Light-Activated Heterotrophic Growth of the Cyanobacterium *Synechocystis* sp. Strain PCC 6803: a Blue-Light-Requiring Process

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A glucose-tolerant strain of *Synechocystis* sp. strain 6803 will not grow on glucose under complete darkness unless given a daily pulse of white light, typically 5 min of $40\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ (light-pulsed conditions). The light pulse is insufficient for photoautotrophy, as glucose is required and growth yield is dependent on glucose concentration. Growth rate is independent of fluence, but growth yield is dependent on fluence, saturating at 40 to $75\ \mu\text{mol m}^{-2}\text{ s}^{-1}$. A *Synechocystis* strain 6803 *psbA* mutant strain grows under light-pulsed conditions at rates similar to those for the glucose-tolerant strain, indicating that photosystem II is not required for growth. The relative spectral sensitivity of the growth of light-pulsed cultures (growth only in blue light, 400 to 500 nm, maximum at 450 nm) precludes energetic contribution from cyclic electron transport around photosystem I. Pulses of long-wavelength light (i.e., 550 and 650 nm) did not support the growth of *Synechocystis* strain 6803 and, when supplied before or after a blue-light pulse, did not inhibit blue-light-stimulated growth of *Synechocystis* strain 6803. We conclude that the required blue-light pulse does not support growth via photosynthetic electron transport but appears instead to function as an environmental signal regulating heterotrophic metabolism, cell division, or other photomorphogenic processes. We have termed the growth of *Synechocystis* strain 6803 pulsed with light and kept otherwise in complete darkness light-activated heterotrophic growth. This observation of a blue-light requirement for the growth of *Synechocystis* strain 6803 represents a novel blue light effect on the growth of a cyanobacterium.

To test the hypothesis that heterotrophic growth can be utilized as a permissive growth condition for selection of cyanobacterial mutants of photosystem (PS) I core polypeptide subunit genes, we began a systematic study of the heterotrophic growth capacity of *Synechocystis* sp. strain PCC 6803. *Synechocystis* strain 6803 is a unicellular cyanobacterium which is naturally competent and is transformable by exogenous DNA (23, 50). This strain can be grown photoheterotrophically, without the need for a functional PS II, at the expense of glucose as a source of reduced organic carbon. The capacities of *Synechocystis* strain 6803 to grow photoheterotrophically and to be transformed have been exploited in the study of structure-function relationships of the PS II reaction center (15, 16, 27, 47, 48). However, mutational analysis by insertional inactivation of cyanobacterial PS I genes has been limited to two genes, *psaD* (10) and *psaE* (7, 9). The *psaD* gene is thought to encode a subunit participating in the docking of ferredoxin to the PS I reaction center. The function of the subunit encoded by the *psaE* gene is unknown. The selection of homozygous cyanobacterial mutants insertionally inactivated in genes encoding PS I subunits that participate directly in electron transport or chlorophyll binding (i.e., *psaA*, *psaB*, and *psaC*) has been unsuccessful under mixotrophic growth conditions (light; air levels of CO₂ and glucose) (1). Heterotrophic growth should eliminate the requirement for a functional PS I, allowing segregation of homozygous mutants of PS I core polypeptide subunit genes.

A number of cyanobacterial strains have been shown to be capable of heterotrophic growth dependent solely on exogenous reduced organic compounds, metabolized via the oxidative pentose-phosphate cycle (35), as a source of both

carbon and energy. Reduced organic compounds support heterotrophic growth of cyanobacteria at a growth rate that is a fraction of their photoautotrophic or photoheterotrophic growth rate (44). Reports exist both refuting (38) and purporting (3, 28, 29, 31) the ability of *Synechocystis* strain 6803 to grow heterotrophically. In a survey of 38 cyanobacterial strains for their ability to grow photoheterotrophically and chemoheterotrophically, Rippka (38) reported that *Synechocystis* strain 6803 is capable of photoheterotrophic but not chemoheterotrophic growth. Chemoheterotrophic growth conditions used in this study were defined as BG-11 medium supplemented with 1% glucose in flask cultures wrapped with aluminum foil and in some cases kept in light-tight cabinets. Astier et al. (3) reported chemoheterotrophic growth (dark, 2% glucose) of *Synechocystis* strain 6803 in liquid cultures with a doubling time of 19 h, but no growth of single colonies on plates under "strictly chemoheterotrophic conditions" was observed unless the plates were supplemented with low-intensity light, 100 to 200 lx (0.18 to $0.36\ \mu\text{mol m}^{-2}\text{ s}^{-1}$). In the absence of glucose, this low-intensity light was not sufficient to support photoautotrophic growth. Similarly, Joset et al. (29) reported heterotrophic doubling times of 25 h for liquid cultures of *Synechocystis* strain 6803 in the dark with 5 mM glucose and "dark growth" of colonies on glucose-containing plates when supplemented with light of "about $3\ \mu\text{E m}^{-2}\text{ s}^{-1}$." Heterotrophic doubling times of 22 h (dark, 1% glucose) (31) and 24 h (28) have also been reported for *Synechocystis* strain 6803. These reports of heterotrophic growth of *Synechocystis* strain 6803 provide little or no description of how dark conditions were maintained or how cultures were sampled for analysis of growth.

In our initial studies on the heterotrophic growth of *Synechocystis* strain 6803, replicate 20-ml cultures in tubes (20 by 175 mm) bubbled with air were wrapped with three

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layers of black cloth and kept in a dark incubator. Daily, one culture was removed from the incubator, unwrapped, and exposed briefly (10 to 20 s) to the ambient room light to check the rate of aeration. Only the culture briefly exposed to the room light grew. It appeared that even brief exposure to dim light could significantly influence the growth of *Synechocystis* strain 6803. We therefore established rigorously controlled dark conditions for our growth studies and examined more closely the effect of brief daily pulses of light on the heterotrophic growth of *Synechocystis* strain 6803. We have found that *Synechocystis* strain 6803 will not grow on glucose under complete darkness unless receiving a daily pulse of dim light. We have termed this light-pulse requirement for growth light-activated heterotrophic growth and present herein characterization of the light-activated heterotrophic growth of *Synechocystis* strain 6803 with respect to growth yield, the intensity and wavelength of light required, and the mode of action of the light pulse.

MATERIALS AND METHODS

A glucose-tolerant strain of *Synechocystis* sp. strain PCC 6803 (50) and a mutant strain inactivated in all three members of the *psbA* gene family and lacking PS II activity (27) were grown in BG-11 medium (39) supplemented with 5 mM TES (*N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid), pH 8.0. When stated, filter-sterilized (0.22- μ m-pore-size filter) glucose was added to autoclaved media at the indicated concentrations. BG-11 medium modified as described by Labarre et al. (31) was supplemented with 6 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.5), and 1% (56 mM) glucose (final concentration) was used where indicated. For growth of the *psbA* mutant, the medium was supplemented with 5 mM glucose, 10 μ M dichlorophenyl dimethylurea in 100% ethanol (filter sterilized through a 0.45- μ m-pore-size filter), kanamycin (5 μ g/ml), streptomycin (10 μ g/ml), spectinomycin (20 μ g/ml), and chloramphenicol (10 μ g/ml) (all filter sterilized through a 0.22- μ m-pore-size filter). Solid medium was BG-11 supplemented with 1.5% (wt/vol) Difco Bacto-Agar, 5 mM TES (pH 8.0), 0.3% (wt/vol) sodium thiosulfate, and when stated 5 mM glucose. Medium for heterotrophic growth on plates was solidified with 1.5% Difco Bacto-Agar purified as previously described (5) and contained 5 mM glucose.

Extreme care was taken to provide rigorously controlled dark conditions and controlled light-pulse conditions for the growth of cultures. Liquid cultures (75 ml in 125-ml Erlenmeyer flasks with cotton stoppers) were maintained on rotary shakers at 150 rpm in specially constructed light-tight boxes supplied with two fans and two air ports all fitted with light baffles. They were grown at $31 \pm 2^\circ\text{C}$ and 40% humidity in a darkroom that is part of a four-darkroom photobiology complex separated by a dark hallway from the nearest lighted hallway. Petri plate cultures were maintained in separate identically constructed light-tight boxes in the same darkroom. Liquid and plate cultures receiving a daily brief pulse of light but maintained otherwise in complete darkness are termed light pulsed, and liquid and plate cultures maintained in continuous darkness are termed dark controls. Where indicated, liquid and plate cultures were illuminated daily with digital-timer-controlled, 5-min white-light pulses at a fluence rate of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the surface of the rotary shaker and the plate shelf from two GE cool white F20T12 CW 20-W fluorescent bulbs mounted inside the top of the light-tight box. When experimental design required different fluence rates or wavelengths, liquid cultures were

illuminated individually in a light-tight box constructed with a 1.5-in. (ca. 3.8-cm)-diameter circular light port in one side fitted with a rubber gasket and an external spring-loaded frame that holds 2-in² (ca. 5-cm²) filters over the port with a light-tight fit. A Kodak 4200 series slide projector with a Sylvania FHS 300W 82V bulb was mounted on the exterior of the box and provided the light source. Different intensities of white light were obtained by varying the distance of the culture from the light source or by the use of neutral density filters (Balzers Corp., Hudson, N.H.) inserted over the light port. Wavelength was defined with interference filters with a 10-nm half-bandwidth (PTR Optics, Waltham, Mass.) and peak transmission at 400, 450, 500, 550, 600, 650, 700, and 750 nm. Radiant energy of monochromatic light was measured with a model 68 Kettering Research radiant power meter. Exposure times were adjusted such that each culture received a 500-J m^{-2} dose of radiant energy of a specific wavelength.

Cultures monitored for growth were inoculated from exponential-phase cultures pregrown for at least one subculturing under light-pulsed conditions. Serial subculturing and plating were performed in a sterile hood in a lighted room. Growth of liquid cultures was routinely monitored by measuring the optical density at 730 nm (OD_{730}) of 0.5-ml samples with a Gilford Response UV/visible light spectrophotometer. To determine CFU per milliliter, appropriate dilutions of liquid cultures were plated on BG-11 medium solidified with 1.5% (wt/vol) Difco Bacto-Agar. Plates for determination of CFU per milliliter were maintained under continuous white light at a fluence rate of $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 31°C for 21 days, and the colonies were counted. Light-pulsed cultures receiving a daily 5-min pulse of white light of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ were sampled for growth analysis during the 5-min irradiation period. Those cultures kept in continuous darkness or pulsed individually with light of varying intensity or wavelength were sampled under a dim green safelight with a light intensity of less than $0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$. Cultures light pulsed with 5 min of white light ($40 \mu\text{mol m}^{-2} \text{s}^{-1}$), light pulsed with monochromatic light (500 J m^{-2} of 400, 450, 500, 550, 600, 700, and 750 nm), and dark control cultures not exposed to the dim green safelight were sampled after 10 days. The final OD_{730} and CFU per milliliter attained by these cultures were indistinguishable from the growth yields attained by cultures exposed daily to the dim green safelight during sampling.

RESULTS

Light-pulsed liquid cultures of a glucose-tolerant strain of *Synechocystis* strain 6803 grew as measured by increase in OD_{730} (Fig. 1), with a doubling time calculated during the mid-exponential phase of growth (days 5 to 8) of 36 h. Dark controls showed a small increase in OD_{730} equivalent to approximately 1.5 doublings (Fig. 1). Serial subcultures of dark control cultures consistently failed to grow (data not shown). Viable cell counts for light-pulsed cultures inoculated to an initial OD_{730} of 0.027 increased with a doubling time calculated during the mid-exponential phase of growth (days 5 to 8) of 26 h to an average of 9.4×10^7 CFU ml⁻¹ during stationary phase. Viable cell counts for dark controls showed a similar increase for the first 6 to 7 days, after which cell viability rapidly declined to approximately 700 times fewer CFU per milliliter at day 14 than when inoculated (Fig. 2). The growth response of light-pulsed cultures showed reciprocity between the fluence rate and duration of exposure (data not shown).

FIG. 1. Growth of a glucose-tolerant strain of *Synechocystis* strain 6803 in liquid media at 31°C with 5 mM glucose under light-pulsed (○) or dark control (●) conditions (seven separate growth experiments, each with $n = 3$) and without 5 mM glucose under light-pulsed conditions (▲) ($n = 3$) and the growth of a *Synechocystis* 6803 *psbA* mutant strain (27) lacking PS II activity with 5 mM glucose under light-pulsed conditions (■) ($n = 3$). Light-pulsed conditions were 5 min of white light of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ daily. Data, presented as a semilog plot, are means $\pm$ standard deviations.

Light-pulsed cultures without 5 mM glucose or any other source of reduced organic compounds failed to grow (Fig. 1) and did not show the small increase in OD_{730} characteristic of cultures containing 5 mM glucose but not pulsed with light. *Synechocystis* strain 6803 was cultured under dark and light-pulsed conditions in a modified BG-11 medium containing 6 mM HEPES (pH 7.5) and 1% (56 mM) glucose, for which a 22-h heterotrophic doubling time for *Synechocystis* strain 6803 has been reported (31). Light-pulsed cultures (Fig. 3) grew in this medium with a doubling time calculated during the mid-exponential phase of growth (days 5 to 8) of 36 h and at stationary phase reached an OD_{730} almost twofold higher than when cultured in BG-11 containing 5 mM glucose. The higher glucose concentration of this me-

FIG. 2. Growth of a glucose-tolerant strain of *Synechocystis* strain 6803 in liquid media containing 5 mM glucose under light-pulsed (○) or dark control (●) conditions at 31°C. Light-pulsed conditions were 5 min of white light of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ daily. Data, presented as a semilog plot, are densities for a typical growth experiment.

FIG. 3. Growth of a glucose-tolerant strain of *Synechocystis* strain 6803 in liquid medium modified as described previously (31) with 1% (56 mM) glucose under light-pulsed (○) or dark control (●) conditions at 31°C. Light-pulsed conditions were 5 min of white light of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ daily. Data, presented as a semilog plot, are means $\pm$ standard deviations for a typical growth experiment with three replicate cultures.

dium had no effect on the growth of dark controls (Fig. 3) as compared with dark controls in our standard medium (Fig. 1).

A *Synechocystis* strain 6803 *psbA* mutant strain lacking PS II activity grows photoheterotrophically with a doubling time of 20 h (data not shown). This *psbA* mutant strain grew as well as the glucose-tolerant strain of *Synechocystis* strain 6803 when cultured under light-pulsed conditions with a doubling time of 32 h (Fig. 1), indicating that PS II activity is not required for light-activated heterotrophic growth of *Synechocystis* strain 6803.

The light requirement for the light-activated heterotrophic growth of *Synechocystis* strain 6803 was further characterized with respect to dependence on light intensity and wavelength. A daily 5-min light pulse at the lowest fluence rate tested, $0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, was sufficient to support the light-activated heterotrophic growth of *Synechocystis* strain 6803 as compared with the dark controls (Fig. 4). The initial slopes of the growth curves at all the fluences tested were approximately the same, and an average doubling time of 35 ± 1.2 h is calculated from these data. However, the growth yield as indicated by OD_{730} of these cultures at stationary phase increased with fluence. A fluence response curve of the average OD_{730} at stationary phase (calculated between days 7 to 10) is presented in the inset of Fig. 4. Light-activated heterotrophic growth in response to a 5-min pulse of white light is saturated at a fluence rate of about 40 to $75 \mu\text{mol m}^{-2} \text{s}^{-1}$, and a half-maximal response is obtained at about $1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$.

The relative spectral sensitivity of light-activated heterotrophic growth of *Synechocystis* strain 6803 was determined by monitoring OD_{730} and CFU per milliliter of cultures pulsed with a 500-J m^{-2} dose of monochromatic light at wavelengths of 400 to 750 nm in 50-nm increments. The OD_{730} and CFU per milliliter (Fig. 5) at day 10 of cultures pulsed with monochromatic light of wavelengths 550 nm and longer were not significantly different from those of dark control cultures. Monochromatic light of 450 nm supported light-activated heterotrophic growth, based on OD_{730} , to 99% of the white-light-pulsed control, with a doubling time (40 h) comparable to that of the control. Monochromatic

FIG. 4. Effect of the fluence of a daily 5-min light pulse on the growth of *Synechocystis* strain 6803 in liquid medium at 31°C containing 5 mM glucose. Cultures were light pulsed with a daily 5-min irradiation of white light at 75 (○), 40 (●), 12 (△), 1 (▲), and 0.5 (□) $\mu\text{mol m}^{-2} \text{s}^{-1}$ or were dark controls (■). Data are means of three separate growth experiments, each with one culture per condition. (Inset) Fluence response curve for the growth yield of light-pulsed *Synechocystis* strain 6803 liquid cultures. Growth yield is the average OD_{730} attained at stationary phase, calculated between days 7 and 10, derived from the growth curves of the cultures pulsed daily for 5 min with white light of 0.5, 1, 12, 40, and 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and for the dark control culture.

light pulses of 400 and 500 nm also supported light-activated heterotrophic growth of *Synechocystis* strain 6803 but to a lesser extent than did pulses of 450-nm light (doubling times of 43 and 48 h, respectively).

An experiment was designed to determine whether *Syn-*

FIG. 5. Relative spectral sensitivity of the growth of *Synechocystis* strain 6803 in light-pulsed liquid cultures at 31°C containing 5 mM glucose. Plotted are the means and standard errors of the culture OD_{730} ($n = 3$) and CFU per milliliter ($n = 2$) at stationary phase (day 10) of cultures pulsed daily with a 500-J m^{-2} dose of monochromatic light at wavelengths of 400 to 750 nm in 50-nm increments, of cultures pulsed with 2,700-J m^{-2} dose (5 min of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$) of white light (WL), and of dark control cultures (D). Cultures were inoculated to an average initial OD_{730} of 0.0284 and average density of $4.2 \times 10^5 \text{ CFU ml}^{-1}$.

TABLE 1. Effect of supplemental 550- and 650-nm light on the growth of *Synechocystis* strain 6803 cultures pulsed with 450-nm light^a

Light pulse condition	OD_{730} at day 7
450 nm.....	0.53 ± 0.14
550 nm.....	0.22 ± 0.04
650 nm.....	0.17 ± 0.01
450 nm, dark 10 s, 550 nm	0.54 ± 0.10
450 nm, dark 10 s, 650 nm	0.53 ± 0.05
550 nm, dark 10 s, 450 nm	0.48 ± 0.08
650 nm, dark 10 s, 450 nm	0.51 ± 0.10

^a Irradiation times were adjusted such that cultures received a 500-J m^{-2} dose of each wavelength of light energy. Three cultures were inoculated to an initial OD_{730} of 0.025.

echocystis strain 6803 did not grow when pulsed with monochromatic light of wavelengths of 550 nm or longer because light of such wavelengths is inhibitory. Control cultures were individually pulsed with a 500-J m^{-2} dose of blue (450 nm), green (550 nm), or red (650 nm) monochromatic light, and experimental cultures were pulsed initially with a 500-J m^{-2} dose of blue light followed by green or red light or initially with a 500-J m^{-2} dose of green or red light followed by blue light. Cultures were inoculated to an initial OD_{730} of 0.025, and growth was monitored by increase in OD_{730} . Growth at day 7 of the experimental cultures (Table 1) was not significantly different from growth of the blue-light control, indicating that light pulses of these longer wavelengths are not inhibitory to light-activated heterotrophic growth and that when applied before or after a blue-light pulse, they do not enhance growth over that with blue light alone.

Standard procedures for the generation and segregation of cyanobacterial mutants require growth of single colonies on solid media. Light-activated heterotrophic growth of single colonies of *Synechocystis* strain 6803 was achieved by inoculating plates of BG-11 supplemented with 5 mM glucose and solidified with 1.5% (wt/vol) Difco Bacto-Agar purified as described previously (5) with cells from liquid cultures preadapted for five serial subcultures to light-activated heterotrophic conditions (results not shown). Light-activated heterotrophic growth was not observed on plates inoculated from mixotrophic cultures. Plates solidified with unpurified Difco Bacto-Agar, unpurified and purified Sigma Gum agar, unpurified Difco Noble agar, USB Ultra-pure agar, or ICN Gel-Gro agar substitute were tested and failed to support light-activated heterotrophic growth of *Synechocystis* strain 6803. Growth of *Synechocystis* strain 6803 in complete darkness on plates was not observed on any of the solid media listed above. Cells derived from light-activated heterotrophically grown plate colonies selected as described above were used in all of the growth experiments reported in this study.

DISCUSSION

We report here the requirement of a daily, dim-light pulse for growth of *Synechocystis* strain 6803 liquid and plate cultures kept otherwise under complete darkness. The increase in culture OD_{730} (Fig. 1) is the result of cell division and maintenance of cell viability (Fig. 2), although a contribution from an increase in cell size cannot be discounted. Growth yield is dependent on glucose concentration (Fig. 1 and 3). Doubling time is independent of fluence (Fig. 4), but growth yield is dependent on fluence (Fig. 4, insert), consistent with limitation of growth yield by self-shading of cul-

tures. These results are consistent with previous observations of a supplemental light requirement for the growth of single colonies of *Synechocystis* strain 6803 on plates (3, 29). In view of the low fluence rate and the short duration of the light pulse required for growth, it is possible that previous reports of heterotrophic growth of *Synechocystis* strain 6803 in liquid culture (3, 28, 29, 31) may be the result of inadvertent culture irradiation during sampling or handling. The stimulation of heterotrophic growth by exposure to dim light has been reported for other cyanobacterial strains. Brief exposure to low-intensity light of 650 nm stimulates growth and promotes the morphological changes associated with the developmental cycle of a strain of *Nostoc muscorum* and is reversed by exposure to green light (32, 33). van Baalen et al. (46) reported that the heterotrophic growth of two cyanobacterial strains (*Lyngbya lagerheimii* Montauk and *Agmenellum quadruplicatum* PR-6), which did not grow on glucose in the dark, was stimulated by continuous illumination with dim light that did not support photoautotrophic growth in the absence of glucose. It is possible that a daily brief flash of light may suffice for growth of these cyanobacterial strains as well.

The light pulse required for growth of *Synechocystis* strain 6803 does not contribute to the energetic metabolism of the cells by the generation of ATP or NADPH via photosynthetic electron transport, as determined from the following evidence. The light pulse is not sufficient for photoautotrophy, as glucose is required for growth and growth yield is dependent on glucose concentration. The growth of the *psbA* PS II mutant strain at rates similar to those of the glucose-tolerant strain under light-pulsed conditions (Fig. 1) clearly indicates that PS II and whole-chain photosynthetic electron transport are not required for growth. This leaves the possibility that cyclic photosynthetic electron transport occurs during the light pulse, generating a proton gradient coupled to ATP synthesis. If cyclic electron transport around PS I were involved, light of the wavelengths absorbed by the PS I antennae, chlorophyll *a*, would be expected to be active in promoting growth. The active spectral component of the light pulse (Fig. 5; Table 1), however, precludes involvement of cyclic electron transport around PS I since no wavelengths of 550 nm or longer, and specifically those in the long-wavelength region contributing to the action spectrum of PS I (49), were capable of supporting growth of *Synechocystis* strain 6803. Finally, light-activated heterotrophic growth of *Synechocystis* strain 6803 has been used successfully in our laboratory to select a homozygous mutant in which the PS I core gene, *psaA*, has been insertionally inactivated and which completely lacks PS I activity (43a). We conclude that the active blue-light pulse does not serve as a source of metabolic energy via photosynthetic electron transport processes.

The blue-light requirement for growth of *Synechocystis* strain 6803 under otherwise complete darkness has been termed light-activated heterotrophic growth. This is to distinguish *Synechocystis* strain 6803, which requires a brief pulse of light for growth, from cyanobacteria that can grow heterotrophically under complete darkness. For example, the filamentous, nitrogen-fixing cyanobacterium *Anabaena variabilis* Kütz. (ATCC 29413), which is reported to grow heterotrophically on 5 mM fructose with a doubling time of 24 h (43), was cultured under complete dark conditions, and we confirm that it did indeed grow well both in liquid and on plates under these strictly heterotrophic conditions (data not shown).

Many cyanobacterial processes are regulated by light

wavelength. For example, complementary chromatic adaptation of the light-harvesting phycobilisome composition (4, 6, 18) and photoinduction and photoreversal of development of filamentous strains (17, 32, 33) are regulated in response to light of green and red wavelengths, and a red-far red light control of spore germination of *Anabaena fertilissima* has been reported (36). The relative spectral sensitivity of light-activated heterotrophic growth of *Synechocystis* strain 6803 reported here is consistent with the participation of a blue-light photoreceptor.

Blue-light controls many developmental processes, including cell growth, cell cycle (21), and synchrony of cell division (26) in fern gametophytes; circadian rhythms in *Neurospora* spp. (40); stomatal opening (2); leaf movement (34); and inhibition of stem elongation (12). Chloroplast differentiation and regulation of nuclear and chloroplast gene transcription in cultured cells (37) and chloroplast gene transcription in intact plants (19, 20, 22) have been shown to be regulated by blue light. In cyanobacteria, the major point of regulation of carbon flow through the oxidative pentose phosphate cycle is at the level of glucose-6-phosphate dehydrogenase, primarily through inhibition by NADP⁺, ribulose-1,5-bisphosphate (42), and light, via thioredoxin (8, 13). Blue light stimulates increased respiration of stored carbohydrate (30) and has been shown to have a generally positive effect on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and to effect a number of glycolytic enzymes in plants and green algae (41).

The blue-light pulse required for the light-activated heterotrophic growth of *Synechocystis* strain 6803 may function as an environmental signal possibly regulating heterotrophic metabolism, cell cycle, or cell division directly at the physiological level or at the level of gene expression. Reports of blue-light effects on cyanobacterial processes are limited to effects on photosynthetic products (24), nitrate reductase (45), and ribulose-1,5-bisphosphate carboxylase/oxygenase (11). Control of cell cycle or cell division by a pulse of blue light may be expected to induce synchronous cell division of light-activated, heterotrophically grown *Synechocystis* strain 6803. Synchronous cell division has been induced in the cyanobacterium *Anacystis nidulans* by light and CO₂ deprivation (25), by alternating light-dark conditions (1a), and with light periods much shorter than the generation time (14). We are currently investigating the possibility that light-activated heterotrophic growth of *Synechocystis* strain 6803 occurs by synchronous cell division. Although further characterization of the mechanism of blue-light action on the light-activated heterotrophic growth of *Synechocystis* strain 6803 is required, the work reported here represents the observation of a novel blue-light effect on the growth of a cyanobacterium.

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