp. strain PCC 7120 (Chastain *et al* 1990; Wei *et al* 1993). In *Anabaena,* the BifA protein was found to bind the target sequence TGT-N_{10}-ACA which is also the target sequence of NtcA in *Synechococcus,* The sequence was found located upstream of several genes known to be differentially expressed in different cell types (Ramasubramanian *et al* 1994). Among the target genus of BifA (NtcA) are the *rbcLS* (two binding sites: between —493 to — 516 bp and between —547 to — 558 bp) encoding ribulose 1,5-bisphosphate carboxylase and expressed only in the vegetative cells, the *xisA* (three binding sites: located between — 152 to — 205 bp) which expresses only transiently in differentiating cells, *nifH* (no clear binding site) expressed only in heterocysts (Elhai and Wolk 1990) and *glnA* (one binding site: —125 to — 148 bp) which expresses both in the heterocysts and the vegetative cells (figure 1). The NtcA binding to these genes was found to decrease in the following order *glnA* > *xisA* > *rbcLS* > *nifH* (Chastain *et al* 1980; Ramasubramanian *et al* 1994). A perfect TGT-N_{10}-ACA sequence is also present 1 kb upstream of the *hetR* gene (see the sequence reported by Buikema and Haselkorn 199 1b) and at — 88 bp in the upstream region of an unnamed gene, the *orf1* (Borthakur *et al* 1990). NtcA may positively regulate the transcription of both *hetR* and *orf1* genes, which are expressed only during nitrogen stepdown and specifically in heterocysts. The *ntcA* gene itself possesses the target sequence, indicating that it may be autoregulated in *Anabaena* sp. strain PCC 7120 (Frias *et al* 1993).

The target sequence of NtcA is the same as that of Nif A, the transcriptional activator of *nif* Operons in all diazotrophs, except that *nifA* and its product have never been identified in cyanobacteria so far and NtcA controls many genes other than *nif* in cyanobacteria (Vega-Palas *et al* 1992; Frias *et al* 1993; Carrasco *et al* 1994). Based on the known expression patterns of the genes it binds to, it was thought that NtcA could act both as activator as well as repressor of different target genes (Wei *et al* 1993) —a common characteristic of the Crp family of regulators (Vega-Palas *et al* 1992). Recently, a *ntcA* null mutant has been obtained in *Anabaena* sp. strain PCC 7120 which has profound negative effects on diazotrophic capabilities of the strain (Carrasco *et al* 1994). The mutant does not grow in combined nitrogen-free media, does not produce heterocysts, fails to rearrange the *nifD* and *fdxN* elements and lacks nitrogenase activity. The mutant also does not express the l 7kb *glnA* transcript which emanates from a "*nif*-like" promoter (at — 90 bp) (Carrasco *et al* 1994). However, it does not affect *rbcLS* expression. These results project NtcA as a transcriptional activator of *xisA* and l·7kb *glnA* transcript. The inability of *ntcA* mutant to develop heterocysts suggests that NtcA is a global nitrogen regulator affecting both heterocyst development (through expression of *hetR?)* and nitrogen fixation, as well as nitrate assimilation. By analogy, therefore, it appears to be a functional equivalent of NtrC in enteric bacteria such as *Klebsiella* (Merrick 1988).

Another DNA-binding protein, termed the factor 2, has also been shown to bind upstream sequences of both the *rbcLS* and *xisA* gene, but not the *glnA* gene, based on gel retardation assays (Ramasubramanian *et al* 1994). More interestingly in the case of *xisA,* its binding domain (—156 to —223bp) completely overlaps the three binding domains (from — 152 to — 205 bp) for NtcA (or BifA). Similarly the binding domain of factor 2 on *rbcLS (*— 519 to — 581 bp) also completely overlaps one of the two binding domains (— 4 9 3 to — 516 bp and — 547 to — 558 bp) of NtcA. The corresponding gene for factor 2 has not yet been cloned. The protein is reportedly found only in the vegetative cells but not in the heterocysts (Ramasubramanian *et al* 1994). This contrasts with

NtcA which occurs both in the vegetative cells as well as in the heterocysts (Ramasubramanian *et al* 1994). This suggests interesting possibilities of differential interaction between these two DNA-binding proteins in different cell types.

5. A model to explain the chronology of events and cell type-specific differential gene expression in *Anabaena*

The principle environmental signal that initiates heterocyst differentiation in *Anabaena* is nitrogen deficiency (Haselkorn 1978; Wolk 1991). Completion of differentiation and formation of mature heterocysts depends on the presence of oxygen in the environment (Rippka and Stanier 1978; Apte 1993; Apte and Prabhavathi 1994). One chronological arrangement of all the important events that eventually result in heterocyst differentiation and constitution of N_2 fixation in *Anabaena* sp. strain PCC 7120 is listed in table 2. How the change in nitrogen levels in the surroundings is sensed in *Anabaena* is still not understood. But in the unicellular cyanobacterium *Synechococcus* sp. Strain PCC 7942 the P_{II} protein, analogue of the well known sensor of nitrogen status in the enteric bacteria, has been identified (Tsinoremas *et al* 1991) and shown to be activated by its phosphorylation (Forchhammer and Tandeau de Marsac 1994). Kinases which can serve this purpose exist in *Anabaena* sp. strain PCC 7120 and are expressed quickly (within 2h) following nitrogen stepdown (Zhang 1993). The *hetR* is one of the earliest genes expressed (2 h) subsequent to nitrogen stepdown and may function as one of the partners in a two-component nitrogen status sensor-transducer system that works early on. The aforesaid early events (table 2) in some way induce certain specific proteases which begin to degrade intracellular nitrogen reserves, especially the hypothetical *N* repressor of development. Degradation of gene products of *hetN, hetM* and *hetI* is likely to occur around this time and HetR may play an important role (Buikema and Haselkorn 1991b). Proteases involved are not known but are unlikely to be Ca^{2+}-dependent proteases since inactivation of genes encoding them does not affect heterocyst differentiation (Maldener *et al* 1991). The HetR and/or the proteases, thus, may help in adjudging the cells (with least content of repressor?) destined to be differentiated (table 2). Soon (3·5 h) *hetR* expression becomes restricted to specific cells—the first visible sign of commitment of cells to differentiation (Black *et al* 1993).

Once the intracellular optimal nitrogen status has been reached (by 6 h) (table 2), expression of NtrC-like transcriptional factors, such as PatA, may occur in committed cells (Liang *et al* 1992). At the same time at least two nitrogen stepdown-specific alternate σ_B and σ_c factors also appear but their functions are not yet known (Brahamsha and Haselkorn 1992). Some of these transcriptional factors trigger expression of heterocyst-specific structural components such as products of *hepA* (envelope polysaccharide) (Wolk *et al* 1993), *hglK* (envelope glycolipid) (Buikema and Haselkorn 1993) and *devA* gene (Maldener *et al* 1994). Expression of another important gene, *hetP,* is also induced at 6h and precedes that of *hepA;* the function of HetP is unknown except that the *hetP* mutants do not differentiate heterocysts (Fernadez-Pinas *et al* 1994). Through the continuing action of proteases, proheterocysts lose the blue phycobiliprotein, phycocyanin (Apte and Prabhavathi 1994), and begin to look pale or greenish. Laying of oxygen-impervious heterocyst walls and loss of phycocyanin results in an anerobic milieu in these cells (table 2). A molecular event that signifies this development is the expression and activation of the Fnr-like transcriptional activator, the

Table 2. Chronology of important events related to nitrogen fixation occurring in *Anabaena* PCC 7120. after nitrogen stepdown.

Time (h)	Important events
0-2	1. Low nitrogen status is sensed probably by P_{II} type of protein which is activated by phosphorylation. 2. Hypothetical repressor of development and N_2 fixation is degraded. 3. The *hetR* expresses in all cells and titrates out the repressor.
3 5	1. First sign of commitment of certain cells (with least amount of the N repressor?) to differentiation is visible. 2. HetR expression localized to such committed cells of the filament. 3. Nitrogen reserves in comitted cells are degraded due to induction of specific proteases.
6-12	1. HetR expression increases further. 2. Expression of *patA* (a "Ntrc-like" transcriptional activator) begins. 3. Expression of *hepA* (coding heterocyst wall polysaccharide) starts. 4. Expression of alternate σ-factor genes *sigB* and *sigC* starts. 5. Expression of *hetN, hetM, hetl* genes is shut-off and probably helps to consolidate the nature of commitment of differentiation. 6. Proheterocysts begin to lose phycocyanin and O_2 evolving capacity and become morphologically discernible. 7. The *devA* (coding periplasmic permease) expresses and trafficking of wall layer components to heterocyst walls starts. 8. Expression of *patB* coding a "Fnr-like" transcriptional activator starts.
12-18	1. Heterocyst envelopes are formed and mature heterocysts appear. 2. PatB activated since optimal redox state is reached in proheterocysts. 3. HetR, SigB and SigC express abundantly. 4. HepA and HglK (coding heterocyst wall glycolipid) express abundantly. 5. The *patA* expression decreases. 6. The *ntcA* gene is expressed strongly. 7. Expression of alternate σ_{nif} capable of interacting with "*nif*-like" promoters probably occurs at this stage.
18-24	1. HetR increases 20-fold. SigB, SigC and PatB express maximally. 2. The three excisase genes *(xisA, xisF, xisC)* are expressed causing gene rearrangements and creating three functional Operons. 3. The 1 7 kb *gln A* transcript appears. 4. Fully developed mature heterocysts are morphologically discernible. 5. Nitrogenase activity commences.
> 24	1. Heterocyst frequency reaches a maximum (about 10%). 2. SigB, SigC and PatB disappear. 3. Expression of HetR, DevA, HepA and HglK decreases. 4. NtcA expresses abundantly. 5. *nifHDK, hupL, glnA* express maximally. 6. Nitrogen fixation reaches a peak at 36-40 h. 7. The *rbcLS* expression is repressed in the heterocysts.

Developmental programme is completed; physiological division of labour is enforced.

PatB, around 12h after nitrogen stepdown (Liang *et al* 1993). The ensuing 6h period witnesses active synthesis of HetR, SigB, SigC, HepA, HglK and DevA but decreased expression of *patA*.

A major development around this time is the strong expression of *ntcA* product (Wei *et al* 1994), which has NifA-like DNA-binding properties and NtrC-like global functions. We propose that another (yet to be found) alternative σ-factor (tentatively called

the σ_{nif} is also expressed around 18h in *Anabaena* sp. strain PCC 7120 heterocysts (table 2). It is an interesting coincidence that NtcA arrives on the scene just when the NtrC-like PatA departs. Appearance of NtcA and σ_{nif} sets in motion the functional differentiation, i.e., the closure of carbon fixation, rearrangements of *nif* and *hup* genes and organization of the nitrogen-fixing apparatus in the mature heterocysts. The important events include expression in heterocysts of the three excisase genes (*xisA, xisF* and *xisC*), followed by all the *nif* genes, the *hupL, fdxH, fdxN* and a new mRNA species of *gln A*. Concomitant repression of *rbcLS* completes the story (table 2).

The global effects of *ntcA* seem to arise from its ability to bind upstream sequences of several genes (figure 1), but its exact role is somewhat enigmatic. Its binding upstream of *hetR, xisA, nifH, orf1,* and *glnA* strongly suggest it to be an activator of transcription while its binding upstream *of rbcLS* befits that of a repressor. This suitably explains the expression of these genes in heterocysts but fails to explain their non-expression in the vegetative cells. The cell-specific expression of these genes appears to be controlled by interactions between the NtcA, another DNA-binding protein factor 2 and the proposed $\sigma_{nif.}$ The late expression of these genes (after 18h) seems to a consequence of late appearance of NtcA on the scene (> 12 h). The possible scheme and chronology of events underlying the constitution of nitrogen fixation apparatus in *Anabaena* heterocysts is outlined in figure 1.

To explain the scheme, we propose that NtcA transcriptionally (i) activates the "*nif*-like" promoters of several genes, especially the *xisA, nifH* and *glnA* in combination with the $\sigma_{nif,}$ and (ii) represses expression of *rbcLS* in *Anabaena* sp. strain PCC 7120. NtcA may also affect expression of *hetR, orf1* and possibly the *xisF* and *xisC* genes, though they lack "*nif*-like" promoters. This suggestion is based on (i) the presence of a target sequence (TGT-N_{10}-ACA) upstream of most of these genes (Borthakur *et al* 1990; Chastain *et al* 1990; Buikema and Haselkorn 1991b; Ramasubramian *et al* 1994), (ii) observed physical binding of NtcA to several of these genes (Chastain *et al* 1990; Ramasubramanian *et al* 1994), and (iii) the Het^-, Nif^- phenotype of the *ntcA* mutant which also lacks the 11 kb and 55 kb rearrangements (Wei *et al* 1994). It may be recalled that a clear NtcA target sequence is not found upstream of *nifH,* though it binds to NtcA (possibly when $\sigma_{nif.}$ is also available). The location of the NtcA-binding site upstream of *glnA* suggests a possible dual role (figure 1), i.e., as an inducer of the "*nif*-like" promoter (at — 90 bp) giving rise to the 1·7 kb transcript typical of nitrogen-fixing cultures, and as repressor of the "*E. coli*-type" promoter (at — 155 bp). The former role appears to be facilitated by σ_{nif} in the heterocysts, there is no evidence that NtcA actually represses *glnA* transcription from the "*E. coli*-type" promoter either in vegetative cells or in the heterocysts. Of the two NtcA-binding sites upstream of *rbcLS,* the one between — 493 to — 416 bp would clearly block the expression from this promoter (at — 504 bp). NtcA, thus, should act as repressor of *rbcLS* operon (Ramasubramanian *et al* 1984) (figure 1).

The factor 2 protein, present only in the vegetative cells, is mainly a positive regulator of the *rbcLS* expression (Ramasubramanian *et al* 1994). We propose that in the vegetative cells, factor 2 may counteract the actions of NtcA by interfering with its binding upstream of *rbcLS* (figure 1). Indeed, Ramasubramanian *et al* (1994) found that in the presence of factor 2, BifA did not bind well to *rbcLS.* It may be emphasised here that factor 2 has very high affinity for *rbcLS* than for *xisA* while the *ntcA* binding follows the order *glnA* > *xisA* > *rbcLS* » *nifH.* It seems very likely, therefore, that factor 2 can outcompete NtcA in its binding to *rbcLS* and cause constitutive expression

of the operon in the vegetative cells. In the heterocysts, which lack the factor 2, the NtcA binds to *rbcLS* and effectively shuts off its transcription (figure 1). That a *ntcA* mutation does not affect the total *rbcLS* expression in *Anabaena* sp. strain PCC 7120 filaments (Wei *et al* 1994) also substantiates the above suggestion.

The reasons for non-expression of *xisA* in vegetative cells appear to be several, viz., transcriptional interference from divergent P_1 and P_2 promoters (Lammers *et al* 1986), repression by binding of factor 2 and/or NtcA at the NRE (Chastain *et al* 1990), and the non-availability of σ_{nif} in the vegetative cells. It seems unlikely that factor 2 can prevent NtcA from binding to *xisA* (as proposed for *rbcLS* above) since (i) NtcA binds to *xisA* much more strongly than factor 2, and (ii) no interference or synergy in binding of either proteins to *xisA* has been reported, so far (Ramasubramanian *et al* 1994). We, therefore, attribute the heterocyst-specific expression of *xisA, nifH* and the l·7kbmRNA of *glnA* primarily to the presence of σ_{nif} exclusively in the heterocysts, and to its absence in the vegetative cells (figure 1). The glutamine synthetase can be synthesized in the vegetative cells from the "*E. coli*-type" promoter and in the heterocysts from the "*nif*-like" promoter.

An important lacuna in the proposed scheme is that the detection and isolation of the proposed σ_{nif} has hitherto evaded several laboratories. More serious attempts in this direction, probably with different methods, would be necessary in future. For example, mobility shift assays with purified DNA-binding proteins using *Anabaena* "*nif*-like" promoter sequences as substrates may prove to be useful. Certain other aspects of the model also need to be corroborated by unambiguous experimental evidence. Thus, the suggested patterns of binding of NtcA and factor 2 to *rbcLS* and also *xisA* (figure 1) can be ascertained in gel retardation assays by setting up competetion between NtcA and factor 2 for binding with these targets. Of particular relevance will be the binding patterns observed at the physiological ratios of these proteins found in the vegetative cells. Another clinching evidence needed to confirm the model is the cell type-specific (vegetative cells versus heterocysts) expression of factor 2 as also of *ntcA*. Studies would also be necessary to fill up the gaps in our understanding of the chain of events (as described in figure 1), especially how the expression of so many transcriptional factors is coordinated in time and linked to the expression of their target genes.

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References

Apte S K 1992 Molecular Biology of Cyanobacterial Nitrogen Fixation: Recent Advances; *Indian J. Microbiol.* **32** 103-106

Apte S K 1993 Cyanobacterial Nitrogen Fixation: Molecular Genetic Aspects; *Proc. Indian Natl. Sci. Acad.* **59** 367-386

Apte S K and Prabhavathi N 1994 Rearrangements of nitrogen fixation (*nif*) genes in the heterocystous cyanobacteria; *J. Biosci.* **19** 579-602

Black T A, Cai Y and Wolk C P 1993 Spatial expression and autoregulation of *hetR,* a gene involved in the control of heterocyst development in *Anabaena; Mol. Microbiol.* **9** 77-84

Black T A and Wolk C P 1994 Analysis of a Het$^-$ mutation in *Anabaena* sp. strain PCC 7120 implicates a secondary metabolite in the regulation of heterocyst spacing; *J. Bacteriol.* **176** 2282-2292

Bohme H and Haselkorn R 1988 Molecular cloning and nucleotide sequence analysis of the gene coding for heterocyst ferredoxin from the cyanobacterium *Anabaena* PCC 7120; *Mol. Gen. Genet.* **214** 278-285

Borthakur D, Basche M, Buikema W J, Borthakur P B and Haselkorn 1990 Expression, nucleotide sequence and mutational analysis of two open reading frames in the *nif* gene region of *Anabaena* sp. strain PCC 7120; *Mol. Gen. Genet.* **221** 227-234

Brahamsha B and Haselkorn R 1991 Isolation and characterization of the gene encoding the principle sigma factor of the vegetative cell RNA polymerase from the cyanobacterium *Anabaena* sp. strain PCC 7120; *J. Bacteriol* **173** 2442-2450

Brahamsha B and Haselkorn R 1992 Identification of multiple RNA polymerase sigma factor homologs in the cyanobacterium *Anabaena* sp. strain PCC 7120: cloning, expression, and inactivation of the *sigB* and *sigC* genes; *J. Bacteriol.* **174** 7273-7282

Brusca J S, Chastain C J and Golden J W 1990 Expression of the *Anabaena* PCC 7120 *xisA* gene from a heterologous promoter results in excision of the *nifD* element; *J. Bacteriol.* **172** 3925-3931

Buikema W J and Haselkorn R 1991a Isolation and complementation of nitrogen fixation mutants of the cyanobacterium *Anabaena* sp. strain PCC 7120; *J. Bacteriol.* **173** 1879-1885

Buikema W J and Haselkorn R 199 1b Characterization of a gene controlling heterocyst differentiation in the cyanobacterium *Anabaena* 7210; *Genes Dev.* **5** 321-330

Buikema W J and Haselkorn R 1993 Molecular genetics of cyanobacterial development; *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44** 33-52

Carrasco C D, Buettner J A and Golden J W 1995 Programmed DNA rearrangement of a cyanobacterial *hupL* gene in heterocysts; *Proc. Natl. Acad. Sci. USA* **92** 791-795

Carrasco C D, Ramaswamy K S, Ramasubramanian T S and Golden J W 1994 *Anabaena xisF* gene encodes a developmentally regulated site-specific recombinase; *Genes Dev.* **8** 74-83

Chastain C J, Brusca J S, Ramasubramanian T S, Wei T F and Golden J W 1990 A sequence specific DNA-binding factor (VFI) from *Anabaena* sp. strain PCC 7120 vegetative cells binds to three adjacent sites in the *xisA* upstream region; *J. Bacteriol.* **172** 5044-5051

Elhai J and Wolk C P 1990 Developmental regulation and spatial pattern of expression of the structural genes for nitrogenase in the cyanobacterium *Anabaena; EMBO J.* **9** 3379-3388

Fernandez-Pinas F, Leganes F and Wolk C P 1994 A third genetic locus required for the formation of heterocysts in *Anabaena* sp. strain PCC 7120; J. *Bacteriol.* **176** 5277-5283

Forchhammer K and Tandeau de Marsac N 1994 The P_{II} protein in the cyanobacterium *Synechococcus* sp. strain PCC 7942 is modified by serine phosphorylation and signals the cellular N status; *J. Bacteriol.* **176** 84-91

Frias J E, Merida A, Herrero A, Martin-Nieto J and Flores E 1993 General distribution of the nitrogen control gene *ntcA* in cyanobacteria; *J. Bacteriol.* **175** 5710-5713

Golden J W, Carrasco C D, Mulligan M E, Schneider G J and Haselkorn R 1988 Deletion of a 55-kilobase-pair element from the chromosome during heterocyst differentiation of *Anabaena sp.* strain PCC 7120; *J. Bacteriol.* **170** 5034-5041

Golden J W, Mulligan M E and Haselkorn R 1987 Different recombination site specificity of two developmentally regulated genome rearrangements; *Nature* (*London*) **327** 526-529

Golden J W, Robinson S J and Haselkorn R 1985 Rearrangement of nitrogen fixation genes during heterocyst differentiation in the cyanobaclerium *Anabaena; Nature (London)* **314** 419-423

Golden J W and Wiest D R 1988 Genome rearrangements and nitrogen fixation in *Anabaena* blocked by inactivation of *xisA* gene; *Science* **242** 1421-1423

Golden J W, Whorff L L and Wiest D R 1991 Independent regulation of *nif HDK* operon transcription and DNA rearrangement during heterocyst differentiation in the cyanobacterium *Anabaena* sp. strain PCC 7120; *J. Bacteriol.* **173** 7098-7105

Haselkorn R 1978 Heterocysts; *Annu. Rev. Plant Physiol.* **29** 275-284

Haselkorn R 1992 Developmentally regulated gene rearrangements in prokaryotes; *Annu. Rev. Genet.* **26** 113-130

Haselkorn R, Basche M, Bohme H, Borthakur D, Borthakur P B, Buikema W J, Mulligan M E and Norris D 1990 Nitrogen fixation in filamentous cyanobacteria; in *Nitrogen fixation: Achievements and objectives* (eds) P Gresshoff, L E Roth, G Stacey and W E Newton (New York: Chapman and Hall) pp 497-504

Holland D and Wolk C P 1990 Identification and characterization of *hetA,* a gene that acts early in the process of morphological differentiation of heterocysts; *J. Bacteriol.* **172** 3131-3137

Houchins J P 1984 The physiology and biochemistry of hydrogen metabolism in cyanobacteria; *Biochim. Biophys. Acta* **768** 227-255

Kuritz T, Ernst A, Black T A and Wolk C P 1993 High-resolution mapping of genetic loci of *Anabaena* PCC 7120 required for photosynthesis and nitrogen fixation; *Mol. Microbiol.* **8** 101-110

Lammers P, Golden J W and Haselkorn R 1986 Identification and sequence of a gene required for a developmentally regulated DNA excision in *Anabaena; Cell* **44** 905-911

Liang J, Scappino L and Haselkorn R 1992 The *pat A* gene product, which contains a region similar to CheY of *Escherichia coli,* controls heterocyst pattern formation in the cyanobacterium *Anabaena* 7120; *Proc. Natl. Acad. Sci. USA* **89** 5655-5659

Liang J, Scappino L and Haselkorn R 1993 The *patB* gene product, required for the growth of cyanobaeterium *Anabaena* sp. strain PCC 7120 under nitrogen limiting conditions, contains ferredoxin and helix-turn-helix domains; *J. Bacteriol.* **175** 1697-1704

Lynn M, Bantle J A and Ownby J D 1986 Estimation of gene expression in heterocysts of *Anabaena variabilis* by using DNA-RNA hydridization; *J. Bacteriol.* **167** 940-946

Madan A P and Nierzwicki-Bauer S A 1993 In situ detection of transcripts for ribulose-l,5-bisphosphate carboxylase in cyanobacterial heterocysts; *J. Bacteriol.* **175** 7301-7306

Maldener I, Fieldler G, Ernst A, Fernandez-Pinas F and Wolk C P 1994 Characterization of *devA,* a gene required for the maturation of proheterocysts in the cyanobacterium *Anabaena* sp. strain PCC 7120; *J Bacteriol.* **176** 7543-7549

Maldener I, Lockau W, Cai Y and Wolk C P 1991 Calcium-dependent protease of the cyanobacterium *Anabaena:* molecular cloning and expression of the gene in *Escherichia coli,* sequencing and site-directed mutagenesis; *Mol. Gen. Genet.* **225** 113-120

Merrick M J 1988 Organisation and regulation of nitrogen fixation genes in *Klebsiella* and *Azotobacter,* in *Nitrogen fixation: Hundred years after* (eds) H Bothe, F J deBruijn and W E Newton (Stuttgart: Gustav Fischer) pp 293-302

Mulligan M E, Buikema W J and Haselkorn R 1988 Bacterial-type ferredoxin genes in the nitrogen fixation regions of the cyanobacterium *Anabaena* sp. strain PCC 7120 and *Rhizobium meliloti; J. Bacterial.* **170** 4406-4410

Ramasubramanian T S, Wei T F and Golden J W 1994 Two *Anabaena* sp. strain PCC 7120 DNA-binding factors interact with vegetative cell- and heterocyst-specific genes; *J. Bacteriol.* **176** 1214-1223

Razquin P, Schmitz S, Fillat M F, Peleato M L and Bohme H 1994 Transcriptional and translational analysis of ferredoxin and flavodoxin under iron and nitrogen stress in *Anabaena* sp. strain PCC 7120; *J. Bactenol.* **176** 7409-7411

Rippka R and Stanier R Y 1978 The effects of anaerobiosis on nitrogenase synthesis and heterocyst development by Nostocacean cyanobacteria; *J. Gen. Microbiol.* **105** 83-94

Scrautemeier B and Bohme H 1985 A distinct ferredoxin for nitrogen fixation isolated from heterocysts of the cyanobacterium *Anabaena variabilis; FEBS Lett.* **184** 304-308

Stewart W D P 1980 Some aspects of structure and function in N_2-fixing cyanobacteria; *Annu Rev. Microbiol.* **34** 497-536

Stock J B, Ninfa A J and Stock A M 1989 Protein phosphorylation; *Microbiol. Rev.* **53** 450-490

Thomas J 1970 Absence of the pigements of photosystem II of photosynthesis in heterocysts of a blue-green alga; *Nature* (*London*) **228** 181-183

Thomas J, Wolk C P, Shaffer P W, Austin S M and Galonsky A 1975 The initial organic products of fixation of ^{13}N-labelled nitrogen gas by the blue-green alga *Anabaena cylindrical Biochem. Biophys. Res. Commun.* **67** 501-507

Tsinoremas N F, Castets A M, Harrison M A, Allen J F and Tandeau de Marsac N 1991 Photosynthetic electron transport controls nitrogen assimilation in cyanobacteria by means of posttranslational modification of the *glnB* gene product; *Proc. Natl. Acad. Sci. USA* **88** 1565-1569

Turner N E, Robinson S J and Haselkorn R 1983 Different promoters for the *Anabaena* glutamine synthetase gene during growth using molecular or fixed nitrogen; *Nature* (*London*) **306** 337-342

Vega-Palas M A, Flores E and Herrero A 1992 NtcA, a global nitrogen regulator from the cyanobacterium *Synechococcus* that belongs to the Crp family of bacterial regulators; *Mol. Microbiol.* **6** 1853-1859

Vega-Palas M A, Madueno F, Herrero A and Flores E 1980 Identification and cloning of a regulatory gene for nitrogen assimilation in the cyanobacterium *Synechococcus* sp. strain PCC 7942; *J. Bacteriol.* **172** 643-647

Wei T F, Ramasubramanian T S and Golden J W 1994 *Anabaena* sp. strain PCC 7120 *ntcA* gene required for growth on nitrate and heterocyst development; *J. Bacteriol.* **176** 4473-4482

Wei T F, Ramasubramanian T S, Pu F and Golden J W 1993 *Anabaena* sp. strain PCC 7120 *bifA* gene encoding a sequence-specific DNA-binding protein cloned by *in vivo* transcriptional interference selection; *J. Bacteriol.* **175** 4025-4035

Wolk C P 1982 Heterocysts; in *The biology of cyanobacteria* (eds) N G Carr and B A Whitton (Berkeley: University of California Press) pp 359-386

Wolk C P 1991 Genetic analysis of cyanobacterial development; *Curr. Opininon Genet. Dev.* **1** 336-341

Wolk C P, Elhai J, Kuritz T and Holland D 1993 Amplified expression of a transcriptional pattern formed during development of *Anabaena; Mol. Microbiol.* **7** 441-445

Zhang C 1993 A gene encoding a protein related to eukaryotic protein kinases from the filamentous, heterocystous cyanobacterium *Anabaena* sp. strain PCC 7120; *Proc. Natl. Acad. Sci. USA* **90** 11840-11844