Comparative study of cyanobacterial state transition induced by high light

複数のシアノバクテリアにおける強光条件生育下で誘導されるステート遷移の比較

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46560　島田和美

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Department of Integrated Biosciences
Graduate School of Frontier Sciences
The University of Tokyo

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46560 Kazumi Shimada

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Abstract

To avoid the photodamage, cyanobacteria regulate the distribution of light energy absorbed by phycobilisome antenna either to photosystem II or photosystem I upon high light acclimation by the process so-called state transition. In *Synechocystis* sp. PCC 6803, it was shown that the mutant of *psaK2* could not perform state transition under high light condition. In this study, to elucidate the role of PsaK in the state transition induced under high light condition, I determined whether state transition could be induced in *Thermosynechococcus elongatus* BP-1, which has only PsaK2-type PsaK, or in *Anabaena* sp. PCC 7120 and *Nostoc punctiforme*, which do not have PsaK2 type PsaK but have another types of PsaK. I found that *Thermosynechococcus elongatus* BP-1 grown under high light condition could perform state transition, while *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* grown under high light could not perform state transition. These results suggest that PsaK2 is the only factor that regulates state transition under high light condition. This PsaK2-deprndent state transition is not specific to *Synechocystis* sp. PCC 6803. Several cyanobacteria seem to conserve this process.

Introduction

Life on the earth depends on photosynthesis, the process converting light energy from the sun to chemical energy. This process is driven by the cooperation of two large transmembrane pigment-protein complexes, photosystem I (PSI) and photosystem II (PSII) in oxygenic photosynthetic organisms, i.e. higher plants, green algae and cyanobacteria. Evolutionarily, PSI is derived from the reaction center of green photosynthetic bacteria and PSII is derived from the reaction center of purple bacteria (Fig. 1.). Using two photosystems connected in series, this type of photosynthesis could utilize H_2O as the electron donor for the subsequent electrochemical reactions. Thus, plants could spread almost all over the world using H_2O which is abundantly present in most of the surface environments of the Earth.

The price of the ability to use H_2O is the necessity to coordinate the two photosystems. Oxygenic photosynthetic organisms must balance the activities of two photosystems under various environmental conditions to optimize the photosynthetic performance and avoid photodamage. To maintain the balance of two photosystems, two types of response to changing light environments have been known. The first mechanism adjusts the stoichiometry between PSII and PSI (PSII/PSI ratio). This regulatory response requires the synthesis of new proteins or degradation of the old ones. This relatively slow response, with a time scale of hours or days, is referred to as long-term response (so called acclimation). The second mechanism provides an adjustment of energy distribution from antenna protein to two reaction centers and is called 'state transition'. Since this process occurs within several minutes upon the change in light

intensity or quality, it is very important process in short-term response of photosynthetic organisms.

In the case of cyanobacteria, state transition regulates the distribution of light energy from the accessory light-harvesting system, phycobilisome, to the two photosynthetic reaction centers (Fig. 2.). State 1 is induced by illumination preferentially absorbed by chlorophyll *a* antenna of photosystem I (PSI) and is characterized by a high efficiency of energy transfer from the phycobilisomes to photosystem II (PSII). State 2 is induced by illumination predominantly absorbed by PSII and is characterized by increased efficiency of energy transfer to PSI (Allen, 1992). State transition is induced not only by the change in light quality but also by the change in light quantity. Exposed to excess light, cyanobacteria change the distribution of light energy between two photosystems. Under normal light condition, the light energy absorbed by phycobilisome is preferentially transferred to PSII. On the contrary, under high light condition, the energy can be transferred not only to PSII but also to PSI (Campbell, 1998). In this case, the physiological significance may not be in the effective photosynthesis but in the avoidance from the photodamage.

Since its discovery in 1969 (Murata, 1969; Bonaventura and Myers, 1969), many research groups have been involved in the research of state transition in cyanobacteria, green algae, and higher plants. In old days, phycobilisome was assumed to act as a light-harvesting antenna solely for PSII. However, energy transfer studies on the wild type (Mullineaux, 1992) and PSII-deficient mutant (Mullineaux, 1994) of *Synechocystis* sp. PCC 6803 indicated that phycobilisome could interact with an efficiently transfer energy to PSI, depending on the growth condition of the cells. The study using the inhibitor of electron transport suggested that state transition

was controlled by the redox state of the plastoquinone pool via cytochrome b_6f complex (Mao, 2002). In cyanobacteria, several components of phycobilisome, such as ApcD, ApcF, and CpcG were reported to be involved in state transition (Ashby and Mullineaux, 1999; Kondo, 2005) (Fig. 3.). In 2005, Fujimori et al. reported for the first time that PsaK2, a subunit of PSI, is a factor that regulates the energy transfer from phycobilisome to PSI, and is essential for the growth of cyanobacteria under high light condition. From the bioenergetics point of view, it is clear that the phycobilisome is able to interact with PSI and transfer energy efficiently to PSI, although, in structural terms, we know little about the direct association of phycobilisome with thylakoid membranes or with reaction centers.

Upon acclimation of cells of *Synechocystis* sp. PCC 6803 to high light, the expression of all of the PSI genes was simultaneously suppressed with only one exception of the *psaK2* gene (Hihara, 2001). The expression of *psaK2* was highly 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 trimmer. 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* (Kjaerulff, 1993). Deduced amino acid sequences of the *psaK1* and the *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 the long N-terminal extension in PsaK2. The two gene products show 42 % homology with each other. Wild type and *psaK1* mutant could induce state transition under high light condition, but *psaK2* mutant could not.

Up to now, 36 different cyanobacterial genomes have been completely sequenced and the genomic information is available or will be available soon. In this study, I examined the state transition induced by high-light condition in several cyanobacteria based on the phylogenetic information obtained from these genomic information to clarify the roles of PsaK. I investigated the functional and structural differences of PsaK1 and PsaK2 to provide new clues for the elucidation of the regulatory mechanism of cyanobacterial state transition under high-light condition.

Materials and Method

Phylogenetic analysis

WUBLAST (http://blast.wustl.edu) was used to search the National Center for Biotechnology Information (NCBI) non-redundant protein database for homologous sequences. An initial multiple sequence alignment was generated using ClustalW (Thompson et al. 1994). The Tree View (version 1.6.6) was used to construct the phylogenetic tree with bootstrap value. The BOOTSTRAP program was used to generate 1000 bootstrap alignments. Distance between sequences was calculated using the P-distance program. PsaK and PsaG of chloroplasts were used as an outgroup, since PsaK is known as an ancestor of chloroplasts PsaK/G.

Strains and growth conditions

Synechocystis sp. PCC 6803 wild type and mutant strains, and *Anabaena* sp. PCC 7120 strain were grown in BG-11 medium (Rippka et al., 1979) with 10 mM TES. Cells in liquid culture 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 400 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 positions 6-70 of amino acid of PsaK1 with the kanamycin-resistant cassette using two *Msc*I sites in the *psaK1* gene (Nakamoto and Hasegawa, 1999). The *psaK2* mutant was constructed by insertion of the kanamycin-resistant cassette into *Age*I site in the *psaK2* gene. The *psaK1* and *psaK2* mutants were maintained with 20 μg/ml kanamycin.

Nostoc punctiforme strain was grown in BG-11 medium with 10 mM TES and 5 mM $NaHCO_3$. Cells in liquid culture 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 400 μmol m^{-2} s^{-1} was regarded as low light and high light, respectively.

Thermosynechococcus elongatus BP-1 strain was grown in BG-11 medium with 10 mM TES. Cells in liquid culture were grown at 55°C in 50 ml glass tubes and bubbled with air under continuous illumination provided by fluorescent lamps. Photon flux density at 50 and 900 μmol m^{-2} s^{-1} was regarded as low light and high light, respectively.

Fluorescence emission spectra determined at 77 K

Low temperature fluorescence emission spectra at 77 K were recorded using a custom-made apparatus (Sonoike and Terashima, 1994). Cells were collected and adjusted to 5 μg chlorophyll/ml in BG-11 medium for measuring state transition to OD_{730}=2.0 for high-light acclimated cells or OD_{730}=1.0 for low-light acclimated cells. Cells were excited with blue light passing through a filter (Corning CS 4-96). 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 the dark incubation of cells for more than 10 min and subsequent freezing in liquid nitrogen in the dark.

Absorption Spectra

Absorption spectra of whole cells of the *Synechocystis* sp. PCC

6803 wild type and mutants, *Anabaena* sp. PCC 7120, *Nostoc punctiforme*, and *Thermosynechococcus elongatus* BP-1 suspended in each grown medium were measured at room temperature using a spectrophotometer (Model 356; Hitachi, Japan) with a cuvette placed just in front of photomultiplier. Concentration of chlorophyll and phycocyanin were calculated by the equations of Arnon et al. (1974).

Results

Using BLAST search program, I searched for PsaK of cyanobacteria and PsaK/PsaG of chloroplasts in databases in order to obtain information on whether two types of PsaK, PsaK1 and PsaK2, are universally observed among cyanobacteria (Table.1). The phylogenetic tree of cyanobacterial and chloroplast PsaK/PsaG is constructed by the neighbor joining method using the ClustalW program (Fig. 4). PsaK/PsaG of chloroplasts were used as the outgroup in its dendrogram. As a results, cyanobacterial PsaK were classified into three groups with high bootstrap values (>90%); *Nostoc* type, PsaK2 type and PsaK1 type. The first group contains two of the three PsaK in *Nostocaceae* cyanobacteria, *Anabaena* sp. PCC 7120 and *Nostoc punctiforme*, and was named *Nostoc* divergent-type. The second group contains PsaK2 of *Synechocystis* sp. PCC 6803, and was named PsaK2-type. The third group contains PsaK1 of *Synechocystis* sp. PCC 6803, and was named PsaK1-type. It is evident that PsaK1-type proteins and PsaK2-type proteins form distinct clades. In the case of *Thermosynechococcus elongatus* BP-1, there is only one *psaK* gene in the genome, which is PsaK2-type. One of the three *psaK* genes in *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* is classified into the PsaK1 type, but the other two genes are classified into the *Nostoc* type (Table 1).

In this study, I 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. I decided to use *Thermosynechococcus elongatus* BP-1 (which only has K2-type of PsaK), *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* (which have both PsaK1 type and *Nostoc* type PsaK) together with the wild type and *psaK* mutants

of *Synechocystis* sp. PCC 6803. State 1 and state 2 were induced by the addition of DCMU and by dark adaptation of the cells, respectively, before the measurements of chlorophyll fluorescence spectra at 77 K. When cells were excited with broad range of blue light (400-600 nm) absorbed by phycobilin and chlorophyll *a*, 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 arises from PSI.

In *Thermosynechococcus elongatus* BP-1 grown under high light condition, relative intensity of PSI fluorescence was much greater in state 2 than state 1, just as in the case of the wild type and the *psaK1* mutant in *Synechocystis* sp. PCC 6803 (Fig.7 and Table.3). This increase of PSI fluorescence in state 2 reflects the enhanced energy transfer from phycobilisome to PSI. Thus, state transition could be induced under high light condition in *Thermosynechococcus elongatus* BP-1. In high light acclimated cells of *Anabaena* sp. PCC 7120 and *Nostoc punctiforme*, the relative intensity of PSI fluorescence in state 2 was very close to that in state 1(Fig. 7. and Table. 3). The result indicates that the high light acclimated cells of the *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* are not able to perform state transition just in the case of *psaK2* mutant in *Synechocystis* sp. PCC 6803. There must be some differences in the roles of PsaK1 and PsaK2 in state transition under high light condition, and PsaK2 is the one that is responsible for state transition.

In the case of low light acclimated cells, the relative intensity of PSI fluorescence was greater in state 2 than in state 1 in all the three

cyanobacteria, *Thermosynechococcus elongatus* BP-1, *Anabaena* sp. PCC 7120, and *Nostoc punctiforme* (Fig. 6, Table. 3). Apparently, state transition in the low light acclimated cells does not depend on the form of PsaK protein.

Although the results obtained above agree with the hypothesis that the PsaK2-type protein is responsible for the high-light induced state transition, there still is the possibility that defect in state transition of high light acclimated cells of *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* is the result of changes in the contents of photosynthetic pigments (chlorophyll and phycocyanin). To investigate this possibility, I determined the concentration of phycocyanin and chlorophyll in these cyanobacterial cells by measuring absorption spectra (Table 4). The concentration of phycocyanin of *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* cells decreased upon high light acclimation, and this decrease is comparable to that observed in *Synechocystis sp.* PCC 6803 cells. The results indicate that inability of high light acclimated cells of *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* to perform state transition is not due to abnormal decrease of phycocyanin concentration in these cells.

Discussion

To date, there were very few reports on the state transition under high light condition, although there were many reports on that under growth light condition. *Synechocystis* sp. PCC 6803 is the only organism that was reported to perform high-light induced state transition, and PsaK2 mutant of this organism is the only one that could not perform state transition under high light condition. In the present study, I studied the ability of high-light induced state transition in several cyanobacterial species. Apparently, PsaK2 is the factor that regulates state transition under high light condition not only in *Synechocystis* sp. PCC 6803 but also in cyanobacteria that have PsaK2 type PsaK. *Thermosynechococcus elongatus* BP-1 (having only one PsaK2-type PsaK) could induce state transition and several other cyanobacteria apparently have PsaK2-type PsaK. On the other hand, I showed the high light acclimated cells of *Nostoc punctiforme* and *Anabaena* sp. PCC 7120 (having no PsaK2-type PsaK but have two *Nostoc* type PsaK and one PsaK1 type PsaK) could not induce state transition under high light condition. For these results, I conclude that all types of PsaK expect for PsaK2-type, i.e. *Nostoc* type and PsaK1 type can not substitute for PsaK2-type PsaK, and PsaK2-type PsaK mediates the state transition induced by high light condition.

In *Anabaena* sp. PCC 7120, the amount of phycocyanin of the cells acclimated to high light condition were decreased to 55.7% of that of the cells acclimated to low light condition in per cell bases, that is comparable to the result obtained for *Synechocystis* sp. PCC 6803 and represents the normal high light acclimation of the phycobilisome. However, the relative height of fluorescence peak of PSI and PSII in state 1 condition (Table 3)

showed that the photosystem stoichiometry (the ratio of PSII and PSI) was not so much affected by high light acclimation as observed in *Synechocystis* sp. PCC 6803. These results suggest that *Anabaena* sp. PCC 7120 could not regulate photosystem stoichiometry under high light condition in addition to the inability of PsaK2-dependent state transition.

This is also true for *Nostoc punctiforme*. The photosystem stoichiometry determined as the fluorescence peak ratio in state 1 condition did not much change by the acclimation to high light. In addition, the aggregation of the cells was observed when *Nostoc punctiforme* was grown under high light condition. *Nostoc punctiforme* is a filamentous cyanobacterium capable of differentiating its normal vegetative cells into nitrogen-fixing heterocysts, motile hormogonia or spore like akinates (Meeks et al., 2002). Like others comprising the subset of strains capable of akinate formation in the order *Nostcales* and *Stigonematales*, akinate of *Nostoc punctiforme* is usually induced in cultures exposed to light limitation or phosphate starvation (Campbell et al., 1996; Wong and Meeks, 2002). Cyanobacterial akinates could survive 5-7 years of desiccation (Yamamoto, 1975; Sili et al., 1994), months of cold (4°C) dark conditions (Sutherland et al., 1979), and have been isolated form sediments as old as 64 years (Livingstone and Jaworski, 1980). Little is known about the molecular basis for such resistance to environmental extremes. At any events, *Nostcaeace* might have another process to response to high light condition, which is not in other species of cyanobacteria, and this may compensate for the absence of state transition under high light condition.

The amount of PsaK1 was high and that of PsaK2 was negligible under low light condition in *Synechocystis* sp. PCC 6803, the amount of PsaK2 increased in acclimation of the cell to high light (Fujimori, et al.

2005). 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 increase during acclimation to high light (Clarke, et al., 1995). The mutant defective in the gene encoding D1:2 is sensitive to photoinhibition because of the failure to exchange D1:1 for D1:2 (Krupa, et al., 1991). A similar interchange of the two PsaK subunits in wild type of *Synechocystis* sp. PCC 6803 might be also induced upon high light acclimation in PSI. In this study, I reported that *Thermosynechococcus elongates* BP-1 which has only PsaK2 could induce state transition by high light. The result may suggest that PsaK2 was not incorporated to PSI complexes in the place of PsaK1. In any event, it is interesting to determine the expression of *psaK2* gene in *Thermosynechococcus elongates* BP-1 under different light condition.

All cyanobacteria that I tested in this study could induce state transition under low light condition. Emlyn-Jones et al. demonstrated that *rpaC* (*r*egulator of *p*hycobilisome *a*ssociation) mutant was unable to perform state transition (Emilyn-Jones, et al., 1999). The expression of *rpaC* was reported to decrease during acclimation to high light (Hihara, et al., 2001). Thus, RpaC is associated with state transition specific to low light condition. All species of cyanobacteria, whose genome was completely sequenced, conserved *rpaC* gene, suggesting that RpaC-dependent state transition is important process in cyanobacteria. It is reasonable to assume that RpaC enables the effective distribution of energy between PSII and PSI in order to maximize the efficiency of photosynthesis under low light condition, while PsaK2 protects photosynthetic machinery against photoinhibition under high light condition.

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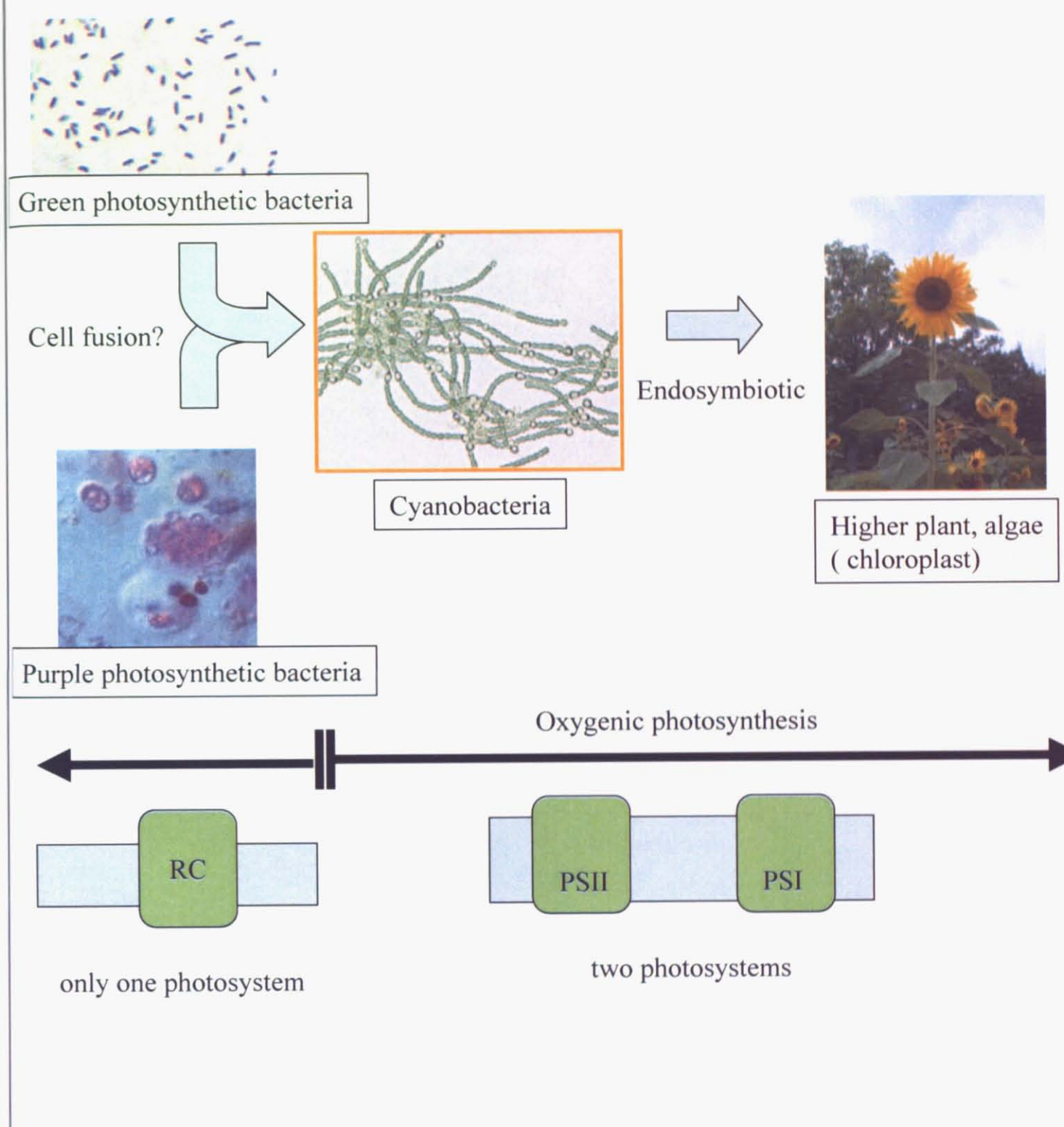

Fig. 1. Evolution of photosynthetic organisms

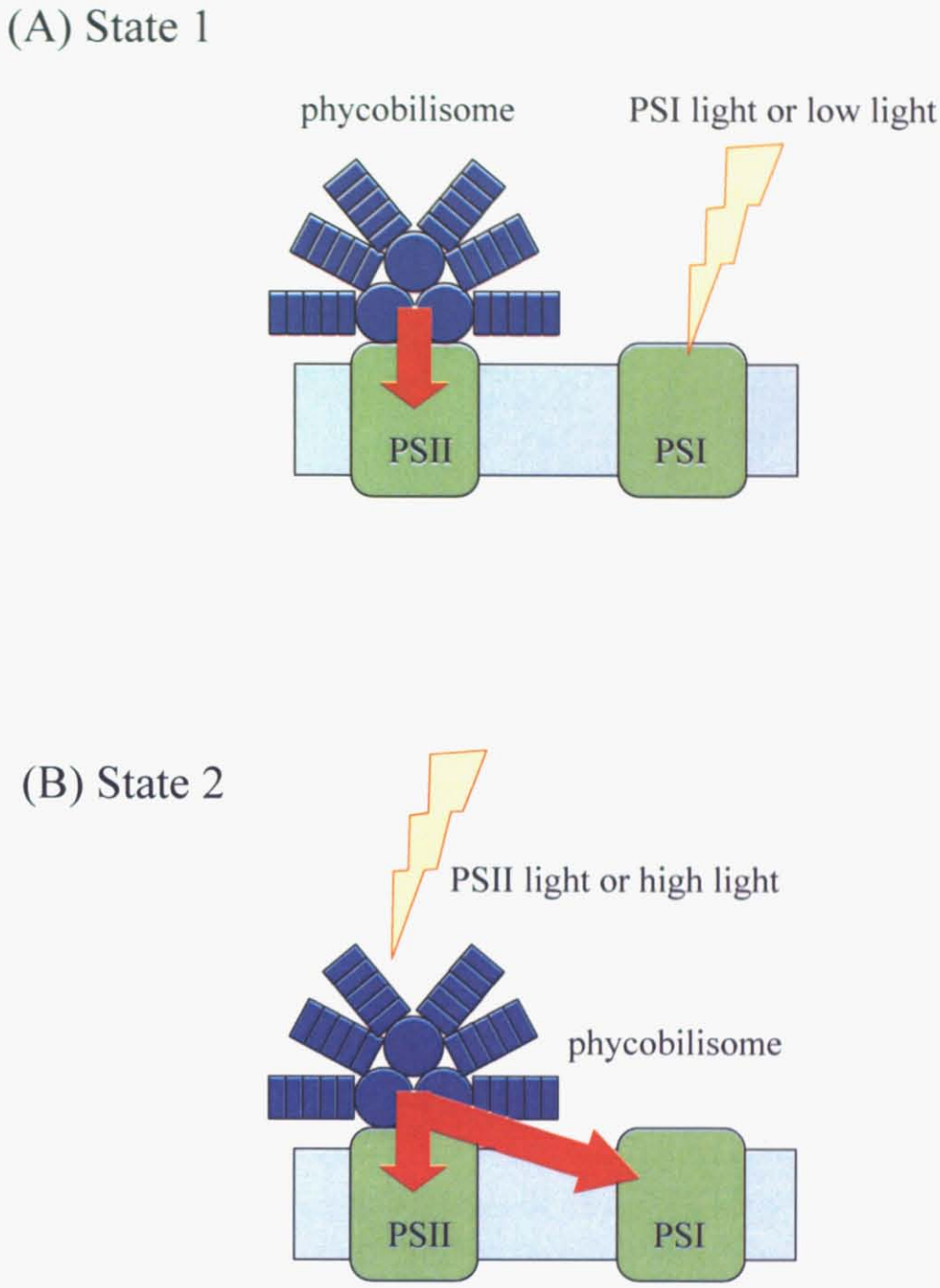

Fig.2. Regulation of energy distribution between PSII and PSI in cyanobacteria. When PSI is preferentially excited, the energy absorbed by phycobilisome is transferred mainly to PSII (state 1). On the contrary, when PSII is mostly activated, it is delivered primarily to PSI (state 2).

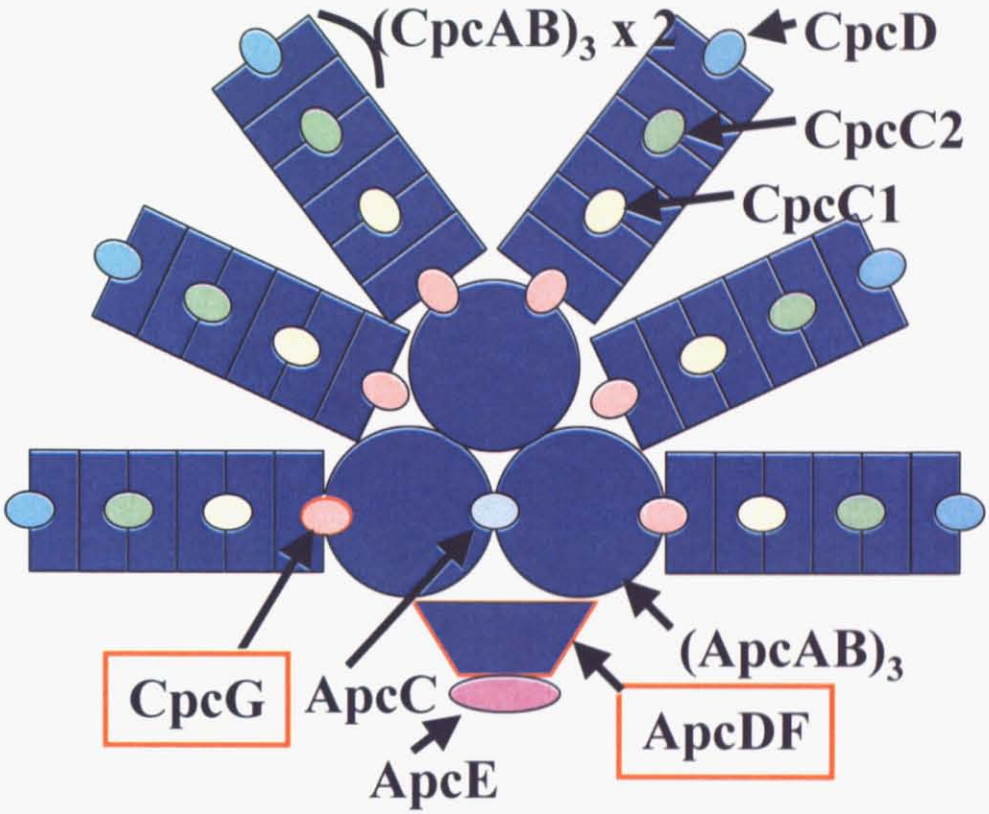

Fig. 3. The structure model of phycobilisome.
ApcD, ApcF, and CpcG are involved in state transition as for proteinaceous factor.

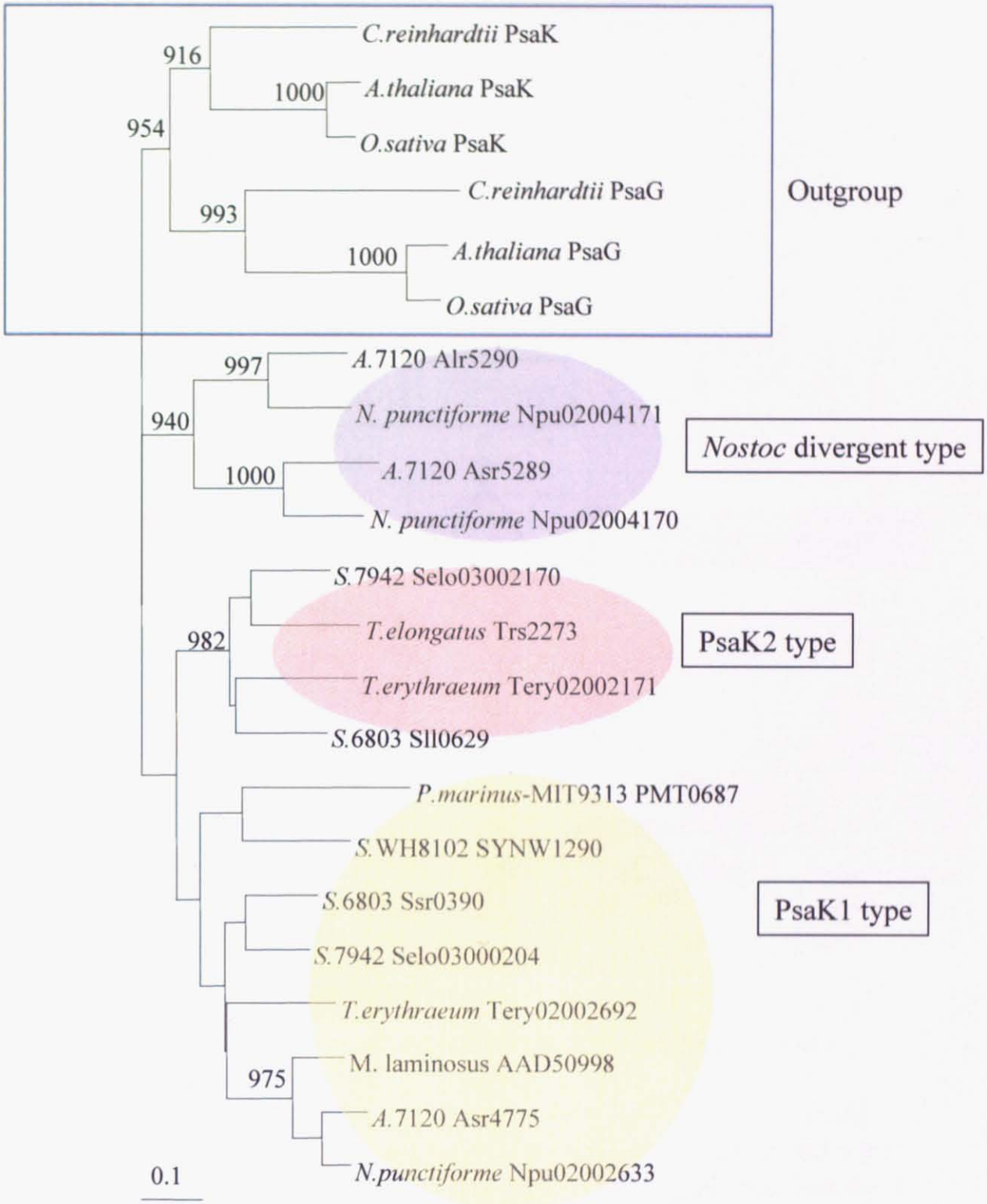

Fig.4. Neighbor-joining tree of cyanobacterial PsaK and PsaK/PsaG of chloroplasts. The phylogenetic tree is constructed by the neighbor joining method and default parameters with ClustalW program.
Bootstrap values (from 1000 bootstrap replicates) are shown at nodes. Amino acid sequences of PsaK/PsaG of chloroplasts were analyzed without removing the region corresponding to transit peptides.The scale bar represents 0.1 substitutions per site. PsaK/PsaG of chloroplasts are used as out group. *C. reinhardtii*, *Chlamydomonas reinhardtii*; *A. thaliana*, *Arabidopsis thaliana*; *O. sativa*, *Oriza sativa*; *A.* 7120, *Anabaena* sp. PCC 7120; *N. punctiforme, Nostoc punctiforme*; *T. elongatus*, *Thermosynechococcus elongatus* BP-1; *S.* 6803, *Synechocystis* sp. PCC 6803; *S.* 7942, *Synechococcus* sp. PCC 7942; *T. erythraeum*, *Trichodesmium erythraeum* IMS 101; *M. laminosus*, *Mastigocladus laminosus*; *S. WH8102*, *Synechococcus* sp. WH 8120; *P. marinus*-MIT, *Prochlorococcus marinus* MIT 9313

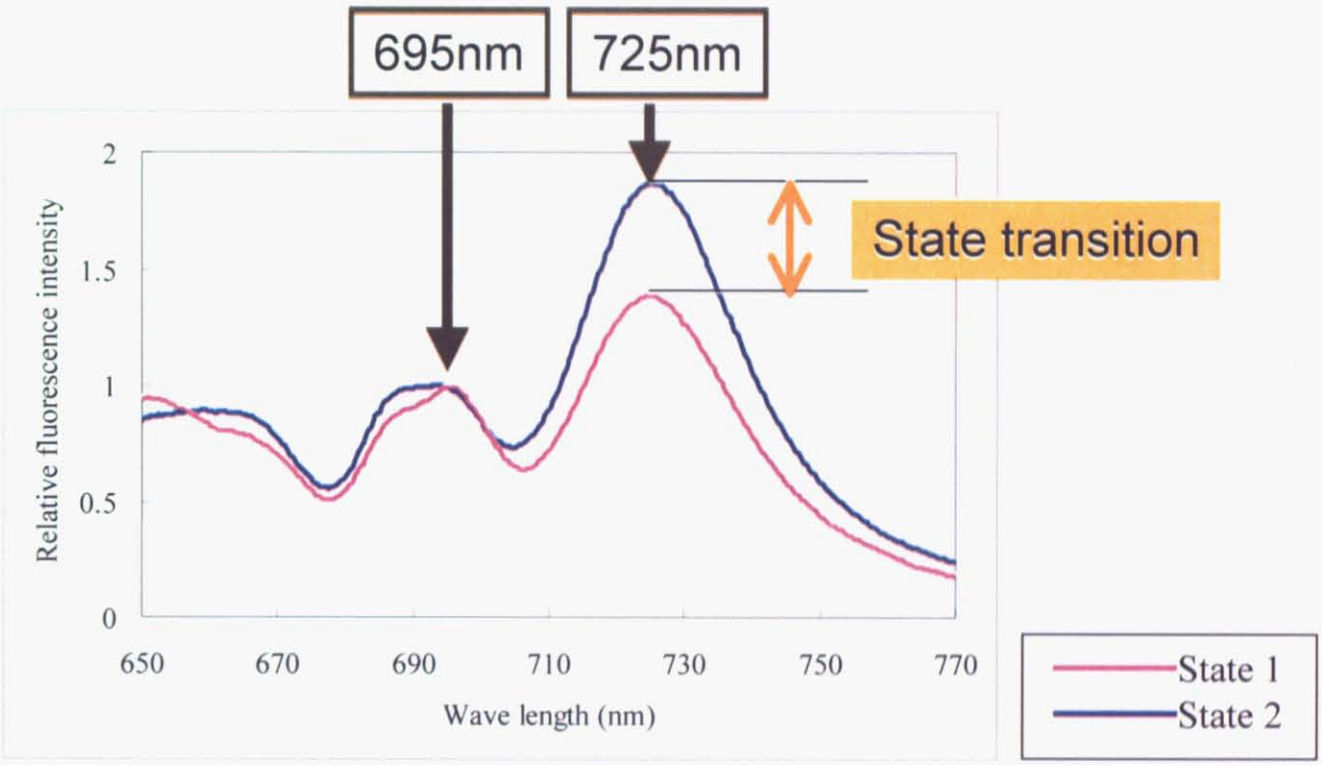

Fig.5. Analysis in state transition estimated by chlorophyll fluorescence emission spectra determined at 77K.

The spectra of several species acclimated to high or low light were measured under state 1 (red line) and state 2 (blue line). Cells were either illuminated with blue light in the presence of 10μM 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), and the state transition was estimated by difference between state 1 and state 2 in the intensity of the fluorescence peak at 725 nm (PSI peak) .

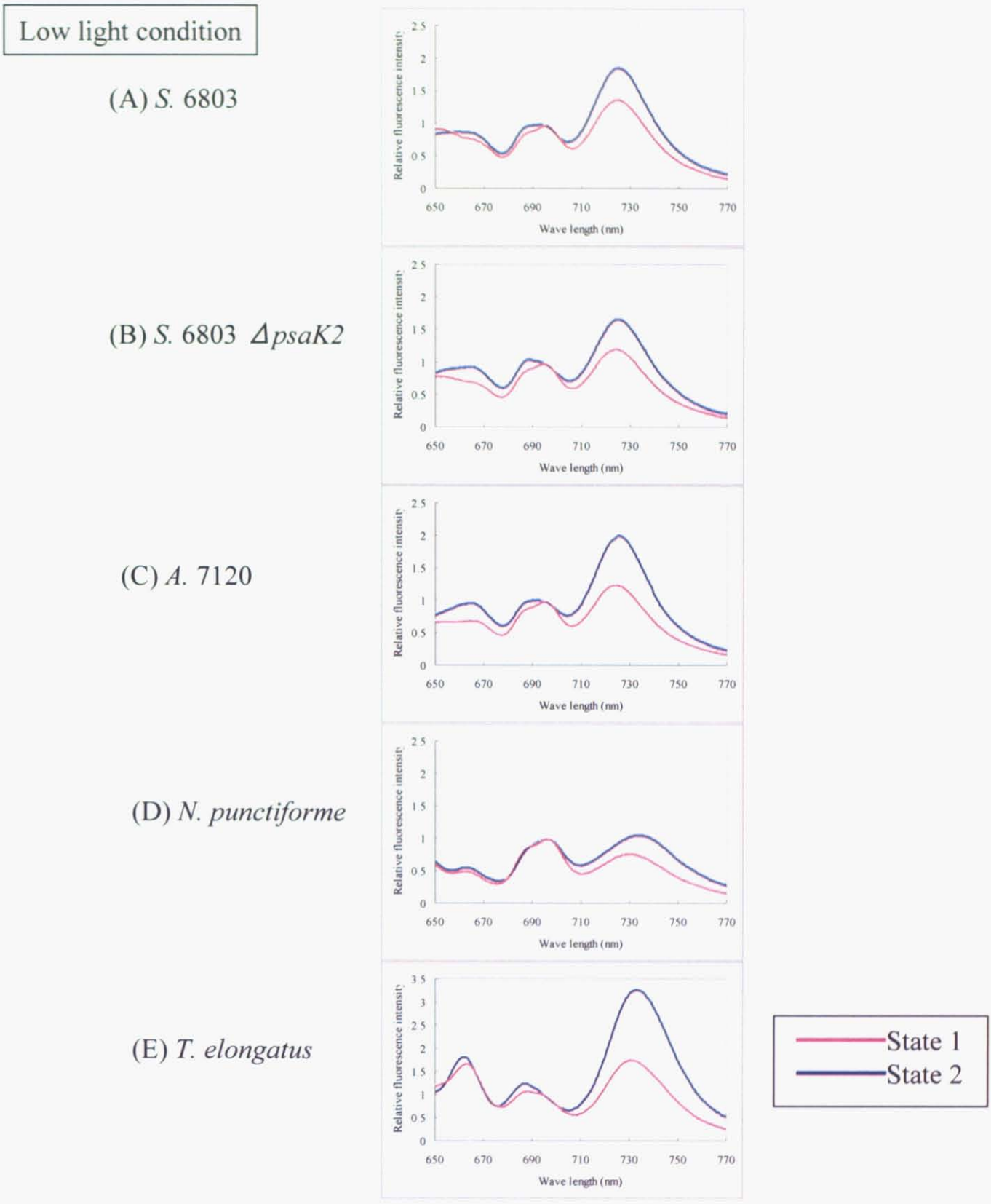

Fig. 6. Chlorophyll fluorescence emission spectra of low light acclimated cells determined at 77 K. The spectra of *Synechocystis* sp. PCC 6803 wild type (A), *Synechocystis* sp. PCC 6803 *psaK2* mutant (B), *Anabaena* sp. PCC 7120 (C), *Nostoc punctiforme* (D), and *Thermocynechococcus elongatus* BP-1 (E) adapted to low light condition were measured under state 1(red line) and state 2(blue line). Cells were either illuminated with blue light in the presence of 10μM 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.

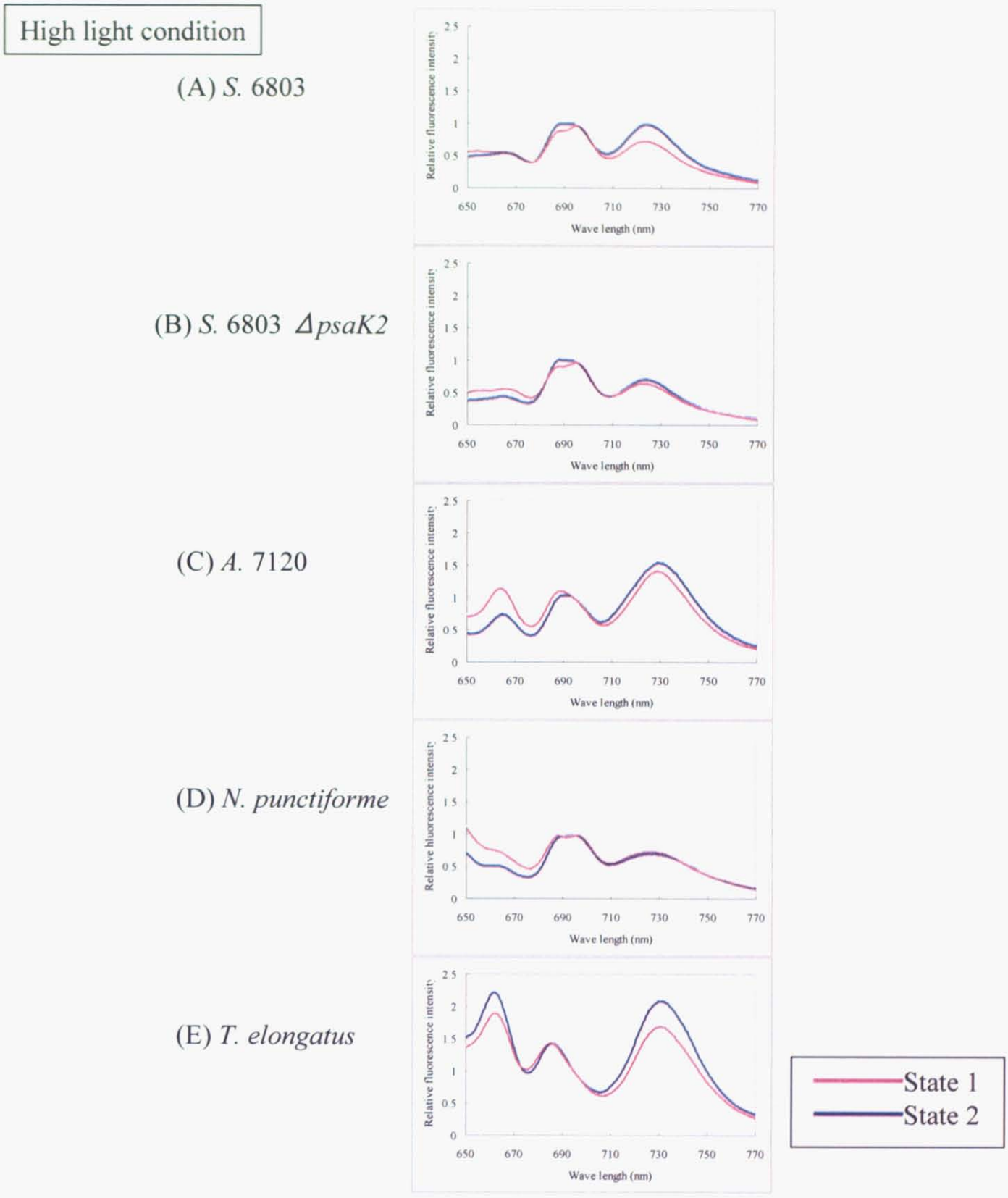

Fig. 7. Chlorophyll fluorescence emission spectra of high light acclimated cells determined at 77 K. The spectra of *Synechocystis* sp. PCC 6803 wild type (A), *Synechocystis* sp. PCC 6803 *psaK2* mutant (B), *Anabaena* sp. PCC 7120 (C), *Nostoc punctiforme* (D), and *Thermocynechococcus elongatus* BP-1 (E) adapted to high light condition were measured under state 1(red line) and state 2(blue line). Cells were either illuminated with blue light in the presence of 10μM 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.

Table. 1. Various kind of PsaK categorized by neighbor-joining tree in several cyanobacteria.

The total PsaK gene in *M. laminosus* has been unclear because its genome project do not go to completion. *S.* 6803, *Synechocystis* sp. PCC 6803; *S.* 7942, *Synechococcus* sp. PCC 7942; *T. erythraeum*, *Trichodesmium erythraeum* IMS 101; *T. elongatus*, *Thermosynechococcus elongatus* BP-1; *M. laminosus*, *Mastigocladus* ; *S. WH8102*, *Synechococcus* sp. WH 8120; *A.* 7120, *Anabaena* sp. PCC 7120; *N. punctiforme, Nostoc punctiforme*; *P. marinus*-MIT, *Prochlorococcus marinus* MIT 9313

	Nostoc divergent -type	PsaK2- type	PsaK1- type	Total
S. 6803		Sll0629	Ssr0390	2
S. 7942		03002170	03000204	2
T. erythreaum		02002127	02002629	2
T. elongatus		Tsr2273		1
M. laminosus			AAD50998	
S. WH8102			SYNW1290	1
*A.*7120	Alr5290 Asr5289		Asr4775	3
N. Punctiforme	02004170 02704171		02002633	3
P. MIT			PMT0687	1

Table. 2. Growth of cultures and strains

S. 6803, *Synechocystis* sp. PCC 6803; *A.* 7120, *Anabaena* sp. PCC 7120; *N. punctiforme, Nostoc punctiforme*;*T. elongatus*, *Thermosynechococcus elongatus* BP-1

strain	growth medium	growth temperature (^{0}C)	photon flux density (μmol $M^{-2}s^{-1}$) low-light	high-light
S. 6803	BG11	30	20	400
S. 6803 *ΔpsaK2*	BG11	30	20	400
A. 7120	BG11	30	20	400
N. punctiforme	BG11 + NaHCO3(5mM)	30	20	400
T. elongatus	BG11	55	50	900

Table. 3. The ratio of F_{725}/F_{695} (fluorescence intensity at 725nm/fluorescence intensity at 695 nm) of low or high light-acclimated cells in either state 1 or state 2 determined by the measurements of chlorophyll fluorescence emission spectra at 77K in these species of cyanobacteria.

Values represent the average ± S.D.with three independent cultures. These cyanobacteria were grown under several photon flux density for 24 hour.

	State1 (725nm/695nm)	State2 (725nm/695nm)	State1/State2
S. 6803			
20μE	1.03±0.03	1.73±0.08	0.75
400μE	0.66±0.08	0.96±0.04	0.68
S. 6803 *ΔpsaK2*			
20μE	1.27±0.07	1.98±0.21	0.65
400μE	0.67±0.08	0.72±0.06	0.92
A. 7120			
20μE	1.37±0.02	2.02±0.02	0.65
400μE	1.36±0.02	1.45±0.02	0.94
N. punctiforme			
20μE	0.74±0.03	0.93±0.04	0.79
400μE	0.77±0.02	0.72±0.03	1.08
T. elongatus			
50μE	1.58±0.12	2.60±0.24	0.61
900μE	1.53±0.15	1.91±0.18	0.80

Table. 4. The concentrations of chlorophyll and phycocyanin in these species of cyanobacteria.

These cyanobacteria were grown under several photon flux density for 24 hour. The concentrations determined by measuring absorption of these species cyanobacteria and normalized at A730.

	Chlorophyll concentration (μg/ ml)	Phycocyanin Concentration (μg/ ml)
S. 6803		
20μE	51.0±0.08	512.4±14.2
400μE	31.6±0.12 (62.0%)	308.2±6.8 (60.1%)
S. 6803 *ΔpsaK2*		
20μE	49.5±0.09	497.6± 9.8
400μE	31.3±0.07 (63.2%)	302.2± 7.5 (60.7%)
A. 7120		
20μE	79.5±0.21	686.3±12.3
400μE	46.7±0.27 (58.7%)	382.5±11.5 (55.7%)
N. punctiforme		
20μE	87.9±1.25	776.7± 9.9
400μE	67.7±2.03 (77.0%)	666.2±11.6 (85.8%)
T. elongatus		
50μE	56.9±1.21	300.5±15.2
900μE	25.4±2.15 (44.6%)	134.8±13.4 (44.9%)