Induction of a Group 2 $\sigma$ Factor, RPOD3, by High Light and the Underlying Mechanism in Synechococcus elongatus PCC 7942*

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Among the $\sigma^{70}$ family bacterial $\sigma$ factors, group 2 $\sigma$ factors have similar promoter recognition specificity to group 1 (principal) $\sigma$ factors and express and function under specific environmental and physiological conditions. In general, the cyanobacterial genome encodes more than four group 2 $\sigma$ factors, and the unicellular Synechococcus elongatus PCC 7942 (Synechococcus) has five group 2 $\sigma$ factors (RpoD2–6). In this study, we analyzed expression of group 2 $\sigma$ factors of Synechococcus at both mRNA and protein levels, and we showed that the rpoD3 expression was activated only by high light (1,500 $\mu$mol photons m$^{-2}$ s$^{-1}$) among the various stress conditions examined. After high light shift, rpoD3 mRNA accumulated transiently within the first 5 min and diminished subsequently, whereas RpoD3 protein increased gradually during the first several hours. We also found that the rpoD3 deletion mutant rapidly lost viability under the same conditions. Analysis of the rpoD3 promoter structure revealed the presence of an HLR1 (high light-responsive element 1) sequence, which was suggested to be responsible for the high light-induced transcription under the control of the NblS (histidine kinase)-RpaB (response regulator) two-component system (Kappel, A. D., and van Waasbergen, L. G. (2007) Arch. Microbiol. 187, 337–342), at +6 to +23 with respect to the transcriptional start site. Here we demonstrated that recombinant RpaB protein specifically bound to HLR1 of the rpoD3 and hliA genes in vitro, and overexpression of a truncated RpaB variant harboring only the phosphoreceiver domain derepressed the transcription in vivo. Thus, we have concluded that phosphorylated RpaB are repressing the rpoD3 and hliA transcription under normal growth conditions, and the RpaB dephosphorylation induced by high light stress results in transcriptional derepression.

The $\sigma$ factor is a key component of the bacteria-type RNA polymerase that controls promoter recognition specificity and transcriptional activity in response to various environmental and physiological changes (2). $\sigma$ factors of the $\sigma^{70}$ family are classified into group 1, 2, and 3 $\sigma$ factors based on their structural and functional features (2, 3). Group 1 $\sigma$ factors, which recognize $\sigma^{70}$-type consensus promoters, are responsible for housekeeping functions and are essential for viability. Group 2 $\sigma$ factors are structurally closely related to group 1 $\sigma$ factors, and both groups recognize similar promoter structures in vitro (4, 5). Meanwhile, group 2 $\sigma$ factors are dispensable at least under optimal growth conditions (5–10). Group 3 $\sigma$ factors share limited sequence homology with group 1 and 2 $\sigma$ factors, and recognize distinct promoter structures that are specific for each subtype (2). There is another type of $\sigma$ factor, the $\sigma^{24}$ family, and they require specific activating proteins for initiation of transcription (11).

Cyanobacteria include a large and diverse group of bacteria, which are specified by oxygenic photosynthesis. Despite the morphological divergence, characteristics of the main metabolic pathways are relatively ubiquitous, and the composition of the main regulatory factors such as the RNA polymerase $\sigma$ factors is well conserved. Compared with other groups of bacteria, the abundance of group 2 $\sigma$ factors is a particular feature of cyanobacteria. Multiple genes encoding group 2 $\sigma$ factors have been found in cyanobacteria that have had their genomes fully sequenced. For example, in Synechococcus elongatus PCC 7942 (hereafter Synechococcus), there are five group 2 $\sigma$ factors encoded in the genome (RpoD2–6, 9, 12–14; RpoD5 is identical to SigC described in Ref. 9 but denoted in this paper as RpoD5). Two of them have corresponding orthologs in each cyanobacterial strain (Table S1). Thus, each subtype of group 2 $\sigma$ factors appears to have been conserved during the long history of cyanobacterial evolution, which suggests specific physiological roles for each subtype.

Physiological functions of cyanobacterial group 2 $\sigma$ factors have been described in recent studies. In Synechocystis for example, SigB and SigD are related to stress responses such as

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The abbreviations used are: Synechococcus, S. elongatus PCC 7942; Synechocystis, Synechocystis sp. PCC 6803; Nostoc, Nostoc sp. PCC 7120; IPTG, isopropyl 1-thio-$\beta$-D-galactopyranoside; PSII, photosystem II.
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temperature shift, salt and osmotic shock, and change in light intensity (8, 10, 19). SigC and SigE function in growth phase-dependent gene expression and regulation of sugar catabolism, respectively (20, 21). Regulatory mechanisms for these σ factors were also investigated and clarified, at least in part (21–23). Involvement of group 2 σ factors in stationary phase- or starvation-induced gene expression was also indicated in Synechococcus sp. PCC 7002 (7) as well as in Nostoc (18).

Although each group 2 σ factor seems to play crucial roles in specific situations, the only specific function that has been assigned in Synechococcus is an effect on circadian clock-regulated gene expression (9, 24). To understand the function of each group 2 σ factor in Synechococcus, we here analyzed the expression of the group 2 σ factors under various stress conditions, and we found that the expression of rpoD3 is activated specifically in response to high light stress. Consistent with this, the rpoD3-defective mutant rapidly showed reduced viability in high light conditions. We also showed that a response regulator RpaB of the OmpR family (25), which is assumed to constitute a two-component signal transduction system with a histidine kinase NblS, negatively regulates rpoD3 transcription.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Media, and Culture—Synechococcus was originally provided by Dr. R. Rippka (Pasteur Institute). Constructions of rpoD deletion mutants were described previously (4, 24). The wild-type strain and derivatives used in this study were grown in BG-11 (26) liquid medium or on BG-11 solid media containing 1.5% (w/v) agar at 30 °C with 2% CO2 aeration under continuous fluorescent light (35 μmol photons m−2 s−1) unless otherwise noted. Cultures were supplemented with 20 μg/ml kanamycin sulfate, 20 μg/ml spectinomycin, or 25 μg/ml chloramphenicol when required. To apply stress conditions, liquid cell cultures were supplemented with 0.5M NaCl or 0.5M chloramphenicol before harvesting.

Construction of the RB-F Strain—For in vivo expression of RpaB with 3× FLAG tags at the C terminus, a DNA fragment was amplified from Synechococcus genomic DNA by PCR using the primers, 5′-ggccgatcatctggacagcttttaaaaattatg-3′ (BamHI site underlined) and 5′-ttgatctctcactgctagtcgctcaatg-3′ (EcoRI site underlined). The amplified fragment was digested with BamHI and EcoRI and inserted into the pBluescript SK+ vector (Stratagene). After insertion of the Ω cassette derived from the pHP451 vector (27) at the HindIII site, the constructed plasmid was used to transform Synechococcus, and the resulting strain was named RB-F.

Construction of the RB-NOX Strain—A sequence encoding the N-terminal receiver domain of RpaB (amino acids 1–123) was amplified from Synechococcus genomic DNA by PCR with the primers 5′-cttggagatcagcaatgaaatggtttcctagctgggtttggtggc-3′ (Ncol site underlined) and 5′-gccatgattccctgccgcttcg-3′ (BamHI site underlined) and digested with Ncol and BamHI. The Ncol-BamHI segment was ligated with Ncol- and BamHI-digested p322Ptrc (28) to obtain p322Ptrc::SR2. The smaller BglII fragment from p322Ptrc::SR2 was inserted into the BamHI site of the pTS2KC (28) to obtain pOX-SR2. The constructed plasmid was used to transform Synechococcus, and the resulting strain was named RB-NOX.

Northern Analysis—The hot phenol method was used to prepare total RNA from Synechococcus cells, and 10 μg of total RNA was subjected to Northern analysis as described (29). DNA probes for rpoD1, rpoD2, rpoD3, rpoD4, rpoD5, rpoD6, and hliA were digoxigenin-labeled after being PCR-amplified using the following primers: rpoD1, 5′-ggccgatcatctggacagcttttaaaaattatg-3′ and 5′-ggccgatcatctggacagcttttaaaaattatg-3′; rpoD2, 5′-cttggagatcagcaatgaaatggtttcctagctgggtttggtggc-3′ and 5′-gtatgtctgggtttggtggc-3′; rpoD3, 5′-ggccgatcatctggacagcttttaaaaattatg-3′ and 5′-gtatgtctgggtttggtggc-3′; rpoD4, 5′-gaggggatcagcaatgaaatggtttcctagctgggtttggtggc-3′ and 5′-gtatgtctgggtttggtggc-3′; rpoD5, 5′-ttccatgctagctgggtttggtggc-3′ and 5′-ttccatgctagctgggtttggtggc-3′; rpoD6, 5′-ttccatgctagctgggtttggtggc-3′ and 5′-ttccatgctagctgggtttggtggc-3′; and hliA, 5′-aattccctggtgcggc-3′ and 5′-aattccctggtgcggc-3′.

Immunoblot Analysis—Total protein prepared from Synechococcus cells (30 μg) was subjected to immunoblot analysis as described elsewhere (5). The antisera against RpoD1 was prepared by immunizing a rabbit with the purified recombinant RpoD1 protein described previously (4). The antisera against RpoD2, RpoD3, and RpoD4 were prepared by immunizing rabbits with N-terminal His6-tagged partial recombinant proteins. In brief, coding regions of the partial RpoD2 (residues 74–320) and RpoD4 (residues 19–225) were cloned between BamHI and HindIII sites of pQE10 (Qiagen), and a coding region of partial RpoD3 (residues 65–247) was cloned between PstI and HindIII sites of pQE9 (Qiagen) to construct pQE-10NB6, pQE-10ND10, and pQE-pNC6 plasmids, respectively. The resulting expression plasmids were transformed into Escherichia coli M15 strain (Qiagen), and the overproduced proteins were purified by nickel-nitrilotriacetic acid affinity chromatography (Qiagen) according to the manufacturer’s protocol. The relative titers of the antisera were analyzed by immunoblot analysis, and the results are shown in Fig. S1.

Primer Extension Analysis—Primer extension analysis was performed as described elsewhere (13). Briefly, 10 μg of total RNA extracted by the hot phenol method from Synechococcus cells was subjected to primer extension analysis using the 32P-labeled primer 5′-cttggagatcagcaatgaaatggtttcctagctgggtttggtggc-3′ (BamHI site underlined) and digested with Ncol and BamHI. The Ncol-BamHI segment was subjected to primer extension analysis using the 32P-labeled primer 5′-gaggggatcagcaatgaaatggtttcctagctgggtttggtggc-3′ (BamHI site underlined). The amplified fragment was digested with BamHI and EcoRI and inserted into the pBluescript SK+ vector (Stratagene). After insertion of the Ω cassette derived from the pHP451 vector (27) at the HindIII site, the constructed plasmid was used to transform Synechococcus, and the resulting strain was named RB-F.

Electrophoretic Gel Mobility Shift Assays—Electrophoretic gel mobility shift assays were performed mainly according to previous reports (1, 30). To prepare DNA probes and competitors, equal volumes of complementary, single-stranded oligonucleotides were heated at 95 °C for 3 min and slowly cooled at room temperature to generate double-stranded DNA fragments. The following sequences, and their complementary sequences, were designed for oligonucleotides in this experiment: rpoD3, number 1 (nucleotides −100 to −51 relative to the translation initiation site), 5′-taagctgtgatcagcaatgaaatggtttcctagctgggtttggtggc-3′; rpoD3, number 2 (−36 to +14 relative to the translation initiation site), 5′-taagctgtgatcagcaatgaaatggtttcctagctgggtttggtggc-3′; hliA, number 1 (−72 to −23 relative to the translation initiation site), 5′-taagctgtgatcagcaatgaaatggtttcctagctgggtttggtggc-3′; and hliA, number 2 (−72 to −23 relative to the translation initiation site), 5′-taagctgtgatcagcaatgaaatggtttcctagctgggtttggtggc-3′.
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Expression analysis of group 1 and 2 σ factors of Synechococcus under various stress conditions. A, Northern analysis. Total RNA (10 μg each) was separated on a 1.5% agarose-formaldehyde gel, and transcripts for each rpoD gene were detected by Northern analysis. Positions of the rpoD1, rpoD2, rpoD3, rpoD4, rpoD5, and rpoD6 transcripts estimated by the molecular weight markers (left) are indicated at the right. Lane 1, control; lane 2, high salt (0.5 M NaCl); lane 3, high osmolality (0.5 M sorbitol); lane 4, high light (1,500 μmol photons m⁻² s⁻¹); lane 5, dark; lane 6, high temperature (42 °C); and lane 7, low temperature (22 °C) stress each applied for 30 min. C, transcript levels of the rpoD3 (with HLR1), hliA (with HLR1), and rpoD1 (without HLR1) genes in response to high light stress in wild-type Synechococcus cells. Total RNAs were prepared from wild type Synechococcus cells exposed to high light and subjected to Northern analysis. Bands corresponding to rpoD3, hliA, and rpoD1 are indicated. Lane 1, 0 min; lane 2, 5 min; lane 3, 15 min; lane 4, 30 min; and lane 5, 120 min exposure to high light. D, RpaB and RpoD3 protein level during the high light stress. Total proteins were prepared from RB-F cells exposed to high light and subjected to immunoblot analysis with either anti-FLAG tag mouse antibody (α) or anti-RpoD3 rabbit antiserum. Bands corresponding to the FLAG-tagged RpaB and RpoD3 are indicated. Lane 1, 0 min; lane 2, 30 min; lane 3, 1 h; lane 4, 2 h; and lane 5, 4 h after the shift to high light (1,500 μmol photons m⁻² s⁻¹).

Expression of the Synechococcus Group 1 and 2 σ Factors in Stress Conditions—Synechococcus cells were grown to mid-exponential phase (OD₇₅₀ = 0.5) under standard growth conditions and shifted to various stress conditions. These included high salt (0.5 M NaCl), high osmolality (0.5 M sorbitol), high light (1,500 μmol photons m⁻² s⁻¹), dark, high temperature (42 °C) or low temperature (22 °C) conditions. We monitored changes of the transcript levels for the rpoD1–6 genes after stress application using Northern analysis (Fig. 1A). The rpoD1 transcript was induced by salt and osmotic stresses and weakly induced by high light. The rpoD2 transcript was detected in all conditions but decreased at high light and low temperature conditions. The rpoD3 transcript was strongly induced in high light conditions and weakly induced in salt and osmotic stress conditions. The rpoD4 transcript was induced in salt and osmotic stress conditions. The rpoD5 and rpoD6 transcripts appeared to be decreased strongly in high light and weakly in other stress conditions. In the same series of experiments, levels of four σ factors (RpoD1–4) were monitored by immunoblot analysis (Fig. 1B). Interestingly, we found that RpoD1 and RpoD4 levels were almost constant irrespective of
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their mRNA level, which may indicate that post-transcriptional regulation maintains the protein level. The protein level increased in parallel with the transcript level in high light conditions only in the case of RpoD3. Thus, we further analyzed in detail the expression and the physiological relevance of rpoD3 in high light conditions. During these experiments, we could not detect RpoD2 protein in any of the conditions tested. The inability to detect RpoD2 under any conditions could have been because of the lower titer of the antiserum. A weak signal detected near the expected size appeared to be a nonspecific band, as it was also detected in the rpoD2-deleted strain (data not shown).

To investigate the time course of rpoD3 expression in response to high light, we examined the transcript level of rpoD3, hliA (high light inducible) and rpoD1 in wild-type Synechococcus cells after exposure to high light (1,500 μmol photons m⁻² s⁻¹). Transcripts of rpoD3 and hliA accumulated similarly within 5 min and rapidly decreased after 30 min after the high light shift (Fig. 1C). In contrast, transcripts of rpoD1 were induced only weakly under the same conditions. In contrast to the transcript level, the RpoD3 protein level continued to increase for at least 120 min after exposure to high light (Fig. 1D).

High Light Sensitivity of the Synechococcus rpoD3 Deletion Mutant—Because both the transcript and the protein levels from the rpoD3 gene increased in response to high light, we hypothesized that rpoD3 has some important role for survival in this condition. Thus, we examined the high light sensitivity of the Synechococcus rpoD3 deletion mutant (4), hereafter D3KM, in comparison with the parental wild-type strain, together with the other group 2 σ factor mutants, D2KM and D4KM (4, 24), in which rpoD2 or rpoD4 was deleted, respectively. Liquid cultures for each strain (OD750 = 1.0–2.0) were diluted serially and spotted onto BG-11 solid medium. After exposure to high light (900 μmol photons m⁻² s⁻¹) for 2 h, plates were transferred to standard growth conditions (35 μmol photons m⁻² s⁻¹) and incubated for 6 days. Compared with other strains, D3KM exhibited an increased sensitivity to high light stress (Fig. 2). Taken together with the results of the expression analysis, we concluded that RpoD3 is a σ factor that is induced and has a role for survival during high light stress.

Promoter Structure of the rpoD3 Gene—To understand the regulatory mechanism for the high light induction of rpoD3, we examined the promoter structure by primer extension analysis. As shown in Fig. 3A, specific signals were detected only from the reaction using RNA from the cells exposed to high light (1,500 μmol photons m⁻² s⁻¹). The rpoD3 gene has a single promoter (Pp30) with the major transcription start site (+1) at 72 bp upstream of the open reading frame, and contains a putative σ⁰-type −10 promoter sequence (Fig. 3A and B). In addition, an HLRI (high light-responsive element) was found at the position +6 to +23 (ttacaaattgac). HLRI is

![FIGURE 2. High light sensitivity of the rpoD3 deletion mutant.](image1)

![FIGURE 3. Promoter structure of the rpoD3 gene.](image2)
RpaB-affects the expression of examined in this study whether the phosphorylation status of the HLR1 element of the RpaB response regulator of the two-component signal transduction system, its corresponding sensor kinase has only recently been identified (1, 25). Kappell and van Waasbergen (1) performed a gel-shift assay using the promoter region of hliB containing HLR1 and cell lysate from low light- or high light-acclimated Synechocystis cells. As a result, the specifically shifted complex was more abundant using cell extracts from low light-acclimated than high light-acclimated cells. Because the NblS homolog is the only histidine kinase (EnvZ-type) retained in some red algal chloroplast genomes, together with the RpaB homolog, they proposed a two-component partnership between NblS and RpaB (32). As they expected, recombinant RpaB bound to the HLR1 element of the hliB gene (1). Therefore, we considered the involvement of RpaB in the high light inducible expression of rpoD3 in Synechococcus. To address this possibility, the easiest approach would have been to construct an rpaB null mutant and to examine rpoD3 expression in this strain. However, because rpaB is an essential gene in Synechococcus we had to adopt an alternative in vitro approach. Thus, we prepared a recombinant RpaB protein using an E. coli system, and we tested for the specific binding to the rpoD3 and hliA promoter regions (Fig. 4, A and B). Our results showed that the RpaB protein bound specifically to the HLR1 element near the transcription initiation point of both rpoD3 and hliA but not to the rpoD1 promoter region (without HLR1) (Fig. 4, C and D). This indicates the involvement of the HLR1 element and the NblS–RpaB two-component system in the high light induction of rpoD3 gene expression.

Negative Regulation of the rpoD3 Transcription by Phosphorylated Response Regulator RpaB—Given that RpaB can bind to the HLR1 element of the rpoD3 and hliA genes, the next question is how the high light signal can induce transcription of these genes. We constructed a cyanobacterial strain (RB-F) in which the chromosomally encoded RpaB is tagged with FLAG protein level. Using this strain, we monitored the RpaB level which the chromosomally encoded RpaB is tagged with FLAG sequence was identified downstream of the transcription initiation site (Fig. 3, A–C). HLR1 was previously suggested to be required for high light-responsive transcriptional regulation and shown to be the binding site for RpaB in Synechocystis (1, 30). Because the position of the HLR1 of the rpoD3 promoter is downstream of the transcription initiation site, and because it was shown in Synechocystis that the activity of the HLR1-binding protein is more enriched in low light conditions than in high light conditions (1), the effect of the RpaB binding is probably to repress transcription. Also in the case of hliA, RpaB binds to the promoter region as shown in Fig. 4. This binding site overlaps the RNA polymerase-binding site, and therefore, RpaB binding appears to repress transcription. In addition, results from the overexpression of the RpaB phospho-receiver domain (Fig. 5) indicate the importance of the phosphorylation of RpaB for the transcriptional repression. It has been extensively reported in OmpR family response regulators that phosphorylation promotes the specific DNA binding activity by enhancing the

3 H. Iwasaki, unpublished data.

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DISCUSSION

RpoD3 Is a High Light-responsive σ Factor in Synechococcus—In this study, we examined changes of the Synechococcus group 1 and 2 σ factors at the transcript and the protein levels in response to various stresses such as salt, osmolarity, light, and temperature. Although the group 1 σ factor and the five group 2 σ factors in Synechococcus exhibited differential expression patterns at the transcript level, only RpoD3 accumulated at a protein level that correlated with its transcript level in high light conditions (Fig. 1, A and B). We also observed high light sensitivity in the rpoD3 mutant (Fig. 2, A and B). Taken together, these results suggest that RpoD3 is a σ factor that is important for survival in high light conditions in Synechococcus. Genes orthologous to rpoD3 are widely conserved among cyanobacteria. Unlike the other group 2 σ factors, the structure of the genomic region around the rpoD3 ortholog is extensively conserved among several cyanobacterial species such as S. elongatus, Anabaena/Nostoc sp., and marine Synechococcus/Prochlorococcus sp. (data not shown). Included in these genomic regions are genes related to DNA repair such as mutT and mpg, genes related to stress response such as groEL, and genes related to PSII, most of which may be related to high light acclimation.

rpoD3 and hliA Genes Are Negatively Regulated by RpaB—From the promoter analysis of the rpoD3 gene, an HLR1-like sequence was identified downstream of the transcription initiation site (Fig. 3, A–C). HLR1 was previously suggested to be required for high light-responsive transcriptional regulation and shown to be the binding site for RpaB in Synechocystis (1, 30). Because the position of the HLR1 of the rpoD3 promoter is downstream of the transcription initiation site, and because it was shown in Synechocystis that the activity of the HLR1-binding protein is more enriched in low light conditions than in high light conditions (1), the effect of the RpaB binding is probably to repress transcription. Also in the case of hliA, RpaB binds to the promoter region as shown in Fig. 4. This binding site overlaps the RNA polymerase-binding site, and therefore, RpaB binding appears to repress transcription. In addition, results from the overexpression of the RpaB phospho-receiver domain (Fig. 5) indicate the importance of the phosphorylation of RpaB for the transcriptional repression. It has been extensively reported in OmpR family response regulators that phosphorylation promotes the specific DNA binding activity by enhancing the
dimer or oligomer formation (33, 34). In the case of OmpR in *E. coli*, it was shown that dephosphorylated OmpR has more than 10-fold lower affinity to its binding site than the phosphorylated form *in vitro* (33). Although excess protein concentration could circumvent the requirement of phosphorylation for the specific DNA binding (Fig. 4, C and D), change of the affinity is assumed to be critical for physiological regulation *in vivo*. Taken together, these results suggest that *rpoD3* and *hliA* transcription was repressed by the binding of phosphorylated RpaB to HLR1 in normal growth conditions, and dephosphorylation
induced by high light stress triggered the derepression and resulted in transcriptional activation.

Control of RpaB Phosphorylation—RpaB phosphorylation is presumably performed by histidine kinase NblS (1, 30). In response to high light stress, RpaB is dephosphorylated by a putative phosphatase, which results in the release from HLR1 and accordingly in transcriptional derepression. This phosphatase could be the NblS itself, because some histidine kinases are able to function as protein phosphatases in response to specific input signals (35). The rpoD3 transcription was transiently activated and subsequently deactivated in response to high light stress (Fig. 1C), whereas the transcript gradually accumulated after the induction of the N-terminal fragment of RpaB (Fig. 5A). Providing that the kinetics of the transcript accumulation correlates with the dephosphorylation status of RpaB, the presence of constitutive kinase activity of NblS in the latter case may explain the gradual accumulation of the transcript.

Tight Control of Gene Expression by the NblS-RpaB System Is Essential in Synechococcus and Synechocystis—Both nblS and rpaB genes are essential for viability in Synechococcus (30). This suggests that repression or activation of genes under the control of this two-component system is critical for viability or growth. In Synechocystis, the dspA/hik33 gene, which has high sequence similarity to nblS, can be deleted in heterotrophic growth conditions but not in autotrophic growth conditions (36, 37). Meanwhile, knockdown of the rpaB gene resulted in decreased efficiency of energy transfer from phycobilisomes to PSII (31). Thus, the loss of viability could result from the defective electron flow from PSII in low light conditions, which is rescued by the supply of exogenous sugar during heterotrophic growth. In the dspA/hik33 deletion mutant, the transcript levels of the high light-responsive hli genes were relatively high even in low light conditions, which could be a consequence of loss of ability to fully repress the expression (36, 38). This kind of leaky gene expression, including hli and rpoD3, might result in low PSII activity and could be the reason for the indispensability of the nblS gene in Synechococcus, which cannot grow heterotrophically.

Presence of HLR1 in Other High Light-inducible Genes—Genes orthologous to rpaB are found in genomes of divergent cyanobacteria and non-green algal chloroplasts. Thus, these genes and relevant HLR1 sequences may be involved in the regulation of high light-inducible genes. In fact, it was already reported that extended HLR1 sequences are found within the intergenic region upstream of hli genes from many cyanobacterial and algal chloroplast genomes (30). In Synechocystis and Synechococcus, the presence of HLR1 sequence is predicted in several high light inducible genes (30). However, bioinformatic prediction of HLR1 from the whole genome sequences was difficult in our hands because of the relatively low sequence conservation. Among high light-inducible genes of Synechococcus, no HLR1 sequence was found in sodB and ccmL genes, indicating some regulatory mechanisms other than the rpaB-related system are working in these cases. As the next step, genome-wide analysis using tools such as microarray and our truncated RpaB construct should be useful to resolve the complicated network regulation.

Function of rpoD3—In this study, we found that the RpoD3 protein did not accumulate after mRNA induction by the RpaB dephosphorylation. This indicates that the rpoD3 expression is
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tightly controlled not only at the transcriptional level but also at the post-transcriptional level (Fig. 6). This kind of regulation in group 2 are factors is also well known in E. coli, where the protein level of RpoS, a unique group 2 σ factor in E. coli, is controlled extensively by post-transcriptional mechanisms (39). Presumably, accumulation of RpoD3 may be inhibitory for cell growth, and therefore its expression should be tightly restricted in normal light conditions. However, once cells are exposed to high light conditions, expression of genes under the control of RpoD3 probably aids their survival. To understand the mechanism by which RpoD3 expression leads to high light stress tolerance, we are currently investigating the genes under control of RpoD3. As described above, the rpoD3 genes have been conserved among cyanobacteria together with their surrounding genes, and these gene clusters could be potential targets for transcriptional regulation.

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