Tracking the Light Environment by Cyanobacteria and the Dynamic Nature of Light Harvesting*

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In nature photosynthetic organisms cope with fluctuating light conditions. Light intensity and quality vary dramatically during the day or from one habitat to another. Photosynthetic organisms sense intensities and wavelengths of light both directly and indirectly. Because light fuels photosynthetic electron transport and CO₂ fixation, it is the primary determinant of levels of NADP/ NADPH, ATP, and carbon metabolites, all of which can serve to modulate cellular processes. Light is also absorbed by photoreceptors that link light cues to cellular metabolism. However, light represents a single environmental cue, and other signals interact with light through a web of regulatory circuits that result in dynamic acclimatory responses. This review focuses on two specific aspects of light-influenced processes in Cyanobacteria; both concern changes in light harvesting structure and biosynthesis. The first part of this review discusses effects of changing wavelengths of light on the biosynthesis of the phycobilisomes (PBS),¹ dominant light harvesting complexes of Cyanobacteria. The other discusses how Cyanobacteria tune light harvesting and photosynthetic function to both light intensity and nutrient availability and how the two responses are integrated.

Light Harvesting and Fluctuating Light Signals

Phycobilisome Structure

PBS are peripheral membrane complexes in Cyanobacteria that efficiently harvest light energy and transfer the energy to photosynthetic reaction centers. PBS, which can comprise 30% of the cellular protein, are organized into two structural domains, the core and rods (Fig. 1). Each of these domains contains pigmented and nonpigmented polypeptides.

All PBS have the chromoproteins (phycobiliprotein) allophycocyanin (AP) and phycocyanin (PC), and many also contain phycerythrin (PE) or phycoerythrocyanin. Phycobiliprotein colors are a consequence of light absorption by linear tetrapyrrole chromophores that covalently associate with the apoproteins (1, 2). Phycobiliproteins are comprised of α and β subunits associated into heterodimers (termed “monomers” in the literature) that aggregate into trimers (αβ)₃ and hexamers (αβ)₆. Nonchromophylated linker (L) polypeptides stabilize PBS, facilitate assembly of phycobiliprotein aggregates, and modulate the absorption characteristics of phycobiliproteins, promoting unidirectional energy flow to photosynthetic reaction centers (1).

PBS cores contain AP trimers along with pigmented and L polypeptides. A high molecular mass core polypeptide, or L_CM, has homology to both phycobiliproteins and L polypeptides (3). The phycobiliprotein-like domain of L_CM binds a tetrapyrrole chromophore and can serve as a PBS terminal energy acceptor. Generally, six rods, each composed of stacks of PC and PE hexamers, radiate from the core, giving PBS a fanlike appearance (Fig. 1) (see Ref. 2 for details).

Complementary Chromatic Adaptation

It was noted over a century ago that Cyanobacteria pigmentation changes with environmental light quality. This light control of pigmentation, shown in the lower half of Fig. 1, was termed complementary chromatic adaptation (CCA). Bennett and Bogorad (4) showed that CCA was the result of altered PBS pigment-protein composition. Development of molecular tools in the 1970s created new opportunities for elucidating PBS regulation, and by the end of the 1980s most genes encoding PBS structural polypeptides were characterized (5).

Genes of the PBS—In the Cyanobacterium Frenemylella diplophon (similar to Calothrix PCC7601) the PE:PC ratio reflects the spectral distribution of light in the environment (6). In red light (RL) the organism has almost no PE, and each PBS rod can have three PC hexamers (and specific L polypeptides). If the Cyanobacterium is moved to green light (GL), new PBS are synthesized with rods having single PC hexamers (core proximal hexamer) and up to three PE hexamers. As the cells replicate in GL, blue-pigmented PBS of RL-grown cells are gradually diluted, and the cells begin to appear red. These light-responsive changes are reversible, and because PC absorbs RL (λmax = 620 nm) and PE absorbs GL (λmax = 560 nm), these changes facilitate efficient absorption of prevalent wavelengths of light in the environment.

Knowledge of genes encoding phycobiliprotein and linker polypeptide has been critical for understanding CCA (see Refs. 5 and 7). Genes encoding α and β subunits of each phycobiliprotein are contiguous on the cyanobacterial genome and are cotranscribed. Often, polycistronic transcripts encode phycobiliprotein subunits and their associated L polypeptides. In F. diplophon, α and β AP subunits (αAP and βAP, respectively), encoded by the apcA1B1 genes, are in an operon that also contains the apcC1 and apcE1 genes; the latter genes encode the core linker polypeptide and the L_CM, respectively (8).

Three distinct operons encode α_PC and β_PC subunits (cpeBA genes in F. diplophon (9–12)). The cpeA1 genes are constitutively transcribed and encode PC subunits (subscript indicates constitutive). This operon also contains cpeE and cpeF, which encode a lyase that attaches the tetrapyrrole chromophores to the α subunit of PC (13). The cpeB2A2 operon is specifically active in RL (inactive in GL) and encodes PC (subscript indicates inducible), which is critical for CCA. Hexamers of PC, comprise the majority of PBS rods when cyanobacterial cells are grown in RL. The genes cpeH2, cpeI2, and cpeD2 (14), encoding L polypeptides associated with PC, are cotranscribed with cpeB2A2. Furthermore, the cpeB2A2H2I2D2 operon is clustered on the F. diplophon genome with cpeB1A1 and cpeE1A1B1C1 (10). A third PC operon, cpeB3A3, plus genes encoding associated L polypeptides, is only active during sulfur-limited growth (12).

The cpeBA operon encodes α and β subunits of PE (15). In contrast to the situation for cpe and apc operons, genes encoding L polypeptides associated with PE are not contiguous on the genome to cpeBA; they are encoded by the cpeCDE operon (16). However, GL activation and RL suppression of cpeBA and cpeCDE are coordinated. Also, cpeBA genes are linked to cpeY and cpeZ, which
encode the lyase that attaches tetrapyrrole chromophores to PE subunits (17).

Expression during CCA—Action spectra for the synthesis of PC and PE in the Cyanobacteria have been measured (18). Maximum PE synthesis and minimum PC synthesis occurred following exposure to 550 nm GL, and maximal PC synthesis and minimal PE synthesis occurred following exposure to 640 nm RL. Hence, photoreceptor(s) controlling CCA absorb RL and GL but elicit different responses in the two light qualities. PC synthesis dominates in RL whereas PE synthesis dominates in GL. Exposure of cells to natural sunlight, a mixture of RL and GL, results in the synthesis of PBS with intermediate PC and PE levels. Photocontrol of PE and PC levels is primarily a consequence of transcriptional regulation of cpeBA and cpcB2A2 operons (19–22).

Mutants in CCA—The dissection of regulatory circuits involving CCA has exploited mutants abnormal for CCA. Several classes of CCA mutants have been isolated (5, 22–24), including the red (FdR), blue (FdB), green (FdG), and black (FdBk) strains. FdR mutants are red under all conditions of illumination and constitutively synthesize PE whereas PC, is never synthesized. These mutants are fixed in a response normally exhibited only in GL, with aberrant regulation of both the cpeBA and cpcB2A2 operons (22). FdB strains are bluer than wild-type cells in RL and require more GL to suppress PC synthesis (25). FdG mutants show normal PC expression, but the cpeBA genes never become active. FdBk mutants have moderate levels of both PE and PC, which remain the same in RL and GL (7, 24).

Initially, an FdR mutant was complemented by rcaC, which encodes a polypeptide of 651 amino acids with sequence similarities to response regulators of two-component regulatory systems (26). It is twice as large (73 kDa) as most response regulators and has two domains among these polypeptides. RcaE, the putative photoreceptor, perceives the light signal. RL causes RcaE to undergo an elevated PE synthesis and depressed PC synthesis. Contiguous to GL wild-type cells likely dephosphorylate Asp-51, which triggers activation of RcaE (7, 24). Two FdR mutants were not complemented by rcaC (29). One was complemented by the putative photoreceptor gene rcaE, and the second by rcaF, which is immediately downstream of rcaE on the F. diplosiphon genome and encodes a small response regulator. RcaF may act as an intermediate in the phosphorelay pathway controlling CCA and facilitate phosphate transfer from its cognate sensor (presumably RcaE) to other response regulators such as RcaC.

Because rcaE is sufficient for complementing both FdBk and FdR mutants, different lesions in rcaE can generate different phenotypes. Furthermore, an FdR phenotype can result from lesions in at least three distinct genes (rcaC, rcaE, and rcaF). The lesions that caused the mutant phenotype in these strains were the result of gene disruption by in vivo transposition (22). Each of the FdBk (rcaE-FdBk) and FdR (rcaE-FdR, rcaF-FdR) mutants characterized contained insertion sequences in the rcaE or rcaF operon (29). In rcaE-FdBk mutants the inserts were located within 200 base pairs of the putative translation start site, and no RcaE protein was detected in mutant cells. The rcaE-FdR mutants contained insertions positioned between the H block and the four conserved motifs critical for histidine kinase activity; this strain appears to synthesize truncated RcaE. The rcaF-FdR mutants contained insertions located ~200 base pairs downstream of the rcaF translation initiation codon.

Constitutive PE and PC synthesis in rcaE-FdBk mutants reflect an intermediate activation state of the system as a consequence of low level phosphorylation of regulatory elements that are no longer under RcaE control. Hence, RcaF likely undergoes low level phosphorylation in the absence of RcaE; this phosphorylation is not controlled by light quality and may result from cross-talk between RcaF and other sensors or phosphoryl transfer from small molecule phosphodonor. It is not unusual that a null mutation in a sensor kinase leads to an intermediate, constitutive activation of the phosphorelay system. In the rcaE-FdR mutants, although the truncated RcaE cannot undergo autophosphorylation, it may bind RcaF and block its phosphorylation by other molecules and/or retain phosphatase activity, which would maintain RcaF in a dephosphorylated state.

Model for CCA—Three regulatory elements critical for CCA are RcaE, RcaF, and RcaC. Although these polypeptides have features of bacterial two-component regulators, the CCA phosphorelay (Fig. 2) is unique because it includes five potential phosphoacceptor domains among these polypeptides. RcaE, the putative photoreceptor, perceives the light signal. RL causes RcaE to undergo an
autophosphorylation followed by transfer of the phosphoryl groups to the response regulator RcaF. In the absence of RcaE, RcaF may interact with other phosphoril donors. RcaF then may transfer phosphoryl groups to the conserved histidine of the H block within RcaC, which can pass it to either the N- or C-terminal receiver domain. The N-terminal receiver domain of RcaC is critical for CCA, whereas the role of the C-terminal receiver domain is unclear. RcaE acts as a phosphatase or blocks phosphotransfer by binding to RcaF; this inhibition causes activation of cpeBA and suppression of cpcB2A2.

Not surprisingly, other regulatory components also appear to be involved in controlling CCA. A class of mutants that only affects cpeBA expression has been identified and is designated turquoise (FdTq). These mutants exhibit normal regulation of cpeB2A2 but cannot activate cpeBA in GL. Complementation of the FdTq strains recovered two genes, trgA and trgB, encoding polypeptides related to protein phosphatases; this finding is interesting, especially because phosphorylation of the putative regulatory protein RcaA has been implicated in the control of cpeBA expression (21).

Integration of Light and Nutrient Signals in PBS

**Biosynthesis**

Specific Nutrient Limitation Responses—Responses of organisms to nutrient availability may be classified as those specific to the limiting nutrient and those that are more general, occurring during any of a number of different nutrient limitation conditions. Specific responses include metabolic changes enabling organisms to efficiently scavenge the limiting nutrient; these responses may include synthesis of high affinity transport systems (30) and production of hydrolytic enzymes that facilitate utilization of alternate forms of the limiting nutrient (31).

**General Responses**—General responses to nutrient-limited growth include changes in cell morphology and metabolism. *Synechococcus* cells starved for iron, nitrogen, or sulfur accumulate low levels of thylakoid membrane, PBS, and ribosomes (32, 33). Nutrient deprivation also causes a rapid loss of O$_2$ evolving activity, reflecting a decline in PSII function (34). A visually dramatic, general response of Cyanobacteria to nutrient-limited growth is the decrease in cellular pigmentation or bleaching (35), which includes an almost complete loss of PBS (36). Degradation of the PBS could provide amino acids or carbon skeletons for production of other cellular constituents required during nutrient deprivation and reduce absorption of excitation energy, making cells less susceptible to photodamage.

Mutants of *Synechococcus* were isolated that could not degrade their PBS during nutrient deprivation. Some of these mutants, designated nbl (nonbleaching), only survived in relatively low light. Complementation of one nbl mutant led to the isolation of nblA (nonbleaching), which encodes a 59-amino acid polypeptide. The nblA transcript only accumulates to high levels in cells starved for nitrogen or sulfur; low levels of the nblA mRNA are observed in cells maintained in phosphorus-free or complete medium. Under all of a number of different nutrient limitation conditions, specific responses include metabolic changes enabling organisms to efficiently scavenge the limiting nutrient; these responses may include synthesis of high affinity transport systems (30) and production of hydrolytic enzymes that facilitate utilization of alternate forms of the limiting nutrient (31).

**Integration of Light and Nutrient Signals in PBS**

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A second nbl mutant was complemented by nblB. NblB has homology to subunits (e.g., CpcE, CpcZ) of lyases that catalyze covalent attachment of phycocromobilin chromophores to apophycobiliprotein subunits (38). This finding suggests that NblB interacts directly with tetrapyrrolyl chromophores attached to phycobiliproteins. Because nblB mutants do not degrade PBS during nutrient limitation, it is reasonable to propose that NblB catalyzes removal of chromophores from holophycobiliprotein subunits and that only after the chromophore is removed can phycobiliprotein subunits be degraded.

A third mutant, nblR, was complemented by a gene encoding a response regulator (39). Cyanobacteria strains null for NblR (a) have ~150% the level of PBS as wild-type cells during nutrient-}

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3 D. M. Kehoe, personal communication.


Minireview: Dynamics of Light Harvesting Biogenesis

and NblA in conjunction with NblB (constitutively expressed) actively degrades PBS. NblR must also activate other processes in the cell (e.g. modification of the photosynthetic apparatus) that favor survival during nutrient stress and HL conditions. During HL exposure, NbsS modulates the activity of many genes encoding components of the photosynthetic apparatus. Regulation of a number of these genes does not require NblR. Hence, in addition to interacting with NblS and NblA in conjunction with NblB (constitutively expressed) NblR must associate with other response regulators that have not yet been identified.

A major question remaining is how may NbsS be sensing the redox state of the cell. During nutrient limitation, when the anabolism of the cell is slowed down or completely arrested, NADP⁺, the final electron acceptor of the photosynthetic electron transport chain, is not recycled as fast as under nutrient-replete conditions and the electron carriers are maintained in a relatively reduced state. Similarly, under HL conditions the electron carriers may be reduced faster than they are re-oxidized. Thus, nutrient limitation and HL result in absorption of excess light energy by photosynthetic pigments, and the overall cellular environment would become highly reduced. The redox state of photosynthetic electron carriers is known to modulate cellular transcription (41), translation (42), state transitions, and changes in the stoichiometry of the photosystems (43). The results discussed above suggest that NbsS/NblR control is linked to the redox state of the cells and possibly couples to the degree of reduction of specific photosynthetic electron carriers. Furthermore, both DCMU, which prevents photosynthetic electron flow beyond QA, and DBMIB, which inhibits electron flow through the cytochrome b6f complex, inhibit PBS degradation and the accumulation of nblA mRNA during sulfur and nitrogen stress. Like PBS degradation and nblA expression, expression of hliA is altered by both DCMU and DBMIB. Interestingly, upon treatment of Synechocystis PCC6803 with cyanide (which inhibits both respiration and photosynthetic electron flow at plastocyanin) hliA is activated in low light. Similarly, in a PSI mutant of Synechocystis PCC6803, there is elevated expression of hli genes even in moderate light.6 These results suggest that hyper-reduction of an electron transport carrier prior to PSI may strongly affect acclimation during HL and nutrient limitation conditions. Factors in addition to the redox state of the photosynthetic apparatus may be important for tuning of NbsS. For example, NbsS control seems to promote PBS degradation during nutrient limitation, but in HL it biases control toward activation of hliA/psbAII/psbAIII/pcpBA. Interactions with a blue light photoreceptor or the direct absorption of blue/UV-A light by NbsS may also bias NbsS action toward the hliA/psbAII/psbAIII regulatory pathway over the nblA pathway. Reactive oxygen species may also serve as regulatory signals that modulate the activity of the nbl system. Several aspects of the model are speculative and/or incomplete. However, it represents an attractive unifying view that predicts global metabolic effects in response to redox status of photosynthetic electron carriers and links nutrient conditions, growth potential, and light to overall regulation of cellular metabolism.

REFERENCES

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6 W. Vermaas, personal communication.