Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms

Nicole Tandeau de Marsac and Jean Houmard

Unité de Physiologie Microbienne (CNRS URA 1129), Département de Biochimie et Génétique Moléculaire, Institut Pasteur, Paris, France

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1. SUMMARY

Dating from the Pre-Cambrian era, cyanobacteria have a long history of adaptation to the Earth’s environment. By evolving oxygen via photosynthetic reactions similar to those of plants and green algae, these prokaryotes were essential to the evolution of the present biosphere. They continue to make a large contribution to the equilibrium of the Earth’s atmosphere by producing oxygen and removing carbon dioxide. To survive in extreme or variable environments, cyanobacteria have developed specific regulatory systems, in addition to more general mechanisms equivalent to those of other prokaryotes or photosynthetic eukaryotes. Specific regulatory systems control the differentiation of specialized nitrogen-fixing cells and of cell types facilitating the dispersion of species. In the past decade, considerable progress has been made towards understanding the expression of the cyanobacterial genome in response to variations in the intensity and spectral quality of incident light and in response to nutritional conditions, especially carbon, nitrogen and sulphur sources. These studies have provided insights into the relationships between carbon and nitrogen intermediary metabolism, and a start towards understanding of the interconnected pathways which lead from the perception of environmental signals to the regulation of enzyme activities and gene expression. Cyanobacterial regulatory mechanisms share common features with those of other prokaryotes, but are unique since these essentially photoautotrophic organisms must maintain a proper cellular C/N balance, in spite of daily variations in incident light. Thus an appropriate coordination between photosynthesis and other metabolic processes must be achieved through control of the catalytic activity of key enzymes by reducing equivalents and ATP produced by photosynthetic or respiratory electron transport. Recently discovered kinases/phosphatases act by post-translational modification of specific proteins which probably act as signal transducers or modulators of gene expression in a manner similar to the well-known two-component regulatory systems described in other bacteria. In this overview, we present our current knowledge on the molecular
aspects of the biology of cyanobacteria, as well as on their mechanisms of resistance to metal ions and their responses to metabolic stress.

2. INTRODUCTION

Although designated for many years as blue-green algae, cyanobacteria in fact constitute a major group of prokaryotes [1]. Found in almost all of the ecosystems examined so far, their wide distribution reflects a large variety of species with diverse morphological and physiological properties; their genetic diversity is retraced by their G + C% contents, which cover the entire range of values found for prokaryotes, while the genome size of the most complex forms far exceeds that of any other bacterial group [2,3]. Besides their prokaryotic nature, the unifying property of the cyanobacteria is their ability to perform oxygenic photosynthesis by a plant-type mechanism. Moreover, some strains are able to fix nitrogen, which may appear paradoxical since the nitrogenase complex, which converts dinitrogen into ammonium, is rapidly and irreversibly inactivated by oxygen, one of the major products of photosynthesis in these prokaryotic organisms. To overcome this difficulty, some filamentous strains have developed the ability to differentiate highly specialized nitrogen-fixing cells, called heterocysts, when facing an environment in which combined nitrogen becomes limiting [4–9]. Such strains thus spatially separate photosynthesis and nitrogen fixation. For filamentous and unicellular strains which do not differentiate heterocysts, the mechanism(s) by which the nitrogenase complex is protected is less well known [6,8–10]. Other differentiation processes, mainly directed towards reproduction and dissemination of the species, resulted in the evolution of three different cell types: akinetes, baeocytes and hormogonia [5,11,12]. Only hormogonium differentiation will be discussed in detail, since no molecular genetic studies have been performed yet for the other two differentiation processes.

The general organization of the cyanobacterial cell was reviewed by Stanier and Cohen-Bazire [13]. Almost all the cyanobacteria possess classical thylakoid membranes with two photosystems (photosystem I and photosystem II). However, unlike most of the photosynthetic eukaryotes, they do not contain chlorophyll b but harvest light energy primarily through unique multimolecular structures, the phycobilisomes. With the exception of the eukaryotic red algae, cyanobacteria are the only known living organisms that possess such structures. Phycobilisomes are regularly arrayed on the stromal surface of the thylakoid membrane and their efficiency in light-harvesting and energy transfer, close to 100%, is quite remarkable [14]. Cyanobacteria also often contain a large variety of intracellular inclusions, some of which are surrounded by a non-unit membrane, and most of which have reserve functions and accumulate under adverse conditions of nutrient imbalance [15–17]. Cyanophycin granules are high-molecular mass non-ribosomally synthesized polymers consisting of equimolar quantities of arginine and aspartate. They are nitrogen reserves unique to, but not universally present in, cyanobacteria [15]. Under certain circumstances, phycobiliproteins, which represent the major components of the phycobilisomes, may also serve as a nitrogen source. Carboxysomes are polyhedral bodies containing carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), the key enzyme required for the net assimilation of inorganic carbon, as well as a few additional enzymes related to carbon metabolism [18]. Polyglucose (glycogen bodies) and poly-/β-hydroxybutyrate are reserves of carbon and energy, while polyphosphate bodies are stores of phosphate. Recently, little attention has been paid to the synthesis and the role of these inclusions, except for carboxysomes, although they may increase the chance of survival in a changing and possibly stressful environment.

Recent works dealing with a molecular description of the structure, function and assembly of the cyanobacterial photosystems and phycobilisomes, as well as of the genes encoding their components, have led to important progress in our knowledge of the biology of cyanobacteria [19–21]. Similarly, the process of heterocyst differentiation and its link to nitrogen fixation has been the subject of an increasing number of stud-
cies, and very interesting genes have recently been isolated. On the other hand, because of the wide distribution of cyanobacteria and of their potential applications, numerous physiological and biochemical studies dealing with the influence of the environment on their properties have been performed during the last 20 years. When growing photoautotrophically, cyanobacteria require only a few nutrients but must adapt to environmental transients, of which changes in light, nitrogen, carbon and sulphur have been most studied.

The purpose of this review is to present current knowledge on the regulation of gene expression in response to changes in the parameters of the environment and to relate physiological data to those obtained through gene characterization.

Fig. 1. Schematic representation of the photosynthetic apparatus from a typical cyanobacterium. Phycobilisomes are arrayed perpendicularly on the external side of the thylakoid. They are composed of two main structures, the core \((A+B+C)\) and the radiating rods (see Table 1 for nomenclature). The \(A\) and \(B\) cylinders lie antiparallel and result from the assembly of four trimeric discs: \((aAPbAP)_2 L_{C}\), \((aAPbAP)_2 \beta_{L_{CM}}\) and \((aAPbAP)_2 L_{C}\). Cylinder \(C\) is composed of four trimers: \(2(aAPbAP)_2 L_{C}\) and \(2(aAPbAP)_2\). The proximal hexamer of each rod \((D)\) is attached to the core through the \(L_{RC}\) and is always composed of phycocyanin \((aPCbPC)\). It is linked to a variable number of hexamers \((E\) and \(F)\), the composition of which is either \((apcflpec)_6\), \((apcflpec)_6\) or \((apcflpec)_6\). From the terminal acceptors, \(L_{CM}\) and/or \(aAPb\), excitation energy goes to photosystem II. Electron flow from photosystem II to photosystem I is represented by solid arrows; proton flows are shown by dashed arrows. Abbreviations: PSII, photosystem II; 9 and 33, subunits of the oxygen-evolving complex; \(Z\), primary electron donor to \(P_680\); the chlorophyll \(a\) reaction centre of PSII; \(Pheo\), pheophytin intermediary electron acceptor; \(Q_{a}\), primary quinone electron acceptor; \(Q_{b}\), bound plastoquinone (PQ) electron acceptor; \(D1\) and \(D2\), reaction centre proteins; \(CP43\) and \(CP47\), integral chlorophyll \(a\) binding proteins; \(Cyt\) \(b_{593}\), cytochrome of PSII (haem indicated by rectangle); \(SIV\), subunit IV of the cytochrome b6/f complex (haem indicated by rectangle); \(FeS\), iron–sulphur centre; \(Cyt\) \(b_{653}\), mobile cytochrome electron carrier (can be replaced by plastocyanin, see text); \(P_{700}\), chlorophyll \(a\) reaction centre of PSI; \(A_0\) and \(A_1\), primary (chlorophyll \(a\) and secondary (phyloquinone) electron acceptors; \(F_{x}\), \(F_{a}\) and \(F_{b}\), iron–sulphur centres; \(Fd\), ferredoxin (can be replaced by flavodoxin, see text); \(FMN\), ferredoxin (flavodoxin) NADP* oxidoreductase. ATPase, ATP synthase or coupling factor CF\(_0\)-CF\(_1\). The extrinsic complex CF\(_1\) is composed of \(\alpha\), \(\beta\), \(\gamma\), \(\delta\), \(\varepsilon\), \(b\) and \(b'\) polypeptides; the intrinsic CF\(_0\) complex is composed of \(c\) and \(a\) polypeptides. See Table 1 and 2 for description of the genes and products of phycobilisomes, photosystems I and II, and cyt b6/f, as well as for nomenclature. (Adapted from [3] with permission of Elsevier, Paris.)
Whenever possible, we shall try to mention the strain used in the experiments, and the divergences that have been observed in the results. Indeed, because of their diversity, not all cyanobacteria are expected to necessarily exhibit the same responses. Throughout the text, cyanobacterial strains will be designated according to the names that are most commonly used. For correspondence between the various names, as well as between the strain numbers in the different culture collections, the reader is referred to Table 1 of ref. [22].

3. ADAPTATION OF THE PHOTOSYNTHETIC APPARATUS TO LIGHT

Modifications in the conditions of illumination are obviously among the changes that all cyanobacteria experience frequently in natural environments. Two parameters, which are never completely independent, are distinguished as components of the incident light: the photosynthetic photon flux density (PPFD, expressed in mol photons m\(^{-2}\) s\(^{-1}\)) and the light wavelength (or spectral quality). Although nowadays PPFD is more and more commonly employed, many papers have often referred to it, using the designations light intensity or light irradiance. In this review, PPFD will be used whenever possible, but light intensity or irradiance will be used when referring to papers in which these terms are employed. Depending on the strain, cyanobacteria can tolerate direct sunlight (approx. 1700 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)) or require PPFD as low as approx. 1–2 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). Most cyanobacteria can adapt to 50–200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\), and maximum growth

<table>
<thead>
<tr>
<th>Polypeptide designation</th>
<th>Gene designation</th>
<th>Protein</th>
<th>Chromophore content</th>
<th>Absorption (\lambda_{\text{max}}) (nm)</th>
<th>Fluorescence (\lambda_{\text{max}}) (nm)</th>
</tr>
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<tbody>
<tr>
<td>Rod components</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha_{\text{PE}})</td>
<td>cpeA</td>
<td>Phycoerythrin</td>
<td>2PEB</td>
<td>565</td>
<td>575</td>
</tr>
<tr>
<td>(\beta_{\text{PE}})</td>
<td>cpeB</td>
<td></td>
<td>3PEB</td>
<td></td>
<td></td>
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<tr>
<td>(\alpha_{\text{PEC}})</td>
<td>pecA</td>
<td>Phycoerythrocyanin</td>
<td>1PXB</td>
<td>568 (590)</td>
<td>625</td>
</tr>
<tr>
<td>(\beta_{\text{PEC}})</td>
<td>pecB</td>
<td></td>
<td>2PCB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha_{\text{PC}})</td>
<td>cpcA</td>
<td>Phycoycyanin</td>
<td>1PCB</td>
<td>615–620</td>
<td>625–645</td>
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<tr>
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<td>cpcB</td>
<td></td>
<td>2PCB</td>
<td></td>
<td></td>
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<tr>
<td>(L_{\text{RC}})</td>
<td>cpcG</td>
<td>Rod-core linker polypeptide</td>
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<tr>
<td>(L_{\text{PC}})</td>
<td>cpcC</td>
<td>PC rod linker polypeptide</td>
<td>1PCB</td>
<td>650</td>
<td>660</td>
</tr>
<tr>
<td>(L_{\text{RC}})</td>
<td>cpcD</td>
<td>PC cap linker polypeptide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Core components</td>
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<td></td>
<td></td>
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<tr>
<td>(\alpha_{\text{AP}})</td>
<td>apcA</td>
<td>Allophycocyanin</td>
<td>1PCB</td>
<td>650</td>
<td>660</td>
</tr>
<tr>
<td>(\beta_{\text{AP}})</td>
<td>apcB</td>
<td></td>
<td>1PCB</td>
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<td></td>
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<tr>
<td>(\alpha_{\text{APB}})</td>
<td>apcD</td>
<td>Allophycocyanin B</td>
<td>1PCB</td>
<td>670 &gt; 618</td>
<td>675</td>
</tr>
<tr>
<td>(\beta_{18.3})</td>
<td>apcF</td>
<td>(\beta_{18.3}) subunit</td>
<td>1PCB</td>
<td>616</td>
<td>640</td>
</tr>
<tr>
<td>(L_{\text{CM}})</td>
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<td>Core-membrane linker polypeptide</td>
<td>1PCB</td>
<td>665</td>
<td>680</td>
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<td>(L_{\text{C}})</td>
<td>apcC</td>
<td>Core linker polypeptide</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Data taken from [3,19,20,521]. Polypeptide designations follow the recommendations of Glazer [537].

\(b\) Abbreviations: PCB, phycocyanobilin; PEB, phycoerythrobilin; PXB, phycobiliviolin (cryptoviolin).

\(c\) Absorption and fluorescence maxima depend slightly on association with linker polypeptides, and on aggregation state (trimer or hexamer), which itself is a function of pH, ionic strength and protein concentration. Number in parentheses denotes a shoulder.
rates ($\mu_{\text{max}}$) are reached with a PPFD of about 50–60 $\mu$mol m$^{-2}$ s$^{-1}$ [23]. As a general rule, small unicellular species and filamentous strains with a narrow cell diameter have the highest $\mu_{\text{max}}$. Because of the selective absorption of radiation above 550 nm by the upper water layers and of water turbidity, almost no radiation above 600 nm can reach a depth of 4 meters; photon flux density is thus an important parameter to consider since it has a major influence on growth and metabolism of many cyanobacteria [24].

Acclimation to light has been the topic of numerous physiological, ultrastructural and biochemical studies which have been performed on a variety of cyanobacterial strains. During photosynthesis, light is harvested by the chlorophyll and by the phycobilisomes that constitute the external, peripheral antennae. Phycobilisomes contain only proteins: phycobiliproteins, chromoproteins which account for about 85% of the total protein content, and linker polypeptides which complete the structure and modulate the spectral properties of the phycobiliprotein aggregates [14,25]. Classically, two sub-structures are distinguished within the phycobilisomes (Fig. 1): the core, which is mainly composed of an assembly of allophycocyanin trimers and is in direct contact with the photosynthetic membrane; and the rods, which are attached to the core and built from hexamers of phycocyanin and, in some cyanobacterial species, of phycoerythrin or phycoerythrocyanin. Altogether, depending on the strain and growth conditions, these polypeptides may represent up to 50% of the total cellular protein in cyanobacteria. Table 1 presents the various known phycobilisome components together with their absorption and fluorescence maxima, if any, as well as the nomenclature of the corresponding genes. Through the overlapping absorption spectra of the different phycobiliprotein complexes, excitation energy is funnelled within the phycobilisome structure from the periphery of the rods to the terminal energy acceptors, the $I_{\text{CM}}$ and the $\alpha_{\text{APB}}$ molecules, located in the basal cylinders of the core (Fig. 1) [19,26]. Exciton energy is then transferred primarily to photosystem II, a chlorophyll–protein complex embedded in the thylakoid membrane. Photosystem II catalyses conversion of exciton energy into chemical energy, by oxidizing water molecules, thereby evolving oxygen and transferring the electrons to plastoquinone. An electron transport chain, consisting of small diffusible proteins and molecules (plastocyanin or cytochrome $c_{553}$, and $b_6/f$), connects photosystem II to photosystem I, where additional energy derived from absorbed light is used to transfer the electrons to ferredoxin. Concomitantly, protons are transported into the thylakoid lumen. The proton gradient is coupled to ATP production by ATP synthase, while ferredoxin transfers reducing equivalents to NADPH [14,27]. Obviously, an efficient coupling between these different multimolecular structures is required to optimize photosynthesis [28]. Figure 1 schematically presents the light-harvesting apparatus, as it can be envisaged for most cyanobacteria. *Gloeobacter violaceus* represents a major exception in that it has no real thylakoid membranes and its bundle-shaped phycobilisomes are directly attached to the cytoplasmic membrane [29].

At present, most of the genes encoding the components of the phycobilisomes and the two photosystems have been isolated and characterized, but only from a limited number of cyanobacteria: two *Synechococcus* species, the strict photoautotroph PCC 6301 (or the very similar species PCC 7942 [30]) and the marine facultative photoheterotroph PCC 7002 (Fig. 2); two closely related facultative photoheterotrophic *Synechocystis* species, PCC 6714 and PCC 6803 (Fig. 2); and three heterocystous filamentous strains, *Anabaena* PCC 7120 (Fig. 6), *Mastigocladus laminosus* PCC 7603 and *Calothrix* PCC 7601 (Fig. 4) [3,19,20].

### 3.1. Adaptation of the photosynthetic apparatus to 'light intensity'

The main changes that occur in cyanobacteria, in response to modifications of the light parameters of the environment, concern the photosynthetic apparatus which occupies most of the cytoplasm in these microorganisms. These effects will be described sequentially following the pathway of the light energy from its harvesting to the production of ATP and reducing equivalents.
As for all photosynthetic organisms, an inverse correlation exists between the amount of light-harvesting pigments and the photon flux density that reaches the cells. In *Synechococcus* PCC 6301, pigment concentration and thylakoid content vary inversely with photon fluency rate [31]. Upon transfer of cells from high to low light, the size of the antenna first increases (by elongation of the phycobilisome rods) followed by an increase in the number of phycobilisomes per unit area of thylakoid membrane, with the ratio of phycobilisomes/photosystem II reaction centres staying almost unchanged [32,33]. The phyco-cyanin/allophycocyanin ratio can vary over a two-fold range, being two times higher at 15 W than at 60 W, as a result of an increase in the number of phyco-cyanin hexamers and of the two rod-associated linker polypeptides (L$_{PC}$) [34]. In *Synechococcus* PCC 7002, similar results have been reported and the photosystem I/photosys-
tem II ratio has also been shown to increase as light intensity decreases [19]. In contrast, upon shift from 20 to 565 $\mu$mol m$^{-2}$ s$^{-1}$, *Microcystis aeruginosa* only modulates the number of phycobilisomes per cell unit with no apparent alteration in their composition or structure [35,36]. Under these conditions, the number of photosynthetic units decreases as a result of changes in both chlorophyll a and phycobiliprotein concentrations, while there is no simultaneous variation in cellular contents of carotenoid, carbon or nitrogen.

Unexpected results have been obtained regarding the expression of the phycobiliprotein genes. In the unicellular *Synechococcus* PCC 6301 and the filamentous *Calothrix* PCC 7601 strains, the amount of mRNAs encoding phycobiliproteins increases with photon flux density while, in contrast, the amount of the corresponding polypeptides decreases [37]. Similar data have been obtained for the transcripts encoding allophycocyanin and phycocyanin, and additionally phycocyanin-2 and phycocerythin in *Calothrix* PCC 7601. These results indicate that there exists, in addition to a transcriptional regulation, a post-transcriptional control that may involve either the inhibition of translation of the abundant mRNAs or a very rapid turnover of the newly synthesized corresponding apoproteins. In contrast, in *Synechococcus* PCC 7002, the relative amount of mRNAs encoding phycobiliproteins follows the abundance of the corresponding gene products [19]. The first studies were performed with *apc*--*lacZ* and *cpc*--*lacZ* fusions, but results obtained with direct quantification of *apc* and *cpc* mRNAs have been reported recently [38]. During a shift from 20 to 1260 $\mu$mol m$^{-2}$ s$^{-1}$, the ratio of phycocyanin/allophycocyanin decreases 1.8-fold and the relative level of the corresponding mRNAs was found to vary in parallel. However, although the ratio of the gene products changed about two-fold between these extreme values, the ratio of mRNAs encoding L$_{PC}$ to that of *cpc*-mRNAs did not vary. The authors concluded that the corresponding *cpcC* gene could be constitutively expressed and that L$_{PC}$ would always be synthesized in excess and never limit rod elongation [38].
chococcus PCC 6301 and Calothrix PCC 7601, an inverse correlation has been observed between the levels of transcripts and of gene products for the cpcBACDE operon in cells grown either at 5 or 40 μmol m⁻² s⁻¹ [39]. In contrast, in Anabaena PCC 7120, a 13-fold increase in pec mRNAs has been observed in cells grown at 40 μmol m⁻² s⁻¹ when compared to the steady state level measured in cells grown at 200 μmol m⁻² s⁻¹, and the intracellular amounts of phycoerythrocyanin (PEC) vary accordingly [40]. These results seem to demonstrate that changes in photosynthetic photon flux density could have different effects on the expression of the operons that encode the core proximal components of the phycobilisome rods (cpcBACDE) on the one hand, and the distal part of the rods (pecBACE) on the other. Unfortunately, many of the data already reported are difficult to compare and relate because of the lack of uniformity in the physiological state of the cells used in the various studies. More attention should also be paid to the standardization of the growth conditions and the interpretations should take into account the diverse properties of the species with regards to generation time, light requirements, etc.

In the cyanobacteria examined so far, the C5-pathway is the source of most (if not all) of δ-aminolevulinate, the sole precursor for all the C and N atoms of the porphyrin skeleton of hemes, chlorophylls and bilins. In this pathway, Glu-tRNA_Glu and glutamate 1-semialdehyde (GSA) are the intermediate products [41]. Charging of the tRNA is mediated by the glutamyl tRNA synthetase (GluRS), the formation of glutamate 1-semialdehyde by the Glu-tRNA reductase (GluTR, encoded by hemA), and the formation of δ-aminolevulinate by the GSA aminotransferase (encoded by hemL) [41,42]. Corresponding genes have recently been isolated from Synechocystis PCC 6803 [43,44] and Synechococcus PCC 6301 [45]. In Synechocystis PCC 6803, the level of Glu-tRNA_Glu seems unaffected by changes in the illumination conditions, and the biosynthesis of heme and phycocyanobilin (PCB) seems to be regulated independently from that of chlorophyll a [43]. However, many studies remain to be performed concerning the level of expression of the different genes and their regulation in conjunction with environmental conditions. At present, the mechanisms of chromophore biosynthesis in cyanobacteria are poorly documented and not fully elucidated, nor is the role of the chromophores in the regulation of the synthesis of the phycobilisome components and their assembly into functional units. Because of the pleiotropic phenotype of Calothrix PCC 7601 CpcF⁻ mutants, we have postulated that an important regulatory role might be played by chromophore molecules [20,46]. The recent work of Glazer and co-workers demonstrates the complexity of these pathways since two gene products from Synechococcus PCC 7002, CpcE and CpcF, form a phycocyanobilin lyase specific for the α subunit of phycocyanin [47–49]. A large number of genes can thus be expected to synthesize the enzymes that attach the right chromophore to the numerous phycobiliprotein subunits. The role of the usually rather lengthy transcribed, but untranslated, regions found in front of many of the mRNAs that correspond to phycobilisome components is also worth examining in relation to the regulation of gene expression.

From physiological and biochemical data, it appears that two photosystem II complexes are generally associated with one phycobilisome and that the phycobilisome/photosystem II ratio remains almost invariant [50,51]. The molecular models recently proposed [26,52] are in agreement with this ratio of two, but the discrepancy with the data reported by Ohki et al. [53], who, by spectroscopic measurements, have found a ratio of one remains to be explained. Whatever the ratio, a coordinated regulation must exist for the synthesis of these multimolecular structures. It has recently been reported that dark-grown (chemoheterotrophic conditions) cells of Synechocystis PCC 6714 have a lower PC and chlorophyll content than light-grown cells and that, in darkness, phycobiliproteins are assembled into complete phycobilisomes which cannot transfer excitation energy to photosystem II chlorophyll molecules. The recovery of energy transfer between dark-synthesized phycobilisomes and photosystem II seems to require light, but neither photosystem II nor de novo protein synthesis are
functioning [54]. Dark-grown cells thus seem to be in a vigilant state, awaiting light to reorganize the structural components of their light harvesting apparatus. Since transcripts corresponding to allophycocyanin and the D1 protein of photosystem II are barely detectable in dark-grown cells of *Synechocystis* PCC 6714, the multimolecular protein substructures must be stable, but detectable transcription of the corresponding genes requires light [54].

Table 2

Components of the thylakoid membrane in cyanobacteria

<table>
<thead>
<tr>
<th>Gene designation</th>
<th>Product</th>
<th>Apparent molecular mass (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Photosystem II</strong></td>
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<tr>
<td><em>psbA</em></td>
<td>D1 protein</td>
<td>32</td>
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<tr>
<td><em>psbB</em></td>
<td>CP 47 protein</td>
<td>47</td>
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<tr>
<td><em>psbC</em></td>
<td>CP 43 protein</td>
<td>43</td>
</tr>
<tr>
<td><em>psbD</em></td>
<td>D2 protein</td>
<td>32</td>
</tr>
<tr>
<td><em>psbE</em></td>
<td>Subunit of cyt b&lt;sub&gt;559&lt;/sub&gt;</td>
<td>9</td>
</tr>
<tr>
<td><em>psbF</em></td>
<td>Subunit of cyt b&lt;sub&gt;559&lt;/sub&gt;</td>
<td>4</td>
</tr>
<tr>
<td><em>psbH</em></td>
<td>10 kDa phosphoprotein (O&lt;sub&gt;2&lt;/sub&gt; evolving complex)</td>
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<td><em>psbI</em></td>
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<td><em>psbK</em></td>
<td>Peripheral component</td>
<td>3.9</td>
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<td><em>psbL</em></td>
<td>Reaction centre component</td>
<td>5</td>
</tr>
<tr>
<td><em>psbM</em></td>
<td>O&lt;sub&gt;2&lt;/sub&gt; evolving core complex</td>
<td>4.7</td>
</tr>
<tr>
<td><em>psbN</em></td>
<td>O&lt;sub&gt;2&lt;/sub&gt; evolving core complex</td>
<td>4.7</td>
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<td><em>psbO</em> (wocA)</td>
<td>Mn-stabilizing protein</td>
<td>33</td>
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<td><em>psaB</em></td>
<td>Chlorophyll a binding P700 apoprotein</td>
<td>82</td>
</tr>
<tr>
<td><em>psaC</em></td>
<td>Iron-sulphur protein</td>
<td>8.9</td>
</tr>
<tr>
<td><em>psaD</em></td>
<td>Subunit II</td>
<td>18</td>
</tr>
<tr>
<td><em>psaE</em></td>
<td>Subunit IV</td>
<td>1.0</td>
</tr>
<tr>
<td><em>psaF</em></td>
<td>Subunit III (plastocyanin binding protein)</td>
<td>18</td>
</tr>
<tr>
<td><em>psaI</em></td>
<td>Subunit IX</td>
<td>3.5</td>
</tr>
<tr>
<td><em>psaJ</em></td>
<td>Subunit VIII</td>
<td>4.8</td>
</tr>
<tr>
<td><em>psaK</em></td>
<td>Homologous to eukaryotic PSI-K?</td>
<td>6.8</td>
</tr>
<tr>
<td>n.i.</td>
<td>Membrane spanning polypeptide</td>
<td>5</td>
</tr>
<tr>
<td><strong>ATP synthase</strong></td>
<td></td>
<td></td>
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<tr>
<td><em>atpA</em></td>
<td>α subunit of F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>54</td>
</tr>
<tr>
<td><em>atpB</em></td>
<td>β subunit of F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>52</td>
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<tr>
<td><em>atpC</em></td>
<td>γ subunit of F&lt;sub&gt;1&lt;/sub&gt;</td>
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<tr>
<td><em>atpD</em></td>
<td>δ subunit of F&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>b′ subunit of F&lt;sub&gt;0&lt;/sub&gt;</td>
<td>16</td>
</tr>
<tr>
<td><em>atpH</em></td>
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<tr>
<td><em>atpl</em></td>
<td>a subunit of F&lt;sub&gt;0&lt;/sub&gt;</td>
<td>31</td>
</tr>
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</table>

* Data taken from [21,63,65–68,72–74,77,78,522–532].

n.i., not isolated.
Most of the genes encoding the components of photosystem II have been isolated from various cyanobacteria (Table 2). However, expression studies mainly concerned the genes psbA (D1 protein), psbC (CP43 protein) and psbD (D2 protein). In every cyanobacterium examined so far, multigene families have been found for both psbA and psbD genes [21]. This is in contrast with the situation in chloroplasts in which only one copy of each gene has been detected. Light regulation of psb gene expression, both directly and using psb–lacZ fusions, has been most thoroughly studied in Synechococcus PCC 7942, which possesses three copies of the psbA gene; two of which (psbAII and psbAIII) encode an identical D1 (32 kDa) protein, Form II, while the third gene (psbAI) encodes Form I which differs at 25 residues, 12 of which are located in the 16 N-terminal amino acids of the protein [55]. The latter accounts for more than 90% of the total psbA mRNAs in cells grown under standard conditions. Similarly, the transcript level of psbDI is about one order of magnitude greater than that of psbDII. The two psbD genes encode an identical product, but psbDI is part of the psbDIC operon while psbDII is a monocistronic unit. Upon a shift to low light (from 125 to 50 μmol m\(^{-2}\) s\(^{-1}\)), it has been demonstrated that the transcription of psbAI is increased while that of psbAII and psbAIII becomes almost undetectable [56,57]. The reverse occurs upon a shift to high light (from 125 to 250–500 μmol m\(^{-2}\) s\(^{-1}\)), psbAII and psbAIII mRNAs increasing about 10-fold within 15 min, while the psbAI transcript level decreases. Simultaneously, the transcription of psbDII increases by a factor of five, but the level of psbDIC mRNA is almost unchanged [58]. As a whole, the amount of psbA mRNAs seems approximately constant, with each copy being expressed to a variable extent. It is interesting to notice that, although the intrinsic half-life of each mRNA species appears to be almost independent of the light intensity, experiments performed in the presence of rifampicin and chloramphenicol led to the conclusion that a factor must exist which is specifically responsible for the turnover of the psbAI and psbAIII transcripts [59]. The transcription of this factor is required at some stage in the pathway between the sensing of light irradiance and the transcriptional regulation of the psbA genes. In Synechococcus PCC 7942, a role for DNA-binding proteins has been proposed in the expression of the psbDII gene but their roles in light acclimation remain to be clarified and they await purification and biochemical characterization [60]. Studying shade adaptation in Synechococcus PCC 6301 at the protein level, Koenig [61] has shown that syntheses of D1 and phycocyanin are inversely correlated: the rate of D1 synthesis is rapid in strong light, but is slow in low light or in high light plus DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), conditions that artificially mimic shade conditions. Based on these results, a role for the D1 protein as a parameter in light acclimation has been postulated. In the very closely related Synechococcus PCC 7942 [30], the composition of the thylakoid membrane in D1 protein corresponds to the level of transcription of the three psbA genes: Form I (PsbAI) decreases 58% and Form II (PsbAII and/or PsbAIII) increases 60% along a gradient of photon flux density from 5 to 482 μmol m\(^{-2}\) s\(^{-1}\). Since Form II is predominant under conditions reported to be photoinhibitory, the authors also postulated that it could play a role in adaptation to high light [62].

The transcriptional regulation of the psbA and psbD genes has also been examined in Synechocystis PCC 6803 although the transcripts for the various copies of the genes (the three psbA and the two psbD) were not distinguished [63,64]. Light shift experiments were performed in the presence or absence of DCMU (which blocks electron transfer from photosystem II to plastoquinone), methyl viologen (which intercepts electrons from PSI and inhibits the cyclic electron transport), and rifampicin [64]. The main conclusions were the following: (i) shifts to high light intensity lead to increased levels of psbA mRNAs, while the transcription of psbA ceases within 5 min after transfer to darkness; (ii) the stability of the psbA mRNAs is considerably lower (half-life = 15 min) under low light than in darkness (half-life = 7 h); (iii) the degradation of the psbA mRNAs does not occur in the presence of light plus DCMU and methyl viologen (half-life > 5 h),...
while the light-stimulated production of psbA transcripts is not affected; the accumulation of psbA mRNAs is solely caused by accelerated transcript production since it does not occur in the presence of rifampicin; and (iv) the stability of the psbD transcript is essentially the same under both dark and low light (half-life = 20 min). Thus, both in Synechococcus and Synechocystis strains, transcriptional and post-transcriptional regulations are involved in the control of psb gene expression by light intensity.

Together with diffusible electron carriers (plastoquinone, plastocyanin, and/or cyt \(c_{553}\)), the cyt \(b_{6-f}\) complex mediates the transfer of electrons from photosystem II to photosystem I (Fig. 1). This complex consists of four proteins, and the corresponding genes (pet) have been isolated (Table 2): petA encodes the cytochrome \(f\), petB the cytochrome \(b_{5}\), petC the Rieske iron–sulphur protein and petD the subunit IV of the complex. In both the unicellular Synechococcus PCC 7002 and the filamentous Nostoc PCC 7906, the petA and petC genes form an operon [65,66]. In Nostoc PCC 7906, and very likely in Synechocystis PCC 6803, the petB and petD genes also form an operon [67,68]. Unfortunately, at present, no studies have been performed regarding light regulation of the transcription of the pet genes.

The electrons finally reach photosystem I complexes, which are affected the most by the control exerted by photosynthetic photon flux density. Changes in the synthesis and assembly of the photosystem I complexes largely account for the modulations of the photosystem I/photosystem II ratio mentioned above, at least in Synechocystis PCC 6714 [69]. Photosystem I particles, which can be obtained upon solubilization of thylakoid membranes by detergents, have been characterized biochemically [70,71]. In cyanobacteria, they are comprised of at least nine polypeptides that have counterparts in higher plants [72]. Most of the corresponding genes (psa) have been isolated (Table 2). A peptide of approx. 5 kDa has been characterized from photosystem I complexes in Anabaena variabilis ATCC 29413 and Synechococcus vulcanus, but the corresponding genes remain to be isolated [72,73]. In both Synechococcus PCC 7002 and Synechocystis PCC 6803, the psaA and psaB genes that encode the two chlorophyll \(a\) binding P700 apoproteins, are co-transcribed [63,74]. In Synechococcus PCC 7002, when photon flux density is decreased from 282 to 119 \(\mu\)mol m\(^{-2}\) s\(^{-1}\), the relative abundance of psaAB transcripts increases, as does that of the cpcBACDEF mRNAs [19]. In Synechocystis PCC 6803, psaAB transcripts can still be detected after 48 h in the dark in the presence of glucose [63], indicating either that transcription continues or that preexisting transcripts are very stable in the dark, as already observed for the psbA mRNAs [64]. The psaD gene constitutes an independent monocistronic unit. As for psaAB, psaD transcripts remain detectable after 48 h in Synechocystis PCC 6803 cells grown in the dark [63]. Although, at present, only few studies have been performed, it seems that both transcription and mRNA stability of psa genes are controlled by light intensity.

Besides regulating the psa gene expression, photosynthetic photon flux density also modulates the activity of the multimolecular complexes and, more precisely, the rate of opening and closing of the photosystem reaction centres [69]. Various mutations in psa genes have been constructed and/or characterized in order to define the role of each polypeptide in the structure and in the functioning of photosystem I units, but the fate of the corresponding transcripts and gene products during acclimation to new environmental parameters remains to be examined. Finally, the photosynthetic apparatus and the ATP synthase complex are connected. Cyanobacteria can couple ATP synthesis to the proton gradient generated by either light-driven electron transport (photophosphorylation) or respiratory electron transport (oxidative phosphorylation) [75]. The cyanobacterial coupling factor is similar in structure and function to the chloroplast enzyme. The genes encoding the nine ATP synthase subunits have been characterized from Synechococcus PCC 6301 and PCC 6716, Synechocystis PCC 6803 and Anabaena PCC 7120. They fall into two clusters not closely linked on the chromosome: the atpBE and atpIHGFDAC group of genes, the latter being adjacent to and divergently transcribed from the apcEABC cluster in Synechococcus PCC 6301.
At present, the regulation of *atp* gene expression has been documented very poorly. The regulation of photosynthesis genes by light intensity deserves many more studies which can now be performed thanks to the very specific probes that can be designed from the various genes already characterized.

3.2. Adaptation of the photosynthetic apparatus to the spectral quality of the light

Because of the difference in their absorption spectra, the various light-harvesting structures preferentially harvest different wavelengths of the visible spectrum. Classically, ‘white light’ is separated into two classes: photosystem I light refers to wavelengths below about 450 nm or above 660 nm, absorbed mainly by chlorophyll *a*, and photosystem II light to wavelengths from about 470 to 660 nm, absorbed mainly by phycobiliproteins. In cyanobacteria, photosystem II absorbs about 80–90% of the latter wavelengths, which mainly correspond to orange and green light, red light being more or less equivalent to long-wavelength photosystem I light [28].

3.2.1. Chromatic adaptation and state transitions

For most cyanobacteria, under orange (photosystem II) light, levels of photosystem I and associated cytochrome (*cyt c*553) increase and photosystem I content becomes larger than that of photosystem II. Upon transfer to red light, preferentially absorbed by chlorophyll *a* (photosystem I light), levels of photosystem I and cytochromes decrease and, in the extreme case, photosystem I content becomes equal to or slightly less than photosystem II content [79,80]. This response is termed inverse chromatic adaptation and occurs in all photoautotrophic organisms [81].

In *Synechococcus* PCC 6301, it has been found that the ratio of photosystem I/photosystem II reaction centres is about 2 in white light and 1 in far-red light. However, the phycobilisomes of wild-type cells have the same size and pigment composition whether grown under white or far-red light [32]. In cells grown under far-red light, the thylakoid area is reduced and the chlorophyll *a* concentration per unit thylakoid area diminishes as a consequence of the decrease in photosystem I reaction centres; however, the phycobilisome number per unit area increases about two-fold, their number remaining proportionally constant with the photosystem II reaction centres [32]. Upon transfer from intense white light to dim red light, the *Synechococcus* PCC 6301 cell first responds by increasing the size of its phycobilisome rods by addition of phycocyanin hexamers (as would occur if the spectral quality of the light was not changed), the number of phycobilisomes increasing only later [33]. Acclimation to light irradiance thus seems to precede chromatic adaptation.

A short-term response to modifications in light wavelength or light intensity is the so-called state transition process [28]. In this process, the distribution of excitation energy between the two photosystems is adjusted in response to excitation of either one [82]. Several models have been proposed, mostly the spill-over model (changes in the energy transfer from photosystem II to photosystem I) and the mobile antenna model (physical dislocation of the phycobilisome from photosystem II) [83]. It has been reported that in *Synechocystis* PCC 6714, during state transitions, each photosystem II remains associated with the phycobilisome and that the primary control of the state transitions is the redox state of a component of the *cyt b*6-f complex rather than that of the plastoquinone pool [84]. Similar conclusions have been reached by other authors who proposed that the signal for light regulation of the photosystems could be elicited by the redox steady state of that *cyt b*6-f complex [85,86]. However, the molecular mechanism that governs state transition is still controversial and both hypotheses, as well as some compromises, have advocates [83].

3.2.2. Complementary chromatic adaptation

In some of the strains that are able to synthesize phycoerythrin, the phycobiliprotein pigment content may change according to the incident light wavelengths, a phenomenon denoted as complementary chromatic adaptation [20,87]. Extensively reviewed recently, complementary chromatic adaptation (CCA) is characterized by a preferential synthesis of light-harvesting pigments...
with absorption spectra complementary to the incident light wavelengths, i.e. phycoerythrin under green light and phycocyanin under red light [20,46,87-90]. Three groups of strains have been distinguished: group I consists of strains that regulate neither phycocyanin nor phycoerythrin; group II contains cyanobacteria that modulate synthesis only of phycoerythrin ('unidirectional adaptation'), phycoerythrin being 3-4 times more abundant in green-light than in red-light grown cells; in strains of group III, the synthesis of both phycoerythrin and phycocyanin is regulated by the spectral quality of the light [89]. In the latter strains, red light promotes the synthesis of a second phycocyanin species, phycocyanin-2, which is repressed by green light, while expression of phycocyanin-1 is light-wavelength independent.

Many physiological and biochemical studies have led to the conclusions that for strains of group III: (i) during the adaptation process, there is no specific turnover of phycocyanin and phycoerythrin, acclimation occurring via de novo synthesis; (ii) only the distal part of the phycobilisomes, the rods, is modified, the cores being unchanged; (iii) under red light, rods contain only phycocyanin-1 and -2 while, under green light, they contain both phycocyanin-1 and phycoerythrin, specific linker polypeptides being associated with each of these phycobiliproteins; (iv) in darkness, the pigment content is equivalent to that found in cells grown under red light; and (v) perception of light spectral quality is a purely photochemical process, since it is temperature independent, and the existence of a photoreversible pigment sharing analogies with the plant-type phytochrome has been postulated [20,89,90]. However, action spectra show that action maxima that trigger phycobiliprotein synthesis are situated at approx. 540 and approx. 640 nm for the cyanobacterial photoreceptor, instead of 660 and 730 nm for phytochrome [88]. At present, all attempts to isolate the photoreversible pigment have failed.

The question of the fate of the newly synthesized components during complementary chromatic adaptation has recently been addressed [91]. It has been found that, after short periods of green illumination followed by dark incubation, every cell of a *Tolypothrix tenuis* (group III adapter) filament synthesizes phycoerythrin which is immediately incorporated into phycobilisomes. Moreover, through phycobilisome purification and separation, phycoerythrin was shown to be almost evenly distributed into all phycobilisomes. The data could not be interpreted as showing insertion of phycoerythrin only in newly formed phycobilisomes. It thus seems that during acclimation to new light wavelengths, phycobilisomes of intermediate composition (containing both phycocyanin-2 and phycoerythrin) can exist within the cell and that, since no free phycoerythrin can be detected, phycoerythrin production must be very well regulated so as to fit the capability of the cell to incorporate it into phycobilisomes. This observation is in agreement with the hypothesis that we formulated to explain the pleiotropic phenotype of *Calothrix PCC 7601* CpcF- mutants [20]. In such mutants, the lack of phycocyanin-1 leads to an inability to elongate rods and, since both *cpeBA* transcript levels and phycoerythrin protein were barely detectable, we had to postulate that a small amount of free phycoerythrin would switch off the transcription of the *cpeBA* operon.

Genes encoding phycobiliproteins and their associated linker polypeptides have been characterized from three chromatic adapters: *Calothrix PCC 7601* and *Pseudanabaena PCC 7409* (group III), and *Synechocystis PCC 6701* (group II) [92]. Most of the studies have concerned *Calothrix PCC 7601*. Using specific probes, designed after characterization of the corresponding genes, it has been possible to demonstrate directly, as already postulated through the use of various metabolic inhibitors, that the regulation operates at least in part at the transcriptional level for the *cpeBA*, the *cpeCD* and the *cpcB2A2H212D2* operons in *Calothrix PCC 7601* [37,93-97]. In contrast, transcripts corresponding to the *apcE-ABC*, *apcD* and *cpcB1A1E* operons have the same size, the same 5' extremity and roughly the same abundance whether they were isolated from green- or red-light-grown cells [26,98-101]. Except for the *cpeCD* operon, genes encoding the linker polypeptides have been found downstream of, and co-transcribed with, the genes corre-
sponding to phycobiliproteins with which they are associated. Segmented transcripts arise from these operons, in agreement with the stoichiometry observed for the different gene products, but segmented transcription does not seem to play any regulatory role in complementary chromatic adaptation. The next step has been to start elucidating the signal transduction pathway from the sensing of light to the switching on or off of the different genes.

Proteins have been identified in *Calothrix* PCC 7601 that bind specifically to the promoter regions of operons expressed either in green light or red light (A. Sobczyk, our laboratory, unpublished results). Two proteins, present only in extracts from cells grown under green light, have been shown by gel retardation assays to bind to a 191-bp fragment that carries the first 110 bp in front of the transcription start site. These two effectors have been designated RcaA and RcaB. Using these partially purified proteins, it has been demonstrated by DNaseI-footprinting experiments that RcaA protects a region of about 20 bp against DNaseI degradation. Within this region, centred at −57/58, is the directly repeated sequence TTG(A)TA-N4-TGTATA which could be the DNA-binding site for RcaA. Downstream of it, centred at about −30, is a region protected by RcaB. Quite interestingly, although it has not yet been possible to define consensus sequences for cyanobacterial promoters, it has been observed that for a given operon there is a higher degree of sequence conservation between strains than between various promoter sequences within a strain [26,37,102,103]. At present, four *cpeBA* operons have been sequenced and the repeated hexanucleotide sequences protected against DNaseI degradation in *Calothrix* PCC 7601 are perfectly conserved except for one base in both chromatic adapters, *Pseudanabaena* PCC 7409 and *Synechocystis* PCC 6701, while they are absent from *Synechococcus* WH7802, a cyanobacterium in which the *cpeBA* operon is constitutively expressed ([104,105]; J. Newman, personal communication). It is worth mentioning that during the characterization of the *Pseudanabaena* PCC 7409 genes, although the authors could not detect a band shift for the promoter of the *cpeBA* operon with extracts from that strain, molecules present in *Calothrix* PCC 7601 extracts from green-light-grown cells were able to bind [104]. This observation is in agreement with the results mentioned above. In *Calothrix* PCC 7601, another protein, which is only detected in extracts from cells grown under red light and designated RcaD, is able to specifically bind to and to protect against DNaseI, a region of the promoter sequence of the red-light-induced *cpcB2A2H2I2D2* operon (A. Sobczyk, our laboratory, unpublished results). In addition, the affinity of both RcaA and RcaD, but not that of RcaB, for their DNA targets is dependent on their phosphorylation state. Thus, both transcriptional and post-translational responses occur at the last stages of the adaptation process. Whether RcaA, RcaB and/or RcaD have to be synthesized de novo to regulate phycobiliprotein mRNA abundance during complementary chromatic adaptation remains to be established. The requirement for de novo synthesis reported for the expression of the *Calothrix* PCC 7601 *cpeBA* and *cpc2* operons [106] may concern RcaA, RcaB and/or RcaD, as well as yet unidentified components. In any event, phosphorylation reactions are very good candidates as primary and rapid sensors of environmental modifications and could very likely represent the first response in the signal transduction pathway.

An alternative approach to the understanding of these regulatory mechanisms is the use of mutants impaired in complementary chromatic adaptation. Such mutants have been isolated from *Calothrix* PCC 7601 and from *Synechocystis* PCC 6701 [89,105,107–109]. However, unravelling the complexity of the pleiotropic phenotypes usually obtained by classical mutagenesis is a real challenge. Thanks to the pioneering work of C.P. Wolk and co-workers, it is now possible to perform reverse genetic experiments with filamentous cyanobacterial strains [110,111]. Through a conjugation system involving *Escherichia coli* partners, exogenous DNA can be introduced into some filamentous cyanobacteria. Although first developed for *Anabaena* /Nostoc* strains, the conjugation system has now been extended to *Calothrix* PCC 7601 by John Cobley (cited in [111]). Many spontaneous pigmentation mutants...
have been obtained with the latter strain and the high frequency with which such mutations arose can now be explained by the presence of mobile insertion elements [112]. Mutants with aberrant regulation of phycobiliprotein synthesis during complementary chromatic adaptation have been examined at a molecular level and it was shown that the presence or absence of the gene product could be correlated directly with the level of corresponding transcripts. Complementation by wild-type DNA of such mutants must be performed to elucidate the signal transduction pathway of photoperception and to isolate and characterize pieces of DNA encoding information required for complementary chromatic adaptation to occur. Preliminary data indicate that a gene, designated rcaC, is able to complement two Calothrix PCC 7601 ‘red’ mutants that constitutively express phycoerythrin and synthesize no phycocyanin-2 (M.R. Schaefer, personal communication). The encoded ORF (RcaC) exhibits strong sequence identity with proteins of the superfamily of positive regulators. This approach is complementary to that which has already allowed the characterization of RcaA, RcaB and RcaD, proteins that directly bind to the promoter region of light wavelength-regulated operons.

Effects of light quality have recently been examined in Spirulina platensis, a strain which does not undergo complementary chromatic adaptation [113]. This species contains neither phycoerythrocyanin nor phycoerythrin. While the concentration of allophycocyanin appears not to vary, phycocyanin content is modulated by the available wavelengths of light. Closer examination revealed that, instead of the classical phycocyanobilin (PCB), some phycocyanin α subunits carry a PXB-type chromophore, usually found associated with α subunits of phycoerythrocyanin. In addition, in Spirulina platensis, the synthesis of the PXB chromophore is higher under green light than under red or white light. The presence of a

Fig. 3. Electron micrographs of intact (A) and uncoiling (B) gas vesicles isolated from Calothrix PCC 7601. Bar markers in A and B represent 100 nm and 10 nm, respectively.
PXB chromophore on phycocyanin α subunits has already been reported in a mutant of the complementary chromatic adapter Calothrix PCC 7601 [109]. This green mutant is no longer able to synthesize phycoerythrin but still modulates its biliprotein content according to available wavelengths. Whether green light acts on phycocyanin apoprotein conformation and/or induces the synthesis of a new isomerase remains to be elucidated, and these results underline the lack of knowledge of chromophore biosynthesis and its role in the light-dependent regulatory mechanisms.

4. BUOYANCY REGULATION AND HORMOGONIUM DIFFERENTIATION

4.1. Gas vesicle structure and synthesis

A number of cyanobacteria have developed an original way to adapt to variation in light intensity in aquatic environments. It allows them to move up and down in the water column and finally to oscillate around the depths where irradiance is optimal for cell growth. The intracellular structures responsible for this adaptation mode are gas vesicles which generally form aggregates, called gas vacuoles, and provide cells with buoyancy [114–117]. These structures consist of a hollow, cylindrical, rigid shell closed by a conical cap at each extremity (Fig. 3). Impermeable to water, but fully permeable to gases which rapidly diffuse in and out, these structures contain gases in constant equilibrium with the surrounding water and generally similar in composition to air at atmospheric pressure. Protein is the only component of gas vesicles. The major structural protein, GvpA, is a small hydrophobic polypeptide of 70 amino acid residues whose sequence is highly conserved among cyanobacteria and other gas vacuolate prokaryotes. The repetition of GvpA molecules all along the structure forms 4–5 nm wide ribs that run at right angles to the long axis of the gas vesicle and most likely represent turns of a spiral; the gas vesicle wall has an average thickness of less than 2 nm. Formation of gas vesicles starts with a bicone which progressively enlarges to a critical diameter and then elongates from the mid-section of the cylinder. The inner surface of the gas vesicle wall is hydrophobic, keeping water out by the effects of surface tension, while the outer surface is hydrophilic, minimizing interfacial tensions which otherwise would tend to collapse the structure.

Gas vesicles vary from 200 nm to 2 μm in maximum length and from 40 to 110 nm in diameter depending on cyanobacterial genera and species, but show little variation within a given species. Generally, resistance of gas vesicles to the cell turgor pressure and to hydrostatic pressure in a column of water is inversely proportional to their diameter. If these pressures exceed the critical pressure resistance of a given type of gas vesicle, the structure collapses [114–116,118]. Collapsed gas vesicles cannot be reinflated by gases, but components may be reused to form new gas vesicles, as shown in Anabaena flos-aquae [119]. A second protein, GvpC, with a molecular mass of approximately 20–22 kDa and whose sequence contains several repeats of 33 amino acids, has been found in gas vesicles of Calothrix PCC 7601 [120,121], Anabaena flos-aquae [122] and Microcystis sp. [122]. According to Walsby and co-workers, the GvpC protein, which represents only 3% of the complete structure, reinforces the structure by binding to the outer surface of the gas vesicle wall [122–125]. However, this hydrophilic protein might be dispensable, since it has not been found in gas vesicles of Pseudanabaena PCC 6901 [121] and the corresponding gene has been detected only in cyanobacteria that form gas vesicles abundantly in a short period of time or during hormogonium differentiation (see section 4.2) [126]. Griffiths et al. [127] recently reported the isolation of the GvpC protein from five additional cyanobacteria and suggested that the low homology found in the partial amino acid sequences that they determined may explain the inability to detect the corresponding genes. However, DNA/DNA hybridization studies on these strains were not presented, and therefore the question of a strict requirement for GvpC-like molecules awaits further analyses.

Gas vesicles allow some cyanobacteria to form
so-called 'water blooms', a phenomenon characterized by a very rapid colonization of a water layer during short periods of the year, generally in spring and autumn, with some oscillations in depth between day and night. Since many gas-vacuolate cyanobacteria synthesize hepatotoxins or neurotoxins, that can cause death to cattle, fish and birds, and can generate dermatitis or gastro-enteritis in humans [128], particular efforts have been put into the study of the mechanisms by which cell buoyancy is regulated in planktonic toxic strains, such as Microcystis sp., Anabaena flos-aquae, Aphanizomenon flos-aquae and Oscillatoria agardhii [116,117]. The main environmental factor which affects buoyancy regulation is light, the level of buoyancy increasing under low photon flux and decreasing under high photon flux [116]. In the dark, gas vesicle formation appears to depend on the prior accumulation of energy reserves [129]. Other factors have also been shown to affect buoyancy, including availability of nitrogen, inorganic carbon, phosphate and temperature, but each of these factors appears to act by affecting the response to light intensity [116,130,131]. Three different mechanisms have evolved that contribute to buoyancy regulation and produce roughly the same result: collapse by turgor pressure, accumulation of carbohydrates, and gas vesicle synthesis. Although these mechanisms are not exclusive, the predominance of one over the other in a given species has been noticed [116]. Collapse of gas vesicles by increase in turgor pressure generated by (a) low-molecular mass products of photosynthesis dissolved in the cellular cytoplasm and (b) light-stimulated uptake of potassium ions has been demonstrated in Anabaena flos-aquae [132–134]. Buoyancy regulation by collapse of gas vesicles also occurs in Aphanizomenon flos-aquae [135], Nostoc muscorum [136], and several green-coloured strains of Oscillatoria agardhii [137]. In Microcystis sp., gas vesicles are too strong to be regulated by turgor pressure; the accumulation of a particulate carbohydrate, in excess of the demands for growth, provides the main mechanism of density regulation [138–140]. In a red-coloured Oscillatoria agardhii, it has been proposed that light intensity controls the synthesis of gas vesicles at the level of gene expression [116], although this has not yet been demonstrated. The molecular demonstration that gas vesicle synthesis can be regulated at the level of gene expression comes from experiments performed with Pseudanabaena PCC 6901 [121]. In this strain, the

Fig. 4. Vegetative filaments (A) and hormogonia (B) from Calothrix PCC 7601. Refractile granules in hormononal cells are aggregates of gas vesicles. Both, phase contrast; bar markers represent 20 μm.
gupA gene has been characterized and used as a probe to demonstrate that the abundance of the gupA mRNA is inversely correlated with photosynthetic photon flux density.

4.2. Hormogonium differentiation

Some filamentous cyanobacteria can reproduce by breakage of the vegetative trichomes into shorter filaments called hormogonia (Fig. 4). These short filaments are distinguishable from mature trichomes by possessing small and rounded cells, often filled with gas vesicles, and by frequently exhibiting gliding motility correlated with the presence of pili on the outer surface of the cell wall [12,141]. Gas vesicles provide hormogonia with buoyancy and contribute to the dispersal of the species in its natural habitat by allowing filaments to float away from the site occupied by the sessile parental colonies. Hormogonia, that represent a transient stage in the developmental life cycle of some filamentous cyanobacteria, are required for the establishment of symbiotic associations between Nostoc sp. and hornworts or liverworts at the initial stage in the infection process [142]. In symbiotic association with the hornwort Anthoceros punctatus L., the induction of hormogonia appears to be mediated by a soluble compound produced by the plant partner when deprived of fixed nitrogen [143]. In free-living cyanobacteria, the differentiation of gas-vacuolate hormogonia has been shown to be induced by changes in various environmental parameters, such as changes in light intensity or spectral light quality, nutrient availability and transfer from aged to fresh medium.

In Nostoc muscorum, both the removal of NaNO₃ and an increased light intensity can induce gas vesicle formation, and the combination of both factors gives a larger induction than either one separately [136]. Replacement of NaNO₃ by different salts in equiosmolar concentration prevents the induction, while glucose and sucrose do not, indicating that induction occurs neither as a result of nitrogen starvation nor of a general osmotic effect. A transfer to higher photosynthetic photon flux density (35 μmol m⁻² s⁻¹) induces a larger increase in gas vesicle formation than occurs at lower photon flux density, and the presence of chloramphenicol causes the disappearance of gas vesicles due to an increase in turgor pressure rise within the cell cytoplasm [136]. In Mastigocladus laminosus, a transfer from liquid medium to solid medium induces hormogonium differentiation and this process appears to require light as well as Ca²⁺ [144]. In Nostoc muscorum A [145] and Nostoc commune 584 [146], red illumination (640–650 nm) promotes aseptate colony breakage and the differentiation of motile hormogonia, while green illumination (approx. 520 nm) reverses the red light effect. Photo-reversibility has been observed, the cyanobacteria responding only to the last green or red irradiation received by the cells [146]. An unidentified substance, unstable to autoclaving and which promotes motility in non-motile cultures, is secreted into the media of hormogonial cultures of both Nostoc muscorum A and Nostoc commune 584. The motility-promoting substances from both cyanobacteria are reciprocally active [146]. On the other hand, Anabaena/Nostoc PCC 7119 secretes in early stationary phase an unstable, but dialysable, compound that inhibits hormogonium differentiation. Removal of this inhibitory compound by cell washing and resuspension in a fresh medium allows hormogonia to differentiate [147].

Recent studies of hormogonium differentiation and gas vesicle formation in Calothrix PCC 7601 have given more insight into these phenomena. In Calothrix PCC 7601, a short period (2 h) of nitrogen deprivation followed by readdition of this nitrogen source triggers hormogonium differentiation suggesting that the metabolic changes leading to differentiation are not associated with a severe nitrogen starvation, but may rather be the result of a more immediate effect involving rapid changes in nitrogen metabolism [147]. Moreover, the succession of events that lead to fully differentiated hormogonia is strongly dependent on the light conditions. 100% differentiation is obtained in 24 h, in a reproducible manner, if cells in early exponential phase are transferred to a fresh medium and further incubated under continuous dim (1 μmol m⁻² s⁻¹) or bright (100 μmol m⁻² s⁻¹) red light, at 25–30°C, for 24 h.
While red light appears essential to promote differentiation, green radiation inhibits this process [141]. The differentiation of hormogonia in *Calothrix* PCC 7601 involves successive events which can be summarized as follows: after 1.5 h, cells start to divide without elongation and without DNA replication, and gas vesicles begin to be synthesized; this is rapidly followed by the formation of pores in the peptidoglycan layer of the cell wall and by the synthesis of pili; after 12–24 h, trichome fragmentation starts to occur and fully differentiated hormogonia are produced; after 72–96 h, mature vegetative filaments are regenerated, pili and gas vesicles are lost, and cells start to elongate and divide [141,147].

In order to follow this differentiation process at a molecular level, the *gvpA* gene encoding the structural GvpA protein has been characterized in *Calothrix* PCC 7601 [148]. The amino acid sequence deduced from nucleotide sequence data definitively established the small size (7375 Da) of the structural protein, which is in agreement with the size predicted from previous crystallographic studies of gas vesicles isolated from *Anabaena flos-aquae* [149]. The *gvpA1* gene has been found to belong to an operon encoding two other genes involved in gas vesicle formation, *gvpA2* (previously designated *gvpB*) and *gvpC*. While *gvpA2* encodes a structural protein identical to the *gvpA1* gene product, the *gvpC* gene encodes the second polypeptide shown to reinforce the gas vesicle structure (see section 4.1) [120]. Another gene, *gvpD*, located approximately 5 kb upstream from the other *gvp* genes and whose predicted amino acid sequence is 82% identical to GvpA, might also contribute to the structure of the *Calothrix* PCC 7601 gas vesicle, but its precise function remains to be determined [37]. The *gvpA1A2C* operon is transcribed as three mRNA species (0.3, 0.8 and 1.4 kb long) that start at the same 5′ extremity and correspond to the transcription of *gvpA1*, *gvpA1A2* and *gvpA1A2C*. In contrast, the *gvpD* transcript is a monocistronic unit (0.6 kb) [150]. In addition, a 0.4-kb anti-sense RNA starts within *gvpA2* and terminates in *gvpA1*. The *gvp* genes are specifically expressed during hormogonium differentiation [141]. The *gvp* transcripts appear 1.5 h after the induction under red light, remain abundant until 9 h and then start to be degraded. After 24–48 h, no transcripts are detectable. During the time the *gvp* genes are abundantly transcribed, the genes encoding subunits of allophycocyanin, phycocyanin-1, phycocyanin-2 and phycocerythrin are turned off. With the exception of the phycocerythrin genes that remain unexpressed if cells are maintained under red light, the genes encoding the different phycobiliproteins are again turned on, while those encoding gas vesicle proteins are switched off. In contrast, the genes encoding the two subunits of Rubisco remain permanently expressed during the differentiation process. Exposure to green light after 3 h of induction under red light represses the expression of the *gvp* genes. This repression is concomitant with an arrest of the differentiation process at an early stage under these conditions [141].

The antagonistic effect of red vs. green light is reminiscent of the effect of the spectral light quality in the phenomenon of complementary chromatic adaptation (see section 3.2.2). However, the two processes display major differences: continuous red illumination is required for the completion of the differentiation of hormogonia; no differentiation occurs in darkness in heterotrophic strains, even in the presence of glucose as an exogenous carbon source, while the synthesis of phycocyanin-2 is maintained under these conditions in vegetative filaments; and the expression of the phycobiliprotein genes is repressed during the differentiation process. Thus, hormogonium differentiation may not depend on the same photoregulatory pathway as complementary chromatic adaptation [20,37,141].

5. ASSIMILATION OF INORGANIC AND ORGANIC CARBON

5.1. Photoautotrophy

Under photoautotrophic growth conditions, the reduction of CO₂ to organic carbon is achieved through the Calvin cycle whose primary step is the condensation of CO₂ molecules with ribulose-1,5-bisphosphate, a reaction catalysed by Ru-
The following steps include the reduction of fixed CO₂ by the 3-phosphoglycerate kinase and the ATP-dependent glyceraldehyde 3-phosphate dehydrogenase, and the regeneration of the CO₂ acceptor molecules via a series of enzymes of the reductive pentose phosphate pathway (Fig. 5A). Some enzymes of this set of reactions require the products of light-driven photosynthetic electron flow, ATP and reduced ferredoxin; this small (11 kDa) non-haem iron–sulphur protein subsequently reduces NADP⁺ to NADPH which then provides reducing equivalents for glyceraldehyde 3-phosphate dehydrogenase, and is also involved in light regulation via thioredoxins of the activity of enzymes of the reductive pentose phosphate pathway, fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase and the ATP-dependent phosphoribulokinase (see below) [13,151–154]. By requiring both ATP and NADPH, the enzyme system required for CO₂ assimilation is closely linked to the photosynthetic electron flow, and cell growth under photoautotrophic conditions consequently depends upon a proper balance between these two systems.

The biochemical and structural properties of Rubisco have been extensively studied in photosynthetic organisms. In cyanobacteria, it generally consists of both large and small subunits which form oligomeric L₈S₈ complexes in vivo [17,153,155]. The rbcL and rbcS genes encoding both types of subunits have been characterized from Synechococcus PCC 6301 [156–158], Anabaena PCC 7120 [159] and Spirulina platensis [160] and have been shown to be co-transcribed [161,162]. When transferred to E. coli, the rbc genes from Synechococcus PCC 6301 are expressed and the polypeptides assembled into a functional enzyme [163–165]. This allowed the purification of the Synechococcus PCC 6301 enzyme and the determination of its three-dimensional structure, which has been recently refined to 2.2 Å resolution [166]. Although the fixation of CO₂ by Rubisco is the first step of the so-called ‘dark reactions’ of photosynthesis, light has been shown to regulate the expression of the rbcLS operon. In the facultative chemoheterotroph Synechocystis PCC 6803, the steady state level of rbcLS mRNAs decreases with light intensity and transcripts fail to accumulate in the dark or in the presence of DCMU, suggesting involvement of the photosynthetic electron flow in the regulation of the expression of this operon [64,167]. These results are in agreement with those reported for the strictly photoautotrophic nitrogen-fixing cyanobacterium Anabaena PCC 7120 in which the rbcL mRNA disappears after an anaerobic induction of heterocysts under argon and in the presence of DCMU, while their abundance does not vary if heterocyst induction only results from resuspension of the cells in a medium lacking combined nitrogen [103]. Whether the effect of light is direct remains to be determined, since transcripts corresponding to rbcLS can be detected in Synechocystis PCC 6803 cells incubated either in darkness in the presence of glucose or under light-adapted heterotrophic conditions (5 min of light every 24 h) [63].

Another recent molecular indication that CO₂ assimilation is controlled by the intracellular photosynthetic electron flow comes from the analysis of a light-sensitive mutant of Synechocystis PCC 6803 which does not survive exposure to light intensity above 3000 lux [168]. The mutation responsible for this phenotype has been shown to correspond to a single nucleotide change in an open reading frame coding for a 39-kDa polypeptide whose amino acid sequence is 60% identical to phosphoribulokinases from photosynthetic eukaryotes, but shares little homology with prokaryotic enzymes. The catalytic activity and the apparent affinity for ATP of the Synechocystis mutated kinase are about one-tenth and one-seventh those of the wild-type kinase, respectively, and the mutated kinase is selectively degraded under high light intensity. This degradation, and consequently cell death under high light, can be suppressed by DCMU. In the presence of DCMU, an impaired enzyme system for carbon reduction does not impede the photosynthetic electron flow which in turn would cause cell death by generating lethal compounds or as a result of damage in essential cellular components [168]. The same conclusions were reached from studies of the prk gene from Synechococcus PCC 7942 (W.E. Borrias, personal communication). However, it remains to be demonstrated if a feedback control
mechanism exists or if the control is only unidirectional which would be surprising since photosynthetic cells generally have to adapt to rapid changes in light intensity in their environment [168].

For the last 20 years, numerous studies have been performed in order to elucidate the structural and functional properties of the different cyanobacterial ferredoxins. Besides their central role in carbon fixation, ferredoxins participate in a variety of other biological processes including cyclic photophosphorylation, sulphite reduction, fatty acid desaturation, hydrogenase, nitrate and nitrite reduction, glutamate synthesis and nitrogen fixation [169–171]. First recognized in cyanobacteria were two plant-type ferredoxins, ferredoxins I and II, which are 95- to 99-amino acid polypeptides characterized by a 2Fe-2S cluster, but which differ in amino acid sequence, net charge and midpoint redox potential. Cyanobacteria usually appear to contain the soluble ferredoxin I as a major species, although the relative proportions between ferredoxin I and II may vary depending on strain or growth conditions [170]. Ferredoxin I is the last component of the photosynthetic electron transport chain. The corresponding gene, petF (also called petFI or fdxV), has been characterized from two filamentous nitrogen-fixing species, Anabaena PCC 7120 [172] and Anabaena variabilis ATCC 29413 [173,174], and from a unicellular non-fixing species, Synechococcus PCC 7942 [174–176]. A gene whose putative product differs from ferredoxin I [177] and possesses an unusual C-terminal extension of eight amino acid residues has also been found to be expressed in Synechococcus PCC 6301 cells [178]. However, the function of this protein is still unknown. In addition to petF, two other ferredoxin genes, fdxH and fdxN, have been identified in Anabaena PCC 7120 [179,180] (see section 6.5). The fdxH gene product is only expressed under conditions of nitrogen fixation; it corresponds to a plant-type ferredoxin which is synthesized in heterocysts and serves as electron donor to nitorgenase [179,181,182]. This 2Fe-2S ferredoxin has been expressed in E. coli and crystallized in order to determine its X-ray structure [183,184]. The fdxH gene has also been recently cloned and sequenced from Calothrix PCC 7601 [185]. The fdxN gene encodes a predicted polypeptide of 116 residues with two cysteine clusters typical of bacterial ferredoxins [180] and of other redox proteins that contain 4Fe-4S clusters, such as psaC, a 9-kDa membrane-extrinsic protein binding the Fα and Fβ iron–sulphur centres which serve as tertiary electron acceptors in the photosystem I core complex [186]. The function of fdxN is not known, although it is linked on the Anabaena chromosome to nifB, a gene involved in nitrogen fixation (see section 6.5). Obviously, more genetic studies associated with biochemical analyses are required to establish the number and relative functions of the different cyanobacterial ferredoxins.

The cyanobacterial ferredoxin-NADP⁺-oxido-reductase is a flavoenzyme (hereafter referred to as FNR) that catalyses the transfer of electrons from reduced ferredoxin to NADP⁺ thus forming

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**Fig. 5.** Assimilation and dissimilation of carbon in the cyanobacterial cell in the light (A) and dark (B), respectively. Black arrows indicate the three enzymes which are activated by reduced thioredoxins (Tdf, f-type thioredoxin; Tdm, m-type thioredoxin; this terminology is taken from chloroplast systems and may not necessarily be valid in cyanobacteria, as explained in the text). The broken arrows indicate that reactions involved in carbon dissimilation proceed at only low rates, or are completely inhibited, in the light (A), whereas those of glycogen synthesis are inactive in the dark (B). ‘IN’ and ‘OUT’ refer to the interior and exterior of the cell. Note that certain enzymes are common to both light and dark metabolism, fructose-1,6-bisphosphatase being the only such enzyme subject to strong redox control mediated via reduced thioredoxin. The enzymes involved are numbered as follows: (1), ribulose-1,5-bisphosphate carboxylase/oxygenase; (2), 3-phosphoglycerate kinase; (3), glyceraldehyde 3-phosphate dehydrogenase; (4), triose phosphate isomerase; (5), fructose-1,6-bisphosphate aldolase; (6), fructose-1,6-bisphosphatase; (7), glyceraldehyde transferase (transketolase); (8), aldolase; (9), sedoheptulose-1,7-bisphosphatase; (10), ribose-5-phosphate isomerase plus ribulose-5-phosphate 3-epimerase; (11), phosphoribulokinase; (12), phosphohexoisomerase; (13), phosphoglucomutase; (14), ADP-glucose pyrophosphorylase; (15), glycogen synthase; (16), glycogen phosphorylase; (17), glucose-6-phosphate dehydrogenase; (18), 6-phosphogluconate dehydrogenase; (19), dihydroxyacetone transferase (transaldolase); (20), glucose (or other sugar) transport system; (21), hexokinase.
NADPH. It has been studied extensively at the enzymatic level, but due to the difficulty of purifying this protein no clear conclusion could be drawn with respect to its molecular properties [170]. The complete amino acid sequence of FNR has been determined for *Spirulina* sp. [187] and the corresponding gene has been characterized from *Anabaena* PCC 7119 [188] and *Synechococcus* PCC 7002 [189]. The properties of the *Synechococcus petH* gene product have been studied in detail, leading to very interesting conclusions which might help to understand the structural and functional properties of this enzyme [189].

The deduced amino acid sequence of the *Synechococcus* protein comprises 402 residues and is thus approximately 110 amino acids longer at the N-terminus than the proteins from other cyanobacteria, including *Spirulina* sp. and *Anabaena* PCC 7119. The authors suggest that this size difference might be due to a particular sensitivity of the protein to degradation and in the case of *Anabaena* PCC 7119 to the determination of an incomplete 5' sequence. Immunoblotting analyses of whole-cell extracts with anti-spinach FNR antibodies revealed two distinct forms of the mature protein with apparent molecular masses of 45 kDa and 43 kDa, corresponding to the predicted molecular mass (44,950 Da) deduced from the nucleotide sequence. The soluble 45-kDa form of FNR is hydrophilic, while the membrane-associated 43-kDa form is hydrophobic. Taking these properties into account, experiments were performed which support the hypothesis that a portion of the FNR protein is posttranslationally modified by fatty acid acylation.

Given the homology between the N-terminal amino acid sequence of the FNR protein and the sequence of the small cap rod linker polypeptide L^PC_\text{R} of cyanobacterial phycobilisomes (Table 1), and the fact that the majority of isolated phycobilisomes contain small amounts of a protein of approximately 45 kDa which, in *Synechococcus* PCC 7942, has a N-terminal sequence presenting homology with FNR, it has been proposed that a part of the FNR enzyme could be associated with the peripheral rods of phycobilisomes at their core-distal ends [189]. Whether the synthesis of the two forms of the FNR protein is regulated by environmental factors remains to be determined.

Thioredoxins are low-molecular mass proteins (approx. 12 kDa) that exist in two oxidation-reduction states which differ by the presence of a disulphide (cystine) in the oxidized form of the protein, and a dithiol (two cysteines) in the reduced protein. In photosynthetic organisms, the reduction of thioredoxins is catalysed by the ferredoxin–thioredoxin reductase (hereafter referred to as FTR). Ferredoxin is the electron donor and the levels of the thioredoxin dithiol form are enhanced by light-driven increases in reduced ferredoxin [190–192]. Reduced thioredoxins activate three key enzymes of the Calvin cycle in the light and simultaneously inhibit the dissimilatory pathway at the level of glucose 6-phosphate dehydrogenase (Fig. 5) [171,193,194]. As changes in catalytic activity of the targeted enzymes are triggered by the reduced/oxidized forms of thioredoxins, cells possess a means of rapidly controlling key metabolic pathways. The ferredoxin-dependent thioredoxins from chloroplasts of higher plants and algae have been classified as either *m* - or *f*-types on the basis of their preferential ability to activate either NADP+-dependent malate dehydrogenase or fructose-1,6-bisphosphatase, respectively [195]. Thioredoxins *m* from spinach and *Chlamydomonas reinhardtii* share 44% and 41%, respectively, with the sequence of the *E. coli* thioredoxin [196,197]. Spinach thioredoxin *f* exhibits only 24% homology with the *E. coli* protein [198]. An *m*-type thioredoxin, renamed T-1 by Gleason [199], has been characterized in *Anabaena* PCC 7119 [200,201] and in *Synechococcus* PCC 7942 [202]. In the latter strain, inactivation of the corresponding *trxM* gene has been shown to be a lethal mutation [202]. Thus, although dispensable in *E. coli*, thioredoxin is required for photosynthetic growth of *Synechococcus* PCC 7942. The amino acid sequences of the *Anabaena* PCC 7119 and *Synechococcus* PCC 7942 proteins, determined by direct sequencing of the purified protein [200] and/or deduced from nucleotide sequence data ([201]; gene *trxM* in [202]) are 84% identical to each other and share approximately 50% identity with the *E. coli* and spinach (type *m*) proteins. By comparison of the sequence of
The second thioredoxin found in the cyanobacterial ferredoxin–thioredoxin system is usually referred to as the f-type thioredoxin by analogy with the nomenclature used for chloroplast thioredoxins [171,194]. However, the situation is more confused for the f-type than for the m-type thioredoxins. Two different thioredoxins have been isolated from Synechococcus PCC 6301, one of approximately 12 kDa and another of higher molecular mass [205]. Yee et al. [206] reported the occurrence of two thioredoxins in Anabaena PCC 7119. Using the same assay procedures as Yee et al., Whittaker and Gleason [207] subsequently purified to homogeneity two proteins that corresponded to the fraction most effective in activating chloroplast NADPH-malate dehydrogenase (thioredoxin m) and fructose-1,6-bisphosphatase (thioredoxin f), respectively. The Anabaena PCC 7119 thioredoxin f has a molecular mass of 25.5 kDa and contains two cysteine residues, but the active site sequence is unknown. This thioredoxin does not activate a partially purified preparation of Anabaena fructose-1,6-bisphosphatase and appears to be a unique protein with no structural or functional properties in common with other thioredoxins [207]. Another unusual thioredoxin has been characterized in Anabaena PCC 7120 [199,208]. This thioredoxin has a molecular mass of 12 kDa as deduced from nucleotide sequence data [208] and it exhibits only 37% identity to thioredoxins from E. coli and Anabaena PCC 7119 (thioredoxin T-1), and 23% identity to the spinach thioredoxin f. This unusual thioredoxin, named T-2 by Gleason [199], occurs at very low level in extracts of Anabaena PCC 7120, PCC 7119 and other cyanobacteria. Anti-Anabaena cross-reaction has been observed neither in extracts of eukaryotic algae, plants and eubacteria nor with an unusual thioredoxin–glutaredoxin produced by bacteriophage T₄. However, the unusual cyanobacterial thioredoxin can be reduced by glutathione and may function in glucose catabolism as a glutaredoxin, another group of disulphide redox proteins [199].

A native FTR enzyme has been purified to homogeneity from Anabaena PCC 7119 [209]. This 4Fe-4S sulphur protein has a molecular mass of 30 kDa similar to those reported for corn and spinach. It consists of two dissimilar subunits: an immunologically conserved subunit of 13 kDa and a subunit of 7 kDa, smaller in size than that reported for corn and spinach (16 kDa and 15 kDa, respectively) [209]. The conserved 13-kDa subunit carries the catalytically active disulphide, which becomes reduced when the enzyme reacts with reduced ferredoxin. These two cysteines become reoxidized when FTR reduces thioredoxin [191,209]. The gene frfV coding for the variable subunit of FTR has been characterized from Synechococcus PCC 6301 [210]. The Synechococ-
**5.2. Facultative heterotrophy**

More than half of the cyanobacterial species tested so far are facultative photoheterotrophs, of which 15–20% are able to grow under chemoheterotrophic conditions (Fig. 5B) (R. Rippka, personal communication). The inability of most strains to grow in the dark at the expense of an exogenous carbon source is likely to be due to a lack of efficient sugar transport systems and/or to a low rate of respiratory ATP synthesis, as well as to an incomplete tricarboxylic cycle which can only function as a biosynthetic pathway and limits the number of utilizable substrates in cyanobacteria [13,151]. Although the capacity to grow photo- or chemoheterotrophically is more widespread among filamentous species, some unicellular strains also possess this property. For example, *Synechococcus* PCC 7002 and *Synechocystis* PCC 6714 can grow on glycerol and glucose, respectively, in the light and in the dark [11]. *Synechocystis* PCC 6803 cells are capable of efficient growth at the expense of glucose in the light, but not under complete darkness unless a 5-min daily pulse of white light (40 μmol m⁻² s⁻¹) is given (light-activated heterotrophic growth conditions) [211]. The active spectral component of the light pulse has been determined to lie specifically in the blue region of the visible light spectrum. Based on evidence that these wavelengths do not serve as a source of metabolic energy via photosynthetic electron transport processes, the authors concluded that light-activated heterotrophic growth of *Synechococcus* PCC 6803 is under the dependence of a blue-light photoreceptor. Two different groups have shown that fructose, which can support growth of a number of cyanobacteria [11], is in fact bactericidal for *Synechocystis* PCC 6803. This toxicity, as well as fructose uptake, are inhibited by glucose and by its non-metabolized analog, 3-O-methyl-D-glucose [212,213]. This suggests that the same permeation system is used for the transport of both sugars, but with a very low affinity for fructose [212]. Such an hypothesis has been confirmed by the isolation of pleiotropic mutants deficient in glucose transport and resistant to fructose. These mutants do not spontaneously revert to the wild-type phenotype, but some of them can be complemented with a DNA fragment carrying the glucose transporter gene [214,215]. This gene, termed **glcP** or **gtr**, has been characterized. It encodes a hydrophobic protein of 49.7 kDa which shares 46–60% amino acid sequence homology and shows similarity in size and predicted secondary structure (12 membrane spanning domains) with a group of non-phosphorylating sugar transporters from mammals, yeasts and *E. coli*. The characteristics of the GlcP (Gtr) gene product agree with its role in glucose–proton symport, but whether it is the only protein involved in this system, as in each of the other sugar transport systems described so far, remains to be determined [214].

**5.3. Light–dark shifts: use of glycogen reserves**

Cyanobacteria synthesize and store glycogen during periods of active photosynthesis. At present, the **glgB** gene that encodes the branching enzyme is the only one of the three enzymes required for glycogen biosynthesis that has been characterized [216,217]. Glycogen reserves are oxidized via the hexose monophosphate shunt and used as carbon and energy source under conditions of energy limitation, such as darkness (Fig. 5B) [151]. Despite the key role played by the hexose monophosphate shunt in cyanobacterial intermediary metabolism, little is known about the molecular mechanisms that control the expression of the genes encoding the different en-
zymes involved in this pathway. The *gnd* gene coding for the 6-phosphogluconate dehydrogenase has only been characterized in *Synechococcus PCC 7942* [218]. Its product (50 kDa) presents 56% homology to the amino acid sequence of its counterpart in *E. coli*. In this cyanobacterium, the specific activities of both the 6-phosphogluconate dehydrogenase and the glucose-6-phosphate dehydrogenase increase about five-fold when light becomes limiting for growth, i.e. during the transition period between exponential growth and stationary phase [219]. Analyses of both *gnd–lacZ* operon and protein fusions, have shown that the induction of the 6-phosphogluconate dehydrogenase results from an increase in the abundance of *gnd* mRNA [219]. The nature of the signal that triggers the growth phase-dependent induction of the 6-phosphogluconate dehydrogenase is unknown. It could be the decrease of either the growth rate or the light intensity. Broedel and Wolf [219] favour the first of these two possibilities, since, as previously shown, the activity of the enzyme is inversely correlated with growth rate in CO₂-limited or light-limited chemostats [220], while it remains constant following a rapid light–dark shift or a DCMU treatment [221]. An opposite response has been obtained for the glucose-6-phosphate dehydrogenase under the same experimental conditions, suggesting that the growth phase-dependent induction of the activity of this enzyme depends upon a different signal, which might be decreasing the light intensity to a level below the threshold required for exponential growth, which results from self-shading during cell growth [219]. Based on previous observations that the activity of the enzyme can be regulated in vitro by thioredoxin [193,222] and in vivo by the NADP/NADPH redox pair [223], it has been proposed that a post-translational regulation might occur rather than a control at the level of gene expression. The recent identification of the *zwf* gene encoding glucose-6-phosphate dehydrogenase in *Synechococcus PCC 7942* should allow rapid resolution of this question [224].

Protein kinase activities have been found to occur in cell-free extracts of the photoautotrophic nitrogen-fixing cyanobacterium *Anabaena PCC 7120* and the pattern of phosphorylation of at least 12 polypeptides has been shown to be regulated by intermediary metabolites and other effectors [225]. Notably, both glucose 6-phosphate and ribulose 5-phosphate considerably reduced the in vitro phosphorylation of a 56-kDa polypeptide by stimulating a protein phosphatase and inhibiting the corresponding protein kinase, respectively. The phosphorylation state of the same polypeptide has also been shown to be regulated in vivo by the supply of combined nitrogen. However, attempts to correlate these effects with a change in glucose 6-phosphate dehydrogenase activity were unsuccessful. The phosphorylation state of several other polypeptides is influenced by the reducing agent dithiothreitol (DTT) and a calmodulin antagonist trifluoperazine (TFP), indicating a control by redox conditions which could be mediated by thioredoxins and possibly indicating the existence of a calmodulin-like mediator [225]. Thus, although the nature of the phosphopolypeptides demonstrated in *Anabaena PCC 7120* remains to be characterized, post-translational modifications may indeed play an important role in the control of carbon metabolism.

5.4. High versus low external concentrations of CO₂

In aquatic environments, the supply of dissolved inorganic carbon (Ci) depends on many parameters including the slow rate of diffusion of CO₂ in water, pH, turbulence and cell densities. To respond to fluctuations in CO₂ availability for photosynthesis, cyanobacteria, like many photosynthetic microorganisms, have developed a C₃-concentrating mechanism which enables cells to cope with these fluctuations in CO₂ concentrations and with the very large difference between the Km(CO₂) of the cyanobacterial Rubisco and the CO₂ concentration of water at equilibrium with air [226–229]. For many years, studies on the C₃-concentrating mechanism in cyanobacteria have focused on physiological aspects, but recently a particular emphasis has been put on the molecular analysis of mutants requiring high CO₂ concentration for growth and on gene cloning, in an attempt to elucidate the control mechanisms
of acclimation to high versus low CO₂ and to clarify the system(s) of transport and accumulation of Cᵢ within the cells. Evidence exist for an active transport of both CO₂ and HCO₃⁻ into the cells, but many aspects are still controversial: does a common carrier or do separate systems transport the two Cᵢ species? What is the nature of the Cᵢ species predominantly taken up and what are the role of Na⁺ and light in the mechanism of Cᵢ transport, its energization and its activation [228]?

A number of mutants that require high CO₂ have been obtained either by chemical mutagenesis or by insertional inactivation of cloned genes from *Synechococcus* PCC 7942 and *Synechocystis* PCC 6803 [226]. Most of these Hcr⁻ mutants are capable of efficient Cᵢ transport but are unable to utilize the intracellular Cᵢ pool for photosynthesis, most likely because they possess aberrant carboxysomes (see below) [230]. Two mutants, RKa and RKb, defective in CO₂ and HCO₃⁻ transport, have been successfully isolated from *Synechocystis* PCC 6803 [231]. In RKa, the mutation has been located in an open reading frame which encodes a very hydrophobic protein of 55.5 kDa and shows high sequence homology to the ndhB gene product (subunit 2 of NADH dehydrogenase in chloroplasts and mitochondria) [232]. Physiological characteristics of the mutant RKa and a specific mutant constructed by inactivation of the cloned gene suggest that the ndhB gene product plays a role in photosynthetic electron transport to energize the Cᵢ-transporting system in *Synechocystis* PCC 6803. Given the hydrophobicity of the ndhB gene product, it is most probably a membrane protein, but its precise location within the cell requires further studies. An open reading frame, designated ictA, encoding another hydrophobic polypeptide has been found to complement the mutation in RKb [233]. This 80-amino acid polypeptide does not show any homology with known protein sequences. The mutation in the ictA gene led to the loss of transport of both CO₂ and HCO₃⁻; however, this does not implicate the existence of a common Cᵢ transport mechanism, since two separate systems could share the same essential IctA component to function.

Exposure of cyanobacterial cells grown under high CO₂ conditions to low CO₂ increases their Cᵢ transport system and photosynthetic affinity for extracellular Cᵢ. One of the specific changes in the polypeptide composition of the cytoplasmic membrane of *Synechococcus* PCC 7942 and PCC 6301 during this acclimation process is the accumulation of a 42-kDa polypeptide, suggesting its potential role in Cᵢ transport [234,235]. However, analysis of high-CO₂ requiring mutants from *Synechococcus* PCC 7942, which do not accumulate the 42-kDa polypeptide and exhibit a very low apparent photosynthetic affinity for extracellular Cᵢ, revealed that this polypeptide does not participate directly in the active transport of Cᵢ [236,237]. One of the mutations, located in a gene of yet unknown function, has been mapped upstream from the rbcLS operon [230], while the other, the cmpA gene, is located in a different genomic region [230,237]. Nucleotide sequence comparison showed that the cmpA gene is in fact identical to the cbpA gene encoding a cytoplasmic membrane carotenoprotein characterized by Reddy et al. [238] in the same cyanobacterium. Accumulation of the cbpA transcripts and the corresponding gene product occur in cells grown under high light intensity [238]. The role of the 42-kDa cytoplasmic protein in cell acclimation and its regulation under different environmental conditions are thus unclear and deserve further studies. Two additional high-CO₂ requiring mutants, D4 and R14, which exhibit photosynthetic characteristics similar to *Synechococcus* PCC 7942 wild-type cells grown under high CO₂ concentration, have been located in an open-reading frame (ORF1) immediately downstream from the rbcLS operon (A. Kaplan, personal communication). ORF1 encodes a putative product of 395 amino acid residues, which shares high homology to the eukaryotic phosphoribosylaminomimidazole carboxylase (AIR carboxylase; ade genes) and the eubacterial subunit II of AIR carboxylase (purK gene). This enzyme catalyses the carboxylation step in the biosynthetic pathway of inosine 5'-monophosphate. Addition of inosine 5'-monophosphate to the culture medium of mutants D4 and R14 enables them to grow under low CO₂ conditions. ORF1 is transcribed as a
1.2-kb species only when cells are transferred to low CO₂ conditions. This transcript is absent in RNA extracted from the mutant cells. These results indicate that the purine biosynthetic pathway might indeed be involved in the process of acclimation of *Synechococcus* PCC 7942 to low CO₂ concentrations, but the precise role of the *purK* gene product remains to be established.

Several lines of evidence support the hypothesis that carbonic anhydrase, the enzyme which catalyses the interconversion of CO₂ and HCO₃⁻ ions and is required for the generation of CO₂ from the intracellular HCO₃⁻ pool, is located in carboxysomes [226-228]. Carboxysomes are polyhedral bodies, surrounded by a protein shell, which contain most of the Rubisco in the cell [17]. According to the quantitative model for Cᵢ flux and photosynthesis in a cyanobacterial cell proposed by Reinhold et al. [239-241], it is postulated that carbonic anhydrase is absent from the cytoplasm and that HCO₃⁻ and CO₂ do not reach equilibrium in this cell compartment. The accumulated HCO₃⁻ ions penetrate into the carboxysomes, where the presence of a low concentration of carbonic anhydrase leads to the generation of CO₂ and subsequently to its fixation by Rubisco [228]. The structure of the carboxysome and the integration of the carbonic anhydrase within the Rubisco matrix would thus provide a means to reduce back flow of CO₂ and to improve the efficiency for the utilization of the Cᵢ pool. The ‘cyanorubrum’ mutant constructed by Pierce et al. [242] provides support to this model. In this mutant, the *Synechocystis* PCC 6803 *rbcL* gene has been replaced by the corresponding gene from a photosynthetic bacterium, *Rhodospirillum rubrum*. As a consequence, the transformed cyanobacterium apparently lacks carboxysomes and exhibits an increased sensitivity to the CO₂/O₂ ratio, no growth being observed in air levels of CO₂ (approx. 0.03%). Other evidence that carbonic anhydrase activity is located inside carboxysomes and that the Cᵢ pool is primarily HCO₃⁻ was provided by Price and Badger [243] who examined the consequences of the expression of the human carbonic anhydrase II within the cytosol of *Synechococcus* PCC 7942 cells. The expression of this protein has dramatic effects on the photosynthetic physiology of the cells and creates a high-CO₂ requiring phenotype. A spontaneous mutant resistant to acetazolamide, an inhibitor of carbonic anhydrase, has been isolated from *Synechocystis* PCC 6803 [244]. Such mutants, affected in the carbonic anhydrase activity, might help in characterizing the relevant gene and subsequently the precise location and function of this enzyme in photosynthesis.

One of the most interesting questions to be solved is the nature of the signal that induces the changes characteristic of adaptation to low CO₂. So far, only speculations have been made on the possible role of a metabolite from the glycolate pathway as a signal perceptor or of a periplasmic protein that could sense ambient CO₂ level [227,228]. On the other hand, it has been observed that the promoter regions of *rbcLS* and *cmpA*, genes whose expression depends on the level of CO₂, contain three highly homologous boxes that are putative regulatory sequences [228]. Most interestingly, the first evidence that the control mechanism which allows cyanobacterial cells to rapidly acclimate to changes in ambient CO₂ concentration involves post-transcriptional modification of specific proteins has been provided by Bloye et al. [245]. These authors showed that several specific polypeptides that were unphosphorylated during HCO₃⁻ limited growth became phosphorylated during the shifts to high CO₂ conditions and to photoheterotrophic growth, as well as following a shift to darkness. Thus, besides transcriptional controls of specific genes, post-translational regulations of enzyme activities might occur and it is tempting to speculate that two-component regulatory systems involving protein kinases/phosphatases come into play in the adaptation of the cells to external Cᵢ supply, as it has been shown to occur in a variety of regulatory processes in bacteria [246,247].

6. FIXATION AND ASSIMILATION OF NITROGEN

The most abundant forms of nitrogen in both aquatic and terrestrial habitats are the inorganic species, dinitrogen (N₂) and, to a lesser extent,
nitrate ($\text{NO}_3^-$) and ammonium ($\text{NH}_4^+$). Generally, all cyanobacteria can utilize nitrate, nitrite and ammonium as the sole nitrogen source for growth [248]. Many unicellular or filamentous species can, in addition, fix dinitrogen when grown under anaerobiosis and many of these are even capable of nitrogen fixation under aerobic conditions [4,6–8,249].

The reduction of nitrate, nitrite and dinitrogen to ammonium occurs in cyanobacteria by the same pathways as in other organisms able to utilize these forms of inorganic nitrogen [248,250]. These processes require ATP and a source of reducing power, which under autotrophic conditions are provided via the photosynthetic electron transport system. Assimilation of ammonium necessitates, in addition, a source of fixed carbon. In cyanobacteria, ferredoxin (or flavodoxin under iron-depleted conditions) is the main physiological electron donor for all the enzymes concerned. Only some cyanobacteria seem to possess NAD(P)H glutamate dehydrogenases, enzymes which provide an alternative ATP-independent route for the incorporation of ammonium into carbon skeletons [249,251,252].

The mechanisms by which the uptake of the different inorganic nitrogen sources and the activities of the enzymes involved are controlled are still controversial and may vary among different cyanobacterial species, in particular depending on the ability of the organism to fix nitrogen. Generally, nitrate is the form of inorganic nitrogen which is the most widely used. However, if different nitrogen sources are available, cells utilize ammonium, nitrate or N$_2$ (for the nitrogen-fixing species), in decreasing order of preference [248].

### 6.1. Nitrate and nitrite utilization

Nitrate assimilation involves nitrate uptake and its sequential intracellular reduction to nitrite by nitrate reductase (a molybdenum-containing enzyme) and to ammonium by nitrite reductase. These two enzymes have been isolated and characterized from *Synechococcus* PCC 6301 [253]. They represent single-polypeptide enzymes of 75 and 50 kDa, respectively. Nitrate reductase has also been partially purified from *Anabaena dolioïdum* [254]. In both non-nitrogen-fixing and nitrogen-fixing strains, the uptake and reduction systems involved in nitrate utilization are suppressed, or at least decreased, when ammonium is present [248,254]. An active transport system sensitive to ammonium appears to drive nitrate and nitrite uptake. A passive diffusion of nitrous acid, insensitive to ammonium, would, in addition, contribute to nitrate uptake [253,255–259]. In unicellular non-nitrogen-fixing strains, the regulation of the activity levels of the nitrate reducing system, nitrate and nitrite reductases, proceeds mainly through a repression promoted by ammonium, which needs to be metabolized through glutamine synthetase to be effective [253,260–262]. Nitrate and nitrite may also have secondary roles in the induction of the enzyme levels in *Synechococcus* PCC 6301 [261,263]. In filamentous strains fixing nitrogen, the regulation is exerted through a combined process involving both induction by nitrate or nitrite and repression by ammonium itself [264,265] or more likely via products of its assimilation [253–255,260,262].

Several genes, which may give a clearer understanding of the regulatory mechanism(s) which control(s) nitrate utilization in cyanobacteria, have been recently identified from the non-nitrogen-fixing unicellular species *Synechococcus* PCC 7942. The $nrtA$ gene encodes a cytoplasmic membrane polypeptide of 48.8 kDa, which is abundantly synthesized in nitrate-grown cells, but absent from ammonium-grown cells. Based on the biochemical characteristics of mutants lacking this protein, it has been concluded that it corresponds to a nitrate transport component [266–269]. Upstream from $nrtA$, an open reading frame has been found whose putative product is 50% identical to nitrite reductase from spinach [268,270]. Three genes involved in the reduction of nitrate to nitrite, $narA$, $narB$ and $narC$, have been cloned [271,272]. Whereas the actual function of the $narA$ and $narC$ genes is presently unknown, $narB$ might correspond to the nitrate reductase structural gene [273]. The $narB$ gene has been found to be located downstream from $nrtA$, but the $narA$ and $narC$ genes seem to reside at different locations on the chromosome [270].
6.2. Ammonium utilization

Two mechanisms have been proposed for the uptake and accumulation of ammonium in cyanobacteria [274]. In species growing in an environment at pH 7–8, such as *Synechococcus* PCC 7942, ammonium ions would cross the cell walls via an active transport system, requiring de novo protein synthesis to be established, while in alkalophilic species, such as *Spirulina platensis*, growing at pH > 10, ammonia would enter the cells by diffusion and would subsequently be protonated in a more acidic cell compartment. Whether ammonium is exogenously supplied by one of these two possible mechanisms or intracellularly produced, the GS/GOGAT pathway is quantitatively the most important enzyme system for the assimilation of this cation [275]. Glutamate synthase (glutamate 2-oxoglutarate amino-transferase; GOGAT) has been poorly studied so far. Most of the work performed concerns glutamine synthetase (GS) that catalyses the ATP-dependent synthesis of glutamine from glutamate and ammonium ions. It represents the connecting step between carbon and nitrogen metabolism. The cyanobacterial enzyme has been purified and characterized from both non-nitrogen-fixing and nitrogen-fixing species [276-278]. It closely resembles that of *E. coli* with 12 subunits of approximately 50 kDa arranged in two superimposed hexagonal rings [276,278-280]. Although studied in detail by different groups, the regulation of the synthesis and activity of GS remains unclear. Divalent cations, amino acids, adenine nucleotides, inorganic pyrophosphate, light/dark shifts and thioredoxin have been shown to modulate this enzyme activity in some cyanobacteria [281-287]. Ammonium-promoted repression of GS synthesis has been observed in many species, but there exists no evidence for a covalent modification of the enzyme by adenylylation as found in enteric bacteria, even in the case of *Synechocystis* PCC 6803, a species reported to display a marked inhibition of GS activity by ammonium and to exhibit a response to nitrogen starvation similar to that of enteric bacteria [278,288-290]. Biochemical analyses of the in vitro reactivation of GS previously inactivated in vitro by ammonium indicate that, in *Synechocystis* PCC 6803, the inactivation occurs via the non-covalent binding of a phosphorylated compound to the enzyme [290]. Although the chemical nature of this compound is still unknown, these studies support previous results and rule out the existence of an adenyllylation/deadenyllylation system for the control of GS activity. By transferring the structural gene (*glnA*) from *Anabaena* PCC 7120 into the chromosome of *Synechocystis* PCC 6803 and further by inactivating the endogenous copy of *glnA*, Mérida et al. [291] have shown that the *Anabaena* GS is functional in *Synechocystis* and subject to ammonium-promoted inactivation. No inactivation is observed in the *Anabaena* strain itself when nitrate-grown cells are supplemented with ammonium [291]. In contrast, inactivation occurs when ammonium is added to cells transferred for 11 h to nitrogen-fixing conditions, i.e. before the differentiation of heterocysts is completed [292]. This suggests that the regulatory mechanisms of GS activity might differ between nitrogen-fixing and non-fixing strains, and might depend on the nitrogen sources provided before their transfer to medium lacking combined nitrogen.

The absence of adenylylation of GS in cyanobacteria has been interpreted for many years as an indication of the lack of a bacterial-like nitrogen regulatory system and thus a lack of the PII protein. In enteric bacteria, the PII protein regulates the activity of GS via adenylylation/deadenyllylation of the protein, and its synthesis via the modulation of the transcription initiation of *glnA* by a two-component system in which NtrB acts as a ‘modulator’ or ‘sensor’ and NtrC as a ‘response regulator’ or ‘transcriptional activator’ [246,293]. In fact, it has recently been demonstrated that cyanobacteria do synthesize a PII protein. This protein has been purified from *Synechococcus* PCC 6301 [294] and the corresponding gene, *glnB*, has been characterized from *Synechococcus* PCC 7942 [295]. It encodes a predicted polypeptide of 12.4 kDa which shares 62–66% sequence identity with the *glnB* gene products of other bacteria. DNA/DNA hybridization experiments indicate that an equivalent of the *glnB* gene is present in all cyanobacteria tested so
far, including *Anabaena* PCC 7120 and *Calothrix* PCC 7601 ([295] and A.-M. Castets, our laboratory, unpublished data). Although it has not yet been demonstrated that the cyanobacterial PII protein is uridylylated, as is its counterpart in other bacteria, it has been shown that the protein is labelled in vivo with radioactive orthophosphate in the absence of ammonium and/or under photosystem II light, i.e. when the functioning of photosystem II is favoured over photosystem I (see section 3.2) [83,295]. This suggests that the photosynthetic electron transport chain may regulate the pathway of nitrogen assimilation via the modification of the PII protein in cyanobacteria. If confirmed, this would mean that the PII protein represents a key component coordinating both carbon and nitrogen assimilation in photosynthetic organisms. In support of such an hypothesis is the report showing that GS activity is subject to a reversible short-term regulation by light/dark transitions in *Synechococcus* PCC 6301 [296]. Moreover, in this case, GS activity appears to be regulated by some metabolic signal(s) probably related to the ammonium assimilation pathway, but neither by glutamine itself nor by a redox process involving thioredoxin, and an active electron flow between the two photosystems seems necessary to maintain the enzyme in its active form [296]. Along the same line is the report by Ohmori and Ohmori [297] who have shown that the nitrogen and carbon supply, as well as the cellular ATP level, determine the metabolism of glutamine.

A gene, designated *ntcA*, which complements a pleiotropic mutant impaired in both nitrate and nitrite reductases, GS and methylammonium transport, has been identified [298,299]. This gene encodes a predicted protein of 25 kDa that shares homology to the *cysR* gene product, a putative regulatory protein involved in sulphur assimilation in the same *Synechococcus* species (see section 7) [300], and to a family of bacterial transcriptional activators, such as Crp [301] and Fnr [302] from *E. coli*, and FixK [303] from *Rhizobium meliloti*. The *ntcA* gene product is proposed to be a global nitrogen regulator required for full expression of proteins subject to repression by ammonium in *Synechococcus* PCC 7942 [298,299].

Comparison of the phenotypic properties of mutants inactivated in *glnB*, *ntcA* or in both genes should provide information on the respective function of the corresponding gene products and reveal any interaction in the control of nitrogen assimilation in *Synechococcus* PCC 7942.

The *glnA* gene has been sequenced from the nitrogen-fixing species *Anabaena* PCC 7120 [304] and *Calothrix* PCC 7601 (K. Elmorjani, our laboratory, unpublished data). Both in vivo analyses and in vitro transcription experiments performed with the RNA polymerase purified from *Anabaena* indicate that, in ammonia-grown cells, the *glnA* gene is transcribed from two promoters, an *E. coli*-like promoter (*P_2*) and an atypical promoter (*P_1*), located at −155 and −273, respectively [304–306]. In cells grown under anaerobic nitrogen-fixing conditions, the major mRNA species starts at the *nif*-type promoter (*P_1*) located at −93. This promoter is not recognized in vitro by the purified *Anabaena* RNA polymerase, suggesting that it requires a specific sigma factor and/or some other effectors to be functional. The 380-bp sequence upstream from the initiation codon in both *Anabaena* and *Calothrix* are 74% identical indicating that these two nitrogen-fixing heterocystous strains share common regulatory mechanisms (N. Tandeau de Marsac, unpublished results). The amino acid sequence of the *Anabaena* and *Calothrix glnA* gene products predicted from nucleotide sequence data are 89% identical and exhibit 53–55% identity with their counterpart in *E. coli*. The *Anabaena glnA* gene has been shown to be expressed in *E. coli* at a level similar to an activated *E. coli glnA* gene, without requirement for the *ntrC* gene product and independently of the ammonium concentration [304]. Identification of the effectors and analysis of their role, including that of the PII protein, in the control of the expression of the *glnA* gene in cyanobacteria would be most interesting, as such information would provide a deeper insight into the regulatory mechanisms of one of the most important steps in nitrogen assimilation. Towards this end, it will be important to further characterize an open-reading frame (OrfA) (S. Liotenberg, our laboratory, unpublished data), located downstream from the *glnA*.
gene of *Calothrix* PCC 7601, and whose predicted amino acid sequence displays approximately 40–50% homology with protein histidine kinases, which, like NtrB, belong to two-component regulatory systems in bacteria [246].

So far little is known about the other cyanobacterial enzymes of the ammonium assimilation pathway [248]. Recently, ferredoxin-dependent GOGAT has been purified to homogeneity and characterized from *Synechococcus* PCC 6301 [307]. The native protein is a monomer of 156 kDa whose chemical and physical properties are very similar to its counterpart from chloroplasts, supporting the hypothesis that it might be the ancestor of the chloroplastic ferredoxin-dependent enzyme. Lightfoot et al. [308] first reported the detection in *Synechococcus* PCC 6301 of an homologue of the *gltB* gene encoding the large subunit of the *E. coli* GOGAT, but the nucleotide sequence of this gene has not been determined. No equivalent of the *gltY* gene encoding the small subunit of this enzyme has yet been found. Both an NADP- and an NAD-specific glutamate dehydrogenase have been purified from *Synechocystis* PCC 6803 [251,252]. The former is composed of four identical subunits and has a molecular mass of 208 kDa for the native enzyme, while the latter is an hexameric complex with a molecular mass of 295 kDa. A mutant lacking the NADP-glutamate dehydrogenase maintains the same activity of NAD-glutamate dehydrogenase demonstrating that the two enzymes are coded by different genes [251]. In *Synechococcus* PCC 6301, neither glutamate dehydrogenase activity nor a gene homologous to the *E. coli* *gdhA* gene have been detected [309]. However, it has been demonstrated that the *E. coli* *gdhA* gene can be expressed in this cyanobacterium and that the transformed cells display minor differences in growth at low light intensities and have an ammonium tolerant phenotype, consistent with the action of their acquired glutamate dehydrogenase activity as an ammonium detoxification mechanism [309].

As a consequence of the incomplete tricarboxylic acid cycle (lacking both 2-oxoglutarate dehydrogenase and succinyl-CoA synthetase activities [13]), 2-oxoglutarate is, in cyanobacteria, a final-step metabolite that provides the carbon skeleton required for ammonium assimilation through the GS/GOGAT system [275]. During CO₂ fixation, 2-oxoglutarate is produced by NADP-isocitrate dehydrogenase. This enzyme has been purified to homogeneity for the first time from *Synechocystis* PCC 6803 [310]. The native enzyme is a homodimer (2 \times 57 kDa). The N-terminal sequence of the subunit exhibits 45% identity with the corresponding region of the *E. coli* isocitrate dehydrogenase. Based on this N-terminal sequence, oligonucleotides can now be synthesized and the corresponding gene cloned and characterized.

6.3. Amino acid biosynthesis and transport systems

While most attention has been paid to glutamine biosynthesis, due to the key role this amino acid plays not only in nitrogen fixation but also in global nitrogen control, information on the biosynthesis of the other amino acids is very scanty. From the physiological and biochemical results obtained so far, it appears likely that both feedback inhibition of enzyme activity and repression of enzyme synthesis contribute to the control of amino acid biosynthesis [311,312]. Based on the determination with labelled compounds of the initial rates of amino acid uptake and on the analysis of mutants resistant to toxic amino acids, Labarre et al. [313] have reported the existence of three different active transport systems (permeases), one for arginine, lysine, histidine and glutamine, another for glutamine and glutamate and a third for all the other amino acids. Thus, with the exception of glutamine, each amino acid would be accumulated by only one major transport system in *Synechocystis* PCC 6803. Even though a common transport system is also involved in glutamine and glutamate uptake in *Anabaena* PCC 7120 [314], the situation might in fact be more complex for other amino acids and depend on cyanobacterial species. According to Flores and Muro-Pastor [315] the transport system for at least two basic amino acids, lysine and arginine, might share some membrane proteins and involve specific binding proteins. Supporting this assumption, both a common high-affinity

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transport system requiring metabolic energy and a low-affinity uptake system for lysine and arginine have been found in *Anabaena* PCC 7120 [316]. In *Anabaena variabilis* ATCC 29413, it has been proposed that general transport systems rather than specific ones might operate since high- and low-affinity systems for the uptake of glutamine and glutamate can also transport several other amino acids [317]. Present results indicate that the low-affinity transport system for leucine uptake in *Anabaena variabilis* ATCC 29413 is also relatively non-specific and differs from the system described in *Synechococcus* PCC 6301 [318,319].

DNA fragments cloned from *Synechococcus* PCC 7002 have been found to complement argE3 (acetylornithine deacetylase), proA2 (γ-glutamyl phosphate reductase), leuB6 (β-isopropylmalate dehydrogenase), leuC171 (α-isopropylmalate isomerase subunit) and thr-1 (threonine biosynthesis) mutations of *E. coli* ([320]; for the different amino acid biosynthetic pathways, see [311]). No complementations of other arg or leu *E. coli* mutations have been obtained, suggesting that the cyanobacterial arg and leu genes are not clustered on the genome. Similar results have been obtained with *Nostoc* sp. strain 7801 but, in contrast, no complementations of the *E. coli* leuC171 and proA mutations could be detected [321]. Besides the glnA genes previously mentioned, only a few cyanobacterial genes involved in the biosynthesis of amino acids have been sequenced so far: *thrB* (homoserine kinase) from *Calothrix* PCC 7601 [322]; *leuB* [323], and *ilv*X and *ilv*Y (two isoforms of acetohydroxy acid synthase) from *Spirulina platensis* [324]; *ilv*X [325] and *metF* (methylene tetrahydrofolate reductase) from *Synechococcus* PCC 7942 (D. Co, our laboratory, unpublished results); *argC* (N-acetylglutamate semialdehyde dehydrogenase) from *Anabaena* PCC 7120 [326]. The *thrB*, *ilv*X, *ilv*Y and *argC* genes complement corresponding *E. coli* mutations [322,326,327]. The characterization of more genes and the analysis of the phenotypic properties of mutants obtained by reverse genetics are obviously necessary to elucidate the control mechanisms involved in the biosynthetic pathways of amino acids.

6.4. Responses to deprivation for sources of combined nitrogen

Cyanobacteria respond to nitrogen starvation by a number of changes in cell structure, cell metabolism and gene expression [15,19]. The first events are a degradation of cyanophycin granules by cyanophycinase, followed by a rapid cell bleaching due to the degradation of the phycobilisome components, phycobiliproteins and linker polypeptides, and an abundant accumulation of intracellular glycogen [15]. Both cyanophycin granules and phycobiliproteins are used as nitrogen reserves under these conditions. The disappearance of the phycobiliproteins is a sequential event, cells first degrading the phycobiliproteins located at the periphery of the phycobilisome structure, subsequently the phycobiliproteins proximal to the core and finally the phycobilisome core itself.

In non-nitrogen-fixing cyanobacteria, providing that no more than approximately 50% of the phycobiliproteins are lost, cells can recover from nitrogen starvation. Upon readdition of a nitrogen source, cells regenerate almost completely in structure and composition, full pigmentation is recovered and glycogen reserves decrease to the level found in nitrogen-replete cells. In contrast, if cells are maintained under nitrogen starvation for a prolonged period of time, both chlorophyll *a* and the thylakoid membranes are degraded and cell division ceases. The process becomes irreversible and leads to cell death [15,328–332]. The effect of nitrogen starvation on carboxysomes and Rubisco remains controversial and its consequence on the photosynthetic and respiratory activities of the cells is poorly documented [15,328,333]. In the facultative heterotrophic species, *Synechocystis* PCC 6803, the rates of oxygen evolution and CO₂ fixation decrease and a lack of variable fluorescence has been observed, suggesting that photosystem II is rendered inactive [334]. Moreover, the decrease of photosynthetic oxygen evolution is more rapid in the presence than in the absence of glucose and an earlier oxygen uptake occurs in the presence of glucose, indicative of a switch to oxidative metabolism [329]. Based on the discovery that a protease that
specifically degrades phycobiliproteins in vitro exists in the nitrogen-fixing *Anabaena* sp. strains [15], the observed inhibition of the degradation of phycocyanin in the presence of glucose has been interpreted as the consequence of an irreversible inactivation of a phycocyaninase by a product of glucose catabolism. However, such a protease has never been purified from *Synechocystis* PCC 6803 and its existence in non-nitrogen-fixing strains is still a matter of debate. In *Synechococcus* PCC 7002, 90% of the phycobiliprotein cell content can disappear in less than 24 h [19]. This degradation is accompanied during the first 3–5 h by an arrest of the transcription of the *cpcBACDEF* and *apcAB* operons, which code for components of the phycobilisomes and the *psaAB* operon, which encodes the chlorophyll α-binding apoproteins of photosystem I reaction centre. In contrast, the transcription of *psbA* encoding the D1 protein of photosystem II does not appear to be affected. Analyses of translational fusions of *lacZ* to *cpcB*, *apcA* and *psaA* promoters have confirmed that the regulation of these genes mainly occurs at the transcriptional level.

Although in diazotrophic species the period of nitrogen starvation is limited to the time required for the nitrogenase complex to be synthesized, cells need to use endogenous reserve materials to survive and may change their pattern of gene expression as do non-fixing strains. Indeed, it has been shown that, in *Anabaena variabilis* ATCC 29413, the transcript abundance of the *cpcBA* and *apcAB* genes rapidly diminishes within 1 h of nitrogen starvation [335]. Transcription of these genes is again initiated as soon as combined nitrogen is provided, first by proteolysis of the intracellular reserve materials and later by nitrogen fixation. Half-lives of the messenger RNAs remain unchanged during these different periods, indicative of a transcriptional regulation. Although no measurements have been performed after 1 h of nitrogen starvation, time-course experiments with samplings at 6 h intervals suggest that the transcriptional levels of *rbcLS*, *psbA*, *psbB* and *glnA* remain constant during the differentiation of heterocysts in *Anabaena* PCC 7120 [103].

Two proteases, which probably provide amino acids for heterocyst-specific protein synthesis during the nitrogen starvation period, have been reported to be present in *Anabaena* sp. cells: a Ca²⁺-requiring soluble protease which can degrade many of the vegetative cell proteins, and another protease, which can degrade phycobiliproteins in vitro [336]. The Ca²⁺-dependent protease (52 kDa) has been purified from *Anabaena variabilis* ATCC 29413 and characterized as a trypsin-like serine protease of apparent molecular mass 52 kDa [337]. The corresponding gene, *prcA*, has been sequenced and the physiological effects of its inactivation examined in both *Anabaena variabilis* and in *Anabaena* PCC 7120 [338]. Surprisingly, in all of the numerous features examined, the mutants did not differ from the wild type strain, suggesting that the Ca²⁺-dependent protease is not essential for the development of functional heterocysts. The role of this protease thus remains to be elucidated.

6.5. Nitrogen fixation in non-heterocystous and heterocystous cyanobacteria

The cyanobacterial ‘conventional’ nitrogenase complex, which converts dinitrogen into ammonium, is highly homologous to its counterpart in other nitrogen-fixing bacteria [4,9]. It consists of a heterotetrameric MoFe-protein encoded by the *nifD* and *nifK* genes and a homodimeric Fe-protein encoded by the *nifH* gene. In this complex, the electron flow, which is coupled to ATP hydrolysis, goes from ferredoxin to the Fe-protein and finally to the MoFe-protein [6,248]. As in other nitrogen-fixing bacteria the cyanobacterial nitrogenase complex is irreversibly inactivated by oxygen. Recent studies have provided evidence for the existence of two ‘alternative’ nitrogenases in *Anabaena variabilis* ATCC 29413, a vanadium-dependent nitrogenase and a Fe-nitrogenase probably containing only Fe-S centres, as prosthetic groups [339,340]. These two enzymes most likely correspond to the alternative nitrogenases encoded by the *vnf* and *anf* genes described in *Azotobacter vinelandii*. A putative *anfH* gene has been detected by DNA–DNA hybridization with...
an *Azotobacter* DNA probe, but its sequence is not yet published [340].

The regulation of the synthesis and activity of the ‘conventional’ nitrogenase complex in cyanobacteria has been the subject of numerous studies presented in several reviews to which the reader is invited to refer for more details and for earlier literature [4,6,7,9,248,249,341]. It is generally accepted that the nitrogenase complex is regulated at the level of both enzyme activity and gene expression. However, in no case are the molecular mechanisms involved completely understood. Dinitrogen is not considered to be an inducer, but nitrate, ammonium and urea, as well as certain amino acids such as glutamine and glutamate have been shown to suppress nitrogen fixation, although the response to these nitrogenous compounds, in particular to nitrate and ammonium, varies depending on the strain.

In filamentous non-heterocystous and unicellular cyanobacteria, the nitrogenase complex is evenly distributed within the cytoplasm and is therefore not separated from the photosynthetic apparatus [342,343]. In such strains, a temporal separation between photosynthesis in the light and nitrogen fixation in the dark, as well as an increase of the respiratory metabolism, contribute to create an appropriate microaerobic environment and to protect the nitrogenase complex from inactivation by oxygen produced by photosynthesis [9]. This metabolic alternation between photosynthesis and nitrogen fixation may not result directly from a light effect. Indeed, when cultures of *Synechococcus* sp. RF-1 are exposed to diurnal light–dark cycles, an endogenous circadian N₂-fixing rhythm develops upon transfer of the cells to nitrate-free medium and incubation under continuous light [344,345]. In the case of the unicellular strain *Gloeoehtece*, however, temporal separation between both biological processes does not seem obligatory [9], and nitrogen fixation is most likely determined by the availability of intracellular glycogen reserves synthesized during the light periods [10]. However, further studies are required to establish if such mechanisms are indeed of importance in nitrogen fixation. In *Oscillatoria* sp., cultures grown photoheterotrophically under alternating light/dark cycles fix nitrogen in both light and darkness, but at higher rates in darkness, indicating that respiration might provide reductant and ATP for nitrogen fixation [346]. The same conclusion had been previously drawn for the obligate photoautotroph *Gloeoehtece* [347]. A slight increase in nitrogenase activity has also been observed in *Plectonema boryanum* grown under photoheterotrophic conditions in the presence of fructose or glucose [348]. In the marine filamentous *Trichodesmium* sp., the nitrogenase complex is regulated at the transcriptional or post-translational level by the presence of urea, but is not regulated by ammonium or nitrate [349]. Moreover, the Fe-protein activity might be regulated by a post-translational modification of the protein as proposed by other groups for the same protein in the heterocystous *Anabaena* strains [350–352]. It has also been proposed that a decrease in the degree of negative supercoiling of the *nif* genes mediates repression of the synthesis of the nitrogenase complex and that a Ca²⁺-dependent process is involved in the protection of nitrogenase against inactivation by oxygen [10,353–355]. Clearly, these results are too dispersed to draw clear-cut conclusions and more studies are needed to resolve the question of the protection of nitrogenase in unicellular and filamentous non-heterocystous cyanobacteria, but these data indicate, nevertheless, that there probably does not exist a universal and general mechanism.

In filamentous heterocystous cyanobacteria grown under aerobic conditions, the expression of the nitrogenase complex is confined to heterocysts whose differentiation specifically occurs in response to a lack of utilizable fixed nitrogen. Nitrogen fixation is thus intimately linked to a developmental process which includes a series of sequential structural and physiological modifications necessary to provide an anaerobic environment for efficient nitrogen fixation [4–9]. Generally not more than 5–10% of the vegetative cells within a filament differentiate into heterocysts. Mature heterocysts, slightly larger than vegetative cells, are surrounded by thickened cell walls which consist of three extra layers external to the vegetative cell envelope and provide a physical barrier to oxygen diffusion into heterocysts. These layers
are made up of glycolipid (innermost laminated layer) and polysaccharide (intermediary homogeneous and outermost fibrous layers). Polar bodies, consisting of cyanophycin deposits, accumulate at the junction with the vegetative cells, and thylakoids are rearranged. Having passed the intermediary stage of differentiation into proheterocysts, which are distinguishable under light microscopy by their lack of fully thickened cell walls and polar bodies, this process becomes irreversible. In mature heterocysts, photosystem II is not functional and respiration is enhanced, thus minimizing intracellular oxygen tension. Under illumination, ATP is mainly supplied via cyclic photophosphorylation within heterocysts, while in darkness it is provided via oxidative phosphorylation. Interconnections between vegetative cells and heterocysts being established, the former provide organic carbon compounds to the heterocysts and the latter glutamine to the neighbouring cells (see Fig. 1 in [250]).

Besides the widely studied effects of various nitrogen sources which have been previously mentioned [4,6,7,9,248,249], other factors such as light irradiance and pH [356], light/dark shifts [287], and availability of external CO$_2$ [357] have recently been reported to have a regulatory effect on nitrogen fixation in heterocystous cyanobacteria. Two groups have shown that the inactivation of the nitrogenase complex in Anabaena variabilis ATCC 29413 [287,351,352] and Anabaena ATCC 33047 [350] is correlated with a post-translational modification of the Fe-protein. According to Ernst et al. [287,352] this modification, being correlated with diurnal regulation of nitrogen fixation, might be involved in the maintenance of a constant C/N ratio, and oxygen (or a metabolic process determined by oxygen) would

Fig. 6. Vegetative filaments of Anabaena PCC 7120 grown in a medium containing nitrate (A; BG-11 medium [11]) or grown under nitrogen-fixing conditions (B and C; BG-11$_0$ medium [11]). Note the presence of heterocysts in B and C. All phase contrast; bar markers represent 10 µm. Arrowheads indicate heterocysts.
be the trigger. Since a product of nitrogen assimilation, such as glutamine, is apparently not necessary for the enzyme modification to occur, the mechanism by which this amino acid leads to an inhibition of nitrogenase activity remains unsolved. Some ions, in particular Ni$^{2+}$, BO$_3^-$, Na$^+$ and Ca$^{2+}$ have also been shown to play a role in nitrogen fixation. Ni$^{2+}$ depletion has been found to delay de novo synthesis of proteins or enzymes required for the synthesis of the nitrogenase complex [358]. This effect, being concomitant with a transient accumulation of cyanophycin, has been postulated to occur as a consequence of a diversion of ammonium into nitrogen reserves. BO$_3^-$ has been proposed to stabilize heterocyst structure [359]. Na$^+$ primarily affects photosynthesis and would have only a secondary effect on nitrogen fixation [360], as do phenoxy acetic herbicides [361]. Ca$^{2+}$ has attracted most attention, since it is in eukaryotes [362]. In Anabaena sp. and Anabaena ATCC 33047, Ca$^{2+}$ has been reported to be involved in the protection of the nitrogenase complex from inactivation by oxygen [363]. In Nostoc PCC 6720, heterocyst frequency and cellular Ca$^{2+}$ content appear to be inversely correlated with incident light irradiance and an effect on the inhibition of the nitrogenase complex has been observed ([364–366]; R.J. Smith, personal communication). Although the relationships between these effects and calmodulin, which seems to be present in cyanobacteria, is still poorly documented, some of these effects could depend on Ca$^{2+}$/calmodulin regulatory processes [367]. Interestingly, abscisic acid, a phytohormone [368], results in an increase of intracellular Ca$^{2+}$ content in Nostoc PCC 6720 [367,369]. It has also been shown to affect growth and to increase both heterocyst frequency and total nitrogenase activity in the same cyanobacterial strain, in a manner comparable to a Ca$^{2+}$ ionophore. Since in cyanobacteria living in symbiotic association with the water fern Azolla and with the hornwort Anthoceros, it has been observed that heterocyst frequency and nitrogenase activity are greatly enhanced [142,370], it would be worth determining whether abscisic acid is involved in the regulation of these processes.

Although the physiological and biochemical aspects of nitrogen fixation and heterocyst differentiation have been studied in various filamentous strains, the most advanced molecular genetic studies have been performed on Anabaena PCC 7120 (Fig. 6). As shown by Haselkorn and co-workers, the structural nif genes are not contiguous in vegetative cells of Anabaena PCC 7120 and this region of the genome undergoes two rearrangements during the late stage of heterocyst differentiation [371–373]. The first one is a precise excision of an 11-kb element that, in vegetative cells, interrupts the nifD reading frame. It occurs via a site-specific recombination between directly repeated 11-bp sequences present at each extremity of this element, restoring the nifHDK operon that can subsequently be transcribed from the nifH promoter as a polycistronic unit [374]. The site-specific recombinase is encoded by the xisA gene located within the 11-kb element [375]. The excised element remains as a stable circular DNA molecule in heterocysts without any detectable amplification [374]. The regulatory region controlling the expression of xisA is a 127-bp sequence located upstream from the gene [376]. A sequence-specific DNA-binding factor (VF1) has been isolated from vegetative cells that may prevent the expression of the xisA gene in this cell type [377]. However, it has also been found to bind weakly to other promoters, such as nifH, rbcL and glnA, genes expressed only in heterocysts, in vegetative cells or in both cell types, respectively. Insertional inactivation of xisA [378,379] or deletion of the 11-kb element [376] demonstrated that the excision of the 11-kb element is required for the expression of the nifK gene, which depends on the nifH promoter, but is independent of heterocyst-specific transcription and has a role neither in heterocyst development nor in spacing. The nifHDK genes are separated in many heterocystous strains, including Anabaena variabilis ATCC 29413 and Anabaena or Nostoc sp. strains isolated from the water fern Azolla sp. or the hornwort Anthoceros punctatus [380–386]. In contrast, the nifHDK genes are contiguous in all the unicellular and non-heterocystous cyanobacterial strains tested so far [380,387–390], as well as in the heterocystous
strain, *Fischerella* ATCC 27929 [385]. The latter observation gives support to the previous conclusion that the 11-kb element has no significance for heterocyst development [378].

In *Anabaena* PCC 7120, the second rearrangement concerns a 55-kb element located between *nifS* (involved in the maturation of the MoFe-protein) and *nifB* (required for the synthesis of the MoFe cofactor). It occurs within the reading frame of the *fxN* gene that encodes a bacterial-type ferredoxin (see section 5.1) [180,391,392]. This rearrangement involves a site-specific recombination between directly repeated 5-bp sequences different from those required for the *nifD* rearrangement. It restores the transcription of the polycistronic operon *orf1-fdxN-nifS-nifU* [392]. The recombinase for the *fxN* rearrangement, which very likely differs from XisA, has not yet been identified and no circular molecule corresponding to the 55-kb element has been detected in heterocysts [391]. In contrast to the *nifD* DNA rearrangement that occurs under aerobic, but not under microaerobic conditions [391], the *fxN* DNA rearrangement occurs under both conditions. The 55-kb element is present neither in the *Anabaena variabilis* ATCC 29413 nor in the *Nostoc commune* genome [381,393]. In the latter species, a gene (*gibN*) whose putative product shares homology with myoglobin has been found between *nifH* and *nifU* [394]. It has been proposed that GibN might scavenge oxygen and thus protect the nitrogenase complex against inactivation.

Approximately 4 kb downstream from the *Anabaena* PCC 7120 *nifK* gene are *nifN, nifX, orf3, nifW, orf1, orf2* and *fxH* [373,395]. The *fxH* gene encodes a plant-type ferredoxin specific for nitrogen fixation (see section 5.1) [179]. The six other genes have been assigned on the basis of their similarity to genes from the nitrogen-fixing eubacteria *Klebsiella* and/or *Azobacter* [373]. Although the function of the open reading frames (ORFs) is not yet well established, analysis of mutants inactivated in *orf1* and complementation experiments with a DNA fragment carrying only *orf1* and *orf2* indicate that at least one of these *orf* is required for efficient nitrogen fixation [395].

In *Anabaena* PCC 7120 and *Anabaena variabilis* ATCC 29413, the mRNA abundance for the structural *nifHDK* genes and for some other genes related to nitrogen fixation appears to be regulated at the transcriptional level by ammonium [179,392,395–398]. Nitrate negatively regulates the nitrogenase mRNA levels in *Anabaena* PCC 7120 in both aerobic and anaerobic incubation conditions [398], but no repression has been observed in *Anabaena variabilis* ATCC 29413 induced under anaerobiosis [396]. A possible explanation for the different results obtained with nitrate is that repression depends on the level of nitrate reductase present in the two strains under the experimental conditions used, as suggested by the absence of regulation in nitrate-assimilation deficient derivatives of *Anabaena* PCC 7120 [398]. Unfortunately, nitrate reductase activity has not been determined in *Anabaena variabilis* ATCC 29413.

Complementation of two Nif" mutants of *Anabaena* PCC 7120 has led to the identification of two genes, *hetA* (mutant EF116) and *hetB* (mutant EF113), involved in the synthesis of the external cell wall layers of heterocysts [399]. Mutant EF116 forms a heterocyst envelope polysaccharide less cohesive than in the wild-type strain, and mutant EF113 retains only a vestigial heterocyst envelope. Subsequently, a mutant lacking the outermost layer of the heterocyst cell wall has been obtained by site-directed inactivation of *hetA* confirming the role of this gene in the synthesis of the envelope polysaccharide [400]. Both *hetA* and *hetB* are distant on the chromosome [401]. The *hetA* gene is a monocistronic unit transcriptionally activated, upon nitrogen deprivation, prior to the activation of the *nifH* promoter and before proheterocysts are produced [402]. The *hetA* gene encodes a 601-amino acid polypeptide sharing no homologies with any known proteins.

As shown by Borthakur and Haselkorn [403], Tn5 transposes in *Anabaena* PCC 7120. In order to identify *Anabaena* genes that respond to removal of fixed nitrogen or to other environmental changes, Wolk and co-workers [404] have constructed a Tn5-derivative that contains the promoter-less *luxAB* genes (encoding bacterial
luciferase from *Vibrio fisheri* as a transcriptional reporter. Such a Tn5-derivative permitted the identification of a gene whose transcription is activated within 1 h in response to nitrogen step-down, as well as the mapping of the position and orientation of the transposon in the genome, facilitating the further cloning of contiguous genomic DNA fragments [404]. Other elegant reporter gene systems, based on promoter fusions to *lacZ* (encoding β-galactosidase in *E. coli*) or to *luxAB* have been used by Wolk and co-workers to monitor the expression of specific genes in time and space during heterocyst differentiation in *Anabaena* PCC 7120 and in a heterocyst-less mutant strain *Anabaena* PCC 7118 [405]. Using recombinant plasmids that lack the cyanobacterial origin necessary for replication, the heterologous DNA has to integrate into the chromosome at a site directed by the cloned region of homology. By this approach, these authors have been able to demonstrate very nicely that, in *Anabaena* PCC 7120, the expression of *PnifHDK-luxAB* gene fusion is exclusively confined to heterocysts, that of *PrbcLS-luxAB* to vegetative cells and that of *PglnA-luxAB* to both cell types, whether cells are deprived of combined nitrogen aerobically or anaerobically. Although faintly evident with the initial construct *PhetA-luxAB*, it has been confirmed, by putting the transcription of the luciferase genes under the control of the T7 RNA polymerase, the latter being placed under the control of the *hetA* promoter, that the expression of *hetA* displays the same spatial pattern as *nifHDK* [406]. In the heterocyst-less mutant strain *Anabaena* PCC 7118, known to express nitrogenase activity when deprived of combined nitrogen under anaerobic conditions in the light, the use of both *PnifHDK-luxAB* and *PnifHDK-lacZ* gene fusions enabled Elhai and Wolk [405] to show that the expression of the *nif* genes is restricted to certain cells within the filament, indicative of a pattern of formation in this strain that corresponds to cryptic heterocyst differentiation. These results provide evidence for a developmental rather than an environmental control of the expression of the *nif* genes during heterocyst differentiation.

An intriguing aspect of heterocyst differentiation, which has been the subject of several hypotheses, is the mechanism(s) determining the number of heterocysts per filament and their spacing [5,406]. In *Nostoc* PCC 6720 and *Anabaena* PCC 7120, heterocysts develop at both extremities and at regular intervals within the filament, approximately every 10 cells. It is believed that heterocysts inhibit the differentiation of adjacent cells into new heterocysts which are always formed midway between the two existing ones, thus maintaining the same spacing when filaments lengthen. Glutamine, or more likely another nitrogen-derived substance(s), has been proposed as the putative diffusible inhibitor(s), whose gradient(s) along the filament would maintain the heterocyst spacing pattern [5,406,407]. To elucidate the molecular bases of this process in *Anabaena* PCC 7120, Haselkorn and co-workers isolated a number of *Fix−* mutants unable to grow aerobically on media lacking fixed nitrogen [408]. One of them is no longer able to differentiate heterocysts (mutant 216) [409] and another differentiates heterocysts mostly at the ends of filaments (mutant PAT-1) [410]. By complementation of these mutants with a cosmid library from the wild-type DNA [409,410], two genes, *hetR* and *patA*, were identified. When transferred back to the wild-type strain the inactivated *hetR* and *patA* genes confer the phenotype initially observed for mutants 216 and PAT-1, respectively.

The predicted HetR protein (299 amino acids) shares no homology with any known regulatory proteins or sigma factors [409]. The *hetR* gene is abundantly transcribed very early in the differentiation process, as two mRNA species 1.4 and 1.9 kb long, and remains transcribed at low level under nitrogen replete conditions, although it is not required for vegetative cell growth. Both mutant 216 and the wild-type strain containing the *hetR* gene on a plasmid produce an increased frequency of heterocysts, even under nitrogen replete conditions. Two different roles have been proposed for the *hetR* gene product: it could be a transcriptional activator for differentiation unless it binds a nitrogen-containing inhibitor, such as glutamine, or a sensor of the fixed carbon to nitrogen ratio in the cell. The second possibility is a more general function that might explain the
presence of DNA fragments hybridizing with hetR in the genome of a number of non-heterocystous nitrogen-fixing cyanobacterial strains [409]. According to preliminary results obtained by Wolk and co-workers [406] using the luciferase promoter–probe vector and transposon mutagenesis, hetR is expressed spatially constitutively but, after 3.5 h of nitrogen starvation, the hetR gene fusion becomes abundantly expressed within particular separated cells, provided that an intact copy of hetR is present. This suggests an autoregulatory control of hetR. Moreover, a control of hetR over the expression of het\textsubscript{4} also seems to occur during the differentiation process in \textit{Anabaena} PCC 7120.

The \textit{patA} gene encodes a predicted protein of 379 amino acids [410] whose C-terminal domain exhibits very high homology with CheY which is involved in the regulation of chemotaxis, as well as with other response regulators of two-component regulatory systems in eubacteria [246,411]. Upon heterocyst differentiation \textit{patA} is expressed as a 1.4-kb mRNA species whose abundance increases with approximately the same timing as the expression of hetR. Like hetR, \textit{patA} remains transcribed at a low level in the presence of fixed nitrogen although the corresponding gene product is not needed for growth under these conditions. Since the PatA\textsuperscript{−} mutant is still able to differentiate terminal heterocysts, this indicates that the \textit{patA} gene product is only required for the differentiation of intercalary heterocysts. Very interestingly, the \textit{patA} mutation suppresses the multiheterocyst phenotype produced by extra copies of the wild-type hetR gene. This suggests that both PatA and HetR are components of the same environmental sensing regulatory circuit in \textit{Anabaena}. Based on the homology between PatA and the CheY family of regulator proteins, it has been proposed that PatA is a transcriptional activator controlled by phosphorylation of an aspartate residue [410]. It should be remembered however, that CheY does not interact with DNA, but activates the flagellar motor and that PatA has its putative phosphorylation site located in the C-terminal domain of the protein, in contrast to the presently known eubacterial transcriptional activators [246,411].

7. SULPHUR ASSIMILATION AND STAR-
VATION

Depending on biotopes, sulphate concentrations may vary considerably: approx. 0.1 mM in fresh water lakes, approx. 0.2 mM in eutrophic lakes and up to approx. 27 mM in sea water [412]. A concentration as high as 2 mM H\textsubscript{2}S can be found in hot springs [413]. Sulphide being readily oxidized by oxygen, its concentration depends on the local oxygenic photosynthetic activity and thus on light conditions, and on the diffusion of oxygen from the overlying atmosphere. Consequently, within a given biotope and with the exception of sea water, important fluctuations may occur seasonally or diurnally [413]. Cyanobacteria have evolved different systems of adaptation to sulphur availability allowing them to colonize and develop in a variety of ecological niches.

A number of cyanobacteria are able to alternatively use H\textsubscript{2}O or H\textsubscript{2}S as a source of reducing power and can thus grow photosynthetically in aerobiosis as well as in anaerobiosis. Anoxygenic photosynthesis is an inducible process driven by photosystem I and independent of photosystem II, the latter being reversibly inactivated by sulphide. In this process, sulphide is oxidized to elemental sulphur and the extracted electrons can be channeled either to CO\textsubscript{2}, hydrogen evolution or nitrogen assimilation. This property of facultative sulphide-dependent anoxygenic photosynthesis appears in different groups of unicellular and filamentous cyanobacteria, but strains exhibit different requirements of sulphide concentration for anoxygenic photosynthesis to be performed. The best known example is the filamentous species \textit{Oscillatoria limnetica} with which most of the ecophysiological and biochemical studies have been performed [412,413]. To date, no molecular studies on the regulatory processes involved in anoxygenic photosynthesis have been reported.

Besides this ability of certain species to switch from an oxygenic to an anoxygenic-type of photosynthesis, cyanobacteria respond differently to sulphate limitation. Freshwater species, such as \textit{Synechococcus} PCC 6301 and PCC 7942, undergo important morphological and physiological
changes when deprived of sulphur: thickening of the cell wall; rapid decrease in cellular phycobiliprotein content followed by a decrease in chlorophyll $a$ levels, accompanied by a loss of photosynthetic membranes; accumulation of glycogen reserves, polyphosphate granules and poly-$\beta$-hydroxybutyrate [16,328,414,415]. Under sulphur deprivation, *Synechococcus* PCC 7942 cells can survive for at least two days without loss of viability due to the activation of the sulphate transport system which involves the synthesis of new soluble and membrane polypeptides [414]. In contrast, in *Synechococcus* PCC 7002, a marine species which is never subjected to variations of sulphur concentration in its natural environment, a deprivation of sulphur leads to an arrest of growth without apparent induction of a specific transport system for sulphate ions [416].

The capacity of *Synechococcus* PCC 7942 to take up exogenous sulphate increases by a factor of 10 within 7 h of sulphur deprivation and protein synthesis is required for acclimation to occur. A cluster of genes, whose transcription is induced under sulphur deprivation, has been characterized [300,417,418]. Based on its strong homology to a polypeptide from the periplasmic transport system of *E. coli* and *Salmonella typhimurium*, the *cysA* gene encodes a membrane-associated ATP-binding protein of the sulphate permease system [417]. Adjacent to *cysA*, but divergently transcribed are the other sulphur-regulated genes [300]. The *sbpA* gene encodes a sulphate binding protein of 37.7 kDa. It contains a typical prokaryotic signal sequence and lacks sulphur-containing amino acids. The SbpA gene product is 40% and 34% identical to the *E. coli* sulphate- and thiosulphate-binding proteins, respectively. The *cysT* and *cysW* genes encode extremely hydrophobic proteins of 30.4 and 30.7 kDa, respectively, that share similarities. Both proteins exhibit homology to almost all membrane-spanning polypeptides of osmotic shock-sensitive transport systems in enteric bacteria, but they are more similar to the polypeptides encoded by the functionally analogous genes in *E. coli*. These proteins may be required to form a channel for the transport of sulphate across the cytoplasmic membrane. The *cysR* gene, located between *cysT* and *cysW*, encodes a 23.8-kDa polypeptide that, like the *ntcA* gene product (see section 6), exhibits some similarities to the DNA-binding regulatory proteins Fnr and Crp from *E. coli* and FixK from *Rhizobium meliloti*. Located downstream from three genes, designated *sbpB*, *cysU* and *cysV*, for which neither sequences nor functions have been reported, is *rhdA* that encodes a predicted periplasmic protein of 33 kDa [418]. This protein shares similarities to bovine liver rhodanese, an enzyme that transfers the thiol group of thiosulphate to a thiophilic acceptor molecule and to a rhodanese-like protein of *Saccharopolyspora erythraea*. However, this RhDA polypeptide displays very low rhodanese activity. In addition, insertional inactivation of *rhdA* significantly lowers neither the rhodanese nor thiosulphate reductase activity in the cell and RhDA$^{-}$ mutants can grow well on sulphate, thiosulphate and tetrathionate. The function of RhDA thus remains to be determined. Inactivation of *sbpA*, *cysT* and *cysW* results in an inability to transport sulphate compounds, in an altered utilization of other sulphur-containing compounds and in cysteine auxotrophy [300,417]. Unlike the CysA$^{-}$ mutant, both the CysT$^{-}$ and CysW$^{-}$ mutants are capable of slow growth on thiosulphate suggesting that this sulphur compound can be transported by two permease systems in which CysA is a common component. SbpA$^{-}$ and CysR$^{-}$ mutants are cysteine auxotrophs and cannot grow on thiocyanate. However, these mutants grow on other sulphur sources, including sulphate and thiosulphate, but they do not exhibit an increase in the $V_{\text{max}}$ for sulphate transport characteristic of wild-type cells grown under sulphur-limiting conditions. The precise function of SbpA and CysR is still uncertain.

Another mode of adaptation to sulphur limitation has been discovered in the filamentous freshwater species *Calothrix* PCC 7601 [20,37,101,419]. Under limited sulphur deprivation, *Calothrix* PCC 7601 cells can maintain continuous growth, although at a slightly lower rate than under sulphur replete conditions, due to the synthesis of a new type of phycocyanin (phycocyanin-3) and associated linker polypeptides. These newly synthesized
proteins, which substitute for the usual phycobilisome rod components found in cells grown under sulphur replete conditions (see section 3.1), are encoded by the cpcB3A3H3I3D3 operon. The physical organization of this operon is identical to that of the red light-induced cpcB2A2H2I2D2 operon with the first two genes encoding the $\beta$ and $\alpha$ subunits of phycocyanin-3 ($\beta^{PC3}$ and $\alpha^{PC3}$) and the last three genes encoding the associated linker polypeptides ($L^{PC3}$) (Table 1). The expression of the cpcB3A3H3I3D3 operon is specifically induced under sulphur limitation and transcribed as three mRNA species, 5.0, 3.5 and 2.0 kb long, corresponding to cpcB3A3, cpcB3A3H3 and cpcB3A3H3I3D3, respectively. The most striking peculiarity of the cpcB3A3H3I3D3 gene products deduced from nucleotide sequence data is the absence of sulphur-containing amino acids, with the exception of three cysteinyl residues, required for the attachment of the phycocyanobilin chromophores on $\beta^{PC3}$ and $\alpha^{PC3}$. The N-terminal methionine has been shown to be absent from the five gene products. In contrast to the operons encoding phycocyanin-1, phycocyanin-2 and phycoerythrin (see section 3.2.2) whose expression is switched off under sulphur limitation, the genes encoding the $\alpha$ and $\beta$ subunits of allophycocyanin remain transcribed. Biochemical and fluorescence data demonstrated that phycobilisomes from sulphur deprived cells are functional with an unchanged core composition. Considering the relative paucity in sulphur-containing amino acids of the allophycocyanin subunits when compared with phycocyanin-1, phycocyanin-2 or phycoerythrin and the fact that N-terminal methionine residues are absent from the mature proteins, this mode of adaptation allows Calothrix PCC 7601 cells to optimize their metabolic capacities to otherwise unfavourable conditions. A comparison of the amino acid sequences of the phycobiliprotein subunits from different cyanobacteria and photosynthetic eukaryotes initially isolated from biotopes either poor or rich in sulphate, indicated a good correlation between the number of sulphur-containing amino acids in the sequences and the ecological origin of the strain considered.

8. RESISTANCE TO METAL IONS AND RESPONSES TO STARVATION

Several metal ions, such as copper, iron, zinc, and cobalt are required for biochemical processes, where they serve as cofactors for many enzymes and are crucial for catalytic activity. They can, however, be toxic when present at high levels. Cadmium, which has no known biological functions, is always found in association with zinc and is highly toxic [420]. Microorganisms have thus developed adaptation mechanisms to face changing concentrations of these ions. In cyanobacteria, much attention has been paid to the effect of iron and copper starvation, in particular on the expression of genes involved in photosynthesis, but very little is known about the molecular mechanisms controlling storage or tolerance to high concentrations of metal ions and nothing is known about metal ion uptake at a molecular genetic level.

The form of iron generally utilized by microorganisms, Fe$^{3+}$, is often limiting due to its low solubility in water at physiological pH. Siderophores, low-molecular mass (0.5–1 kDa) iron transport compounds, are known to be produced by cyanobacteria and to play an important role in increasing the solubility and uptake of ferric ions, but these compounds have been biochemically characterized only in Anabaena sp. [421]. A recent report by Brown and Trick [422] has presented evidence for the existence of two different siderophores, a hydroxamate-type and a catechol-type, in Oscillatoria tenuis. These authors also found that the production of these siderophores is expressed at two different iron levels in the medium, but no molecular genetic studies have been performed.

Throughout the living kingdom, storage and buffering of iron are achieved by ferritins, a class of multimeric proteins able to store up to 4500 iron atoms in their large (approx. 80 Å in diameter) internal cavity [423]. Heavy metal ions can be complexed in polynuclear thiol cluster arrangement by metallothioneins, cysteine-rich polypeptides of low molecular mass [424–426]. Metallothioneins are diverse in structure. Class I and II
metallothioneins are proteins encoded by structural genes, while those belonging to Class III are secondary metabolites. The precise physiological role(s) of metallothioneins is still a matter of discussion. They have been proposed to play a role in the storage of certain essential heavy metal ions and the detoxification of supra-optimal concentrations of essential and non-essential ones, as well as in cell development and in different cellular functions in eukaryotes. In mammalian cells, transcription of the metallothionein genes is induced by exposure to high concentrations of a number of metals, hormones and other factors associated with chemical and physical stress, while in the fungi, *Saccharomyces cerevisiae* and *Neurospora crassa*, the regulation is less complex with the metal regulatory elements responding only to elevated concentrations of copper ions [424, 425].

In cyanobacteria, the first amino acid sequence of a metallothionein has been reported by Olafson’s group [427, 428] for *Synechococcus* PCC 6301. This 56-amino acid polypeptide, classified as a Class II metallothionein, has a primary structure which differs substantially from the known eukaryotic proteins. However, the molecule complexes cadmium, zinc and copper, and has a high thiol content with cysteine-containing sequences similar to those observed in eukaryotic metallothioneins [427]. Moreover, predicted secondary structures indicate some similarity between the *Synechococcus* and the eukaryotic metal-thiolate clusters [428]. Previous physiological experiments performed with *Synechococcus* strain RRIMP N1 suggested that cyanobacterial metallothionein synthesis is regulated at the transcriptional level in response to increasing concentrations of cadmium and zinc as in eukaryotic cells [429]. A gene encoding a metallothionein (*smtA*) has been cloned and characterized in *Synechococcus* PCC 6301 and PCC 7942 [430]. The *smtA* gene product is identical to the polypeptide previously purified and sequenced from the same cyanobacterial species, with the exception of Cys32 substituted for Ser and two additional codons in C-terminal position in the *Synechococcus* PCC 7942 polypeptide ([428]; N. Robinson, personal communication). Exposure of the *Synechococcus* PCC 6301 cells to elevated concentrations of cadmium, zinc and copper ions leads to an increased abundance of *smtA* transcripts, indicative of an induction of transcription initiation analogous to the regulation occurring in eukaryotic cells [431] or of changes in mRNA stability [430]. *Synechococcus* PCC 6301 cells tolerant to cadmium concentrations up to 3.2 μM have been selected by stepwise adaptation to increasing concentrations ([432]; N. Robinson, personal communication). Tolerant cell lines arose by amplification of the chromosomal gene and rearrangement of the *smt* locus (N. Robinson, personal communication). The amplification phenomenon could be analogous to the observed amplification of the eukaryotic metallothionein genes [433]. Whether such an adaptation mechanism involving amplification and rearrangement occurs in the genome of cyanobacterial strains inhabiting metal-polluted environments is worthy of examination. The polypeptide from *Synechococcus* PCC 7942 has been expressed in *E. coli* as a C-terminal extension of glutathione-S-transferase (GST) (N. Robinson, personal communication). The recombinant *E. coli* cells show no detectable increase in cadmium, copper and zinc tolerance, but a three-fold increase in the accumulation of zinc and a slight increase in the accumulation of copper is observed when cells are grown under low concentrations of these metals. The GST-SmtA fusion protein displays high affinities for zinc, cadmium, mercury and copper, and the metal ions bind to the SmtA portion of the fusion protein. Taken together, these experiments provide the first evidence that *Synechococcus* cells do indeed synthesize a metallothionein in response to increased concentrations of metal ions.

Laulhere et al. [434] reported the first purification and characterization of a ferritin from *Synechocystis* PCC 6803. The cyanobacterial ferritin has a molecular mass of 400 kDa and is composed of 19-kDa subunits. It contains 2300 atoms of iron and 1500 molecules of phosphate per molecule of protein, and 0.25 haem residue per subunit. The α-peak of the cytochrome has a maximum of absorption at 559 nm. The protein shares some important functional features with all known ferritins, such as the ability to take up
iron in vivo and in vitro, as well as a high iron content per molecule. However, it appears to be more closely related to bacterioferritins than to plant or animal ferritins, since it displays the same spectrophotometric characteristics and has a similar Fe/P ratio. The N-terminal amino acid sequence (54 residues) of the *Synechocystis* PCC 6803 ferritin subunit is approximately 40–50% identical to that of *E. coli* or *Azotobacter vinelandii*. This subunit shares neither immunological determinants nor amino acid sequence homology with plant ferritins within the first 54 residues sequenced. In plant cells, ferritins are found in chloroplasts and other plastids where they assume an iron-storage function, and their synthesis is regulated at the level of transcription by iron [435–438]. In *Synechocystis* PCC 6803, ferritin is synthesized whatever the iron status of the cells. Moreover, labelling experiments performed with cells incubated under different iron concentrations showed that the primary role of the cyanobacterial ferritin is most likely to be related to the buffering of excess iron, while low-molecular-mass molecules (approx. 10 kDa), would assume the storage function [434]. Thus, the role of ferritin in *Synechocystis* PCC 6803 appears to be similar to that described for *E. coli* and different from that found in plants. Considering the likely cyanobacterial origin of plant chloroplasts [439,440], these results raise interesting questions about the evolutionary origin of the plant ferritin. At present the common origin of prokaryotic and eukaryotic ferritins is a matter of debate. According to Andrews et al. [441,442], the structural and functional similarity of the two classes of proteins reflect convergent evolution; according to Grossman et al. [443], however, all key residues specifying the unique structural motifs of eukaryotic ferritin being conserved in prokaryotic sequences, they form a single protein family potentially obligate for aerobic metabolism. It would thus be important to characterize the cyanobacterial gene as it would pave the way for further investigations on the structure, function and regulation of the synthesis of this protein at a molecular level. It might also help to clarify the evolutionary pathway(s) of these proteins.

The effects of iron limitation on cyanobacterial cell structure and physiology is well documented [27,421,444–452]. Among the most important changes are the alterations in the composition and functioning of the photosynthetic apparatus. Iron-deprived cells have fewer photosynthetic membranes, phycobilisomes and generally fewer carboxysomes, but accumulate large amounts of glycogen reserves [446]. Differences in the polypeptide composition of the photosynthetic and outer membranes from iron-replete and iron-deprived cells have been reported [446,447,450]. Under limited iron starvation cyanobacteria, like red algae, synthesize new polypeptides which can, at least in part, assume essential functions for cell growth. The best known example is the replacement of ferredoxin by flavodoxin, a low-molecular-mass (approx. 20 kDa) FMN-containing flavoprotein, reported to occur in several cyanobacteria [453–455]. Natural isolates of *Microcystis aeruginosa* have been found which contain either ferredoxins I and II, or only ferredoxin I, or only flavodoxin; this was apparently due to different environmental growth conditions [456]. In the filamentous strain, *Nostoc* sp. strain MAC, grown under laboratory conditions in a medium containing less than 2 μM Fe, ferredoxin II becomes virtually absent and flavodoxin is synthesized, but ferredoxin I is still formed at about 30% of the level found in iron-replete cultures [453,457]. The two filamentous heterocystous *Anabaena* strains ATCC 29413 and PCC 7119 behave similarly in response to iron limitation [455], ferredoxin being replaced by flavodoxin in vegetative cells only. As also shown in a unicellular strain, *Synechocystis* PCC 6714, which does not fix nitrogen, the substitution of redox proteins does not significantly affect photosynthetic electron transport reactions and cell growth [454]. In contrast, in *Anabaena* nitrogen fixation decreases, probably due to an iron-limited synthesis of active nitrogenase complex since, as shown in *Anabaena* PCC 7119, flavodoxin remains constitutively synthesized in heterocysts regardless of the iron supply. In *Anabaena* PCC 6309 cells, the amount of ferredoxin is dependent on the concentration of iron in the medium, but ferredoxin is not replaced by the flavoprotein [455,458]. Being unable to adapt its metabolism to changes in
iron concentration, a decline in the photosynthetic activity of the cells, correlated with a decrease in chlorophyll $a$ and phycocyanin, as well as a decline in cell growth under iron limitation, is observed in *Anabaena* PCC 6309.

Structural and chemical properties of flavodoxin purified from *Anabaena* PCC 7119 have been characterized in detail [459,460]. The N-terminal sequence determined with the purified protein is strictly identical to the amino acid sequence deduced from the corresponding gene cloned from *Anabaena* PCC 7120 [461]. The *Anabaena* flavodoxin amino acid sequence exhibits 69.8% homology with the predicted sequence from *Synechococcus* PCC 7942 [462]. In *Synechococcus* PCC 7942, the flavodoxin gene (isiB) is single copy and encodes a predicted polypeptide of 18.7 kDa [462]. The isiB gene is transcribed as part of a larger operon and located at the 3' end of a 1900-nucleotide message induced by iron stress. Located immediately upstream from isiB is an open-reading frame designated isiA which is transcribed as a 1000-nucleotide iron stress-induced message [462]. The expression of both the isiA and isiB genes appears to be rapidly repressed by iron due to an arrest of the initiation of transcription or to a rapid turnover of the transcripts [462]. Upstream from the isiAB operon transcription start site are three 17-bp sequences which resemble the iron-regulated *fur* operator of *E. coli* [463,464]. The isiA gene encodes a putative polypeptide of 36.8 kDa [463]. The same isaab operon has also been characterized in *Synechococcus* PCC 7002 [465]. The predicted amino acid sequence of IsiA shows strong similarities to the psbC gene product of higher plants and cyanobacteria and has been proposed to correspond to the 36-kDa polypeptide of photosystem II described by Pakrasi et al. [466] and by Riethman and Sherman [467]. This polypeptide, isolated as part of the chlorophyll $a$-protein complex CPVI-4, which is induced in iron-deficient cells and possibly functions as light-harvesting antenna as replacement for phycobilisomes lost due to iron deficiency, could thus be the counterpart of the CP43 chlorophyll $a$-binding protein complex found in iron-replete cells [444,446,467]. However, in view of the results obtained by Reddy et al. [468], the 36-kDa polypeptide of the complex CPVI-4 is in fact a cytoplasmic membrane protein encoded by the *irpA* gene, while a 34-kDa polypeptide corresponds to the chlorophyll $a$-binding apoprotein of this complex. Whether this 34-kDa polypeptide corresponds to the isiA gene product or whether there exists another chlorophyll $a$-binding polypeptide having the same electrophoretic mobility as the *irpA* gene product remains to be clarified. The *irpA* gene encodes a predicted polypeptide of 38.6 kDa containing a putative N-terminal sequence of 44 amino acid residues. The inability of an IrpA$^{-}$ mutant to grow under iron-deficient conditions led the authors to suggest that IrpA corresponds to a protein involved in iron acquisition or storage [468]. Moreover, the upstream region of the *irpA* gene contains, like isiA, a sequence similar to the *fur* operon of *E. coli* indicative of a possible regulatory control by iron [468]. The petF1 gene encoding a 10.5-kDa ferredoxin has been characterized from both *Anabaena variabilis* ATCC 29413 and *Synechococcus* PCC 7942 [174,175]. In contrast to the flavodoxin-encoding isiB gene, the expression of the *Synechococcus* petF1 gene is controlled at the translational or post-translational level in response to iron availability [174,462].

Although participating in fewer biochemical reactions than iron, copper constitutes the prosthetic group of a number of redox proteins and its concentration appears to affect a variety of enzymatic activities in cyanobacteria [469]. Plastocyanin, one of the mobile electron carriers connecting photosystem II to photosystem I, contains one copper atom per molecule. It catalyses the electron transfer between the membrane-bound cytochrome $b_{6}/f$ complex and the $P_{700}$ reaction centre. Although plastocyanin has been found in many cyanobacteria, its presence depends on copper availability and is not universal; for example, species forming abundant water-blooms generally lack this protein. When absent, either due to a lack of genetic information or to an arrest of synthesis under copper starvation, plastocyanin is usually replaced by cytochrome $c_{553}$, another electron carrier which in fact appears to be predominantly present among cyanobacteria [470–
The petE gene encoding plastocyanin has been identified in Synechocystis PCC 6803 [478] and Anabaena variabilis ATCC 29413 [477,479]. In both strains plastocyanin appears to be synthesized as a preprotein probably involved in the translocation of preplastocyanin over the thylakoid membrane. This preprotein consists of 126 amino acids in Synechocystis PCC 6803 and 139 amino acids in Anabaena variabilis ATCC 29413, with a 29- and 34-amino acid signal peptide, respectively. These predicted sequences share 56% identity. Both petE genes are transcribed as a single specific mRNA species approximately 740 bases long, but while the expression of the Synechocystis PCC 6803 gene is controlled either at the translational or post-translational level, the Anabaena variabilis ATCC 29413 gene is regulated at the level of transcription in response to copper concentration. Bovy et al. [479] have isolated the gene encoding cytochrome c553 (designated cytA in [480]) from Anabaena variabilis ATCC 29413. The predicted gene product is 111 amino acids long with a putative 25-amino acid N-terminal signal sequence. Interestingly, the same authors [481] observed a parallel copper-dependent increase in plastocyanin and decrease in cytochrome c553 mRNA levels. In both cases the mRNA stability being unaltered under varying copper concentrations and the transcription being abolished by chloramphenicol, they concluded that both genes are regulated at the level of transcription initiation and that de novo synthesis of at least one trans-acting element is required to control their mRNA levels. Upon transfer to the genome of Synechococcus PCC 7942 which lacks plastocyanin, the petE1 gene from Anabaena variabilis ATCC 29413 becomes constitutively expressed, regardless of the amount of copper available [477]; it is thus tempting to postulate the occurrence of a repressor for the control of the expression of petE1 in Anabaena variabilis ATCC 29413. In Synechococcus PCC 7942, the cytA gene is transcribed as a single 480-bp mRNA species which encodes a predicted precursor polypeptide 111 amino acids long with a 24-amino acid N-terminal signal sequence, which shares 43% identity with its counterpart in Anabaena variabilis ATCC 29413 [480]. However, in contrast to Anabaena, the expression of the Synechococcus cytA gene is not regulated by copper availability. More surprisingly, a deletion mutant constructed by replacing the first half of cytA by a streptomycin cassette has been shown to exhibit a decreased efficiency of cytochrome f oxidation, but still displays a photosynthetic activity similar to that of the wild-type strain [480]. Since this cyanobacterium is believed not to be able to synthesize plastocyanin, an alternate system that remains to be determined must exist to donate electrons to the P-700 reaction centre.

9. RESPONSES TO HEAT SHOCK OR TO OTHER STRESSES

Living cells subjected to heat shock or to other deleterious environmental changes respond by reducing their growth rate as a consequence of deep modifications in their general metabolism and pattern of gene expression. Although the molecular bases of the stress response phenomenon is poorly documented in cyanobacteria so far, there exists increasing evidence for the synthesis of stress proteins upon cell exposure to rapid changes in temperature, high salinity, desiccation, heavy metals, nutrient starvation, darkness, ultra-violet light, phage infection etc. [333,417,482-494]. A number of these proteins are general stress species or specific for a given stress and have molecular masses corresponding to those of the E. coli heat-shock proteins [482,495]. In addition, cyclic nucleotides (cAMP and cGMP), guanosine tetraphosphate and pentaphosphate (ppGpp and pppGpp), as well as bisnucleoside polyphosphates, have been found to be present and to accumulate under specific environmental stress conditions [484,496-507]. Recently, an adenylate cyclase has been partially purified from Anabaena PCC 7119 [508]. This enzyme shows an apparent molecular mass of 183 kDa and is activated by Ca2+ and calmodulin, as well as by endogenous calmodulin-like activity. Since the functions of the cyanobacterial nucleoside phosphates are largely unknown, it remains to be demonstrated that they indeed act as signal molecules for the modulation of cyanobacterial
responses to various environmental types of stress, including light, as it is generally proposed.

The two best known cyanobacterial heat-shock proteins are the molecular chaperones GroEL and GroES, members of a class of chaperonins found in prokaryotes, mitochondria and chloroplasts [509–512]. In chloroplasts, GroEL is an abundant protein implicated in the assembly of the oligomeric Rubisco enzyme [510]. Very elegant experiments performed by Goloubinoff and co-workers [513, 514] have demonstrated both in vivo and in vitro that the assembly of foreign prokaryotic Rubiscos, including the LsS 8 enzyme from *Synechococcus* PCC 6301 known to be functional in *E. coli* [163, 165], is in fact facilitated by the *E. coli* GroEL and GroES proteins. The cyanobacterial groEL and groES genes have been identified in both *Synechococcus* PCC 7942 [483] and PCC 6301 [76, 515] and found to be located adjacent to an ATP synthase locus. Although groEL has been only partially sequenced in *Synechococcus* PCC 6301, it is likely that both genes are identical in these two very closely related strains. The groES and groEL genes form an operon transcribed as a 2.4-kb mRNA species in *Synechococcus* PCC 6301, it is likely that both genes are identical in these two very closely related strains. The groES and groEL genes form an operon transcribed as a 2.4-kb mRNA species in *Synechococcus* PCC 6301 and encode two predicted polypeptides of 10.7 and 58 kDa, respectively. The cyanobacterial GroES and GroEL amino acid sequences display approximately 50% identity with their prokaryotic and eukaryotic counterparts. The groESL transcripts are barely detectable in cells grown at 30°C and accumulate in cells exposed to 45°C for 20 min, but the abundance of the transcript diminishes after 60–120 min. The mapped promoter region of the groESL operon shares some similarity to the *E. coli* heat shock consensus promoter at both the −10 and the −35 positions, but differs markedly from the promoter regions of other genes cloned from the same cyanobacterium, suggesting the existence of an alternate RNA polymerase sigma subunit involved in transcriptional initiation of the heat-inducible genes. Interestingly, although not yet explained, GroEL has been found to be strongly bound to the membranes of iron-stressed cells [483]. Among the four heat shock proteins (apparent molecular masses of 70, 64, 15, 14 kDa) induced by heat-treatment of *Synechocystis* PCC 6803, the two most abundant species are HSP70 and HSP64, which cross-react immunologically with DnaK and GroEL from *E. coli*, respectively [485]. The HSP64 protein has been purified to homogeneity and its N-terminal sequence confirmed that it corresponds to GroEL. Immunocytochemical studies of *Anabaena* PCC 7120 cells with antibodies raised against the *E. coli* GroEL protein showed that, although GroEL is present in both vegetative cells and heterocysts, this chaperonin is primarily detected in carboxysomes and in the thylakoid region of the cytoplasm in vegetative cells [516]. Various functions have been proposed for the cyanobacterial chaperonin: a participation in protein secretion, similar to the *E. coli* GroEL protein, a role in the thermoprotection of the thylakoid membrane in cells subjected to heat stress, and in the assembly of Rubisco within carboxysomes and possibly of phycobilisomes [485]. The last hypothesis is of particular interest in view of the so far unexplained result that phycobilisomes from the chromatically adapting cyanobacterium *Nostoc* sp. strain MAC (see section 3.2.2) have the same phycobiliprotein composition in cells grown either under green light at 30°C or under white light at 39°C [517] and that incubation of *Synechococcus* PCC 6301 cells at 0°C causes reversible dissociation of the phycobilisome from one photosystem II complex [50]. Obviously, however, one has to demonstrate a direct effect of GroEL on these phenomena before drawing clear-cut conclusions.

*Anabaena variabilis* M-3 responds to a shift from high to low temperature (38°C to 22°C) by changing the composition of fatty acids and lipid molecular species [518–520]. No genes corresponding to the desaturases involved in these processes have yet been identified. However, during a search for such genes, a gene of different function, designated *ltl2*, has been isolated [533]. The deduced amino acid sequence of *ltl2* (552 amino acid residues) shares significant homology to α-amylases from both prokaryotes and eukaryotes and could correspond to a cyanobacterial glucotransferase. This gene is a monocistronic unit encoding a 2-kb mRNA species whose abundance increases 40-fold within 1 h after a shift
from 38°C to 22°C and then slowly decreases to a low steady state level. However, kinetics of induction of the \(lti2\) transcripts tend to exclude the \(lti2\) gene product being a heat shock protein, but these results are reminiscent of the cold shock protein encoded by \(cspA\) of \(E. coli\) [534]. Nevertheless, no homology has been detected between the predicted \(lti2\) and \(cspA\) gene products, or with cold-acclimation plant genes, leaving open the question of the role of \(lti2\) during temperature acclimation.

The physiological and biochemical aspects of salt acclimation are rather well documented in cyanobacteria [535,536,538–543]. Two salt-acclimation strategies have been elucidated: (i) avoidance of toxic internal amounts of inorganic ions by means of active export systems accompanied by enhanced respiratory enzyme activities and ultrastructural changes [540–542,544–551]; (ii) synthesis and accumulation of compatible solutes, low-molecular mass hydrophilic compounds which substitute ions and are less toxic than ions even at high concentrations [535,536,538,543,552]. Three main groups of cyanobacteria are distinguished according to the osmoprotective compounds accumulated: strains with the lowest salt tolerance synthesize sucrose and trehalose, intermediate strains use glucosylglycerol and strains with the highest tolerance accumulate glycine betaine and glutamate betaine. Recent reports indicate that abscisic acid is produced and can be released from cyanobacteria in response to different types of stress, including salt stress [553–557]. However, nothing is known about the possible role of the cyanobacterial phytohormone release, nor about the consequences it may have on other organisms in natural environments. In eubacteria osmotic stress has been widely studied at a genetic level [558], while in cyanobacteria there is a dearth of information. However, the pioneering work of Apte and Haselkorn [490] should provide genetic information in the near future. These authors have reported the cloning of salinity stress-induced genes from \(Anabaena torulosa\) and estimated, based on the analysis of the transcription of eleven independent recombinant cosmids, that a substantial portion of the genome, probably more than 100 kb, is involved in salt responsiveness.

As early as 1722, de Réaumur [559] wonderfully described his observations on the ability of the terrestrial ‘\(Nostoch\)’ sp. to survive seasonal cycles of desiccation and rehydration in nature. Surprisingly, more than two centuries later, very little progress has been made on the understanding of the desiccation/rehydration process in cyanobacteria. Some reports concern physiological, biochemical and structural aspects of desiccation tolerance in field samples of \(Nostoc commune\) and in the laboratory strain \(Nostoc commune\) UTEX 584 [491,560–572]. Under natural conditions, growth of \(Nostoc commute\) results in the formation of macroscopic colonies (0.5–3 mm thick), aggregates of cells embedded in a water-absorbing sheath composed of carbohydrates [491]. When subjected to repeated cycles of drying and rehydration, field samples of \(Nostoc commute\) specifically accumulate acidic proteins of apparent molecular masses between 30 and 39 kDa [491]. Three of these proteins (33, 37 and 39 kDa) have been purified. Peptide mapping, amino acid composition and immunological studies using polyclonal antibodies raised against the 39-kDa species revealed that the 33-kDa polypeptide results from in vivo proteolysis of the largest polypeptide. An apparent correlation between the preservation of intact vegetative cells and heterocysts, and the presence of a high amount of water stress proteins suggests a protective function at a structural level [491,562]. The known N-terminal sequence of the 39-kDa polypeptide provides a useful tool for beginning molecular studies of the water stress proteins [491].

Despite the crucial role of phosphate in cell metabolism, structure and regulation, in recent years little attention has been paid to the effects of deprivation in this nutrient in cyanobacteria. In natural environments, phosphate is often present in forms which are not readily taken up and is thus frequently limiting. As in other prokaryotes, phosphate limitation significantly affects cyanobacterial cell growth and leads to an increased expression of alkaline phosphatase activity, as well as to an elevated transport of phos-
phate into the cell [573–575]. In Synechococcus PCC 7942, alkaline phosphatase is located in the periplasmic space but is likely to be associated with the cell wall or cytoplasmic membranes in contrast to the E. coli enzyme [576]. Unlike other known alkaline phosphatases, the cyanobacterial enzyme has a subunit molecular mass of 145 kDa and has been shown to be irreversibly inhibited by zinc [573,576]. The corresponding phoA gene is the only cyanobacterial gene involved in phosphate metabolism which has been characterized so far [577]. The predicted phoA gene product (1344 amino acids long) does not display significant homology with previously identified alkaline phosphatases, but exhibits some sequence similarities with the α subunit of the ATPase from bacteria and chloroplasts, and the UshA sugar hydrolase of E. coli. Although the cyanobacterial enzyme is a periplasmic protein, there is no N-terminal amino acid signal sequence. However, the N-terminal region forms a hydrophobic domain which may function in transport of the protein across the inner cytoplasmic membrane. High levels of transcripts have been shown to accumulate after 8–12 h of phosphate deprivation and to be maintained for at least 24 h under these conditions. Similar kinetics for transcript disappearance upon readmission of phosphate to deprived cultures have been obtained, indicative of an almost immediate arrest of phoA transcription. The large size of the transcript species (approximately 11 kb) suggests that other phosphate regulated genes are present upstream from phoA. No typical E. coli-like promoter sequence and a pho box only weakly homologous to that involved in the transcription of the E. coli pho regulon [246] have been found. Insertional inactivation of the cyanobacterial phoA gene results in the loss of extracellular, phosphate-regulated alkaline phosphatase activity but does not alter the capacity of the cell for phosphate uptake. Cyanobacterial cells under phosphate limitation are also characterized by an abundant and rapid accumulation of cyanophycin granules, accompanied by a degradation of glycogen reserves, phycocyanin and chlorophyll a [15,577–580]. A decrease in the assimilation rate of nitrate and a reduced rate of ammonium transport have been reported, attributable at least in part to a decline in the intracellular ATP pool contributed by photosystems II and I [579,580]. Under phosphate limitation, the expression of the cpcBACDEF and psaAB operons diminishes, but the arrest of transcription is delayed when compared with nitrogen-starvation conditions [19]. Studies on phosphate regulation are still at an early stage in cyanobacteria, but with the increasing evidence of the important role of phosphorylation in cyanobacterial regulatory circuits emerging from both biochemical and molecular genetic studies, one can expect that rapid progress will soon be made in our knowledge of the consequences of phosphate limitation in these organisms.

10. FURTHER PROSPECTS

Cyanobacteria are unique in that they possess physiological properties otherwise found only either in eukaryotic (oxygenic photosynthesis) or prokaryotic (nitrogen fixation, for example) organisms. In addition, they perform their various functions without differentiation of true tissues, heterocysts being a particular cell type that not all cyanobacteria can form. As shown throughout this review, many different environmental stimuli are sensed by cyanobacteria, and these stimuli often elicit pleiotropic responses. The challenge is to understand, in molecular terms, every individual regulatory mechanism and to identify their interactions. At a molecular level, the most studied parameters affecting cyanobacterial growth and metabolism are light and sources of carbon, nitrogen and sulphur.

At present, very little solid information is available concerning signal perception, the first step of every regulatory mechanism. Light energy can be harvested and absorbed by the several hundred pigment molecules present in each photosynthetic unit, and a redox control at the level of photosynthetic electron transport (plastoquinone and/or cytochromes) has been hypothesized although the precise mechanism is still controversial. Isomerizations of chromophores are good candidates for the sensing of light signals because light could directly provide
the energy which is necessary for the transition. Recently, more studies have been directed towards the specific attachment of chromophores to the apoproteins, and also to a lesser extent towards the elucidation of the pathways involved in the biosynthesis of the numerous tetrapyrrrole molecules, either hemes, chlorophylls or linear bilins (see section 3.1). Isomerization of any of these molecules could modify enzymatic activities and modulate chromophore availability which, in turn, might lead to a pleiotropic cellular response. Some of the properties of the photoreversible pigment involved in complementary chromatic adaptation have been described, but the molecule itself remains to be isolated. Since only some of the cyanobacteria can undergo complementary chromatic adaptation (see section 3.2.2), the corresponding photoreceptor cannot be considered as the main and ubiquitous light sensor. The receptors for nitrogen (see section 6), sulphur and carbon sensing (see sections 5 and 7), have not yet been identified.

Following perception comes the transduction of the signal, which very often involves multiple steps, the number of which is not easy to define by a strictly biochemical approach since it implies precise determination of the number of protein–protein and/or protein–ligand interactions. The GlnB or PII protein represents the first example of a signal transducer found in cyanobacteria [295]. Its pleiotropic role remains to be understood but PII could be a central component in the cross-talk between various regulatory pathways by precisely monitoring the intracellular C/N ratio. This protein exists in two forms, one of them being modified to an XMP derivative. The nucleotide transferase that post-translationally modifies PII, as well as the kinase(s) required for the phosphorylation of the proteins (RcaA and RcaD) involved in complementary chromatic adaptation, still need to be characterized. Because they are very rapid, phosphorylation reactions may well be involved in many short-term adaptations, activation or inactivation of enzymes or sets of enzymes. Such reactions will also likely be found to act as intermediates allowing the cell to control the expression of the genes whose products are necessary in the newly altered environment. Two-component systems will surely play an important role in signal transduction, as already found in a number of different bacteria [246]. Histidine kinases belonging to this family have indeed been found (M.R. Schaefer, personal communication; our laboratory, unpublished data) and their number will probably increase very rapidly. Two regulatory proteins, NtcA [299] and CysR [300], sharing homology with the E. coli Crp and Fnr, and the Rhizobium meliloti FixK, have been identified in Synechococcus PCC 7942. Other members of this family will certainly be found. These regulatory proteins have been identified either by sequencing regions adjacent to structural genes or by cloning pieces of DNA complementing characterized mutations. The latter approach will be used more and more now that DNA transfer into cyanobacteria through transformation for some unicellular strains, or conjugation for filamentous strains, has become possible and since an increasing number of genetic tools are becoming available: shuttle vectors, promoter probe vectors, reporter genes, insertion elements and transposons [22,111]. A systematic use, via reverse genetics, of the many mutants characterized in various laboratories and of specifically engineered ones will undoubtedly increase our knowledge on the regulatory mechanisms that exist in cyanobacteria.

The last step, the control of gene expression, is the most documented at a molecular level. At the moment, many useful techniques are available, and the major enzyme involved in this process, RNA polymerase, can be purified. This enzyme has been characterized from Anabaena PCC 7120 [305] and Calothrix PCC 7601 (G. Schyns, our laboratory, unpublished results). It resembles its chloroplast counterpart in that, in addition to the classical prokaryotic subunits \( \alpha_2\beta\beta' \), it has a \( \gamma \) subunit [305]. The same composition has been found in the other cyanobacterial enzymes examined to date [581]. Some of the genes encoding the RNA polymerase subunits have been isolated, namely those for the \( \gamma \), \( \beta \) and \( \beta' \) subunits (rpoBC1C2) of Nostoc commune UTEX 584, and for the \( \gamma \) and \( \sigma \) subunits (rpoC1 and sig4) of Anabaena PCC 7120 [582–584]. Biochemical studies aimed at defining the role of the different
subunits in promoter recognition, as well as in the transcription process, are urged. Preliminary results obtained via in vitro transcription run-off experiments are encouraging [306] and the RcaA, RcaB and RcaD proteins (regulators for complementary chromatic adaptation) have been identified via gel retardation assays and footprinting experiments (see section 3.2.2). Through such in vitro approaches, the isolation of either one of the partners of a nucleic acid–protein interaction is often feasible. These studies are not only important from a fundamental point of view but are required to obtain an operational definition of cyanobacterial promoters, a prerequisite for better industrial uses of these pluripotential microorganisms.

Results already obtained through molecular genetics on hormogonium differentiation (see section 4), heterocyst differentiation (see section 6) and resistance to metal ions and other types of stress (see sections 8 and 9) pave the way to further developments and to a more comprehensive understanding of the many physiological and biochemical data that have been accumulated. Symbiotic associations involving cyanobacteria were empirically used for centuries to improve crop production before they attracted the attention of the scientific community. The possibility for cyanobacteria to develop in associative symbiosis with eukaryotic plants is almost entirely restricted to heterocystous-forming species, most of them being infective as hormogonia [585]. Symbiotic N$_2$-fixing associations have been reported with various eukaryotic partners. Contrary to the case of the specific *Rhizobium*–legume symbioses, where a given *Rhizobium* species can only associate with members of one genus of angiosperms, cyanobacteria belonging to the same genus, primarily *Nostoc* sp., can form symbiotic associations with algae, fungi, bryophytes, ferns, pteridophytes, gymnosperms, and angiosperms. The development of a symbiosis is also a response to the perception of a signal that is emitted by the plant partner, and it will be of interest to compare the molecular mechanisms involved in this process with those mentioned above. Such approaches are now possible since reconstructions of the symbiosis, from isolated partners, have been successful for *Blasia*, *Phaeoceros*, *Gunnera* and *Anthoceros* with *Nostoc* sp., for example [586–589].

Cyanobacteria are recognized as secreting a variety of compounds that have potential biotechnological applications, as well as different toxins harmful to animals and humans [128,590]. The control of cyanobacterial populations, either for biomass production or growth limitation is no doubt of great importance. Useful information will surely emerge from the current burst of studies directed towards the understanding of regulation of gene expression, but also from the forthcoming molecular studies on the symbiotic associations that cyanobacteria can develop, and their secretory mechanism(s). Finally, we shall have to take into account circadian rhythms, since this phenomenon has been shown to occur in cyanobacteria [344,345,591], but has been mostly neglected so far.

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