Disruption of a *Synechocystis* sp. PCC 6803 gene with partial similarity to phytochrome genes alters growth under changing light qualities

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Abstract A gene that may encode a novel light sensing histidine protein kinase, designated *plpA* (phytochrome-like protein), was isolated from the cyanobacterium *Synechocystis* sp. PCC 6803. The 200 COOH-terminal amino acids of the gene product show homology with conserved domains of several bacterial histidine kinases and the ethylene response gene *etr1* of *Arabidopsis*, whereas its central region is similar to the chromophore attachment site of plant phytochromes. Interruption or partial deletion of *plpA* yielded mutants unable to grow under blue light.

Key words: Two-component system; Phytochrome; Light quality; *Synechocystis* 6803

1. Introduction

In bacteria, protein phosphotransfer reactions play a central role in signal transduction pathways, regulating a variety of adaptive responses to changing environmental conditions. Autophosphorylating histidine kinases are part of the two-component signaling systems of bacteria. They can be identified on the basis of their conserved sequences that generally extend for approximately 200 residues and correspond to a domain that catalyzes the phosphorylation of a histidine [1]. This phosphate is then transferred to an aspartate residue in a response regulator, the second component of this regulatory system. Although members of these two families have been identified in a wide range of bacterial taxa, eukaryotic two-component regulators have been found only recently, including putative members of two-component systems in *Saccharomyces cerevisiae* [2,3], *Neurospora crassa* [4], the ethylene response genes *etr1* and *ers* in *Arabidopsis* [5,6] and their homologues in tomato [7]. In addition, bacterial sensor protein-like sequences were noted in some plant phytochrome genes [8,9]. Phytochrome is a photoreceptor that is capable of detecting and processing information from the natural light environment. The red/far red reversible chromoprotein regulates a wide variety of photomorphogenic responses in plants which include leaflet movement, seed germination as well as induction of flowering. The presence of multiple phytochrome genes and the complex pattern of phytochrome action on the plant organism led several researchers to use more simple systems, like mosses or green algae [9]. However, the molecular basis of signal transduction by phytochrome remains to be elucidated.

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Abbreviations: WL, white light; FRL, far red light; RL, red light; BL, blue light; Chl, chlorophyll; PC, phycocyanin; PS, photosystem; PBS, phycobilisome

There was no evidence for the presence of homologues to phytochrome in phototrophic bacteria, since a sequencing project on the cyanobacterium *Synechocystis* sp. PCC 6803 revealed a putative histidine kinase of 748 amino acids [10], exhibiting 45% identity to 170 residues surrounding the chromophore binding site in plant phytochromes. Comparatively little is known about the nature of different signal transduction pathways in cyanobacteria, especially regarding light responses. Cyanobacteria respond to changes in both the intensity and spectral quality of light. These responses to changes in light quality include long-term adaptation mechanism regulating the stoichiometry of photosystem (PS) I and PSII in the thylakoids, complementary chromatic adaptation and state transitions controlling the distribution of excitation energy transfer from the light-harvesting antenna complexes to the two PS. With the help of these regulating mechanisms cells are able to compensate for the imbalance of light absorption by the two PS (reviewed in [11,12]).

The cyanobacterial strain *Synechocystis* sp. PCC 6803 used in this study is naturally transformable by exogenous DNA [13] and can grow (photo)heterotrophically without photosynthetic activity [14,15]. Using gene replacement via homologous recombination, many directed mutations have been introduced into photosynthetic genes [16–18] as well as genes with unknown function [19].

In this paper, we describe the isolation and inactivation of a new *Synechocystis* 6803 gene, which encodes a putative histidine kinase exhibiting a limited sequence similarity to residues 238–384 spanning the chromophore attachment site of tobacco *phyB* gene. The data on initial characterization of the constructed *plpA* mutants show that interruption of this gene leads to an altered response of *Synechocystis* 6803 cells to different light qualities.

2. Materials and methods

2.1. Growth conditions

Synechocystis sp. PCC 6803 wild type and mutant strains were grown at 30°C in BG-11 medium [20] in batch culture. Transformants of *Synechocystis* 6803 were selected on media with increasing amounts of kanamycin (10–50 μg ml^{-1}) [19]. Growth of the wild type and mutant strains was followed under photoautotrophic and photoheterotrophic (5 mM glucose) conditions by monitoring the optical density at 750 nm (Lambda 2, Perkin Elmer, Germany) and cell number by light microscopy (hemocytometer, Thoma, Bad Blankenburg, Germany). Cells were grown under white light (WL) at a light intensity of approximately 30 μmol photons m^{-2} s^{-1}. Different light qualities were obtained by a couple of blue and red plastic cutoff filter foils (VEB ORWO, Germany) opening from 690 nm with τ1/2 at 719 nm (far red light (FRL), 31 μmol photons m^{-2} s^{-1}) and opening from 620 nm with τ1/2 at 666 nm (red light (RL), 8.7 μmol photons m^{-2} s^{-1}). Blue light (BL) (30 μmol photons m^{-2} s^{-1}) had about 400 nm to 510 nm bandwidth peaking at 472 nm. Cultures were always inoculated from WL precultures during exponential growth at an initial cell

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concentration of 8.0×10^6 cells ml^{-1} and allowed to adapt to the new light regime for a minimum of 52 h. Irradiance was measured with a high-resolution spectroradiometer SR-9910 (Macam Photometrics Ltd, Livingston, UK).

2.2. DNA isolation and hybridization procedures

Genomic DNA of *Synechocystis* 6803 was extracted as described by Franche and Damerval [21]. After digestion of DNA by restriction endonucleases and electrophoresis, gel blotting to nylon membranes (Hybond N, Amersham, Braunschweig, Germany) was performed. DNA probes were labeled with [α-^{32}P]dCTP (Amersham, Braunschweig, Germany) by the random priming method as recommended in the instructions of the manufacturer. DNA gel blot hybridizations were carried out as described [22] with a mixture of gene-specific *Eco*RI and *Eco*RI/*Hin*dIII fragments of *Synechocystis* DNA.

2.3. Cloning, sequence analysis and inactivation

Restriction endonucleases were obtained from Amersham (Braunschweig, Germany) and used according to the manufacturer's recommendations. The *plpA* sequence was identified in a *Lorist6* cosmid genomic library of *Synechocystis* 6803 [23]. Sequence reactions were carried out using a thermal cycle amplification system (BioRad, Hercules, CA), and analyzed on an ABI 373 automatic DNA sequencer (Applied Biosystems Inc., Perkin Elmer, Fullerton, Ca). The nucleotide sequence of a 4697-bp region of the *Synechocystis* 6803 genome containing the *plpA* gene has been submitted to GenBank as accession number U67397 (cross-reference: sll1124, according to the CyanoBase at Kazusa DNA Research Institute).

A 5.7 kb *Hin*dIII/*Pst*I fragment containing a part of *plpA* was subcloned into pSPORT 1 (Gibco, BRL) and used to construct cyanobacterial mutants (Fig. 1). The 1.2 kb *Eco*RI fragment from pUC4K [24] served as a kanamycin resistance gene cartridge. *Synechocystis* cells were transformed following the protocol described in [25].

2.4. Absorption measurements

In vivo absorption spectra of whole cells of wild-type *Synechocystis* 6803 and its *plpA* mutants M58 and M57 suspended in BG-11 medium were measured at room temperature using a Lambda 2 spectrophotometer, equipped with an integrating sphere 'Labsphere' (Perkin-Elmer, Germany). The phycocyanin (PC) to chlorophyll (Chl) ratios were calculated from the absorption spectra using the equations of Myers et al. [26].

3. Results and discussion

3.1. Sequence analysis

A *Synechocystis* 6803 gene that shows partial homology to plant phytochrome genes was isolated from a genomic library by using a maize iojap gene [27] as a probe under conditions of very low stringency. Subsequent sequencing revealed an open reading frame of 4113 bp. The amino acid sequence of the predicted protein is outlined in Fig. 2. The putative gene product would comprise 1371 amino acids with a calculated mass of 154.6 kDa and an isoelectric point of 5.1. From the hydropathy profile it appears to be a soluble protein. The sequence was used to search DNA databases. There was no similarity to the sequence originally used as a probe. Instead, the central 708–820 amino acid residue region exhibited 21–25% identity and about 45% similarity to the conserved NH_2-terminal chromophore domain of known phytochromes. Therefore, the protein has been designed phytochrome-like protein, *plpA*. The COOH-terminus shows striking similarity to the bacterial two-component histidine kinase family (Fig. 2). The relatedness of a COOH-terminal section of plant phytochrome sequences to bacterial sensor proteins was first noted by Schneider-Poetsch et al. [8]. Lagarias et al. [9] mentioned a new structural motif in moss phytochromes indicating evolutionary relatedness to several bacterial sensor kinases, and to a family of eukaryotic regulatory proteins. This conserved motif is also present in *plpA* (amino acid residue

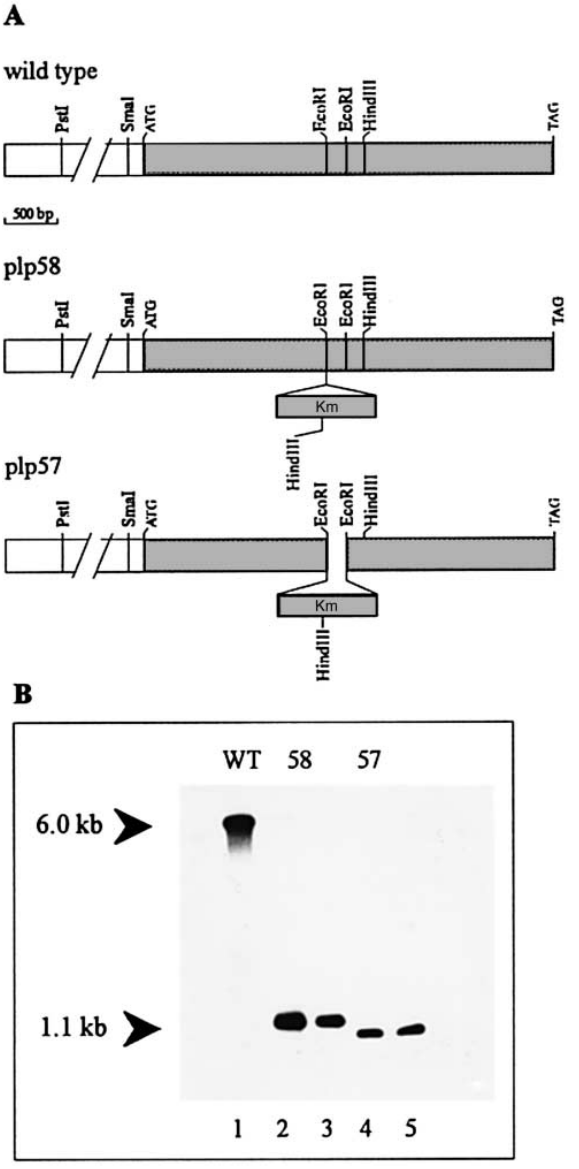

Fig. 1. Inactivation of *plpA* in *Synechocystis* 6803. A: Restriction maps of the wild-type *plpA* gene fragment and strategy for constructing the donor plasmids with inactivated (plp58) and deleted (plp57) *plpA* copies. Km^r, kanamycin resistance. B: DNA gel blot analyses of *Hin*dIII-digested genomic DNA isolated from the *Synechocystis* 6803 wild type (WT, lane 1), *plpA* mutants M58 (58, lanes 2, 3) and M57 (57, lanes 4, 5). The intragenic 395-bp *Eco*RI and *Eco*RI-*Hin*dIII fragments were mixed and used as a probe.

877–919). From homology searches it can be deduced that *plpA* is composed of four major domains, the 700 NH_2-terminal residues, that have no similarity to known genes, the central region with homology to phytochromes, the COOH-terminal histidine kinase domain and a region that links the putative chromophore and the histidine kinase domain. Interestingly, this linking domain also shows sequence similarity to the *Arabidopsis* gene *etr1*.

Recently, Kehoe and Grossman [28] reported on a similar gene, *rcaE*, in the cyanobacterium *Freymella diplosiphon* that they expect it to be involved in regulation of complementary chromatic adaptation. Since *Synechocystis* 6803 is not able to undergo this kind of light adaptation, the functional role of *plpA* should be different. Interestingly, the recently completed sequence of the *Synechocystis* 6803 genome revealed the pres-

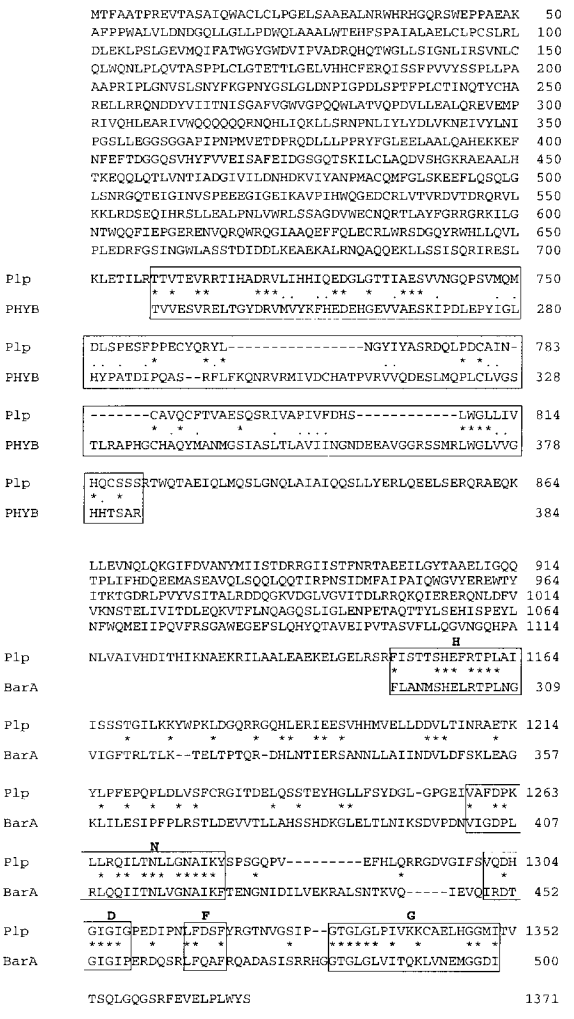

Fig. 2. Comparison of the deduced amino acid sequence of *plpA* with the chromophore attachment domain of tobacco PHYB [32] and with the histidine kinase domains of *E. coli* BarA [33]. Asterisks indicate identical residues, points indicate conservative substitutions.

ence of some more genes with a certain degree of similarity to phytochrome genes. Most intriguing was the discovery of a putative phytochrome gene that exhibits a well conserved histidine kinase domain at its COOH-terminus as well [10].

3.2. *Inactivation of Synechocystis 6803 plpA gene*

To create mutants lacking *plpA*, a kanamycin resistance cassette was inserted into the *plpA* open reading frame in two different positions. The strategy for inactivation of *plpA* is outlined in Fig. 1. Disruption or partial deletion of *plpA* within the chromosome was confirmed by DNA gel blot analyses (Fig. 1). After four restreakings of transformants, no wild-type copies of the gene were detected in the transformed cells. One of five transformants with disrupted *plpA* sequence (named M57) and one of seven deletion mutants (M58), which were tested for homozygoty, were chosen for further studies. Disruption of a bacterial gene could have (polar) effects on the expression of neighboring genes, in particular if cotranscribed into a polycistronic messenger. According to the sequence of the *Synechocystis* 6803 chromosome, there is an open reading frame potentially encoding 256 amino acids overlapping with 4 bp at the 5′ end of *plpA*. There is no indication of a possible function of this open reading frame [10]. Even if expressed, it is not very probable that the mutation of *plpA* could have an influence on the expression of this putative open reading frame located about 1900 bp upstream of the inactivation site. A gene for a ribosomal protein (*rps20*) is located about 100 bp downstream of *plpA* [10]. Ribosomal function seems to be unaffected in our mutants.

3.3. *Analysis of plpA mutants*

Both independently constructed mutant strains showed an identical phenotype. Interestingly, mutants M57 and M58 were very mucous, in particular when growing in medium supplemented with glucose. Under all light regimes used, photoheterotrophic conditions induced fairly increased growth rates since the strains were grown under limited light conditions in order to facilitate the detection of light effects. When grown under WL photoheterotrophic conditions, no significant differences were found in the growth rates between the wild type and any of the mutant strains. In the absence of glucose, however, mutants grew considerably slower than the wild type, with a more than 25% increase in their doubling time as shown for mutant M57 in Fig. 3. Surprisingly, mutants could not grow under BL, whilst wild-type cells did. Photoheterotrophic conditions restored the ability of the mutants to grow under BL, though photoheterotrophic growth rates remained distinctly lower than observed for the wild type. Under RL (not shown) and FRL the doubling rates of the wild type and mutants were almost the same (Fig. 3).

Whole-cell absorption spectra were used to monitor the pigmentation of the cells in exponential growth (at $OD_{750} = 0.4–0.6$). In the case of the mutants, which did not grow under BL, measurements were made after 52 h of adaptation to BL conditions (Fig. 4). OD_{680} reflects mainly Chl and OD_{628} mainly PC absorption. The PC to Chl ratios for WL-grown cultures were 0.88 ± 0.02 and 0.99 ± 0.01 for the mutant M58 (M57 gave comparable data in all experiments) and the wild type, respectively. Under FRL (for which Chl is the principal absorber) mutant and wild type showed nearly the same PC to Chl ratios (0.82 ± 0.02). However, the differences in the PC to Chl ratios between WL and FRL conditions are rather small for the mutants compared with the wild type.

Generally, the acclimatization to different light qualities is a long-term response that includes the adjustment of the PS stoichiometry. That is accompanied by significant alterations in the pigment composition of the organisms [29]. Kim et al. [30] suggested that signal perception for PS stoichiometry adjustment occurs in plants as well as in cyanobacteria at the thylakoid membrane level as differential sensitization of the pigments associated with the two PS. It remains to be shown

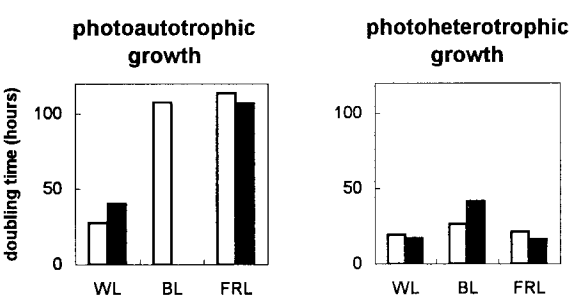

Fig. 3. Growth of wild type (empty bars) and mutant M57 (black bars) adapted to WL, BL and FRL under photoautotrophic and photoheterotrophic conditions.

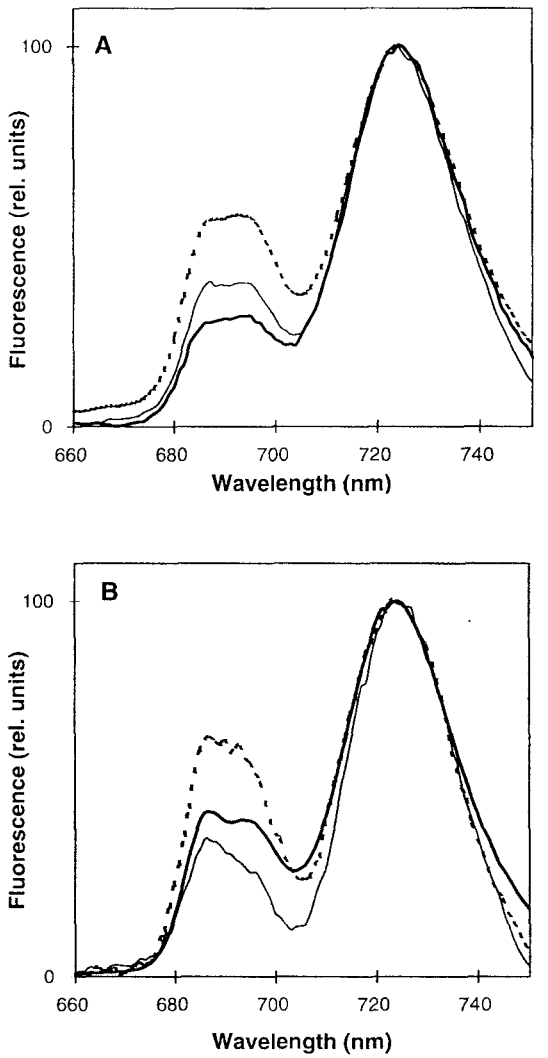

Fig. 4. In vivo absorption spectra of the wild type (A) and mutant M58 (B). The spectra were normalized to the Chl *a* absorption peak at 680 nm. Spectra were measured using whole cells acclimatized to WL (thick solid line), BL (dotted line), RL (gray line) and FRL (solid line).

if the altered acclimatization of *plpA* mutants to FRL (in relation to WL) is a direct or a secondary effect of the mutation. Interestingly, under BL, also primarily absorbed by Chl, the PC to Chl ratios of the wild type and the mutant strains are increased by 13% in comparison to WL. This indicates that the mutants are able to adapt to BL in a similar way as the wild type does, i.e. this adaptation is independent of the function of the *plpA* gene. However, the data described above indicate that *plpA* is needed for growth under BL. *Synechocystis* 6803 needs light (several minutes per day) for growth even if supplied with glucose [15] and the *plpA* product might act as one of several light receptors involved in light-dependent regulation of growth. Clearly, more work is needed to elucidate the nature of this putative photosensor and the signal transduction processes leading to the cell responses.

Recent studies [10,28] and our results establish that cyanobacteria contain phytochrome-related genes which seem to be members of two-component systems of signal transduction. According to the endosymbiont hypothesis [31] cyanobacteria are the ancestors of chloroplasts. Therefore, it is tempting to speculate that one or more of the discovered cyanobacterial genes may represent the evolutionary origin of plant phytochrome genes.

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