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Review article

P_{II} Signal Transduction in Cyanobacteria

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Abstract

P_{II} signalling proteins are pivotal players in almost all aspects of nitrogen regulation in bacteria. The P_{II} signal transduction system in cyanobacteria displays some profound differences compared to the P_{II} signalling systems of other bacteria characterised so far. This review summarises the advances in functional and mechanistic insights in P_{II} signal perception and transduction in unicellular cyanobacteria. A model is proposed which provides a rational basis for the observed synergy between the effector molecules ATP and 2-oxoglutarate in controlling the phosphorylation state of P_{II}. The function of P_{II} in global nitrogen control of cyanobacteria is discussed in the view of novel results.

Keywords: Nitrogen regulation, NtcA, nitrate uptake, protein phosphorylation

1. Introduction: Global Nitrogen Control in Cyanobacteria

Cyanobacteria constitute one of the largest groups of Gram-negative bacteria, and due to their wide distribution, abundance and their metabolism, they play

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a dominant role in the global nitrogen- and carbon cycle. Their metabolism is based on oxygenic photosynthesis, a process that provides reducing equivalents and ATP to assimilate simple inorganic nutrients from the environment. All cyanobacteria are able to utilise ammonium, nitrate or nitrite as nitrogen sources. However, only a subset of cyanobacterial species are able to fix gaseous dinitrogen. Among these, a monophyletic group of filamentous cyanobacteria fixes nitrogen within specialized cells, the heterocysts, which are differentiated upon the onset of nitrogen starvation (for recent reviews, see Meeks and Elhai, 2002; Wolk, 2000; Adams and Duggan, 1999). Other diazotrophic strains are able to fix N_2 without the need of specialised cells, employing different strategies to protect nitrogenase from oxygen (Berman-Frank et al., 2001; for review see Gallon, 2001; Sherman et al., 1998). Ammonium, the preferred source of combined nitrogen, depresses the utilization of alternate nitrogen sources. It causes a general decrease in the abundance of nitrogen assimilatory enzymes and inhibits the activity of combined nitrogen transport systems, a process known as global nitrogen control (reviewed by Flores and Herrero, 1994; Herrero et al., 2001). Global nitrogen control is operating in diazotrophic as well as in non-diazotrophic strains. In contrast to the diazotrophic strains, the non-diazotrophic cyanobacteria respond to the absence of a combined nitrogen source by a process known as chlorosis, characterised by a loss of pigmentation (Allen and Smith, 1969; Collier and Grossman, 1992). In the model organism *Synechococcus elongatus* strain PCC 7942 it had been demonstrated that chlorotic cells are able to survive nitrogen starvation for extended periods of time (up to 2 years) in a dormant like state (Görl et al., 1998; Sauer et al., 2001; for review see Forchhammer, 2002).

An essential gene in global nitrogen control was discovered in the early 90th in the group of Flores and Herrero in the unicellular strain *Synechococcus* sp. PCC 7942 (Vega-Palas et al., 1992) and by Wei et al. (1993) in *Anabaena* sp. PCC7120. It encodes a transcription factor of the CRP/FNR family which was termed NtcA (nitrogen control). Meanwhile, NtcA is recognized to be the major transcription factor responsible for the transcriptional activation of genes subject to global nitrogen control (reviewed by Herrero et al., 2001). In addition, NtcA represses various genes under conditions of nitrogen shortage. Whereas the DNA-binding sites of NtcA are clearly established (Luque et al., 1994), the mode of regulation of NtcA activity is not yet fully understood. Recent *in vitro* studies demonstrated that 2-oxoglutarate binding of NtcA plays a crucial role in activating the binding of NtcA to its DNA targets (Vazquez-Bermudez et al., 2002) and in stimulating transcriptional initiation at NtcA-dependent promoters (Tanigawa et al., 2002). Our recent studies on the nitrogen-starvation response in *Synechococcus* PCC 7942 revealed that in addition, the PII signal transduction protein is required for activating NtcA-dependent gene expression following nitrogen step-down (see below).

2. The P_{II} Signalling Protein in Cyanobacteria

The family of P_{II} signal transduction proteins consists of one of the most widely distributed signalling proteins in nature. They are pivotal players in various aspects of nitrogen regulation (for review, see Arcondeguy et al., 2001). In all cyanobacteria investigated so far, P_{II} homologues, exhibiting approx. 50% to 65% amino acid identity to P_{II} paralogues from proteobacteria could be identified (Tsinoremas et al., 1991; Palinska et al., 2002). Sequence alignments revealed a distinct amino acid signature in the cyanobacterial P_{II} homologues which is centred around the T-loop region of the protein (see below) (Palinska et al., 2002). The entirely sequenced cyanobacterial genomes each contain only one *glnB* encoding gene, indicating the absence of multiple P_{II} paralogues in these organisms. The first cyanobacterial P_{II} protein was identified by Harrison et al. (1990) in the non-diazotrophic strain *Synechococcus* PCC 6301 and the gene was subsequently cloned from the highly similar strain *Synechococcus* PCC 7942 (Tsinoremas et al., 1991). Subsequent biochemical investigation of the modification of the *Synechococcus* P_{II} protein (*Sc* P_{II}) revealed that, in contrast to the hitherto characterized proteobacterial P_{II} homologues, *Sc* P_{II} is phosphorylated at a seryl-residue (Forchhammer and Tandeau de Marsac, 1994). The phosphorylation site could be identified as Ser 49, located only two amino acids away from the site of uridylylation of the *Escherichia coli* P_{II} (Tyr 51) (Forchhammer and Tandeau de Marsac, 1995b). In the crystal structure of the P_{II} proteins GlnB and GlnK from *E. coli* (Carr et al., 1996; Xu et al., 1998), this position is located at the tip of a solvent exposed flexible loop, termed T-loop, which plays a key role in the interactions of P_{II} with target proteins (Jiang et al., 1997). The fact, that *Sc* P_{II} forms heterotrimers with the *E. coli* GlnK and GlnB proteins indicates that their structures are highly similar (Forchhammer et al., 1999). Four different forms of the trimeric *Sc* P_{II} protein can be resolved by non-denaturing electrophoresis, the unmodified form, and the P_{II} trimer containing one, two or three phosphorylated subunits (designated P_{II}^0, P_{II}^1, P_{II}^2 and P_{II}^3). Analysis of the phosphorylation state of P_{II} *in vivo* showed a strong correlation with the status of nitrogen and carbon assimilation (Fig. 1). *Sc* P_{II} was mostly non-phosphorylated in ammonium-grown cells whereas the degree of phosphorylation markedly increased when the cells utilized nitrate as N-source, provided that the cells were sufficiently CO_2 supplied. Limiting CO_2 supply led to a marked decrease in the phosphorylation state of P_{II}. Following nitrogen-step down, the unphosphorylated form of P_{II} disappeared completely, P_{II} being present in a highly phosphorylated state. Treatments with inhibitors affecting photosynthetic electron transport led to a decrease of P_{II} phosphorylation (Forchhammer and Tandeau de Marsac, 1994; Hisbergues et

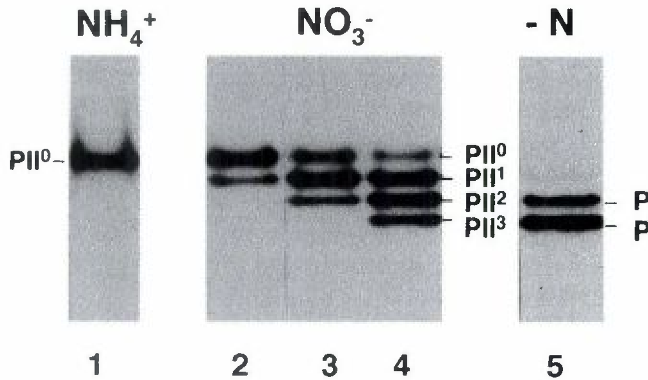

Figure 1. The phosphorylation state of *Sc* P_{II} depends on the cellular nitrogen and carbon supply: Extracts of *Synechococcus* PCC 7942 cells grown under different conditions were separated by non-denaturing gel-electrophoresis to separate the differentially phosphorylated forms of P_{II} and the position of the P_{II} forms was detected by immuno-blotting as described by Forchhammer and Tandeau de Marsac (1994). P_{II}^0, P_{II}^1, P_{II}^2 and P_{II}^3 designate the trimeric P_{II} protein containing no, one two or three phosphorylated subunits. Lanes: 1, Cells grown in the presence of ammonium; lanes 2–4: cells grown in the presence of nitrate; 2, without aeration and agitation (carbon limited); 3, aeration with air (0.03 % CO_2); 4, aeration with air enriched with 1% CO_2. lane 5: cells deprived for a combined nitrogen source for 3 hours.

al., 1999). Hisbergues et al. (1999) interpreted this correlation as an indication that P_{II} might also respond to changes in the cellular redox-state.

3. Functional Analysis of P_{II} Proteins in Cyanobacteria

To reveal the function of P_{II} in cyanobacteria, attempts were made to create *glnB* knockout mutants in different cyanobacterial strains. Completely segregated mutants were obtained in the unicellular strains *Synechococcus* PCC 7942 (Forchhammer and Tandeau de Marsac, 1995a) and *Synechocystis* PCC 6803 (Hisbergues et al., 1999). However, complete segregation of the mutant *glnB* allele failed in the filamentous heterocyst-forming strains *Nostoc punctiforme* (Hanson et al., 1997) and *Anabaena* PCC 7120 (C.C. Zhang, personal communication), suggesting that the *glnB* gene is essential in these organisms. The P_{II}-deficient *Synechococcus* PCC 7942 strain MP2 showed a variety of pleiotropic defects, such as a global intracellular nitrogen-carbon

imbalance and an impairment to rapidly adapt to changing conditions of illumination and of nitrogen and carbon supply (Forchhammer and Tandeau de Marsac, 1995a). The nitrate/nitrite transport system was deregulated and was no more inhibited by CO_2 shortage and ammonium treatment (Forchhammer and Tandeau de Marsac, 1995a; Lee et al., 1998). P_{II} mutants, in which the phosphorylated seryl residue was changed to alanine, showed a constitutive repression of nitrate/nitrite uptake, suggesting that the unmodified form of P_{II} was inhibitory to nitrate/nitrite uptake (Lee et al., 2000). Nevertheless, ammonium repression of various NtcA regulated genes was unaffected in the P_{II}-deficient mutant, suggesting that P_{II} was not involved in regulation of NtcA activity (Forchhammer and Tandeau de Marsac, 1995a).

The P_{II}-deficient mutant of *Synechocystis* PCC 6803 displayed a similar phenotype than the *Synechococcus* PCC 7942 mutant. Furthermore, the high affinity bicarbonate uptake system was constitutively active in the absence of a functional P_{II} protein (Hisbergues et al., 1999), suggesting that P_{II} is also involved in regulating bicarbonate transport.

4. The *Synechococcus* P_{II} Protein is a Sensor of the Cellular 2-oxoglutarate Levels under ATP Replete Conditions

Biochemical studies of the *Synechococcus* P_{II} protein yielded insights into the mechanism of signal perception of P_{II}. Ligand binding studies showed that *Sc* P_{II} binds ATP and 2-oxoglutarate in a mutually dependent manner (Forchhammer and Hedler, 1997; reviewed in Forchhammer, 1999). The binding characteristics are similar to those reported for the *E. coli* GlnB protein (Kamberov et al., 1995): Binding of ATP is strongly enhanced in the presence of 2-oxoglutarate and vice versa, 2-oxoglutarate binding to P_{II} is stimulated by ATP. In the absence of 2-oxoglutarate, one ATP molecule binds to the trimeric P_{II} protein with an affinity of 37 μM. However, in the presence of 5 mM 2-oxoglutarate, the stoichiometry of ATP binding to *Sc* P_{II} increases by 2.2 fold (Forchhammer and Hedler, 1997). The crystal structure of the trimeric *E. coli* P_{II} protein revealed three putative ATP binding sites, which are located in the cleft at the interface of two subunits (Xu et al., 2001). The observed stoichiometry in the absence of 2-oxoglutarate thus suggests that binding of one ATP molecule to *Sc* PII exerts a strong anticooperativity towards the other two ATP binding sites. Therefore, only one site per trimer can be occupied with high affinity.

Using partially purified extract from a P_{II}-deficient mutant of *Synechococcus* PCC 7942, P_{II} kinase and phosphatase activities could be characterized. Addition of a kinase extract to unphosphorylated P_{II} caused a partial phosphorylation of the substrate in a strictly ATP and 2-oxoglutarate

dependent manner. However, no phosphorylation was observed using other low molecular weight effectors (Forchhammer and Tandeau de Marsac, 1995b). Conversely, dephosphorylation of phospho-P_{II} (P_{II}-P) was catalyzed by a Mg^{2+}-dependent activity (Irmler et al., 1997). This reaction could be inhibited by the addition of ATP and 2-oxoglutarate. In contrast to the bifunctional P_{II} modifying UTase/UR enzyme in proteobacteria, *Sc* P_{II} kinase and phosphatase are distinct enzymes, since the activities could be separated by chromatography (Irmler et al., 1997). Activity of the phosphatase extract towards other substrates, such as phosphocasein, was not affected by ATP and 2-oxoglutarate. This fact, together with the reciprocal regulation of kinase and phosphatase activities by ATP and 2-oxoglutarate and the corresponding binding properties of these effectors to P_{II} (see above) suggested that P_{II} regulates its phosphorylation and dephosphorylation through conformational changes caused by the binding of these effector molecules. Therefore, we suggested that 2-oxoglutarate is the primary signal which indicates the state of nitrogen and carbon assimilation in cyanobacteria (Forchhammer, 1999).

5. Identification and Properties of the P_{II}-P Specific Phosphatase from *Synechocystis* PCC 6803

The biochemical studies suggested that P_{II}-P phosphatase displays characteristic features of protein phosphatases of the PP2C family (Irmler et al., 1997). The genome of the cyanobacterium *Synechocystis* PCC 6803 encodes eight genes with homology to members of the PP2C family (Kaneko et al., 1996). To identify the P_{II}-P phosphatase at the molecular level, we inactivated various phosphatase encoding genes from this organism and analysed the phenotype of the resulting mutants with respect to P_{II}-P dephosphorylation (Irmler and Forchhammer, 2001). The mutant in open reading frame sll1771 showed the phenotype expected for a P_{II}-phosphatase mutant: it was unable to rapidly dephosphorylate P_{II}-P in response to ammonium treatment, CO_2 shortage or other stresses which cause P_{II}-P dephosphorylation in the wild-type. The gene, which we designated *pphA* encodes a 28.5 kDa protein that exhibits the 11 conserved motifs present in the catalytic core of the members of the PP2C family (Shi et al., 1998). N- or C-terminal domains that could act as substrate binding or regulatory domains, as known from eukaryotic PP2C members, are missing in PphA. Sequence comparison with the database revealed that PphA is member of a large, but hitherto uncharacterized group of bacterial PP2C homologues, most of which share the small size with PphA (Irmler and Forchhammer, 2001). Closely related homologues of PphA can be found in the genomic data from the heterocystous strains *Anabaena* PCC 7120 and *Nostoc punctiforme*. Surprisingly,

no PphA homologue was found in the genome of the prochlorophyte *Prochlorococcus marinus* PCC 9511. In agreement, biochemical studies showed that its P_{II} protein is not modified by phosphorylation (Palinska et al., 2002), despite the high sequence identity to *Sc* P_{II}, including the phosphorylation site Ser 49.

PphA from *Synechocystis* PCC 6803 was purified and its enzymatic properties were characterized (Ruppert et al., 2002). It showed the typical features of PP2C phosphatases, dephosphorylating a broad range of phosphorylated substrates in a Mg^{2+}/Mn^{2+}-dependent manner. Comparison of the reactivity towards different substrates revealed a clear preference for substrates in which phosphorylated seryl- and threonyl-residues are embedded in a large peptide environment, since dephosphorylation of phosphocasein is more efficient by three orders of magnitude than that of various phosphopeptides. In the absence of effector molecules, reactivity towards P_{II}-P is similar to that towards phosphocasein. However, P_{II}-P dephosphorylation is strictly regulated by effector molecules, whereas phosphocasein dephosphorylation is not affected. In agreement with the previous biochemical data using partially purified extracts (see above), this suggests that the effector molecules act through binding to P_{II}, thereby altering its reactivity towards PphA. Thus, dephosphorylation of P_{II}-P by PphA in a purified system can be used as a highly sensitive indicator of the liganded status of P_{II}-P.

6. Probing P_{II}-P-effector Molecule Interactions through Protection towards Dephosphorylation

Addition of ATP, GTP or ADP in the mM range to a P_{II}-P dephosphorylation assay partially protected P_{II}-P from dephosphorylation by PphA (Ruppert et al., 2002). Addition of 2-oxoglutarate alone had no effect on the dephosphorylation reaction, but in combination with ATP it strongly enhanced the protection from dephosphorylation (Fig. 2). By contrast 2-oxoglutarate had no, or only a marginal, effect in the presence of GTP or ADP. Half maximal P_{II}-P protection in the presence of 1 mM ATP required approx. 20 µM 2-oxoglutarate. At these or at lower 2-oxoglutarate concentrations, protection of P_{II}-P is not fully established and a further increase of ATP concentration increased P_{II}-P protection. However, at 2-oxoglutarate concentrations above 0.1 mM, P_{II}-P protection was already fully established by ATP concentrations above 0.5 mM (Fig. 2). This suggested that variations of the ATP concentrations within the physiological mM range may effect the *in vivo* reactivity of P_{II}-P with PphA when the intracellular 2-oxoglutarate concentrations are low. Besides 2-oxoglutarate, also oxaloactetate enhanced P_{II}-P protection by ATP, although

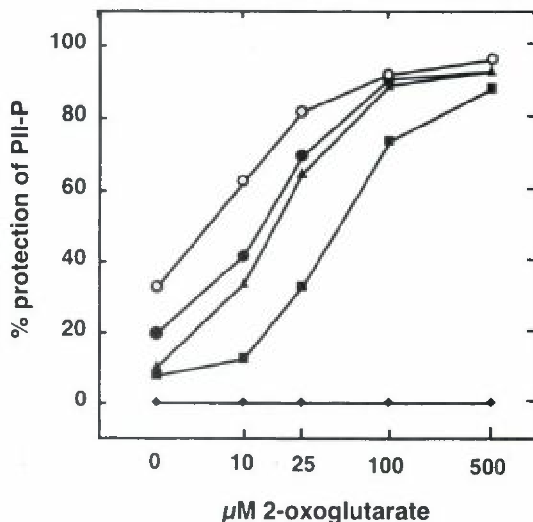

Figure 2. ATP and 2-oxoglutarate-dependent protection of P_{II}-P from dephosphorylation by PphA. Series of P_{II}-P dephosphorylation reactions were conducted in the presence of none (closed diamonds), 0.1 mM (closed squares), 0.5 mM (closed triangles) 1 mM (closed circles) or 2.5 mM (open circles) ATP, together with the indicated amounts of 2-oxoglutarate (0, 10 μM, 25 μM, 100 μM and 500 μM). Subsequent to the analysis of the phosphorylation state of P_{II}, protection of P_{II}-P from dephosphorylation was calculated. Experimental details are described by Ruppert et al. (2002).

less efficiently than 2-oxoglutarate. These results refine our model of the signal perception of P_{II} in cyanobacteria: the primary signal was confirmed to be 2-oxoglutarate. Furthermore, P_{II} integrates signals from the related metabolite oxaloactetate and under certain conditions may also be a sensor of the energy charge of the cells.

A clue to the mechanism of ATP-mediated inhibition of P_{II}-P dephosphorylation and the synergistic effect of 2-oxoglutarate came from experiments with non-hydrolyzable ATP analogues (Ruppert et al., 2002). P_{II}-P protection by AMP-PNP was very efficient but was not stimulated by 2-oxoglutarate. By contrast, AMP-PNP and ATP-γ-S showed a 2-oxoglutarate dependent P_{II}-P protection, like ATP. This suggests that there is a critical difference between ATP and AMP-PCP, which affects their binding to P_{II} and the synergism of 2-oxoglutarate. The only difference between these molecules is the atom between the β- and γ-phosphate, O being replaced by C. This affects the hydrogen bonding properties and the steric orientation of the γ-phosphate

group. From these findings, and in accord with the binding characteristics of *Sc* P_{II} (see above), we propose the following model: When one ATP molecule is bound to the trimeric P_{II} protein (exhibiting three potential ATP binding sites), anticooperative interactions, in which the γ-phosphate group of ATP plays a critical role, cause a conformational change which reduces the affinity of binding sites 2 and 3. Upon binding of 2-oxoglutarate, anticooperativity between the ATP binding sites is reduced, allowing sites 2 and 3 to be occupied by ATP with higher affinity. A higher occupation of P_{II}-P with ATP finally results in protection of P_{II}-P from dephosphorylation by PphA. This model suggests a direct or indirect interaction of 2-oxoglutarate with the γ-phosphate group of ATP to alleviate the anticooperativity between the ATP binding sites. The subtle differences between ATP and AMP-PCP around the γ-phosphate would be sufficient to reduce these interactions in the case of AMP-PCP binding. In accord with this model, the crystal structure of *E. coli* PII with ATP shows that the γ-phosphate group is indeed highly co-ordinated (Xu et al., 2001). Recently, Benelli et al. (2002) proposed a potential 2-oxoglutarate binding site in P_{II}, based on structural comparisons with other 2-oxoglutarate binding proteins. According to this model and in agreement with our suggestion, the 2-oxoglutarate binding site is co-ordinated by the same amino acids, which are involved in co-ordination of the γ-phosphate group of ATP, namely Lys 90 and Arg 101.

7. A Proposed Function for P_{II} Phosphorylation

In the PphA deficient mutant, P_{II} remains in its highly phosphorylated state upon treating the cells with ammonium, lowering the CO_2 supply or inhibiting photosynthetic electron transport (Irmler and Forchhammer, 2001). Nevertheless, the mutant showed no apparent growth phenotype on ammonium supplemented medium or when growing them under various conditions of CO_2 supply (Irmler and Forchhammer, unpublished; Irmler 2001). Therefore, dephosphorylation of P_{II} may not be essential for the regulation of nitrogen metabolism by ammonium. A similar conclusion was drawn from a study of P_{II}-mutants in *Synechococcus* PCC 7942, in which Ser 49 was replaced by either by Ala (P_{II}-A) to yield a P_{II} protein which can not be phosphorylated, or by Asp (P_{II}-D) or Glu (P_{II}-E), which mimic the phosphorylated protein (Lee et al., 2000). Whereas P_{II}-A constitutively blocked nitrate/nitrite uptake, regulation of nitrate/nitrite uptake was not affected in the mutants P_{II}-D and P_{II}-E. This suggested that relief of nitrate/nitrite transport inhibition by P_{II} requires not only phosphorylation of Ser 49 (or substitution by Asp or Glu), but also additional effectors, whose concentration depends on the nitrogen/carbon supply. These effectors are most likely the P_{II} effector molecules 2-oxolutarate,

oxaloacetate and nucleotides. By analogy with the regulation of PphA-P_{II}-P interaction, a conformational change caused by their binding to P_{II}-P (or P_{II}-D or P_{II}-E) might be necessary, in addition to P_{II}-phosphorylation, to relieve the inhibitory effect of P_{II} on nitrate/nitrite transport, although the mechanistic details of this interaction have not been investigated in detail hitherto. Lowering of the effector molecule concentration would, in turn, immediately inhibit nitrate/nitrite transport by a conformational switch. Subsequent dephosphorylation of P_{II}-P by PphA would then arrest P_{II} in a state in which nitrate transport is inhibited regardless of the effector molecule concentration, until P_{II} is again phosphorylated by the P_{II} kinase under appropriate conditions. According to this model, the allosteric interactions of P_{II} with effector molecules would be the first level of P_{II} regulation and phosphorylation of P_{II} would be a second level, which could be used to fine tune the signal transduction system. The lack of P_{II} phosphorylation in *Prochlorococcus marinus* PCC 9511 implies that in this organism the first level of P_{II} regulation is sufficient. Regulation of P_{II} activity only at the first level, without the need of covalent modification, is reminiscent to the situation in enteric bacteria, where control of NifL activity by the P_{II} paralogue GlnK was shown to be independent of uridylylation of Tyr 51 (He et al., 1998; Arcondeguy et al., 1999).

8. P_{II} Regulates NtcA Activity during Nitrogen-Deprivation in *Synechococcus* PCC 7942

The P_{II}-deficient mutant in *Synechococcus* PCC 7942 (MP2) displayed a variety of pleiotropic phenotypes, indicating that P_{II} is involved in the regulation of a wide range of cellular functions (Forchhammer and Tandeau de Marsac, 1995a). Analysis of the response of this non-diazotrophic strain towards nitrogen-starvation revealed that the expression of a nitrogen-starvation specific glutamine synthetase (GSIII) was severely impaired in MP2 following nitrogen-step down (Sauer et al., 2000). Subsequent investigation of *de-novo* protein synthesis by *in vivo* ^{35}S-labelling and 2D-PAGE analysis revealed that the MP2 cells were unable to induce almost all proteins which were specifically induced by nitrogen step-down in wild-type cells. The same defect was observed in a mutant in which the *ntcA* gene was inactivated (Sauer, 2001). This result suggested a regulatory link between P_{II} and the activation of NtcA-dependent gene expression. To study further the relationship between P_{II} and NtcA, fusions of the promotorless *luxAB*-reporter genes with the NtcA-regulated *glnB* gene were constructed. Following transformation into wild-type, MP2 and MNtcA cells, *luxAB* expression under different growth conditions was determined by measuring luciferase-activity of the recombinant strains

(Aldehni et al., 2002). In the presence of ammonium, a strong repression of the NtcA-dependent promotor could be observed in both wild-type and P_{II}-deficient background. This confirms the previous data, which suggested that ammonium-repression of NtcA activity is not impaired in a P_{II}-deficient mutant (Forchhammer and Tandeau de Marsac, 1995b; Lee et al., 2000). However, following nitrogen deprivation, a striking difference between the strains could be observed. Whereas in the wild-type background, a more than 100-fold increase of expression from the NtcA dependent start-site could be observed, the P_{II}-deficient mutant was unable to increase the activity of the reporter gene, the same as observed in the NtcA-deficient mutant. Therefore, we conclude that P_{II} is required to activate NtcA-dependent gene expression following nitrogen step-down, whereas it is dispensable to promote repression of NtcA activity in the presence of ammonium. The P_{II}-independent repression of NtcA might reflect the direct response of NtcA towards 2-oxoglutarate (see above). An investigation of metabolite pools in *Synechocystis* PCC 6803 has demonstrated that in ammonium grown cells, 2-oxoglutarate levels are indeed low and correlate with the repression of NtcA-dependent genes (Muro-Pastor et al., 2001). However, NtcA is apparently unable to directly perceive nitrogen-starvation through 2-oxoglutarate binding, since P_{II} is required for its activation. Until now, the mechanism of P_{II} mediated NtcA activation is not understood. P_{II} may directly interact with NtcA, thereby modulating its activity. Alternatively, P_{II} may interact indirectly with NtcA, through a so-far unidentified protein. Indirect regulation of transcription factors by P_{II}-like proteins is found in the regulation of NtrC through NtrB (reviewed by Ninfa and Atkinson, 2000) or NifA through NifL in γ-proteobacteria (reviewed by Arcondeguy et al., 2001). This issue is currently under investigation.

9. Concluding Remarks

In the past few years, our understanding of the mechanisms underlying nitrogen control in cyanobacteria has significantly advanced. Several lines of evidences support the view that 2-oxoglutarate is the key regulatory metabolite which signals the cellular balance of nitrogen- and carbon assimilation. In autotrophic organisms, 2-oxoglutarate is a central metabolite in anabolic reactions, providing the carbon skeleton for incorporation of ammonium. The cells use the P_{II} signalling protein and NtcA to integrate this signal into a co-ordinated cellular response. However, important parts of the puzzle are still missing, such as the P_{II} kinase. Attempts to identify this enzyme by using genetic or biochemical approaches have failed, so far. The P_{II} kinase may respond to further signals, which could be integrated into the P_{II} regulatory pathway. The targets of P_{II} regulation are so far poorly understood:

no molecular study has so far addressed the question of the interaction of P_{II} with nitrate permease; the mode of P_{II}-NtcA interaction remains to be elucidated and further targets of P_{II} may exist in various cyanobacteria. With the advance of the powerful tools of global analysis such as transcriptome and proteome analysis, new insights are to expect in near future.

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