Effects of Deficiency and Overdose of Group 2 Sigma Factors in Triple Inactivation Strains of *Synechocystis* sp. Strain PCC 6803

Maija Pollari,‡ Susanne Rantamäki, Tuomas Huokko, Anna Kårlund-Marttila, Virpi Virjamo,§ Esa Tyystjärvi, and Taina Tyystjärvi*

*Molecular Plant Biology, Department of Biochemistry and Food Chemistry, University of Turku, FI-20014 Turku, Finland*

Received 2 September 2010/Accepted 13 October 2010

Acclimation of cyanobacteria to environmental changes includes major changes in the gene expression patterns partly orchestrated by the replacement of a particular σ subunit with another in the RNA polymerase holoenzyme. The cyanobacterium *Synechocystis* sp. strain PCC 6803 encodes nine σ factors, all belonging to the σ*54* family. Cyanobacteria typically encode many group 2 σ factors that closely resemble the principal σ factor. We inactivated three out of the four group 2 σ factors of *Synechocystis* simultaneously in all possible combinations and found that all triple inactivation strains grow well under standard conditions. Unlike the other strains, the Δ**sigBCD** strain, which contains SigE as the only functional group 2 σ factor, did not grow faster under mixotrophic than under autotrophic conditions. The SigB and SigD factors were important in low-temperature acclimation, especially under diurnal light rhythm. The Δ**sigBCD**, Δ**sigBCE**, and Δ**sigBDE** strains were sensitive to high-light-induced photoinhibition, indicating a central role of the SigB factor in high-light tolerance. Furthermore, the Δ**sigBCE** strain (SigD is the only functional group 2 σ factor) appeared to be locked in the high-fluorescence state (state 1) and grew slowly in blue but not in orange or white light. Our results suggest that features of the triple inactivation strains can be categorized as (i) direct consequences of the inactivation of a particular σ factor(s) and (ii) effects resulting from the higher probability that the remaining group 2 σ factors associate with the RNA polymerase core.

Cyanobacteria are eubacteria capable of oxygen-producing photosynthesis, and the chloroplasts of plants and algae evolved from cyanobacteria (26). *Synechocystis* sp. strain PCC 6803 (here *Synechocystis*) is a unicellular cyanobacterium commonly used as a model organism (11). The glucose-tolerant strain of *Synechocystis* is especially popular, as genetic manipulations and found that all triple inactivation strains grow well under standard conditions. Unlike the other strains, the Δ**sigBCD** strain, which contains SigE as the only functional group 2 σ factor, did not grow faster under mixotrophic than under autotrophic conditions. The SigB and SigD factors were important in low-temperature acclimation, especially under diurnal light rhythm. The Δ**sigBCD**, Δ**sigBCE**, and Δ**sigBDE** strains were sensitive to high-light-induced photoinhibition, indicating a central role of the SigB factor in high-light tolerance. Furthermore, the Δ**sigBCE** strain (SigD is the only functional group 2 σ factor) appeared to be locked in the high-fluorescence state (state 1) and grew slowly in blue but not in orange or white light. Our results suggest that features of the triple inactivation strains can be categorized as (i) direct consequences of the inactivation of a particular σ factor(s) and (ii) effects resulting from the higher probability that the remaining group 2 σ factors associate with the RNA polymerase core.

* Corresponding author. Mailing address: Molecular Plant Biology, Department of Biochemistry and Food Chemistry, University of Turku, FI-20014 Turku, Finland. Phone: 358-2-333-5797. Fax: 358-2-333-5549. E-mail: taitgy@utu.fi.

‡ Present address: VTT Technical Research Centre of Finland, FI-02044 Espoo, Finland.

§ Present address: Department of Biology, University of Eastern Finland, FI-80101 Joensuu, Finland.

† Supplemental material for this article may be found at http://jb.asm.org/.

‡ Published ahead of print on 22 October 2010.
factor, together with the SigB and SigE factors, is involved in light regulation both in light-dark transitions and upon light intensity changes (12, 13, 24, 25, 29, 42). The SigE factor has a role in sugar metabolism and is required for light-activated heterotrophic growth (23). All group 2 σ factors affect the acclimation of Synechocystis cells to osmotic stress conditions (25).

In the present study, we constructed strains in which three group 2 σ factor genes were interrupted simultaneously in all possible combinations, leaving only one group 2 σ factor gene active in each strain. Our results point to two distinct features of the inactivation strains: (i) direct consequences of the inactivation of a particular σ factor(s) and (ii) effects resulting from an overdose of the remaining group 2 σ factor.

### MATERIALS AND METHODS

Construction of triple inactivation strains of group 2 σ factors in Synechocystis. The glucose-tolerant strain Synechocystis sp. PCC 6803 (38) was used as a control strain (CS). To construct the triple inactivation strains, the sigC and sigE genes were amplified by PCR using the primers shown in Table 1. The PCR products were cloned into a pCR Blunt II-TOPO vector (Invitrogen). pCR Blunt II-TOPO-sigC was digested with SpeI and EcoRV, and the sigC fragment was ligated into an XbaI and SmaI double-digested pUC19, pCR Blunt II-TOPO-sigE was digested with PstI and EcoRI, and the sigE fragment was ligated into PstI and EcoRI double-digested pUC19. The fragment containing chloramphenicol (Cm) resistance was obtained from pKRPl0 (CVector) by BamHI digestion and subsequently ligated into BamHI-digested pUC19-sigC or pUC19-sigE. The resulting inactivation plasmid, pUC19-sigC-Cm′, was used to transform the ΔsigBD, ΔsigBE, and ΔsigDE strains (25) to obtain the ΔsigBCD, ΔsigBCE, and ΔsigCDE strains, respectively, and to obtain the ΔsigBDE strain, the ΔsigBD strain was transformed with pUC19-sigC-Cm′ (see Fig. S1 in the supplemental material for constructs of the triple inactivation strains). Selective agar plates contained kanamycin (Kn) (50 μg/ml), streptomycin (Str) (20 μg/ml), spectinomycin (Spc) (10 μg/ml), and Cm (10 μg/ml).

PCR analysis was used to verify the complete replacement of the native gene with the inactivated gene (Fig. 1A). Genomic DNA was isolated according to the method of Williams (38), and the sigB, sigC, sigD, and sigE genes were amplified using gene-specific primers (Table 1).

### Growth of Synechocystis

Standard growth conditions for Synechocystis were BG-11 medium (25) supplemented with 20 mM HEPES-NaOH, pH 7.5; a concentration of 20 μmol m−2 s−1 of light was provided for 12 h every 24 h as continuous light.

### FIG. 1. Triple inactivation strains of group 2 σ factors in Synechocystis.

**(A)** PCR analysis of the ΔsigBCD, ΔsigBCE, ΔsigBDE, and ΔsigCDE inactivation strains. Genomic DNA was isolated from the inactivation strains, and each sig gene was amplified by PCR. The expected size of each PCR product is shown under each gel. Std, standard. (B) Growth of strains. The A750 of the cultures was set to 0.1, and the cells were grown in BG-11 medium, pH 7.5, at a PPFD of 40 μmol m−2 s−1 in continuous light (solid lines) or in a 12-h light/12-h dark rhythm (dashed lines) at 32°C. Each data point represents the mean of at least three biological replicates with independent liquid cultures, and the error bars indicate standard errors (SE). CS, control strain. (C) Doubling times of the strains in the presence and absence of 5 mM glucose at a PPFD of 40 μmol m−2 s−1. The doubling times were calculated on the basis of growth during the first 24 h. Each data point represents the mean of at least four biological replicates with independent liquid cultures, and the error bars indicate SE. The asterisks denote significant differences between the growth rate in the absence and in the presence of glucose (P < 0.01; Student’s t test).

### TABLE 1. Primers used for amplification of sig genes

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>sigB forward</td>
<td>5’-ATGGTAACAGTGCACHTAT-3’</td>
</tr>
<tr>
<td>sigB reverse</td>
<td>5’-TATGCTTTGGCCATCTGTTA-3’</td>
</tr>
<tr>
<td>sigC forward</td>
<td>5’-ATGACGATAACCGAAATTTCCTGC-3’</td>
</tr>
<tr>
<td>sigC reverse</td>
<td>5’-TCATAAACCCTTTGAGGGGCG-3’</td>
</tr>
<tr>
<td>sigD forward</td>
<td>5’-ATGAGCGATATGTCTTCCCT-3’</td>
</tr>
<tr>
<td>sigD reverse</td>
<td>5’-CTATAAACCCTTTGAGGGGCG-3’</td>
</tr>
<tr>
<td>sigE forward</td>
<td>5’-ATGAGCGATATGTCTTCCCT-3’</td>
</tr>
<tr>
<td>sigE reverse</td>
<td>5’-ATGAGCGATATGTCTTCCCT-3’</td>
</tr>
</tbody>
</table>
tions of photosynthetic photon flux density (PPFD) of 40 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}; 32^\circ\text{C} and ambient CO_2. The BG-11 agar plates of triple mutants were supplemented with K_n (50 \mu \text{g} \text{ml}^{-1}), \text{Str} (20 \mu \text{g} \text{ml}^{-1}), \text{Spc} (10 \mu \text{g} \text{ml}^{-1}) and \text{Cm} (10 \mu \text{g} \text{ml}^{-1}) and those of single mutants with K_n (50 \mu \text{g} \text{ml}^{-1}). For the measurements, cells were grown in liquid media without addition of antibiotics.

For growth measurements, the \(A_{\text{m}}\) of liquid cultures was set to 0.1 (30 ml cell culture in a 100-ml Erlenmeyer flask), and the \(A_{\text{m}}\) was measured every 24 h. Samples of dense cultures were diluted so that the \(A_{\text{m}}\) did not exceed 0.3, and the dilutions were taken into account when the final results were calculated. Similar relationships between the \(A_{\text{m}}\) and cell numbers can be expected in the control strain and in all inactivation strains because cells harvested on an \(A_{\text{m}}\) basis contain similar amounts of chlorophyll, RNA, and proteins and have the same photosynthetic activity in all strains. Furthermore, the whole-cell absorption profiles of all strains are virtually identical.

Cells were grown under standard conditions under continuous illumination or in a 12-h light/12-h dark rhythm. In addition, growth was measured at 22\(^\circ\text{C}\) under continuous illumination (PPFD, 40 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}) or in a 12-h light/12-h dark rhythm. For light rhythm measurements, the experiments were started in the middle of the light period. Cells were also grown under continuous blue or orange light. Blue light was obtained by illuminating the cultures through a filter with a transmission peak at 425 nm (HT 119 Dark Blue; Lee Filters) and orange light by using filters 105 Orange (Lee Filters) and Rosco number 89; this combination has a transmission peak at 568 nm. Mixotrophic growth conditions were obtained by adding 5 mM glucose to the growth medium under otherwise standard conditions. The growth during the first day was used for the doubling-time calculations.

**Determination of photosynthetic and PSII capacities.** Light-saturated photosynthetic activity in vivo was measured from 1 ml of cell suspension (10 \mu \text{g} \text{Chl/ml}) in BG-11 medium supplemented with 10 mM NaHCO_3 under saturating light (500 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}) with a Clark-type oxygen electrode (Hansatech) at 32\(^\circ\text{C}\). For PSII capacity measurements, 0.7 \mu \text{mol} \text{m}^{-2} \text{s}^{-1} (Zymed) and the CDP star chemiluminescence kit (New England Biolabs) were used as an artificial electron acceptor, and 0.7 \mu \text{mol} \text{ferricyanide} was added to keep the quinone in an oxidized form.

**Photoinhibition treatments in vivo.** In the photoinhibition experiments, cell cultures containing 10 \mu g Chl/ml were illuminated at a PPFD of 1.500 \mu \text{mol} \text{m}^{-2} \text{s}^{-1} with a slide projector at 32\(^\circ\text{C}\). One-milliliter samples were drawn for PSII measurements from untreated cultures and after 15, 30, and 45 min of illumination.

**Chl \(a\) fluorescence measurements.** For nonphotochemical quenching (NPO) measurements, cells were grown under standard growth conditions and concentrated to an \(A_{\text{m}}\) of 2.5. Then, the cultures were further incubated for 1 h under standard growth conditions or illuminated with blue light (HT 119 Dark Blue filter) at 40 \mu \text{mol} photons \text{m}^{-2} \text{s}^{-1}. The cell suspension (1 ml) was placed in a temperature-controlled cuvette, and chlorophyll \(a\) fluorescence was measured with a PAM-2000 fluorometer (Heinz Walz GmbH) at 32\(^\circ\text{C}\). The suspension was first incubated in the dark for 3 min, the initial fluorescence level (\(F_0\)) was measured, and then the maximum fluorescence level (\(F_{\text{m}}\)) was measured using a saturating flash of 0.4 s and a PPFD of 5.000 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}. Illumination with blue-green light (PPFD, 1.000 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}) was initiated, and the maximum fluorescence level in the light (\(F_{\text{m}}^\prime\)) was measured at 40-s intervals by firing a saturating flash. For state transition measurements at room temperature, the cells were taken from standard growth conditions and incubated in the dark for 3 min. Thereafter, the cell suspension was illuminated for 135 s with blue-green light (PPFD, 80 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}) obtained by filtering the output from a slide projector through a Corion long-pass filter and for 195 s with orange light (PPFD, 20 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}), obtained by filtering through a 580-nm Corion long-pass filter. A saturating flash was fired every 30 s throughout the procedure to measure \(F_{\text{m}}^\prime\).

**77 K emission spectroscopy.** For state transition measurements with 77 K emission spectroscopy, cells (50 \mu g Chl/ml; 50-\mu l samples) were rapidly frozen with liquid nitrogen directly from growth conditions or cells were successively illuminated with blue light (450-nm Corion long-pass filter) at 40 \mu \text{mol} photons \text{m}^{-2} \text{s}^{-1} for 5 min, kept in the dark for 5 min, and illuminated again with blue light for 5 min and then frozen. Fluorescence emission spectra were measured at 77 K with an S2000 spectrometer (Ocean Optics) by exciting the sample with orange light obtained by filtering output from a slide projector through a 580-nm narrow-band filter (Corion). The spectra were corrected by subtracting a low background signal, smoothed with a moving median using a 2-\text{nm} window and normalized by dividing by the peak value of PSII emission at 723 nm.

**In vivo absorption spectra.** In vivo absorption spectra were measured with a UV-3000 spectrophotometer (Shimadzu, Japan) from 350 nm to 800 nm.

**Western blotting.** The cells (25-ml cell culture; \(A_{\text{m}} = 1\)) were harvested directly from growth condition light changes after 1-h treatments at high PPFD, 1,000 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}) or by blue light (HT 119 Dark Blue filter; 40 \mu \text{mol} photons \text{m}^{-2} \text{s}^{-1}) by centrifugation at 10,000 \times g for 5 min at 4\(^\circ\text{C}\). In the photoinhibition experiments, cell samples using a 1:1,500 dilution of an OCP-specific antibody, a generous gift from Diana Kirilovsky. Commercial antibodies (Agrisera) were used to detect the PSI reaction center protein PDI (AS06 124A) and the PSI reaction center protein PsaA (AS06 172) from thylakoid samples. Both antibodies were used at 1:1,000 dilution. The goat anti-rabbit IgG (H+L) alkaline phosphatase conjugate (Zymed) and the CDP star chemiluminescence kit (New England Biolabs) were used for detection in Western blotting. The immunoblots were quantified with a FluorChem image analyzer (Alpha Innotech Corp.).

**RESULTS**

**Triple inactivation strains of group 2 \(\sigma\) factors in *Synechocystis*.** We constructed all possible triple inactivation strains of group 2 \(\sigma\) factors in *Synechocystis*. The sigC and sigE genes were inactivated with a chloramphenical resistance cassette, and transformation of the \(\Delta\text{sigBD}, \Delta\text{sigBE},\) and \(\Delta\text{sigDE}\) double inactivation strains (25) resulted in the \(\Delta\text{sigBCD}, \Delta\text{sigBCE}, \Delta\text{sigBE},\) and \(\Delta\text{sigCDE}\) triple inactivation strains. The complete segregation of the inactivated gene from the native gene was confirmed by PCR analysis (Fig. 1A). Two lines that were descendants of independently raised colonies on the first selection plate were used in measurements to minimize the possibility that secondary mutations would affect the results. However, the two lines behaved similarly, and the results are shown for one line only.

The growth curves of all triple inactivation strains (Fig. 1B) were similar to that of the control strain under standard growth conditions (BG-11 medium, pH 7.5; continuous light; PPFD of 40 \mu \text{mol} \text{m}^{-2} \text{s}^{-1}; 32\(^\circ\text{C}\); and ambient CO_2).

**TABLE 2. Photosynthetic and PSII activities of triple inactivation strains of group 2 \(\sigma\) factors in *Synechocystis* sp. strain PCC 6803**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Light-saturated photosynthetic activity (\mu\text{mol} \text{O}_2 \text{mg} \text{Chl}^{-1} \text{h}^{-1})</th>
<th>Light-saturated PSII activity (\mu\text{mol} \text{O}_2 \text{mg} \text{Chl}^{-1} \text{h}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125.8 ± 7.8</td>
<td>387.4 ± 7.6</td>
</tr>
<tr>
<td>(\Delta\text{sigBCD})</td>
<td>125.3 ± 3.0</td>
<td>413.1 ± 3.0</td>
</tr>
<tr>
<td>(\Delta\text{sigBCE})</td>
<td>124.6 ± 2.8</td>
<td>392.1 ± 9.6</td>
</tr>
<tr>
<td>(\Delta\text{sigBDE})</td>
<td>126.6 ± 1.4</td>
<td>387.0 ± 20.1</td>
</tr>
<tr>
<td>(\Delta\text{sigCDE})</td>
<td>132.2 ± 5.3</td>
<td>427.5 ± 13.2</td>
</tr>
</tbody>
</table>

Downloaded from http://jb.asm.org/ on June 26, 2017 by guest
tion without affecting the viability and growth of the cells under standard conditions. All triple inactivation strains grew well in a 12-h light/12-h dark rhythm (Fig. 1B).

In laboratory batch cultures of *Synechocystis*, a short exponential growth phase is followed by a phase of linear growth and finally a stationary phase (4, 25). The doubling times during the first day of growth were measured for autotrophically and mixotrophically growing cells under continuous illumination (PPFD, 40 μmol m⁻² s⁻¹) at 32°C. The control and the ∆sigBCE, ∆sigBDE, and ∆sigCDE strains grew faster under mixotrophic than under autotrophic growth conditions (Fig. 1C), while the ∆sigBCD strain grew similarly under autotrophic and mixotrophic conditions.

SigB and SigD factors are involved in acclimation to low-temperature stress. The roles of σ factors in acclimation to low-temperature stress were not tested previously in cyanobacteria. We used 22°C; at this temperature, cells of the control strain grow, but slowly, with a doubling time of circa 19 h. We first tested the single inactivation strains and found that none of them grew as well as the control strain at 22°C, and especially, the ∆sigB and ∆sigD strains grew slowly (Fig. 2A). In accordance with the results obtained with single inactivation strains, the triple inactivation strains containing only SigC (∆sigBDE) or only SigE (∆sigBCD) grew poorly at 22°C, whereas those strains containing only SigD (∆sigBCE) or only SigB (∆sigCDE) grew like the control strain (Fig. 2B). These results point to the importance of the SigB and SigD factors for cold acclimation.

Next, low-temperature stress was combined with a light/dark rhythm. All single mutants grew more slowly at low temperature in a light rhythm than the control strain; the ∆sigB strain was the most sensitive (Fig. 2C). The importance of the SigB and SigD factors was obvious under light rhythm at 22°C, as strains that contained only SigC (∆sigBDE) or SigE (∆sigBCD) hardly grew at all (Fig. 2D), while strains with only SigB (∆sigCDE) or only SigD (∆sigBCE) grew only somewhat more slowly than the control strain, at a rate similar to that of the ∆sigB strain. The SigB and SigD factors showed the highest mutual similarity among all group 2 σ factors (25), and these results suggest that they have functional redundancy under low-temperature conditions. We have also noticed redundancy in their functions under high light, under high-temperature stress, and when very dilute cell cultures are grown (25).

High-light acclimation is disturbed in triple inactivation strains. The sensitivities of the strains to high light were studied by subjecting the cells to 1,500 μmol photons m⁻² s⁻¹. Photoinhibition was determined as loss of PSII capacity compared to untreated samples. The ∆sigBCD, ∆sigBCE, and ∆sigBDE strains lost their PSII activities 20% faster than the control strain, but the ∆sigCDE strain behaved like the control strain (Fig. 3). These results indicate that the SigB factor is important in bright-light responses in *Synechocystis*.

The ∆sigBCE strain has defects in light quality acclimation. The light quality acclimation capacities of the triple inactivation strains were tested by growing the cells under continuous orange or blue light. The PPFD of blue and orange light was adjusted to 40 μmol photons m⁻² s⁻¹, the same as the PPFD of the white light of standard growth conditions. The doubling times of the control strain were 12 h, 15 h, and 19 h under white, orange, and blue light, respectively. These growth rates
are in agreement with higher in vivo photosynthetic activity of *Synechocystis* cells under orange than under blue light (34). The ΔsigBCE strain grew more slowly than the control or the other triple inactivation strains in blue light, with a doubling time of 47 h (Fig. 4A). In orange light, however, all triple mutants grew like the control strain (Fig. 4B).

In cyanobacteria, the OCP is essential for phycobilisome-associated NPQ, a safety mechanism that reduces energy transfer from the phycobilisomes to the photosystems (39, 40). As NPQ in cyanobacteria is induced by blue-green light (3) and slow growth of the ΔsigBCE strain is a blue-light-specific phenomenon, we compared the OCP content and NPQ in the control and ΔsigBCE strains. Western blot analysis revealed that the amounts of the OCP were similar in the control and ΔsigBCE strains under standard growth conditions but that after a 1-h treatment in high (1,000 mol photons m\(^{-2}\) s\(^{-1}\)) or blue (40 mol photons m\(^{-2}\) s\(^{-1}\)) light, the OCP contents of the ΔsigBCE strain were 108% and 107%, respectively, of that measured for the control strain under the same conditions (Fig. 5A). For NPQ measurements, cells were first illuminated for 1 h with blue light (40 mol photons m\(^{-2}\) s\(^{-1}\)), F\(_0\) and F\(_{\text{M}}\) were measured with a PAM fluorometer from dark-adapted cells of the control strain (B) or the ΔsigBCE strain (C) during illumination with blue-green light (PPFD, 1,000 μmol m\(^{-2}\) s\(^{-1}\)). Each peak corresponds to a saturating flash. rel, relative.

FIG. 3. Photoinhibition of the triple inactivation strains. Cell cultures were illuminated at a PPFD of 1,500 μmol m\(^{-2}\) s\(^{-1}\), and light-saturated PSII activity was measured after 0, 15, 30, and 45 min with a Clark-type oxygen electrode at 32°C using 0.7 mM 2,6-dichlorobenzoquinone as an artificial electron acceptor. PSII activity is expressed as a percentage of the activity measured from untreated control samples. Each data point represents an average of five independent experiments, and the error bars denote SE. After 45 min of illumination, the difference between the control strain and the ΔsigBCD, ΔsigBCE, and ΔsigBDE strains was statistically significant (P < 0.05; Student’s t test).

FIG. 4. Growth of the control strain and the triple inactivation strains in blue light, preferentially absorbed by PSI (A), or in orange light, preferentially absorbed by PSII (B). The PPFD was 40 μmol photons m\(^{-2}\) s\(^{-1}\). Each data point represents the mean of three independent cultures, and the error bars denote SE.

FIG. 5. Orange carotenoid protein content and nonphotochemical quenching in the control and ΔsigBCE strains. (A) Western blot showing the amounts of the orange carotenoid protein under standard growth conditions (standard), after 1 h of illumination at a PPFD of 1,000 μmol m\(^{-2}\) s\(^{-1}\) (high light), and after 1 h of illumination with blue light at 40 μmol photons m\(^{-2}\) s\(^{-1}\) (blue light). (B and C) Chlorophyll a fluorescence yield measured with the PAM fluorometer from dark-adapted cells of the control strain (B) or the ΔsigBCE strain (C) during illumination with blue-green light (PPFD, 1,000 μmol m\(^{-2}\) s\(^{-1}\)). Each peak corresponds to a saturating flash. rel, relative.
when NPQ was measured for cells grown under standard conditions (data not shown).

As no differences in NPQ between the ΔsigBCE and control strains were detected, we continued by studying the state transitions that function to balance energy distribution between PSI and PSII. To follow state transitions, fluorescence emission spectra at 77 K were measured from the control and ΔsigBCE strains. For the measurements, cells taken from standard growth conditions or cells that had passed a treatment consisting of 5 min in blue light, 5 min in the dark, and finally illuminated for 5 min with blue light (blue 2). Light at 590 nm was used for excitation, and the data were normalized by dividing by the height of the photosystem I emission peak at 723 nm. Each spectrum represents an average of four independent experiments. (C) Absorption spectra of control and ΔsigBCE cells grown under standard conditions. The spectra were normalized to the Chl a absorption peak at 440 nm. (D) Amounts of PSII reaction center protein D1 and PSI reaction center protein PsaA. Proteins of isolated thylakoids (0.5 μg of Chl a) were separated by SDS-PAGE, and the amounts of the reaction center proteins were determined by Western blotting using D1 and PsaA protein-specific antibodies. (E and F) Fluorescence yields from dark-adapted control (E) and ΔsigBCE (F) cells were measured with a PAM-2000 fluorometer. The illumination protocol consisted of darkness, blue-green light (PPFD, 80 μmol m⁻² s⁻¹), orange light (PPFD, 20 μmol m⁻² s⁻¹), and finally darkness, as indicated. A saturating pulse was fired every 30 s to measure Fₘ'.
photosystem stoichiometries in the control and ΔσgBCE strains under standard conditions. Thus, the photosynthetic machinery of the ΔσgBCE strain appears to be locked in state 1.

The state transitions were further studied at room temperature, using a PAM fluorometer. In these measurements, the control strain showed a clear increase of FM in blue-green light and a decrease of FM in orange light (Fig. 6E). The blue-light-induced increase in FM did not occur in the ΔσgBCE strain, and only a very small decrease of FM occurred during illumination with orange light (Fig. 6F), confirming that the ΔσgBCE strain is not able to perform normal state transitions.

DISCUSSION

To our knowledge, this is the first time that a complete series of inactivation strains with only one group 2 factor left active has been constructed and examined in a cyanobacterium. Summerfield and Sherman (29) constructed a ΔσgBDE triple inactivation strain in Synechocystis and, similar to our results, found no differences in growth under standard conditions between the control and inactivation strains. Double inactivation strains of Synechocystis (25, 29), Anabaena sp. strain PCC 7120 (16), and Synechococcus elongatus PCC 7942 (20) have also been shown to grow well under standard conditions.

Previous studies with the sigE gene inactivation strain revealed that the SigE factor is important for light-activated heterotrophic growth, and SigE has been shown to activate some genes involved in sugar catabolic pathways (23). The ΔσgBCD strain, containing SigE as the only functional group 2 factor, was not able to utilize glucose in the light like the other strains (Fig. 1). As all σ factors compete for binding to the same RNA polymerase core, inactivation of three group 2 σ factors could actually promote the binding of the remaining group 2 σ factor. Thus, our finding may suggest that inactivation of the other three group 2 σ factors leads to an overdose of the SigE factor in the RNA polymerase holoenzyme, and this overdose disturbs the utilization of glucose under continuous light. The data strengthen the idea that the SigE factor is an important regulator of sugar metabolism.

According to the data presented here, group 2 σ factors, particularly SigB and SigD, are important for acclimation to cold and light stresses in Synechocystis. The results are summarized in Fig. 7. Maximal growth of the triple inactivation strains containing either SigB or SigD as the only group 2 σ factor and the slightly retarded growth of the ΔσgB and ΔσgD strains suggest that either SigB or SigD is sufficient to ensure optimal growth at low temperatures if the factor does not need to compete with other group 2 σ factors for a limited pool of RNAP core enzymes. In Escherichia coli K-12, the only group 2 σ factor, RpoS, is involved in acclimation to low temperatures. Recent microarray analysis revealed that 7% of the genes in E. coli K-12 are upregulated under cold stress conditions, and 40% of those genes have been shown to be controlled by RpoS under at least one stress condition or during the stationary growth phase (37).

Cyanobacteria are unique among eubacteria in having endogenous circadian rhythms (2). This phenomenon has been investigated in detail in the cyanobacterium S. elongatus PCC 7942. Inactivation of any of the four group 2 σ factor genes individually or in pairs or overproduction of the group 2 σ factors altered the circadian expression of different reporter genes in S. elongatus PCC 7942, indicating that group 2 σ factors are involved in circadian regulation but that none of them alone is responsible (20). In Synechocystis, all triple inactivation strains grow well under a diurnal light rhythm at optimal temperature, but they are more sensitive to low tem-
temperatures under a light rhythm than under continuous light, suggesting that group 2 sigma factors might also be involved in circadian regulation in *Synechocystis*, although none of them alone is crucial (Fig. 7).

For light quantity acclimation, the SigB and SigD factors seem to be the most important. The sigB and sigD genes are upregulated upon transfer of *Synechocystis* cells to high-light conditions (9, 10). A close homolog of SigD, RpoD3 of *Synechocystis* elongatus S. conditions (9, 10). A close homolog of SigD, RpoD3 of the growth light intensity (24).

Under high-light conditions (PPFD, 1,500 μmol m⁻² s⁻¹), the half-life of the D1 protein is only 20 min (33) and the survival of the cells is crucially dependent on efficient repair of photoinhibited PSII. We have previously shown that the double inactivation strain ΔsigBD is sensitive to high light (24). The PSII repair cycle of the ΔsigBD strain does not function efficiently, because transcription of the psbA genes encoding the PSII reaction center protein D1 is not upregulated normally (24). The results of this study show that the SigB factor alone is sufficient to provide normal tolerance for high-light-induced photoinhibition. However, the ΔsigD strain can tolerate high-light treatment as well as the control strain (24), suggesting that, together with the SigC and SigE factors, the SigD factor is able to substitute for the SigB factor under high-light stress.

Another aspect of light acclimation is acclimation to different qualities of light. The slow growth of the ΔsigBCE strain in blue light (with a doubling time 2.5 times longer than in the control strain) might be due to an increased probability of having the SigD factor in the RNA polymerase holoenzyme because the other group 2 σ factors are missing. For a more comprehensive picture of the blue-light phenomenon, growth of the ΔsigBD, ΔsigB, and ΔsigD strains was also measured in blue light. The growth of the ΔsigB strain was slightly reduced (doubling time, 1.14 times longer than in the control strain). In the ΔsigBCE strain, SigD is the only group 2 σ factor, and in the ΔsigB strain, the absence of the SigB factor might promote binding of the other group 2 σ factors, including the SigD factor, to the RNA polymerase core (Fig. 7). In *S. elongatus* PCC 7942, overproduction of group 2 σ factors under an IPTG (isopropyl-β-D-thiogalactopyranoside)-induced promoter has been shown to affect circadian regulation of reporter genes (20).

The ΔsigBCE strain lacks the capacity for normal state transitions induced by blue light (Fig. 6). Obviously, the absence of state transitions leads to an inability to balance the functions of the two photosystems in blue light, although the ΔsigBCE strain appears to be locked in state 1, which is the state normally induced by blue light. On the other hand, another light-balancing system, the OCP-related NPO, appears to function normally in the ΔsigBCE strain (Fig. 5). Thus, our results with the ΔsigBCE strain fully agree with the conclusion, drawn from experiments with the ΔOCP strain and phycobilisome and PSI mat-25; conditions (9, 10). A close homolog of SigD, RpoD3 of *Synechocystis* elongatus S. conditions (9, 10). A close homolog of SigD, RpoD3 of the growth light intensity (24). The results of this study show that the SigB factor alone is sufficient to provide normal tolerance for high-light-induced photoinhibition. However, the ΔsigD strain can tolerate high-light treatment as well as the control strain (24), suggesting that, together with the SigC and SigE factors, the SigD factor is able to substitute for the SigB factor under high-light stress.

Another aspect of light acclimation is acclimation to different qualities of light. The slow growth of the ΔsigBCE strain in blue light (with a doubling time 2.5 times longer than in the control strain) might be due to an increased probability of having the SigD factor in the RNA polymerase holoenzyme because the other group 2 σ factors are missing. For a more comprehensive picture of the blue-light phenomenon, growth of the ΔsigBD, ΔsigB, and ΔsigD strains was also measured in blue light. The growth of the ΔsigB strain was slightly reduced (doubling time, 1.14 times longer than in the control strain). In the ΔsigBCE strain, SigD is the only group 2 σ factor, and in the ΔsigB strain, the absence of the SigB factor might promote binding of the other group 2 σ factors, including the SigD factor, to the RNA polymerase core (Fig. 7). In *S. elongatus* PCC 7942, overproduction of group 2 σ factors under an IPTG (isopropyl-β-D-thiogalactopyranoside)-induced promoter has been shown to affect circadian regulation of reporter genes (20).

The ΔsigBCE strain lacks the capacity for normal state transitions induced by blue light (Fig. 6). Obviously, the absence of state transitions leads to an inability to balance the functions of the two photosystems in blue light, although the ΔsigBCE strain appears to be locked in state 1, which is the state normally induced by blue light. On the other hand, another light-balancing system, the OCP-related NPO, appears to function normally in the ΔsigBCE strain (Fig. 5). Thus, our results with the ΔsigBCE strain fully agree with the conclusion, drawn from experiments with the ΔOCP strain and phycobilisome and PSI mat-25; conditions (9, 10). A close homolog of SigD, RpoD3 of *Synechocystis* elongatus S. conditions (9, 10). A close homolog of SigD, RpoD3 of the growth light intensity (24).


