**REVIEW
ARTICLE****Protein phosphorylation in cyanobacteria**

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The post-translational modification of proteins by phosphorylation may take a variety of forms, but in essence it is always a component of a process of adaptation that enables cells to sense and respond to changes in their external or internal environments. This review presents evidence that not only do the various forms of protein phosphorylation occur in the cyanobacteria, but that they are central to many of the adaptive responses exhibited by these organisms (for review see Tandeau de Marsac & Houmard, 1993) and, indeed, may play a key role in the integration of metabolism. Early studies on protein phosphorylation in prokaryotes were largely confined to phosphate monoester formation with hydroxyl-containing amino acid residues. It was originally thought that protein acyl phosphates and amido phosphates only occurred as intermediates of enzyme catalysis. However, it is now clear that these forms of protein phosphorylation are of enormous significance in the process of sensory transduction via two-component sensory systems involving members of the conserved histidine protein kinase and response regulator families of proteins (for reviews see Stock *et al.*, 1989; Parkinson & Kofoid, 1992).

The dominant nutritional mode of cyanobacteria is photoautotrophy, and thus growth, with its reliance on light as the primary energy source, proceeds via the fixation of CO₂ and the acquisition of other simple inorganic nutrients. This would lead one to suggest that the central adaptive responses of these organisms might be to environmental variables such as the intensity and spectral quality of light, coupled with the availability of key inorganic nutrients. A potentially lethal consequence of their phototrophic lifestyle is the risk of photo-inhibition, when the rate at which light energy is harvested exceeds the rate at which it can be converted into useful metabolic energy. This emphasizes the importance of inorganic nutrients, since limitation for one or more greatly increases the non-productive excitation of photosynthetic reaction centres and photoinhibition may be greatly increased. Furthermore, under conditions where CO₂ fixation is restricted, photosynthetic electron transport may lead to the extensive production of

superoxide radicals with the potential for extensive oxidation damage to the cell. These factors emphasize the critical importance to the physiology of cyanobacteria of integrating the central metabolic process of light harvesting with inorganic nutrient acquisition and consequently this review also examines the possible role of protein phosphorylation in this process of integration.

Evidence for protein phosphorylation

There are several reports which describe the *in vivo* labelling of polypeptides, using [³²P]orthophosphate, in various species of cyanobacteria, including *Synechococcus* sp. PCC 6301 (Allen *et al.*, 1985), *Calothrix* sp. PCC 7601 (Schuster *et al.*, 1984), *Anabaena* sp. PCC 7120 (Mann *et al.*, 1991), *Synechocystis* sp. PCC 6803 (Bloye *et al.*, 1992; Hagemann *et al.*, 1993) and *Synechococcus* sp. PCC 7942 (Forchhammer & Tandeau de Marsac, 1994). Such studies reveal of the order of 10 labelled polypeptides, but this must be taken as very much a minimum number since only the most abundant phosphoproteins would be discovered by such an approach and, furthermore, only polypeptides phosphorylated as monoesters would have been detected by the methods employed. An additional caveat which must be borne in mind when assessing the results obtained using this approach is that in addition to phosphorylation, other forms of protein modification may be detected, such as ADP-ribosylation and adenylation. A complementary approach to the *in vivo* labelling of cells with ³²P-orthophosphate has been to study protein phosphorylation at the expense of [γ -³²P]ATP *in vitro*. Such experiments have been carried out with cell-free extracts from *Calothrix* sp. PCC 7601 (Schuster *et al.*, 1984), *Synechococcus* sp. PCC 6301 (Allen *et al.*, 1985), *Anabaena* sp. PCC 7120 (Mann *et al.*, 1991) and *Synechocystis* sp. PCC 6803 (Bloye *et al.*, 1992; Hagemann *et al.*, 1993) and numerous phosphorylated polypeptides detected. The caveat with this approach is that while only the phosphorylation of proteins is detected, many more polypeptides become labelled than can be detected by the *in vivo* approach and, thus, there must be concern that the phosphorylation of some of these polypeptides may be artifactual and has no genuine physiological significance.

Light

Cyanobacteria, like higher plants, exhibit oxygenic photosynthesis and possess two distinct photosystems with associated protein complexes within the thylakoid membranes, which are usually quite distinct from the cytoplasmic membrane. Chlorophyll *a* is involved in the reaction centres of both photosystems, but cyanobacteria lack light-harvesting complex II (LHCII) and, instead, phycobiliproteins, organized into macromolecular complexes known as phycobilisomes, represent the primary light harvesting antennae (for review see Grossman *et al.*, 1993). Cyanobacteria are capable of responding to changes in both the intensity and spectral quality of light and protein phosphorylation has been implicated in the response to spectral quality. Among the cyanobacterial responses to changes in spectral quality are state transitions, which involve a redistribution of excitation energy between PSI (photosystem I) and PSII (photosystem II), and chromatic adaptation, in which the complement of light-harvesting phycobiliproteins becomes modified. Both processes enable the organism to optimize its photosynthetic capacity in response to a particular regime of illumination.

Thylakoid phosphoproteins

Since much of the scientific interest in cyanobacteria is focused on their photosynthetic processes, it follows that many analyses of the roles of protein phosphorylation in cyanobacteria have been concerned with the organisms' responses to light, but only in very few cases have known components of the photosynthetic complexes been unambiguously identified as being subject to phosphorylation. In higher plants the *psbH* gene product, a component of PSII, is known to be phosphorylated on a threonine residue at position 2 in the polypeptide, but the cyanobacterial *PsbH* lacks the phosphorylation site (see Allen, 1992). However, Race & Gounaris (1993) have established immunologically that the *Synechocystis* sp. PCC 6803 *psbH* gene product is present in the thylakoid as a 6 kDa phosphoprotein. Harrison (1990) has tentatively identified the β -phycocyanin as a phosphoprotein in *Synechococcus* sp. PCC 6301 following *in vivo* labelling with [32 P]orthophosphate, extensive protein purification and amino-terminal sequencing of an 18.5 kDa phosphoprotein. In this laboratory (unpublished results) *in vitro* labelling experiments using cell-free extracts of *Synechocystis* sp. PCC 6803 have led to the same conclusion. However, though few specific thylakoid proteins have been reported as being subject to phosphorylation, there is considerable evidence of a less direct nature that a number of thylakoid and/or phycobilisome proteins are subject to phosphorylation. A variety of *in vitro* labelling experiments using [γ - 32 P]ATP have indicated the phosphorylation of thylakoid proteins in thylakoid preparations, of greater or lesser purity, from *Calothrix* sp. PCC 7601 (Schuster *et al.*, 1984), *Synechococcus* sp. PCC 6301 (Allen *et al.*, 1985), *Synechococcus* sp. PCC 7942 (James *et al.*, 1992) and *Synechocystis* sp. PCC 6803 (Bloye *et al.*, 1992). *In vivo* labelling studies using white-light-grown *Calothrix* sp. PCC 7601 cells revealed the phosphorylation

of three thylakoid proteins on serine or threonine residues (Schuster *et al.*, 1984). Similar experiments with *Synechococcus* sp. PCC 6301 revealed the light-dependent phosphorylation of a 15 kDa polypeptide exclusively associated with the membranes and a predominantly cytoplasmic polypeptide of 18.5 kDa (Allen *et al.*, 1985). The 18.5 kDa protein could also be found in a purified phycobilisome fraction after *in vivo* labelling of *Synechococcus* sp. PCC 6301 cells (Sanders & Allen, 1987) and presumably, on the basis of the results of Harrison (1990), may be β -phycocyanin.

State transitions

Cyanobacteria, like higher plants, are capable of short-term responses to fluctuations in the spectral quality of incident light that lead to an imbalance in the energy harvested by PSI and PSII. These state transitions result in a readjustment of the distribution of excitation energy between the two photosystems so as to minimize the imbalance. The regulation of light-induced state transitions in cyanobacteria and the associated phosphorylation of thylakoid proteins has amassed a very considerable and, at times, contradictory literature, which could not be dealt with in this short review. Fortunately this topic has recently been the subject of an excellent and detailed treatment by Allen (1992). The essence of the debate concerns the models which have been proposed to explain two key aspects of the state transitions, namely control of the process and the mechanism of energy redistribution. As regards control of the process it is variously proposed that the control of state transitions are determined by (i) the redox state of components of the intersystem chain, (ii) localized electron gradients around PSI and PSII, and (iii) cyclic electron flow around PSI. The opposing ideas concerning energy redistribution centre round spill-over from PSII to PSI or redirection of energy harvested by the phycobilisome directly to PSI. Furthermore, although several thylakoid membrane proteins are subject to phosphorylation both *in vivo* and *in vitro*, correlative rather than direct evidence implicates protein phosphorylation in the state transition process. In addition to the information reviewed by Allen (1992), it has been shown more recently in the case of *Synechococcus* sp. PCC 7942 (Forchhammer & Tandeau de Marsac, 1994) that the *in vivo* phosphorylation of certain polypeptides was influenced by the spectral quality of the light used for growth. Before leaving this topic it is important, for reasons relating to the crucial relationship between light harvesting and inorganic nutrient acquisition, to note that state transitions, and/or closely related phenomena, may not be exclusively determined by the spectral quality of the irradiance and that nitrogen metabolism, inorganic carbon (C_i) limitation and salt stress have all been shown to be involved (see below).

Chromatic adaptation

In cyanobacteria the light-harvesting antenna pigments for PSII are the phycobiliproteins, primarily phycocyanin, phycoerythrin and allophycocyanin, organized as

components of the phycobilisome. Many cyanobacteria, though not all, exhibit an ability to alter the phycobiliprotein composition of their phycobilisomes in response to the spectral quality of the incident illumination, a process known as chromatic adaptation (for reviews see Grossman *et al.*, 1993; Tandeau de Marsac & Houmard, 1993). Group I organisms do not alter their phycobiliprotein composition, whereas group II organisms alter the abundance of phycoerythrin. In group III organisms such as *Fremyella diplosiphon*, complementary chromatic adaptation occurs, in which red light promotes the synthesis of an inducible phycocyanin (PC_i) and represses the synthesis of phycoerythrin, whereas green light stimulates the synthesis of phycoerythrin and represses the synthesis of PC_i. Accumulation of the different phycobiliproteins is a direct reflection of transcript abundance.

Sobczyk *et al.* (1993) have identified factors that interact with the promoter region of the phycoerythrin operon. Two proteins (designated RcaA and RcaB) were found only in extracts of cells of *Calothrix* sp. PCC 7601 grown under green light and appeared to behave as positive transcriptional activators of the *cpeBA* (phycoerythrin) operon. The ability of RcaA, but not RcaB, to bind to the promoter region was lost upon treatment with alkaline phosphatase, strongly suggesting that the binding activity of RcaA was phosphorylation dependent. The RcaA binding site contained the motif TTGTTA-N₄-TTGTTA. A protein (PepB) with the same binding specificity was detected by Schmidt-Goff & Federspiel (1993), but PepB appeared to be present under both red and green light and no information is available on whether PepB is subject to phosphorylation. Sobczyk (1994) suggests that the discrepancy between these results may possibly be explained by differences in culture conditions and by the fact that the SF33 strain used by Schmidt-Goff & Federspiel (1993) is a mutant of *Calothrix* sp. PCC 7601. Another protein (RcaD), which could only be isolated from red-light-grown cells, bound to the promoter region of the red-light-induced *cpcB2A2H2I2D2* operon, also in an apparently phosphorylation-sensitive fashion (Sobczyk *et al.*, 1994).

Chiang *et al.* (1992) have characterized a gene, *rcaC*, which complements a mutation in *F. diplosiphon* that causes the organism to be unable to respond to red light. The polypeptide encoded by *rcaC* is thought to be a negative regulator and is unusual since it exhibits two regions of strong similarity to the response regulator family of proteins, including the two potential phosphorylation sites; in this sense it resembles FrzZ of *Myxococcus xanthus* (see Parkinson & Kofoed, 1992). Chiang *et al.* (1992) point out that its N-terminus (extending up to approximately residue 240) exhibits similarity to the N-terminus of *Bacillus subtilis* PhoP. The C-terminal domain of RcaC (extending from approximately residue 530 to 633) exhibits strong similarity to the C-terminus of *Escherichia coli* CreB, also a response regulator. We have also noted a region (130 residues) of lower similarity (20.8%) to SscC (resolvase integrase) in the intervening domain. Another mutant, FdB1, of *F. diplosiphon*, which exhibits con-

stitutive synthesis of PC_i, has been complemented by a fragment of genomic DNA containing three ORFs, two of which encode polypeptides exhibiting strong homologies to the *E. coli* histidine protein kinase NR_{II}. The third ORF exhibits homology to rat myosin light-chain kinase (Grossman *et al.*, 1993). The role of these kinases, however, is still open to doubt since re-complementation of the mutant with the DNA fragment carrying these ORFs was not successful. This evidence for a phosphorylation cascade, modulated by photo-perception, controlling complementary chromatic adaptation has been incorporated into a model described in full in the review by Grossman *et al.* (1993).

Most cyanobacteria also exhibit inverse chromatic adaptation in which under PSII light there is a relative increase in PSI components and a decrease under PSI light (see Tandeau de Marsac & Houmard, 1993). *In vivo* labelling of *Synechococcus* sp. PCC 6301 cells revealed enhanced phosphorylation, under PSII light, of a 19 kDa polypeptide in the phycobilisome fraction (Sanders *et al.*, 1989). Harrison (1990) established that β -phycocyanin is subject to phosphorylation *in vivo* and suggested that the redox control of β -phycocyanin phosphorylation on a tyrosine residue may be involved in a photoreceptor mechanism involved in modulating long-term changes in the composition of the photosynthetic complexes and that the phosphorylation produces a photochromic form of the pigment. Such a model would be consistent with the changes involved in inverse chromatic adaptation. Indeed, it is worth noting that sequence analysis of a 14.9 kb fragment of DNA from *Synechococcus* sp. WH8020 encoding the major phycobiliprotein rod components revealed a partial ORF, adjacent to *rpeF*, which would encode a predicted polypeptide exhibiting homology to the N-terminal sequence of a low molecular mass cytosolic acid phosphatase from bovine liver (Wilbanks & Glazer, 1993). The liver enzyme shows phosphotyrosine phosphatase activity and a particular feature of the homology is a 12 residue stretch of identical amino acids between the two proteins believed to be the active site of the bovine enzyme. The polypeptide encoded by this ORF could be a candidate for a phosphatase controlling the suggested tyrosine phosphorylation of β -phycocyanin.

Inorganic nutrients

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Quantitatively CO₂ is the most important inorganic nutrient for cyanobacteria and it frequently becomes the limiting nutrient, during periods of high insolation, in the surface layers of freshwater environments which have a significant cyanobacterial population. In the presence of O₂, cyanobacterial ribulose-1,5-bisphosphate carboxylase/oxygenases exhibit a relatively low affinity (effective K_m approximately 600 μ M) for CO₂ (Pierce & Omata, 1988) and under conditions of low CO₂ concentration many species induce a bicarbonate-concentrating system (see Badger, 1987; Miller, 1990). Following a shift from bicarbonate-limited conditions to excess bicarbonate supply, *Synechocystis* sp. PCC 6803 exhibited a

rapid reduction in bicarbonate uptake rate and this was accompanied by the phosphorylation of a number of polypeptides ranging in size from 15–70 kDa (Bloye *et al.*, 1992). Similar changes in the profile of phosphorylated polypeptides were also observed when bicarbonate-limited cultures were shifted into the dark or were provided with exogenous glucose. Matching patterns of phosphorylation were obtained *in vitro* using the membrane fraction. Further studies (unpublished results – this laboratory) have localized the majority of these phosphorylated membrane proteins to the thylakoid fraction and it may be that some of these changes in the pattern of protein phosphorylation are associated with a loss of the partially uncoupled state of PSII, which is a characteristic of the C_i -limited condition (Miller & Calvin, 1987). In keeping with this is the observation that CO_2 transport was driven by ATP produced by cyclic or pseudocyclic photophosphorylation (Miller *et al.*, 1988) and the response to C_i availability might represent a nutritionally induced state transition. Thus C_i limitation may induce a transition to state 2, which would favour the ATP production required to energize C_i concentration.

Nitrogen metabolism and heterocyst formation

Nitrogen is quantitatively the second most important inorganic nutrient for cyanobacteria. Nitrate and ammonium, as well as a range of other inorganic and organic nitrogen sources, are utilized, and many species, including some unicellular strains, are capable of fixing dinitrogen. Furthermore, when nitrate is the nitrogen source for growth there will be a significantly increased demand for reductant in competition with photosynthetic CO_2 fixation. *Anacystis nidulans* strain 1402-1 cells exhibited stimulation of non-cyclic electron flow in response to nitrate, presumably in response to enhanced demand for reductant (Romero & Lara, 1987). The same organism exhibited non-photochemical fluorescence quenching, resembling a state 1 transition, in response to ammonium, but not nitrate (Romero *et al.*, 1992). A 13 kDa protein in *Synechococcus* sp. PCC 6301 was shown to become labelled with P_i *in vivo* exclusively under PSII light; subsequent purification and N-terminal sequencing revealed it to have strong homology to the GlnB (P_{II} protein) of *E. coli* (see Allen, 1992). In *E. coli* GlnB is subject to uridylation as a response to decreasing glutamine concentration relative to 2-oxoglutarate concentration and in turn activates an adenylyltransferase which deadenylylates glutamine synthetase causing its activation. Uridylation of GlnB also serves, by a cascade of phosphorylation reactions, to lead to transcription of the σ^{54} -dependent *glnALG* operon resulting in increased *de novo* synthesis of glutamine synthetase (for review see Magasanik, 1988). Using probes based on this N-terminal sequence information, Tsinoiremas *et al.* (1991) cloned and sequenced a *glnB* homologue from *Synechococcus* sp. PCC 7942 and demonstrated that the *in vivo* modifying group was sensitive to phosphodiesterase, consistent with uridylation. The modification state of the cyanobacterial GlnB homologue in *Synechococcus* sp. PCC 6301 was shown to be sensitive not only to ammonium ion concentration,

but also to light quality (Tsinoiremas *et al.*, 1991). Incubation under light exciting PSII increased the degree of modification. Thus, it was proposed that photosynthetic electron transport might regulate nitrogen assimilation via the modification state of the GlnB protein.

Recently, Forchhammer & Tandeau de Marsac (1994) have shown that the GlnB protein of *Synechococcus* sp. PCC 7942 is not in fact uridylylated, rather it is phosphorylated on a serine residue. It appears that the cyanobacterial GlnB protein is a trimer and consequently can potentially exist in four states, ranging from unphosphorylated to all three subunits phosphorylated. These studies, however, conflict with earlier studies regarding the effect of light quality. Forchhammer & Tandeau de Marsac (1994) found that the nitrogen status of the cell was the primary determinant of GlnB modification and that light-dependent variations were mediated through nitrate metabolism. The apparently contradictory results may reflect physiological differences between the strains used.

There is indirect evidence for protein phosphorylation playing a role in heterocyst pattern formation. Liang *et al.* (1992) isolated a mutant (PAT-1) of *Anabaena* sp. PCC 7120 exhibiting an altered pattern of heterocyst formation and poor growth under nitrogen-fixing conditions. The wild-type gene *patA*, which complements the lesion in the mutant, encodes a 379 amino acid polypeptide consisting of three domains. The carboxyl-terminal domain exhibits considerable homology to the response regulator CheY.

Phosphate

Phosphate is quantitatively the next most important inorganic nutrient for cyanobacteria after nitrogen, but in fact is more commonly the limiting nutrient in many freshwater environments. It has been suggested, as a result of studies on phosphate acquisition by *Synechococcus* sp. PCC 6301, that the uptake system may respond to changes in the external phosphate concentration by some form of sensory system (Falkner *et al.*, 1989). Indeed, there is now rapidly increasing evidence for a response to restricted phosphate availability which involves components exhibiting a marked similarity to the key elements of the extensively characterized enterobacterial phosphate (Pho) regulon (see Rao & Torriani, 1990). Aiba *et al.* (1993) cloned a chromosomal fragment from *Synechococcus* sp. PCC 7942 which was able to phenotypically complement a double mutant of *E. coli* that had lesions in the *phoR* and *creC* (histidine protein kinases) genes and was phenotypically $PhoA^-$ (alkaline phosphatase negative) as a consequence of being unable to induce the phosphate regulon. The cloned fragment carried two genes, designated *sphS* and *sphR*, encoding polypeptides which, respectively, exhibited strong similarities to the histidine protein kinase and response regulator families of proteins. A purified truncated version of SphS exhibited autophosphorylation activity consistent with its identification as a histidine protein kinase. Deletion mutants of *Synechococcus* sp. PCC 7942 lacking both *sphS* and *sphR* failed to induce several

proteins in response to phosphate limitation that were observed with the wild-type, and also failed to induce significant alkaline phosphatase activity. More recently, Nagaya *et al.* (1994) have shown that purified SphR is phosphorylated by phosphotransfer from SphS and binds to two distinct sites upstream from the alkaline phosphatase promoter, thus establishing their unambiguous identification as a histidine protein kinase/response pair. Similar evidence has been obtained with a phycoerythrin-containing marine strain, *Synechococcus* sp. WH7803. Two closely linked ORFs exhibiting high similarities (41 % and 26 % identity, respectively) to PhoP of *B. subtilis* and PhoR of *E. coli* have been characterized, and transcription of these genes was markedly increased under conditions of phosphate limitation (G. M. F. Watson, this laboratory – unpublished results).

A second similarity with the Pho regulon was established with *Synechococcus* sp. WH7803. This strain has been shown to contain a gene (*pstS*) encoding a presumptive periplasmic phosphate-binding protein which is transcribed under conditions of phosphate limitation (Scanlan *et al.*, 1993). No homologue of the *pstS* gene could be detected in freshwater cyanobacterial strains, nor could the PstS protein be detected immunologically. However, Aiba & Mizuno (1994) recently described a 33 kDa protein, under the control of SphS/R, which was induced in *Synechococcus* sp. PCC 7942 by phosphate limitation. Sequence analysis of the cloned gene *sphX* revealed an ORF of 337 amino acids which had a putative bacterial-signal-like sequence at the N-terminus. Wagner *et al.* (1994) reported the induction in the freshwater cyanobacterium *Anacystis nidulans* (*Synechococcus* species) strain 1402-1 of a polypeptide with an apparent size of 28–35 kDa in response to phosphate-limited growth which appeared to localize to the cytoplasmic membrane. The protein, which was described as a high affinity phosphate-binding protein, could be functionally reconstituted into liposomes. Amino-terminal sequence analyses of tryptic fragments of the protein were carried out and the two sequences reported, SFVNFYLQNA and VYAPGTDSGTYDYFNEAILNK, are identical to two regions of SphX. Mann & Scanlan (1994) have proposed that SphX and PstS are related and are both members of a family of phosphate-binding proteins. Thus it seems that the cyanobacterial response to phosphate limitation involves a system bearing marked similarities to the enterobacterial Pho regulon and that the regulation of expression of the proteins involved in the response is under the control of phosphorylation of a response regulator protein.

Chemotaxis

Many cyanobacteria have been shown to exhibit chemotactic responses, and Morgan *et al.* (1993) have demonstrated the presence of proteins antigenically related to the methyl-accepting chemotaxis proteins of *E. coli* in *Synechocystis* sp. PCC 6803 and *Oscillatoria tenuis*. The *patA* gene product of *Anabaena* sp. PCC 7120 shows marked homology in its carboxyl-terminal domain to the

response regulator chemotaxis protein CheY; however, *patA* is involved in the control of heterocyst pattern formation (Liang *et al.*, 1992). Recently though, sequence analysis of a genomic fragment from the phycoerythrin-containing marine strain *Synechococcus* sp. WH7803 revealed two ORFs for which the predicted translation products exhibited very high homologies to CheA and a methyl-accepting chemotaxis protein (G. M. F. Watson, this laboratory – unpublished results), suggesting that at least this strain may possess a phosphorylation-dependent signal transduction pathway related to chemotaxis. Waterbury *et al.* (1985) have shown that several of the marine phycoerythrin-containing *Synechococcus* strains are capable of a swimming motility which is thought to enable them to respond chemotactically to nutrient-enriched micropatches.

General stress responses

Salinity

The one general stress response in cyanobacteria in which protein phosphorylation has been implicated is that of salt stress (Hagemann *et al.*, 1993). Both hyper- and hypo-osmotic salt shocks led to very rapid alterations in the *in vivo* patterns of protein phosphorylation in cultures of *Synechocystis* sp. PCC 6803. These effects seem to be specific to NaCl since they could not be achieved with glycerol at the same osmotic concentration. Similarly, alterations in the pattern of phosphorylation were detected *in vitro* using either extracts of salt-shocked cells or upon addition of salt to extracts from unshocked cells. There is no information regarding the localization or identity of the phosphorylated species. The adaptation to salt stress in *Synechocystis* sp. PCC 6803 involves a diminished energy transfer from the phycobilisomes to PSII (Schubert & Hagemann, 1990) and in that sense resembles a state transition. Moreover, this process of adaptation involves increases in electron transport rates involving both PSI and cytochrome oxidase activity (Jeanjean *et al.*, 1990). Thus, the evidence that protein phosphorylation is involved mechanistically in these changes is only correlative.

Two-component sensory systems

Sensory transduction pathways involving two highly conserved families of proteins, histidine protein kinases and response regulators, have been extensively characterized from many genera of bacteria and are involved in adaptive responses to an enormously wide range of environmental variables (for reviews see Stock *et al.*, 1989; Parkinson & Kofoed, 1992). Several genes have been characterized from cyanobacteria which appear to encode polypeptides that exhibit marked sequence similarities to either the histidine protein kinase or response regulator families of proteins (see Table 1). Some of these genes have already been mentioned, i.e. in the case of phosphate acquisition and chromatic adaptation. In addition, Schluchter & Bryant (1992) have described an incomplete ORF downstream from the *petH* (ferredoxin-NADP⁺ oxidoreductase) gene of *Synechococcus*

Table 1. Cyanobacterial genes and ORFs encoding putative members of the histidine protein kinase or response regulator families of two component sensory systems

Organism	Gene or ORF designation	Predicted/observed size of polypeptide (amino acids)	Histidine protein kinase/response regulator	Homologies	Suggested function	Reference/source/ accession number
<i>Fremyella diplosiphon</i>	<i>rcaC</i>	632	RR	PhoR <i>B. subtilis</i>	Chromatic adaptation	Chiang <i>et al.</i> (1992) S27576
<i>Synechocystis</i> sp. PCC 6803	<i>dfrA</i>	663	HPK	PhoR <i>B. subtilis</i>	—	X72856
<i>Synechococcus</i> sp. PCC 7942	ORF	153	RR	PhoP <i>B. subtilis</i>	—	Chitnis <i>et al.</i> (1989)
	<i>spbS</i>	415	HPK	PhoR <i>B. subtilis</i>	Pho regulon	Aiba <i>et al.</i> (1993)
	<i>spbR</i>	257	RR	PhoP <i>B. subtilis</i>	Pho regulon	D13172
	<i>sasA</i>	387	HPK	RcsC <i>E. coli</i>	—	Nagaya <i>et al.</i> (1993)
	<i>srrA</i>	516	RR	OmpR rhodophyte	—	Anandan <i>et al.</i> (1993) and S. S. Golden (personal communication)
<i>Synechococcus</i> sp. PCC 7002	<i>srrB</i>	237	RR	OmpR rhodophyte	—	Schluchter & Bryant (1992) JO5366
<i>Synechococcus</i> sp. WH7803	ORF	Incomplete	RR	ArcA (dye) <i>E. coli</i>	—	G. M. F. Watson (unpublished results; this laboratory)
	<i>phoR</i>	383	HPK	PhoR <i>B. subtilis</i>	Pho regulon	
	<i>phoB</i>	234	RR	PhoP <i>B. subtilis</i>	Pho regulon	
<i>Anabaena</i> sp. PCC 7120	ORF	Incomplete	HPK	CheA <i>E. coli</i>	Chemotaxis	
	<i>patA</i>	379	RR	CheY <i>E. coli</i>	Heterocyst pattern	Liang <i>et al.</i> (1992)

sp. PCC 7002 which encodes a polypeptide with 32–42% similarity to the response regulators RscC, PhoB, the dye-resistance protein, and SprA. Shestakov *et al.* (1993) have described the isolation of a mutant of *Synechocystis* sp. PCC 6803 resistant to the herbicide difunon which is simultaneously resistant to chlorpromazine. Sequence analysis showed the mutation to affect a large ORF (663 amino acids) potentially encoding a polypeptide (Dfr) of 74.5 kDa and the construction of insertion mutations in this ORF gave rise to strains which were difunon resistant. The C-terminal end of Dfr exhibits considerable homology with PhoR of *B. subtilis* and therefore may be the histidine protein kinase element of a two-component sensory system. Nagaya *et al.* (1993), using a similar approach to that of Aiba *et al.* (1993), have cloned a gene, designated *sasA*, from *Synechococcus* sp. PCC 7942 which at high copy number is able to complement lesions in an *envZ* mutant of *E. coli* and also in a *phoR creC* double mutant. The *sasA* gene encodes a polypeptide of 387 amino acids which exhibits autophosphorylation and appears to be a member of the histidine protein kinase family with particular similarity to RcsC, EnvZ and PhoR of *E. coli*. It is interesting to note that the N-terminal domain of SasA lacks any significant potential membrane-spanning domains and therefore may be cytoplasmic. Two further genes, *srrA* and *srrB*, have been isolated from *Synechococcus* sp. PCC 7942, both of which appear to encode response regulators (Anandan *et al.*, 1993). SsrA appears to have an extra N-terminal domain in addition to the typical C-

terminal response regulator domain (S. S. Golden, personal communication). The large endogenous plasmid of *Synechococcus* sp. PCC 7942 also appears to encode a histidine protein kinase response regulator pair (D. Laudenbach, personal communication). Finally, Chitnis *et al.* (1989) have found an ORF immediately upstream from the PSI gene *psaE* which potentially encoded a protein with considerable similarity to the *B. subtilis* response regulator PhoP. However, the ORF was transcribed from the opposite strand to *psaE*.

Other protein kinases and phosphatases

Whilst there has been progress in analysing the physiological role of histidine protein kinases, particularly with respect to phosphate acquisition, comparatively little has been established about the nature of the kinase and phosphatases involved in determining the phosphorylation states of proteins which are subject to phosphorylation as monoesters. This is despite the fact that several species of cyanobacteria, including *Synechococcus* sp. PCC 6301 (Allen *et al.*, 1985), *Calothrix* sp. PCC 7601 (Schuster *et al.*, 1984), *Anabaena* sp. PCC 7120 (Mann *et al.*, 1991) and *Synechocystis* sp. PCC 6803 (Bloye *et al.*, 1992), have been shown, directly or indirectly, to contain a number of proteins which are subject to phosphorylation via the formation of monoesters with the hydroxyl-containing amino acids serine, threonine and tyrosine. Using an approach initially developed with *Mycobacterium xanthus*,

Zhang (1993) has characterized a gene (*pknA*) from *Anabaena* sp. PCC 7120 which encodes a protein containing marked similarities in amino acid sequence to the catalytic domain of the eukaryotic serine/threonine protein kinases. As in the case of *M. xanthus*, it would appear that there is a cyanobacterial family of related kinases. Inactivation of the gene led to a pleiotropic phenotype which included a reduced heterocyst frequency under N_2 -fixing conditions. Mention has already been made of the putative protein-tyrosine-phosphatase-encoding ORF adjacent to the genes encoding the major phycobilisome rod components in *Synechococcus* sp. WH8020 (Wilbanks & Glazer, 1993) and to the possible serine/threonine protein kinase of *F. diplosiphon* (Grossman *et al.*, 1993). A polypeptide of approximately 60 kDa associated with the thylakoids of *Synechocystis* sp. PCC 6803 is immunologically cross-reactive to antiserum raised against a preparation of higher plant LHCII kinase (unpublished results – this laboratory). Potts *et al.* (1993) have characterized a gene, designated *ihpP*, from *Nostoc commune* UTEX 584, encoding a polypeptide of approximately 30 kDa (294 amino acids), which included a sequence motif characteristic of certain protein tyrosine phosphatases from eukaryotes. The *ihpP* gene, on expression in *E. coli*, yields a protein with both tyrosine and serine phosphatase activities against artificial substrates. Somewhat surprisingly the amino acid sequence predicted for the *ihpP* gene product includes at its amino terminus a sequence of 24 amino acids which constitute a potential signal sequence, and indeed the gene when expressed in *E. coli* does appear to give rise to an extracellular product. Potts *et al.* (1993) also demonstrated by immunoblotting the presence of a tyrosine phosphorylated protein of approximately 85 kDa in *N. commune* grown in the presence of combined nitrogen which was not detectable after growth in nitrogen-deficient media. However, if the *IhpP* protein is also subject to secretion in *N. commune* it is difficult to reconcile this with the protein having a role in the regulation of nitrogen metabolism and perhaps it functions rather in phosphate nutrition. Sim & Mudge (1993) report that naturally occurring cyanobacterial scums containing predominantly *Anabaena circinalis* produced significant amounts of a glycogen phosphorylase phosphatase activity.

The activities of several of the protein kinases and phosphatases in cyanobacteria can be modulated by metabolites at physiologically significant concentrations and other factors such as the redox potential. The phosphorylation state of several *Synechococcus* sp. PCC 6301 thylakoid proteins is determined by redox conditions, in particular the redox state of plastoquinone (and/or the cytochrome b_6/f complex) (see Allen, 1992). In the case of *Anabaena* sp. PCC 7120, glucose 6-phosphate and ribulose 5-phosphate modulated the phosphorylation state of a 56 kDa protein by respectively stimulating a phosphatase and inhibiting a kinase, and the phosphorylation of other proteins could be affected by dithiothreitol (Mann *et al.*, 1991). Similarly, glucose 6-phosphate and ribulose 5-phosphate are key effectors, together with $NAD^+/NADH$ and $NADP^+/NADPH$,

of the two thylakoid-associated protein kinases of *Synechocystis* sp. PCC 6803 (unpublished results – this laboratory). In the case of these two kinases the redox state of plastoquinone and/or the cytochrome b_6/f complex was also an effector of activity.

Comments

It is clear that protein phosphorylation plays a role in several key aspects of cyanobacterial physiology and that two-component sensory systems are implicated in several of these areas. However, it now seems possible that protein phosphorylation may be involved in the overall integration of metabolism. Cyanobacteria must respond not only to changes in light quality and quantity which will affect the relative production of ATP and NADPH, but also to changing metabolic demands for energy and reductant and indeed to the switch between dark respiratory metabolism and light photosynthetic metabolism. Thus, growth under conditions of low C_i availability would lead to increased requirement for ATP, while growth on nitrate as a nitrogen source would lead to increased requirement for reductant. Consequently, the cell must regulate its photosynthetic activity, both in terms of absolute rate and of the pathways of photosynthetic electron flow (linear, cyclic and pseudocyclic). In the longer term the adaptation process may rely, at least in part, on changes in the pattern of transcription. For example in the case of persistent shifts in light quality, the stoichiometry of PSI/PSII in the thylakoids is altered by adjusting the absolute amounts of PSI (see Murakami & Fujita, 1993). Protein phosphorylation is likely to be involved in the short-term adaptive responses that permit balanced metabolism prior to the transcriptional adaptation or where the environmental parameter varies over time-scales too short for transcriptional responses. Evidence has already been described for short-term changes in the pattern of protein phosphorylation in response to such factors as C_i availability, light quality, nitrogen source, salt stress, etc. and in some of these cases the changes in the pattern of phosphorylation have been shown to involve thylakoid-associated proteins. Thus, it is tempting to speculate that the thylakoids are not just involved in regulating state transitions but are the site of metabolic integration for a range of disparate signals and that the mechanism involved in integration is protein phosphorylation. This being the case, the thylakoid-associated protein kinases would need to be sensitive to a wide range of metabolic factors which would include the redox state of photosynthetic electron transport pathway components, the energy charge, the redox state of the pyridine nucleotide pools, and the concentration of key metabolites of photosynthetic and respiratory metabolism. Such a degree of versatility is seen in the range of signals responded to by the isocitrate dehydrogenase kinase/phosphatase of *E. coli* (see Holms, 1986). There is some evidence to support this notion of the phosphorylation of thylakoid-associated proteins being a key process in metabolic integration. The redox control of protein phosphorylation in cyanobacterial cell-free

extracts has been reported (Mann *et al.*, 1991) and this is also true for partially purified thylakoid systems (see Allen, 1992). Furthermore, the regulation of protein phosphorylation by key metabolites of photosynthetic and dark respiratory metabolism, in particular ribulose 5-phosphate and glucose 6-phosphate, has been detected in cell-free extracts of *Anabaena* sp. PCC 7120 and partially purified thylakoids from *Synechocystis* sp. PCC 6803 (unpublished results – this laboratory). *In vivo* studies on protein phosphorylation in *Synechocystis* sp. PCC6803 showed that the protein phosphorylation profile of cells growing under conditions of low C_i availability were altered in the same fashion by three distinct environmental changes, i.e. increase in C_i availability, addition of exogenous glucose and transfer into the dark (Bloye *et al.*, 1992). The majority of these proteins were membrane associated. The phosphorylation state of the GlnB protein involved in controlling nitrogen metabolism is responsive, at least in some strains, to the spectral quality of the incident light (Tsinoremas *et al.*, 1991; Forchhammer & Tandeau de Marsac, 1994). This model for metabolic integration emphasizes the importance of identifying cyanobacterial thylakoid-associated proteins and characterizing the kinases and phosphatases that determine their phosphorylation state.

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