PsaK2 SUBUNIT IN PHOTOSYSTEM I IS INVOLVED IN STATE TRANSITION UNDER HIGH-LIGHT CONDITION IN THE CYANOBACTERIUM *Synechocystis* SP. PCC 6803

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Running Title: The role of PsaK2 subunit in state transition

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In order to avoid the photodamage, cyanobacteria regulate the distribution of light energy absorbed by phycobilisome antenna either to photosystem II (PSII) or to photosystem I (PSI) upon high-light acclimation by the process so called state transition. We found that an alternative PSI subunit, PsaK2 (*sll0629* gene product), is involved in this process in the cyanobacterium *Synechocystis* sp. PCC 6803. Examination of the subunit composition of the purified PSI reaction center complexes revealed that PsaK2 subunit was absent in the PSI complexes under low-light condition, but was incorporated into the complexes during acclimation to high light. The growth of the *psaK2* mutant on solid medium was inhibited under high-light condition. We determined the photosynthetic characteristics of the wild type strain and the two mutants, the *psaK1* (*ssr0390*) mutant and the *psaK2* mutant, using pulse amplitude modulation (PAM) fluorometer. Non-photochemical quenching (qN), which reflects the energy transfer from phycobilisome to PSI in cyanobacteria, was higher in high-light grown cells than in low-light grown cells, both in the wild type and the *psaK1* mutant. However, this change of qN during acclimation to high light was not observed in the *psaK2* mutant. Thus, PsaK2 subunit is involved in the energy transfer from phycobilisome to PSI under high-light condition. The role of PsaK2 in state transition under high-light condition was also confirmed by chlorophyll fluorescence emission spectra determined at 77 K. The results suggest that PsaK2-dependent state transition is essential for the growth of this cyanobacterium under high-light condition.

The effective absorption of light energy is the first step in photosynthesis. All oxygenic photosynthetic organisms share common core antenna pigments of about 40 chlorophyll *a* in PSII and about 100 chlorophyll *a* in PSI (1). Cyanobacteria have an additional light-harvesting system, phycobilisome, which is primarily associated with PSII (2, 3). Thus, cyanobacteria use two kinds of antenna pigments with totally different absorption wavelengths.

Since neither light quality nor light quantity is constant in natural environments, cyanobacteria have to distribute the light energy absorbed by antenna pigments to two photosystems so as to optimize the photosynthetic performance in response to changing light environments. When cells are exposed to illumination favoring either PSII or PSI, the distribution of light energy between two photosystems would be adjusted (4). When cells have been pre-illuminated with light mainly absorbed by phycobiliproteins (PSII light), the energy transfer to PSI increases while that to PSII decreases (state 2). Pre-illumination of cells with light mainly absorbed by chlorophyll *a* (PSI light) causes a reverse effect leading to the increase of energy transfer to PSII and the decrease of that to PSI (state 1) (5). Distribution of light energy to two photosystems is regulated in response to light regime not only in cyanobacteria but also in green algae and higher plants. This regulation of energy distribution between PSII and PSI has been generally called ‘state transition’. Since its discovery in 1969 (6, 7), many research groups have been involved in the research of state transition in cyanobacteria, green algae and higher plants.

State transition is induced not only by the change in light quality but also by the change

in light quantity. When exposed to excess light, cyanobacteria are able to change the distribution of light energy between two photosystems. Under normal-light condition, the light energy absorbed by phycobilisome is preferentially transferred to PSII. Under high-light condition, on the contrary, the energy can be transferred not only to PSII but also to PSI (8). In this case, the physiological significance may not be in the effective photosynthesis but in the avoidance from the photodamage.

In old days, phycobilisome was assumed to act as a light-harvesting antenna solely for PSII. However, energy transfer studies on the wild type (9) and a PSII-deficient mutant (10) of *Synechocystis* sp. PCC 6803 indicated that phycobilisome could interact with and efficiently transfer energy to PSI, depending on the growth condition of the cells. Phycobilisome was found to be able to move rapidly on thylakoid membranes (11, 12). The study using inhibitor of electron transport suggested that state transition was controlled by the redox state of the plastoquinone pool via cytochrome b_6f complex (13). Oxidation of plastoquinone induces state 1 while reduction induces state 2. As for proteinous factors, it was reported that ApcD and ApcF, phycobilisome core components, were involved in state transition (14). RpaC (regulator of phycobilisome association) was also shown to function in state transition (15): The *rpaC* gene was isolated by screening for mutants displaying no changes in fluorescence yield in response to changes in light quality. The deduced amino acid sequence of the *rpaC* gene has no recognizable sequence motifs. The *rpaC* mutant can perform state transition upon excitation of chlorophyll but not upon excitation of phycobilisome. At very low photon flux densities of yellow or white light, the growth of the *rpaC* mutant was slower than that of the wild type. However, actual function of this protein is unknown. Moreover, we have no information on the possible interaction of phycobilisome with PSI under different light regimes.

Upon acclimation of cells of *Synechocystis* sp. PCC 6803 to high light, the expression of all the PSI genes was simultaneously suppressed, with only one exception of the *psaK2* gene (16). The expression of *psaK2* was eminently induced after the shift of cells to high light, implying that PsaK2 might be involved in high-light acclimation. PsaK is a subunit of PSI complexes and localized on the outside edge of cyanobacterial PSI trimer (17). The genomic DNA of *Synechocystis* sp. PCC 6803 contains two unlinked *psaK* genes, *psaK1* and *psaK2*. Cyanobacterial *psaK* seems to be the common ancestor of higher plants *psaK*/*psaG* (18). Cyanobacterial genomes contain one to three *psaK* genes while only one *psaK* gene has been found in green algae and higher plants so far. Deduced amino acid sequences of the *psaK1* and *psaK2* genes in *Synechocystis* sp. PCC 6803 are notably different in length (86 amino acids for the PsaK1 protein and 128 amino acids for the PsaK2 protein) due to long N-terminal extension in PsaK2. The two gene products show 42% homology with each other. In the past studies, both the *psaK1* and *psaK2* mutants were characterized, but clear phenotype was not observed under normal growth condition (19, 20). In this study, we investigated the physiological role of PsaK2 in *Synechocystis* sp. PCC 6803 under high-light condition. The results indicate that PsaK2 is involved in the energy transfer from phycobilisome to PSI under high-light condition, and that PsaK2-dependent state transition is essential for the growth of this cyanobacterium under high-light condition.

Experimental Procedures

Growth of cultures and strains - Wild type and mutant strains of *Synechocystis* sp. PCC 6803 were grown in BG-11 medium (21) supplied with 10 mM TES. Liquid cultures were grown at 30˚C in 50 ml glass tubes and bubbled with air under continuous illumination provided by fluorescent lamps. Photon flux density at 20 and 200 µmol m^{-2} s^{-1} was regarded as low light and high light, respectively. The *psaK1* mutant was constructed by the replacement of nucleotide sequence corresponding to 6th to 70th amino acid of PsaK1 with the kanamycin-resistant cassette using two *Msc*I sites in the *psaK1* gene (19). The *psaK2* mutant was constructed by insertion of the kanamycin-resistant cassette into *Age*I site in the *psaK2* gene. The *rpaC* gene was disrupted by insertion of the spectinomycin-resistant cassette into *Sty*I site in the *rpaC* gene. Mutants were usually maintained with kanamycin and/or spectinomycin at the final concentration of 20 µg ml^{-1}.

Analysis of chlorophyll fluorescence - Chlorophyll fluorescence was measured with a pulse-amplitude modulation chlorophyll fluorometer (PAM 101/102/103, Heinz Waltz,

Effeltrich, Germany) with a high sensitivity emitter-detector unit (ED-101US, Heinz Waltz, Effeltrich, Germany) as described in Sonoike et al. (22). Cells were dark-adapted for 5 min and then the measuring light was turned on to obtain the minimal fluorescence level, Fo. The stable level of fluorescence (Fs) was determined during exposure of cells to actinic light with defined photon flux density (KL 1500, Schott, Wiesbaden, Germany). The fluorescence level with fully reduced Q_A (Fm') was obtained by applying multiple turnover flashes (XMT 103, Heinz Waltz, Effeltrich, Germany) under actinic light. The far-red light from a photodiode (FR 102, Heinz Waltz, Effeltrich, Germany) was applied just after turning off the actinic light to determine Fo'. The maximum fluorescence (Fm) was obtained by adding 10 μM DCMU to the sample under actinic light. Photochemical quenching (qP) and non-photochemical quenching (qN) were calculated as (Fm'-Fs)/(Fm'-Fo') and 1-[(Fm'-Fo')/(Fm-Fo)], respectively.

Fluorescence emission spectra - Low temperature fluorescence emission spectra at 77 K were recorded using a custom-made apparatus as described by Sonoike and Terashima (23). Cells acclimated to low- or high-light condition for 24 h were collected and concentrated by centrifugation. Cells were adjusted to a concentration of OD_{730}=2.0 (high-light acclimated cells) or OD_{730}=1.0 (low-light acclimated ones) with BG-11. State 1 conditions were achieved by illumination of cells with blue light (100 μmol m^{-2} s^{-1}) in the presence of 10 μM DCMU for 2 min and subsequent freezing in liquid nitrogen under the same light condition. State 2 conditions were achieved by dark incubation of cells for more than 10 min and subsequent freezing in liquid nitrogen in the dark.

Isolation of thylakoid membranes and purification of PSI complexes - Cyanobacterial cells of the wild type and the mutant strains were harvested and suspended in 0.4 M sucrose, 10 mM NaCl, 1 mM $CaCl_2$, 0.2 mM phenylmethylsulfonyl fluoride, 5 mM benzamidine, and 50 mM 3-morpholinopropanesulfonic acid (MOPS) (pH 7.0) (24). Cells were then broken with a bead-beater (Model 1107900, Biospec Products, Bartlesville, America). Unbroken cells and debris were removed by low speed centrifugation at 4,700 x g. Thylakoid membranes were pelleted by centrifugation at 140,000 x g for 45 min and resuspended in 50 mM MOPS (pH 7.0). After incubation for 30 min at room temperature with 1 mM $CaCl_2$ in 50 mM MOPS (pH 7.0) to stimulate trimerization of PSI complexes, thylakoid membranes were solubilized with β-dodecyl-maltoside (DM) for 15 min on ice. The ratio of chlorophyll to β-dodecyl-maltoside was 1:15. The solubilized thylakoid membranes were centrifuged at 15,000 x g for 15 min and the resulting supernatant was layered on a 10-30% step gradient of sucrose in 10 mM MOPS (pH 7.0) and 0.05% β-dodecyl-maltoside (25). Three distinct bands were resolved after ultracentrifugation at 140,000 x g for 4 h with an angle rotor (70T, Hitachi, Yokohama, Japan). The upper orange band contained carotenoid, the middle green band contained PSII complexes and monomers of PSI complexes, and the lower green band contained trimers of PSI complexes. The lower band was collected and subjected to non-denaturing gel electrophoresis (26) to purify the trimer form of PSI complexes. The band of PSI trimer was cut out and homogenized in 50 mM MOPS (pH 7.0). Gel fragments were removed by centrifugation at 1350 x g for 10 min, and the purified PSI complexes in the supernatant was pelleted by centrifugation at 550,000 x g for 1 h, and resuspended in 50 mM MOPS (pH 7.0). All the centrifugations were carried out at 4˚C. The purity of PSI was confirmed by the measurement of low temperature fluorescence emission spectra (data not shown).

SDS-PAGE - Subunits of the purified PSI complexes were resolved by SDS-PAGE using 16-22% acrylamide gel containing 7.5 M urea (27). Before electrophoresis, the samples were treated with 5% lithium dodecyl sulfate and 60 mM dithiothreitol for 1 hr at room temperature. Sample equivalent to 1 μg chlorophyll was loaded on each lane. Chlorophyll *a* concentrations were determined after extraction with 100% methanol (28). After electrophoresis, gel was stained using a Silver Stain Plus Kit (BioRad, Hercules, CA).

RESULTS

Although the *psaK2* gene expression specifically increased during acclimation to high light (16), the amounts of PsaK1 and PsaK2 proteins in PSI complex of high-light acclimated cells were unknown. We purified PSI complex

from the wild type cells grown under low- (20 μmol m^{-2} s^{-1}) and high-light (200 μmol m^{-2} s^{-1}) conditions, and also from the *psaK1* and the *psaK2* mutant cells grown under high-light condition. Proteins in the purified PSI complexes were resolved by SDS-PAGE and visualized by silver staining (Fig. 1). PSI complex isolated from low-light acclimated wild type contained a considerable amount of PsaK1 subunit with only a trace amount of PsaK2. High-light treatment increased the amount of PsaK2 in PSI complexes. In PSI complexes from high-light acclimated *psaK1* or *psaK2* mutant, PsaK1 or PsaK2 subunit was missing, respectively, while the amount of the other subunits of PSI was not affected, being good agreement with a previous report (20). Furthermore, we confirmed that the protein which increased under high-light condition was PsaK2 subunit by using MALDI-TOF mass spectrometry (data not shown).

To examine whether the failure to accumulate PsaK2 subunit in PSI affects the growth under high-light condition, we examined the growth property of the wild type as well as the *psaK1* and the *psaK2* mutants. 10 μl of the liquid culture was spotted on an agar plate, and grown under low- or high-light condition for three days. Little differences in the growth between the wild type and the two mutants were observed under low-light condition (Fig. 2A). Under high-light condition, the growth of the *psaK2* mutant was considerably slower compared with that of the wild type and the *psaK1* mutant (Fig. 2B). These results demonstrate that the accumulation of PsaK2 in PSI is essential for the growth of this cyanobacterium under high-light condition.

We then determined the photosynthetic characteristics of the wild type and the *psaK1* and the *psaK2* mutants grown under low- and high-light conditions for 24 h using PAM fluorometer. The photon flux density of actinic light was fixed at 200 μmol m^{-2} s^{-1} in the determination both for low- and high-light acclimated cells. In low-light acclimated cells, we observed no significant differences between the wild type and the mutants in relation to non-photochemical quenching (qN) as well as photochemical quenching (qP) (Table 1). In high-light acclimated cells, however, qN in the wild type and the *psaK1* mutant was notably higher than that in the *psaK2* mutant, although qP were invariable between the wild type and the mutants. In the fluorescence emission trace, the difference between Fm' and Fm reflected the decrease in fluorescence intensity because of qN (Fig. 3). Thus, the increase of qN upon high-light acclimation, which reflects state transition in cyanobacteria, was specifically suppressed in the *psaK2* mutant.

Since the previous result suggested the role of PsaK2 in state transition, we examined the energy transfer from phycobilisome to two photosystems by monitoring 77 K emission spectra of chlorophyll fluorescence from cells either in state 1 or in state 2. When cells were excited with blue light (400-600 nm) absorbed by phycobilin and chlorophyll *a*, the fluorescence emission spectra for cells adapted to either state 1 or state 2 had emission peaks at 663, 685, 695 and 725 nm. The peak at 663 nm arises from phycobilisome. The peak at 685 nm arises from PSII and possibly from the terminal emitters of phycobilisome. The 695 nm peak arises from PSII and the 725 nm peak from PSI. In the wild type and the *psaK1* mutant grown under high-light condition, relative intensity of PSI fluorescence was much greater in state 2 than in state 1 (Fig. 4A and B; Table 2). This increase of PSI fluorescence in state 2 reflects the enhanced energy transfer from phycobilisome to PSI. In the *psaK2* mutant, relative intensity of PSI fluorescence in state 2 was very close to that in state 1 (Fig. 4C; Table 2). These results indicate that high-light acclimated *psaK2* mutant is not able to perform state transition, supporting the conclusion drawn from the analysis of non-photochemical quenching (Table 1). The relative intensity of PSI fluorescence was greater in low-light acclimated cells than in high-light acclimated cells (compare Fig. 4 and Fig. 5) due to the regulation of photosystem stoichiometry in acclimation to the changes in photon flux density (29). In low-light acclimated cells of the wild type and the *psaK1* and the *psaK2* mutants, the relative intensity of PSI fluorescence was greater in state 2 than in state 1, indicating that the *psaK2* mutant as well as the wild type can perform state transition under low-light condition (Fig. 5; Table 2). PsaK2-dependent state transition is apparently specific to high-light condition.

We also examined state transition of the *psaK2rpaC* double mutant by chlorophyll fluorescence emission spectra at 77 K. In high-light acclimated cells, the *psaK2rpaC* double mutant displayed the same phenotype as the *psaK2* mutant (data not shown). In low-light

acclimated cells, the double mutant can partially perform state transition (data not shown). Thus, there seems to be third mechanism for state transition, which is independent both of PsaK2 and RpaC.

Using BLAST search program, we searched two types of PsaK proteins (PsaK1 type and PsaK2 type) in databases in order to examine whether this mechanism is conserved in other cyanobacteria. Two other cyanobacteria, *Synechococcus elongatus* PCC 7942 and *Trichodesmium erythraeum* IMS 101 have two types of PsaK proteins as in *Synechocystis* sp. PCC 6803 (Fig. 6). Black boxes in the figure show the amino acid residues conserved only in PsaK1 type or in PsaK2 type protein. These amino acid residues might be responsible for the specific function of each protein. When unrooted dendrogram is constructed by Neighbor Joining method by Clustal W program (http://clustalw.genome.jp/), it is evident that PsaK1-type proteins and PsaK2-type proteins form distinct clades (Fig. 7). In the genomes of *Nostoc punctiforme* and *Thermosynechococcus elongatus* BP-1, there is only one *psaK* gene in each genome, PsaK1 type in the former and PsaK2 type in the latter. For the marine type cyanobacteria, *Prochlorococcus* and marine *Synechococcus*, only one *psaK* gene is present in distinct clade which is closer to PsaK1-type clade. One of *psaK* genes in *Anabaena* PCC 7120 is PsaK1 type, but other two genes are rather divergent and form the forth clade of cyanobacterial PsaK. PsaK and PsaG of higher plants seem to be equally distant from cyanobacterial PsaK as previously suggested (18).

DISCUSSION

Upon state transition of cyanobacteria, energy flow from phycobilisome is redirected to PSI. In cyanobacteria, several components of phycobilisome such as ApcD and ApcE, were reported to be involved in state transition (14, 15). However, the factors on PSI side have not been reported. Here, we reported for the first time that PsaK2, a subunit of PSI, is a factor that regulates the energy transfer from phycobilisome to PSI under high-light condition.

Cyanobacterial PsaK subunit of PSI has weak homology with both PsaK and PsaG of higher plant PSI (18). Crystal structure of higher plant PSI revealed that PsaK and PsaG are located on the outer edge of PSI complex, and may serve for the association of light-harvesting complex I (LHCI) with core complex (30). Since the place for PsaG is open in the crystal structure of cyanobacterial PSI (17), it is tempting to assume that PsaK2 expressed in *Synechocystis* sp. PCC 6803 occupies the place for PsaG in higher plants under high-light condition. Although energy transfer studies imply that phycobilisome can interact with PSI and transfer energy efficiently to PSI, in structure terms we know little about the direct association of phycobilisome with thylakoid membrane or with reaction centers. PsaK2 might become a clue to the elucidation of the interaction of phycobilisome with PSI in *Synechocystis* sp. PCC 6803.

In higher plants and green algae, state transition is induced by the dissociation of LHCII from PSII complex. The redox state of the plastoquinone pool controls the activation of the thylakoid kinase responsible for phosphorylation of LHCII proteins (4). The binding of LHCII to PSI requires PSI-H, -I -L or -O (31-33). Chemical cross-linking study suggested that the docking sites for LHCII in PSI might be PSI-H, -I, and –L. Although the absence of PSI-G or -K also indirectly retards state transition to some extent (33-35), the main function of these two subunits seems to be related to the binding of LHCI judging from the crystal structure of PSI (30). Apparently, PsaK/G proteins serving for state transition in cyanobacteria are recruited for the binding of LHCI in higher plants, and PsaH protein is newly evolved for LHCII-type state transition of higher plants (31).

Although SDS-PAGE analysis clearly showed the increase of PsaK2 subunit in PSI under high-light condition, whether PsaK1 decreased under high-light condition or not is unclear from the silver stained gel presented in Fig. 1. At present, we could not deny the possibility that PsaK2 protein incorporated to PSI complexes in place of PsaK1 protein. In *Synechococcus* sp. PCC 7942, high light induces the interchange between two forms (D1:1 and D1:2) of D1 protein, a reaction center subunit of PSII. D1:1 is predominant under low-light condition and D1:2 increases during acclimation to high light (36). The mutant defective in the gene encoding D1:2 is sensitive to photoinhibition because of the failure to exchange D1:1 for D1:2 (37). Similar interchange of two PsaK subunits might be induced upon high-light acclimation also in PSI.

In any event, the incorporation of PsaK2 subunit into PSI complex is essential for the proper regulation of state transition under high-light condition. The growth of *psaK2* mutant was severely suppressed under high-light condition compared to the wild type and the *psaK1* mutant (Fig. 2B). The result clearly indicates that state transition has the physiological importance under high-light condition. It was reported that the mutant incapable of state transition under relatively low light conditions could grow even under the light of specific quality such as yellow light albeit at the reduced rate (15). The situation may be totally different for the high-light condition, and the proper state transition mediated by PsaK2 is essential for the growth under high-light condition. It must be noted that previously identified regulatory factors are all involved in the light-quality induced state transition. To our knowledge, PsaK2 is the only factor that regulates state transition under high-light condition.

Interestingly, no differences in state transition between the wild type and the *psaK2* mutant were observed under low-light condition (Fig. 5). This implies that at least two kinds of mechanisms for state transition might exist such as light-quality and light-quantity or low-light and high-light dependent state transition. Under low-light or biased light (PSII or PSI light) condition, RpaC-dependent state transition might enable the effective distribution of energy between PSII and PSI in order to perform photosynthesis as maximally as possible. Under high-light condition, PsaK2-dependent state transition might allow cells to be protected against the photodamage. It seems that PsaK2-dependent state transition is the only mechanism of state transition under high-light condition. In this study, the *psaK2rpaC* double mutant showed the same phenotype as the *rpaC* mutant reported by Emlyn-Jones et al. (15) under low-light condition (data not shown). Thus, there must be third mechanism of state transition under low-light condition independent of *psaK2* or *rpaC*. Although the *rpaC* mutant could not perform state transition upon phycobilisome excitation, the wild type and the mutant did show some kind of regulation of energy transfer from PSII to PSI under chlorophyll excitation (15). Small but significant state transition observed in the *psaK2rpaC* double mutant under low-light condition may be ascribed to this chlorophyll-dependent state transition, since we used the actinic light absorbed by both phycobilin and chlorophyll *a*.

The mechanism of state transition revealed in this study is not specific to *Synechocystis* sp. PCC 6803, since at least two other cyanobacterial species also have both *psaK1* type and *psaK2* type genes in their genome (Fig. 5). Apparently, marine cyanobacteria such as *Prochlorococcus marinus* or *Synechococcus* WH8102 have only one *psaK* which is more or less similar to PsaK1 type (Fig. 6). These cyanobacteria may have different mechanism of state transition under high-light condition. Interestingly, *Nostoc punctiforme* has only PsaK1 type gene and *Thermosynechococcus elongatus* BP-1 has only PsaK2 type gene in their genomes as far as we searched in CyanoBase (http://www.kazusa.or.jp/cyano/). The regulatory mechanism of state transition in these species must be examined in near future.

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FOOTNOTES

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[1]The abbreviations used are: PAM, pulse amplitude modulation; qN, non-photochemical quenching; qP, photochemical quenching; ϕII, effective quantum yield of electron transport through PSII; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PSII, photosystem II; PSI, photosystem I; Fm, the maximum level of fluorescence; Fo, the minimal level of fluorescence; Fs, the stable level of fluorescence; LHCII, light harvesting complex II; LHCI, light harvesting complex I; DCMU, dichlorophenyldimethylurea; MOPS, 3-morpholinopropanesulfonic acid; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

FIGURE LEGENDS

Fig. 1. Subunit composition of PSI complexes isolated from the wild type and the mutant cells of *Synechocystis* sp. PCC 6803 grown under low- and high-light conditions. Subunits of PSI complexes equivalent to 1 µg chlorophyll were separated by SDS-PAGE and visualized by silver staining. The fraction enclosed by dot line in Panel A was enlarged in Panel B with higher contrast.

Fig. 2. The growth of the wild type, the *psaK1* mutant and the *psaK2* mutant cells under low- (A) and high-light (B) conditions. 10 µl liquid-cultured cells (OD_{730}=0.5, OD_{730}=0.25 and OD_{730}=0.05) grown under low-light condition were spotted on a plate of solid BG-11medium. The cells were then grown for 3 days under low- or high-light condition.

Fig. 3. Fluorescence emission traces for quenching analysis. The traces of the wild type (A and D), the *psaK1* mutant (B and E) and the *psaK2* mutant (C and F) acclimated to low (A, B and C) or high light (D, E and F) were measured by PAM fluorometer. The fluorescence measurement initiated by applying modulated measuring light (ML on) after 5 min dark adaptation of cells. Subsequently a series of multi-turnover flashes (MT flashes) was applied to temporally close PSII for obtaining Fm'. After actinic light was turn on (AL on), the fluorescence settled down at steady state level (Fs). Actinic light was turned off (AL off) and far-red light was turned on (FR on) to obtain the minimal fluorescence level under actinic light (Fo'). Finally, DCMU was added to bring cells to full state 1 in exposure to actinic light to obtain Fm.

Fig. 4. Chlorophyll fluorescence emission spectra of high-light acclimated cells determined at 77 K. Spectra of the wild type (A), the *psaK1* mutant (B) and the *psaK2* mutant (C) acclimated to high light were measured under state 1 (solid line) and state 2 (thin line). Cells were either illuminated with blue light in the presence of 10 µM DCMU (state 1) or incubated in the dark for more than 10 min (state 2). The spectra were normalized to the intensity of the fluorescence peak at 695 nm (PSII peak).

Fig. 5. Chlorophyll fluorescence emission spectra of low-light acclimated cells determined at 77 K. Spectra of the wild type (A), the *psaK1* mutant (B) and the *psaK2* mutant (C) acclimated to low light were measured under state 1 (solid line) and state 2 (thin line). Cells were either illuminated with blue light in the presence of 10 µM DCMU (state 1) or incubated in the dark for more than 10 min (state 2). The spectra were normalized to the intensity of the fluorescence peak at 695 nm (PSII peak).

Fig. 6. PsaK proteins in cyanobacteria. The amino acid sequences of PsaK1 (GI:16329311) and PsaK2 (GI:16332131) in *Synechocystis* sp. PCC 6803 were compared with PsaK1 (GI:46129567) and PsaK2 (GI:53763010) in *Synechococcus elongatus* PCC 7942 and PsaK2 (GI:48893268) in *Trichodesmium erythraeum* IMS 101. Judging from the similarity to PsaK1 in other cyanobacteria, start methionine of PsaK1 in *Trichodesmium erythraeum* IMS 101 seems to be located 23 amino acids upstream of that predicted by Joint Genome institute (GI:48894022). Re-annotated sequence was shown in this figure. Black boxes show the amino acid residues conserved only in PsaK1 type protein or in PsaK2 type protein. Gray boxes indicate the amino acid residues conserved in both PsaK1 type and PsaK2 type proteins.

Fig. 7. Unrooted phylogenetic tree of cyanobacterial PsaK and PsaK/PsaG of higher plants. The phylogenetic tree is constructed by Neighbor Joining method with slow/accurate pairwise alignment and default parameters with Clustal W program (http://clustalw.genome.jp/). Amino acid sequences of PsaK/PsaG of higher plants were analyzed without removing the region corresponding to transit peptides. The Re-annotated sequence was used for PsaK1 in *Trichodesmium erythraeum* IMS 101 as described in Fig. 5. *S*. WH 8102, *Synechococcus* sp. WH 8102; *P*. MIT 9313, *Prochlorococcus marinus* MIT 9313; *P*. SS120, *Prochlorococcus marinus* SS120; *P*. MED4, *Prochlorococcus marinus* MED4; *A*. 7120, *Anabaena* sp. PCC 7120; *N. punctiforme*, *Nostoc punctiforme*; *S*. 6803, *Synechocystis* sp. PCC 6803; *S*. 7942, *Synechococcus* sp. PCC 7942; *T. elongatus*, *Thermosynechococcus elongatus*BP-1; *M. laminosus*, *Mastigocladus laminosus*; *T. erythraeum*, *Trichodesmium erythraeum*; *A. thaliana*, *Arabidopsis thaliana*; *O. sativa*, *Oriza sativa*; *C. reinhardtii*, *Chlamydomonas reinhardtii*.

Table 1. Fluorescence characteristics of the wild type, the *psaK1* mutant and the *psaK2* mutant grown under low- (A) or high-light (B) condition for 24 h. Photochemical quenching (qP) and non-photochemical quenching (qN) were measured by PAM fluorometer. The photon flux density of actinic light was fixed at 200 μmol m^{-2} s^{-1} both for low- and high-light acclimated cells. Values represent the average ± standard deviation with three independent cultures.

(A)

	qP	qN
WT	0.55±0.02	0.24±0.03
Δ*psaK1*	0.56±0.06	0.25±0.02
Δ*psaK2*	0.58±0.05	0.21±0.03

(B)

	qP	qN
WT	0.61±0.03	0.37±0.05
Δ*psaK1*	0.51±0.02	0.35±0.05
Δ*psaK2*	0.55±0.0	0.22±0.07

Table 2. The ratio of F_{725}/F_{695} (fluorescence intensity at 725 nm / fluorescence intensity at 695 nm) of low- (A) or high-light (B) acclimated cells under state 1 and state 2 determined by chlorophyll fluorescence emission spectra at 77 K. Values represent the average ± standard deviation with five independent cultures.

(A)

	state 1	state 2
WT	1.235±0.152	2.054±0.288
Δ*psaK1*	1.300±0.048	2.131±0.071
Δ*psaK2*	1.265±0.072	1.977±0.207

(B)

	state 1	state 2
WT	0.643±0.024	0.889±0.118
Δ*psaK1*	0.647±0.086	0.859±0.137
Δ*psaK2*	0.608±0.126	0.667±0.061

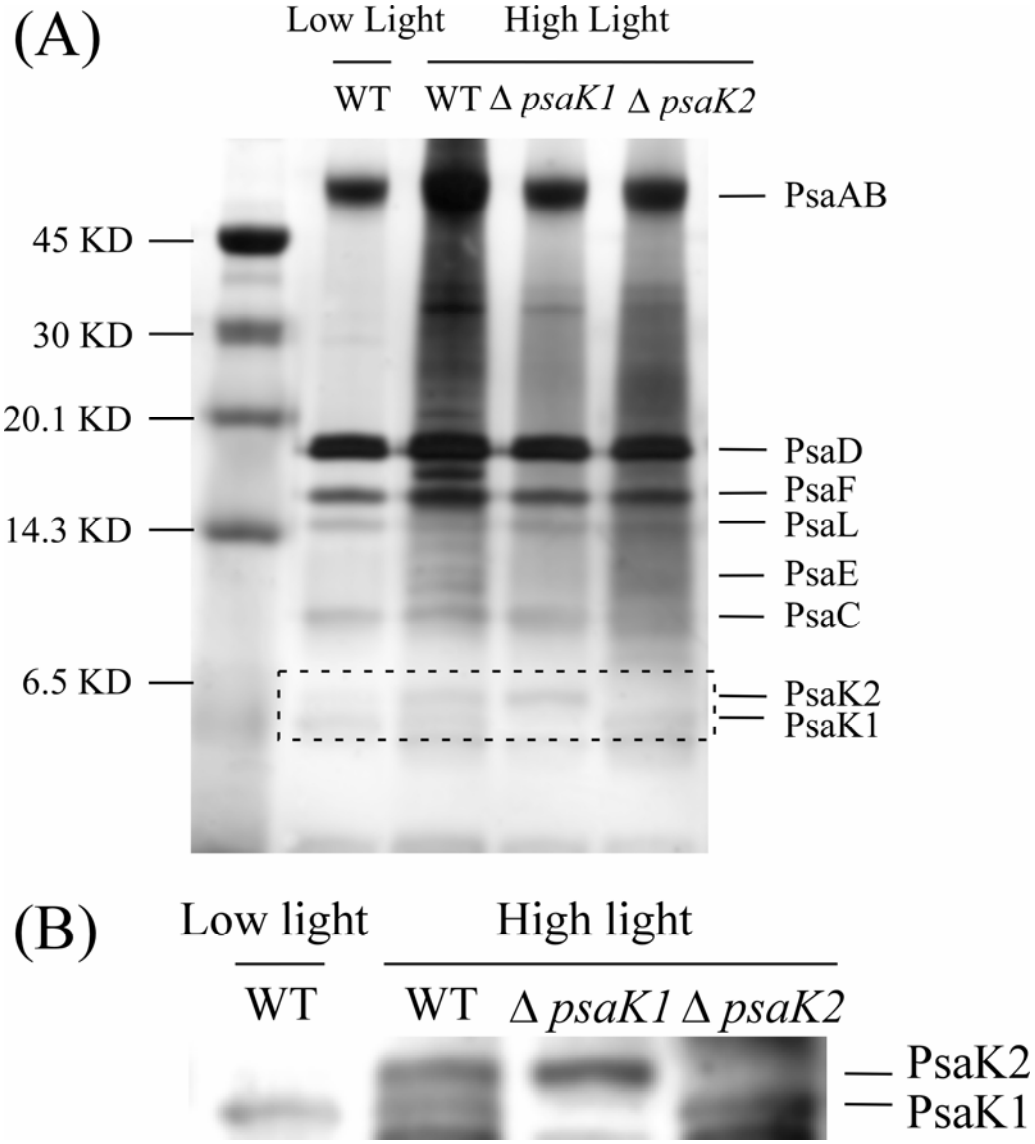

(A)
Low Light
High Light
WT
WT
Δ psaK1
Δ psaK2
45 KD
30 KD
20.1 KD
14.3 KD
6.5 KD
PsaAB
PsaD
PsaF
PsaL
PsaE
PsaC
PsaK2
PsaK1
(B)
Low light
High light
WT
WT
Δ psaK1
Δ psaK2
PsaK2
PsaK1

Fig. 1

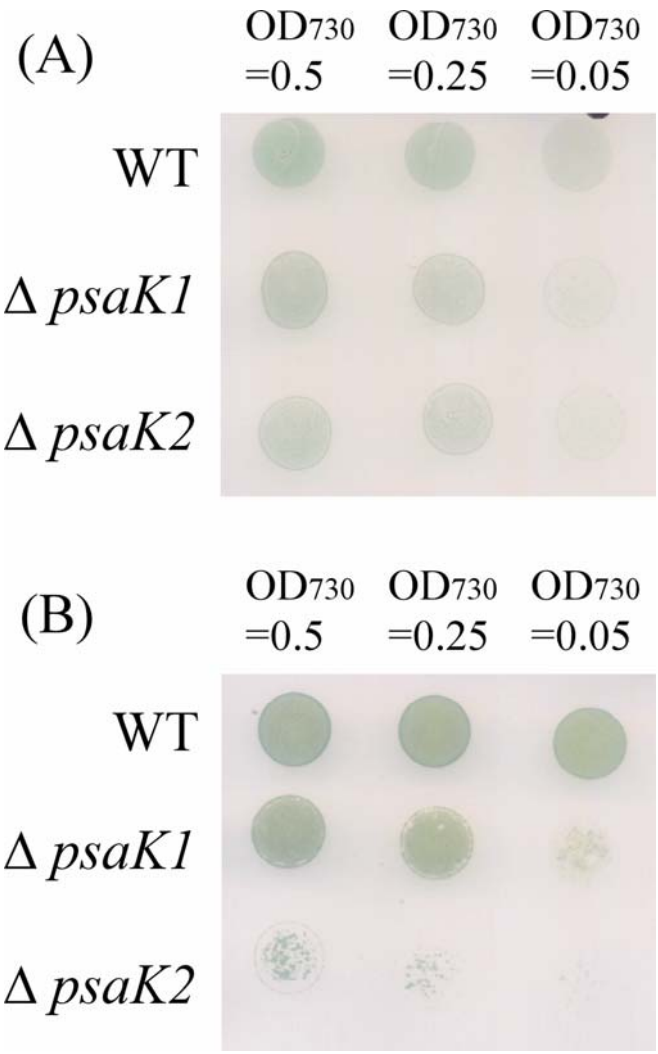

(A)
OD730 =0.5
OD730 =0.25
OD730 =0.05
WT
Δ psaK1
Δ psaK2
(B)
OD730 =0.5
OD730 =0.25
OD730 =0.05
WT
Δ psaK1
Δ psaK2

Fig. 2

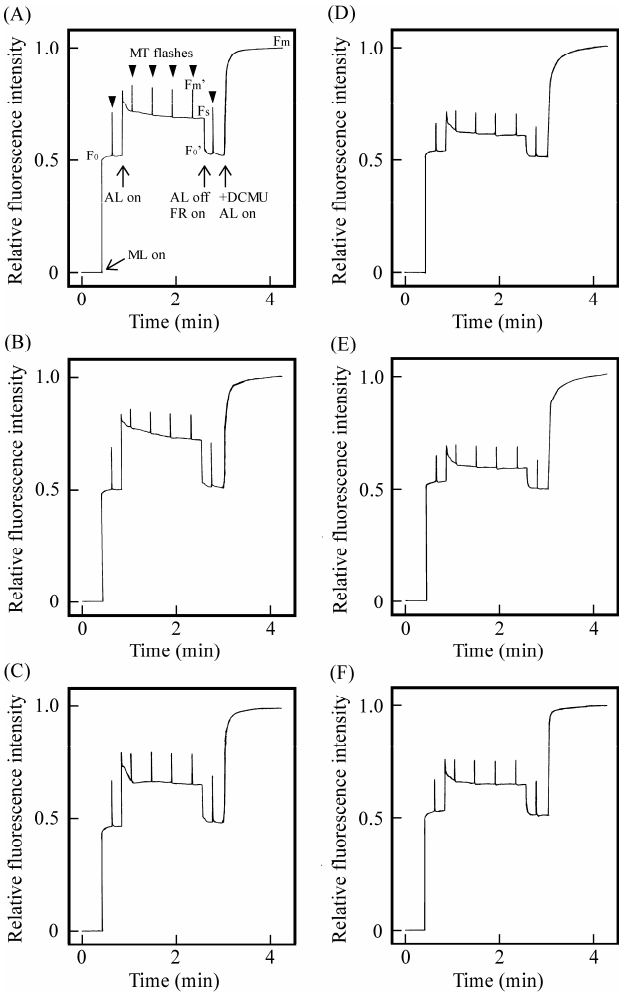
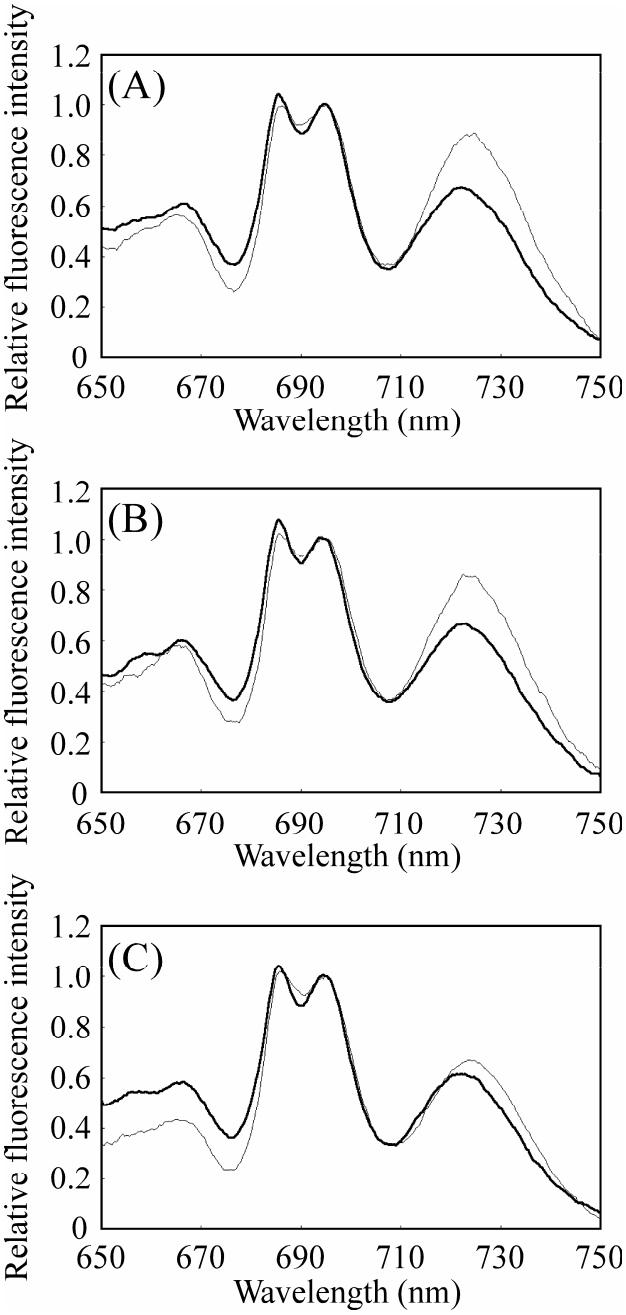

(A)
(B)
(C)
(D)
(E)
(F)
Relative fluorescence intensity
Time (min)
MT flashes
Fm
Fm'
Fs
Fo'
Fo
AL on
AL off
FR on
+DCMU
AL on
ML on

Fig. 3


(A)
(B)
(C)
Relative fluorescence intensity
Wavelength (nm)
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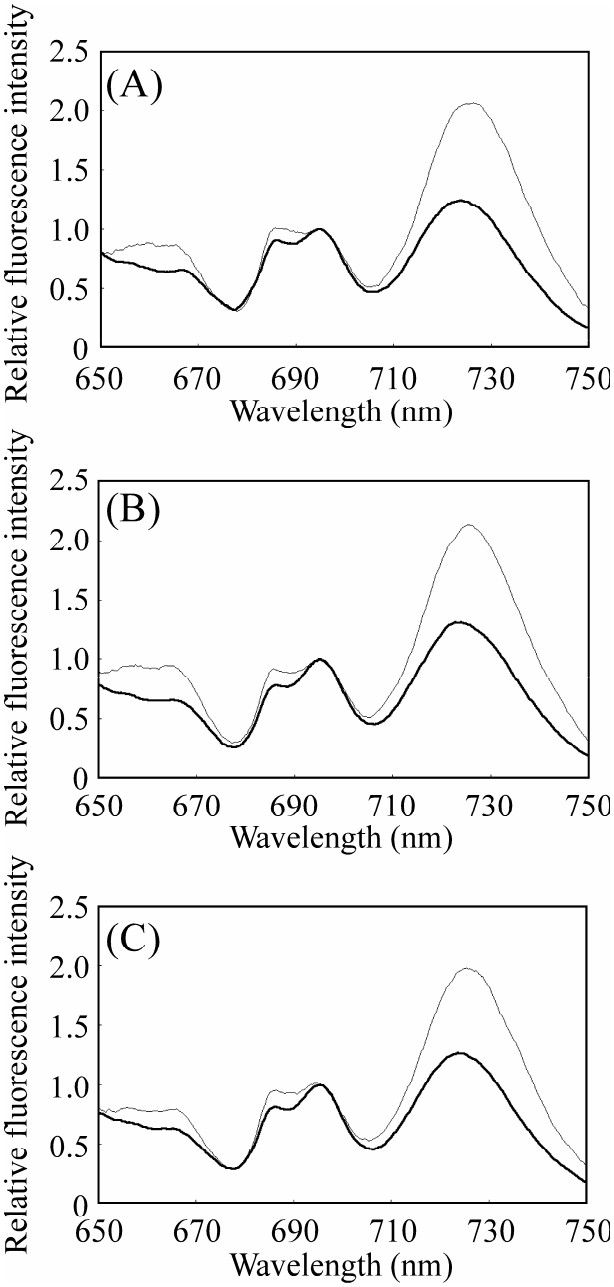
0.4
0.6
0.8
1.0
1.2
650
670
690
710
730
750

Fig. 4


(A)
(B)
(C)
Relative fluorescence intensity
Wavelength (nm)
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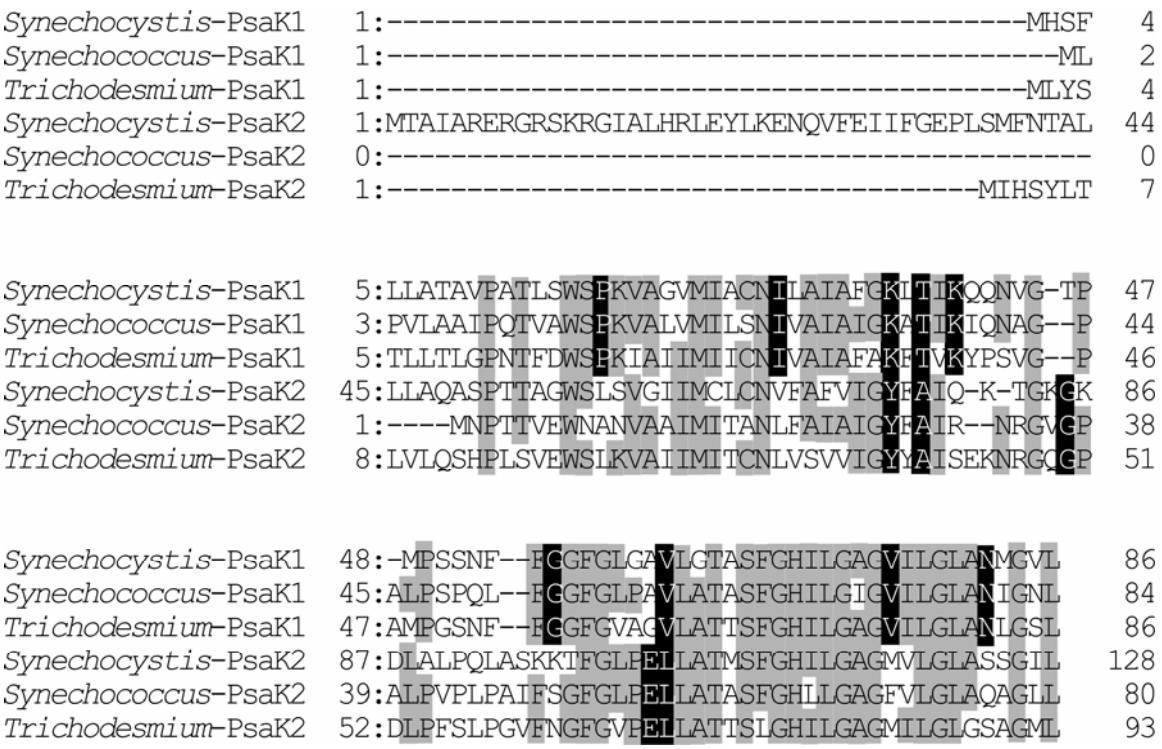
0.5
1.0
1.5
2.0
2.5
650
670
690
710
730
750

Fig. 5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Trichodesmium-PsaK1 5:TLLTLGPNTFDWSPKIAIIMIICNIVAIAFAKFTVKYPSVG--P 46
Synechocystis-PsaK2 45:LLAQASPTTAGWSLSVGIIMCLCNVFAFVIGYFAIQ-K-TGKGK 86
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Trichodesmium-PsaK2 8:LVLQSHPLSVEWSLKVAIIMITCNLVSVVIGYYAISEKNRGQGP 51
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Synechococcus-PsaK1 45:ALPSPQL--FGGFGLPAVLATASFGHILGIGVILGLANIGNL 84
Trichodesmium-PsaK1 47:AMPGSNF--FGGFGVAGVLATTSFGHILGAGVILGLANLGSL 86
Synechocystis-PsaK2 87:DLALPQLASKKTFGLPELLATMSFGHILGAGMVLGLASSGIL 128
Synechococcus-PsaK2 39:ALPVPLPAIFSGFGLPELLATASFGHLLGAGFVLGLAQAGLL 80
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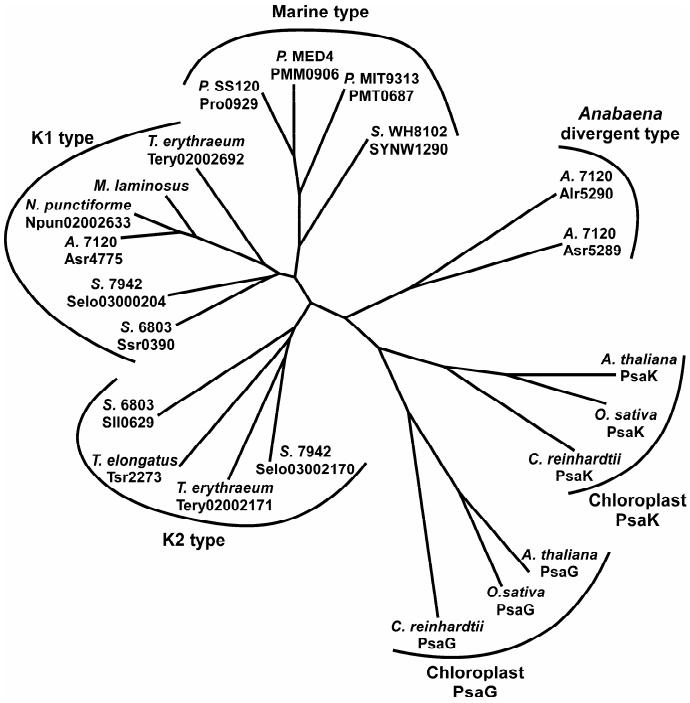
Fig. 6


Marine type
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P. MIT9313
PMT0687
P. SS120
Pro0929
S. WH8102
SYNW1290
K1 type
T. erythraeum
Tery02002692
M. laminosus
N. punctiforme
Npun02002633
A. 7120
Asr4775
S. 7942
Selo03000204
S. 6803
Ssr0390
Anabaena
divergent type
A. 7120
Alr5290
A. 7120
Asr5289
A. thaliana
PsaK
O. sativa
PsaK
C. reinhardtii
PsaK
Chloroplast
PsaK
S. 6803
Sll0629
T. elongatus
Tsr2273
T. erythraeum
Tery02002171
S. 7942
Selo03002170
K2 type
A. thaliana
PsaG
O.sativa
PsaG
C. reinhardtii
PsaG
Chloroplast
PsaG

Fig. 7