Supporting Information

Boulay et al. 10.1073/pnas.1002912107

SI Materials and Methods

Overexpression of FRP in *Synechocystis*. To overexpress the FRP with an N- or C-terminal HisTag in *Synechocystis*, *slr*1964 was amplified by PCR. The 64NterNdeI/psbA2HPAI (GCATTCCATATGGGC TTCTCGTTGCAGAGCAGGC/GCATCGGTTAACGGTGT-ACACCTACAGCAAGCAA) oligonucleotides pairs were used. The 506-bp resulting fragment was introduced (after a NdeI-HpaI digestion) into the pPSBA2 plasmid (1). To overexpress OCP followed by FRP, the plasmid containing a DNA fragment including the *slr1963* and *slr1964* genes cloned into the pPSBA2 plasmid (2) was used. The His-Tag was added to the 3′ and 5′ ends of *slr1964* by directed mutagenesis (Quickchange XL site-directed mutagenesis kit; Stratagene) using the oligonucleotides F64Nter-His/R64NterHisII (CACCACCACCAC CACCACGGCTTCTC-GTTGCAGAGCAGGCTCACTTTTT/GTGGTGGTGGTGGT-GGTG CATATGGTTATAATTCCTTATGTATTTGTCG) and F64CterHis/R64CterHis (GTGCA CCACCACCACCACCACT-GATTCTAGTAAATATCTAAAACTCCAC/GTGGTGGTGG T-GGTGGTGCACCCGGGCCAGGGCCTTAATTTAGATTGC), respectively. A 2,000-bp spectinomycin and streptomycin resistance cassette was then inserted into the unique restriction site HpaI of these plasmids (Fig. S2 *A* and *B*). All these plasmids were used to transform the ΔFRP strain (2). Complete segregation was tested by PCR amplification (Fig. S2*C*).

Overexpression of FRP in *E. coli*. To overexpress the long FRP with an N-terminal His tag in *E. coli*, the *slr1964* gene (beginning in the GTG coding for M1) from *Synechocystis* PCC6803 was amplified by PCR using the 64NterNdeI/64CterBamHI oligonucleotides (GCATTCCATATGGGCT TCTCGTTGCAGAGCAGGC/ATTTTAGGATCCTCACAGCCGGGCCAGGGCCTTA). The 405-bp resulting fragment was introduced in the pET-15b plasmid (Novagene) after a digestion with NdeI and BamHI and the construct was named pCB9. The ultracompetent XL10-gold strain (Stratagene) was transformed with pCB9, then, the plasmid was extracted and used for the transformation of the BL21strain (Stratagene) adapted for protein overexpression. To overexpress the short FRP containing a His6 tag attached to its N terminus in the *E. coli* strain BL21 (DE3), the *slr1964* gene (beginning in the ATG coding for M26) was amplified by PCR using the PETM26for/PETM26rev oligonucleotides (GTGCCGCGCGG-CAGCCATATGTTACAAACCGCCGAAGCACCTTGG/AT-GGCTGC CGCGCGGCACCAGGCCGCTGCTGTGATGAT-GATGATGATG) and the pCB9 plasmid as template. All plasmids were checked by sequencing (MWG).

Purification of the Long His-Tagged FRP Overexpressed in *E. coli*. *E. coli* cells (375 mL) were grown at 37 °C and FRP overexpression was induced at a DO_{600nm} of 0.5 with 0.8 mM isopropyl-β-D-thiogalactoside. After 12 h incubation, the cells were centrifuged (6 min for 3,000 × *g* at 4 °C) and resuspended in 50 mL of a solution containing 20 mM Tris-HCl, pH 8, 100 mM NaCl, 5 mM caproic acid, 5 mM benzamidine, 2 mM $MgCl_2$, 0.5% Triton X-100, and 50 μg/mL of DNase I. The cells were broken by sonication. The long overexpressed N-terminal His-tagged FRP in *E. coli* forms was obtained as inclusion bodies. The inclusion bodies were collected by centrifugation (20,000 × *g*) and washed three times. The pellet containing the washed inclusion bodies was resuspended in 15 mL of a solution containing 100 mM Tris-HCl, pH 8, 12.5% sucrose, 5 mM benzamidine, 5 mM caproic acid, and 2% LiDS. The LiDS was precipitated by adding 150 mM KCl + 6 M urea + 0.03% β-DM. After centrifugation, the supernatant was dialyzed for 12 h against a buffer containing 40 mM Tris-HCl, pH 8, and 0.03% β-DM. The solubilized FRP was purified on a Ni-ProBond resin (Invitrogen) preequilibrated with a buffer containing 40 mM Tris-HCl, pH 8, 150 mM K-phosphate buffer, 500 mM NaCl, 10% glycerol, and 0.03% β-DM. The FRP was eluted with 160 mM imidazole in the equilibration buffer. The eluted fraction was dialyzed against 40 mM Tris-HCl, pH 8, and 0.03% β-DM at 4 °C for 12 h. The FRP quantity was then estimated using a Bradford test, and its quality was checked on SDS/PAGE gels and by Western blot.

Purification of the Short His-Tagged FRP Overexpressed in *E. coli*. Culture (100 mL) of *E. coli* cells overexpressing the short FRP grown overnight at 37 °C was used to inoculate 2.4 L of new growth media. Protein expression was induced at an OD_{600} of 0.3 by adding 1 mM isopropyl-β-D-thiogalactoside and 2% ethanol. After 12 h of growth at 20 °C, the cells were harvested by centrifugation at 4,000 × *g* for 20 min at 4 °C and resuspended in ice-cold lysis buffer (40 mM Tris-HCl, pH 8, with 10% glycerol, 1 mM EDTA, 0.25 M NaCl, and 2 mM $MgCl_2$) containing 5 mM caproic acid, 5 mM benzamidine, 1 mM of PMSF, and 50 U/mL of DnaseI (bovine pancreas type IV; Sigma). Cells were lysed by two cycles through a French Press at 700 psi. The lysate was centrifuged at 20,000 × *g* for 45 min at 4 °C. The supernatant was loaded on a column of Ni-ProBond resin (Invitrogen) pretreated with binding buffer (40-mM Tris-HCl, pH 8, 150 mM K^+ phosphate buffer, 500 mM NaCl, and 10% glycerol). The FRP was eluted with binding buffer containing 300 mM imidazole. The elution fraction was dialyzed against 40 mM Tris-HCl, pH 8, at 4 °C for 24 h and concentrated. The purity of the protein was checked by SDS/PAGE and stored at −80 °C.

Size Exclusion Chromatography. Purified short FRP or long FRP was injected into a Superdex 75 HR 10/30 column (Pharmacia) equilibrated with 40 mM Tris-HCl, pH 8, 150 mM NaCl. The Akta FLPC system was equipped with a UV detector at 280 nm and was running at a flow rate of 0.5 mL/min. The gel filtration standards (Biorad and Sigma) included thyroglobulin (670 kDa), bovine γ-globulin (158 kDa), bovine albumin (66 kDa), chicken ovalbumin (45 kDa), carbonic anhydrase (29 kDa), equine myoglobin (17 kDa), and vitamin B_{12} (1.35 kDa).

1. Lagarde D, Beuf L, Vermaas W (2000) Increased production of zeaxanthin and other pigments by application of genetic engineering techniques to Synechocystis sp. strain PCC 6803. *Appl Environ Microbiol* 66:64–72.
2. Wilson A, et al. (2008) A photoactive carotenoid protein acting as light intensity sensor. *Proc Natl Acad Sci USA* 105:12075–12080.

```
Microcystis NIES-843       -----MSAKRVMVVQKPKIIIPKTKIMPMSEIIWSDTEQEIAQTAFQKAYQRETSTLIEH
Microcystis PCC 7806       --------------------------MPMSEIIWSDTEQEIAQTAFQKAYQRETSTLIEH
Lyngbya sp. PCC 8106       -------------------------MIPVSDIEWSTAEKEIAREAFEKAYQREITALIKV
Anabaena sp. PCC 7120      --------------------------MYVSEAEWSDIEKTIARTAFDQAYKREIEALLKQ
Anabaena ATCC 29431        --------------------------MHVSEAEWTDIEKKIARTAFDQAYKREIEALLKQ
Synechocystis PCC 6803     MGFSLQSRLTFFIMVMIITNQYHKLMLQTAEAPWSQAETQSAHALFRKAYQRELDGLLAT
Arthrospira CS-328         ----------------------------MSEINWSEQDKNAAVDALHKAYEREVKILIDE
Cyanothece sp. CCY0110     --------------------------MKTNTQEWSQTDQKIAYEALTKAYARETEALIAE
Cyanothece sp. ATCC 51142  --------------------------MKTNTQQWSQTDQKIAQEALGKAYARETEALVAE
Synechococcus sp. 7002     -----------------------MLTMPTANTQWSLQEKTIAKEAFELSYRREIAAVMAE
Synechococcus sp. WH5701   -------------------------MVESALITWSDDEQAIAEEAFSCAYARAVQTVIST
Synechococcus sp. WH7805   ----------------------------MSAADWTHQEQSVAKHAFETGKQRSIEALIVI
Synechococcus sp. WH7803   ----------------------------MSAADWTHQEQSIAKDAFETGKQRSIEALIVI
Synechococcus sp. WH8102   ----------------------------MSATDWTSEEQTVARTAFELGKQRSITALIST
Synechococcus sp. RS9917   ----------------------------MSATDWSQDEIVVARHAFERGNQKSIEVLIAS
Synechococcus sp. RCC307   ----------------------------MSANEWTEQEKTIAEQAFESGNKKAMEVLIRS
Synechococcus sp. CC9902   ----------------------------MTPADWSSQEEQVARHAFDNGRNRSIESLIST
Synechococcus sp. BL107    ----------------------------MTPADWSSQEEQVARHVFDNGRRRSIESLISV
Synechococcus sp. CC9311   ------------------------MNHVMTQVDWPSAETEIARKAFQKGNERAVTVLISV
                                                            *.  :   *   :  .  :    ::

NIES-843     IKEQSGQITVLDDIWQMHDYLSARRHQIDGKYDYRYTSLIFVFAQLLKEGWLKLEDLNGLEKDKLAKIAALSRM-
PCC 7806     IKEQSGQITVLDDIWQMHDYLSARRHQIDGKYDYRYTSLIFVFAQLLKEGWLKLEDLNGLEKDKLAKIAALSRM-
PCC 8106     VREQAGEITKLDDIWLLHDFLSARRHDIDGKYDHRDSLLIFSFAQLVKEGWLHLDELKGLDAEKISKVSVLTRM-
PCC 7120     VQKEASTLVELDELWQLHDFLSARRHEIEGKYDYQYSALLFVFAGLVKDGWLHVNELEGLSKDKLSKVSALARM-
ATCC 29431   VQKEASTLVELDGLWQLHDFLSARRHEIEGKYDYQYSALLFVFAGLVKDGWLHVNELEGLSKDKLSKVSALARM-
PCC 6803     VQAQASQITQIDDLWKLHDFLSAKRHEIDGKYDDRQSVIIFVFAQLLKEGLVQAEELTFLAADKQSKIKALARL-
CS-328       VKQKAFNLTEIDEVWKLHDFLSARRHDIDGKYDYRDAASVFVLATLVKQGWLSLEELQVLGQDKLAKISALTRL-
CCY0110      INQKASQVNHIDEIWALNDYLSSKRYNIDGKYDFRESASVFVLAQLVKEKWLTLDELTDLTPSTKAKVAALTKMS
ATCC 51142   INQKASQITHINDIWALNDYLNSKRYHIDGKYYSSDATSMFVLAQLVKEKWLTLDELTGLTPSTRAKVAALAKMS
PCC 7002     AKRQATLAADPADLWQLNDFLSARRHYLDGKYDFSPESLVFTFAQLIKEGWLELQELDGLAAEKLSKIRILTLM-
WH5701       VRREADSINNAQSLWRLHDYLSTQRHVMEGRFDFRLEGILFVFASLVKENLLDLQELDGLDADKLAKIKAMSRF-
WH7805       LREQSALLDTPESIWKFHDFLSSERFQYEGRSEFDTANILFTLADMIKQNLINYDDLEGLEKTKLSKIKAMAMF-
WH7803       LRKQSALLDTPESIWRYHDFLSSERYQYEGRAEFDTANILFNLADMIKQNLITYEDLEGLDTTKLSKIKAMAMF-
WH8102       LQEQSSTLDTPESVWKLHDHLSTERYLYEGRAEFDEGNVLFVLADMIKQRLIALDDLQGLDQTKVSKVKAMSMF-
RS9917       LQEQVNSLNTAESIWKLHDFLSTERFDYEGRSEFDDAKILFILADMVKRKLISRQDLVGLSDKKISKIKAMAMF-
RCC307       LQELSLRLDSPESVWKFHDYLSSERYAYEGRSAFDEANALFSLAEMVKLKLIVLENLQGLDQSKIGKIKAMSMF-
CC9902       LQAKCQELNTDESVWNMHDYLSTERHLFEGRSEFDYGNILFTLAELLKQQLITLDELNGLDPKKLAKIKAMSMF-
BL107        LQTKCQELSTDESVWSMHDYLSTERHIFEGRSEFDYNNILFTLADLLKQQLITLEELEGLDPKKLAKIKAMSMF-
CC9311       IQNMSQSLSTLESVCSLHDYLSTERFEIEGRMEFNHDTILFSLAEMMKRNLIEANDLQGLDPKKVSKIKAMSLF-
              .           :*  :*.*.:.*.  :*:        :* :* ::*   :  ::*  *   . .*:  :: :
```

Fig. S1. Sequence alignment of 19 predicted proteins encoded by *slr*1964 homologue genes in 19 cyanobacteria. Blue color and a period indicate amino acid weakly similar, green color and a colon indicatye amino acid strongly similar, red color and an asterisk indicate identical amino acid. The most conserved parts are underlined in yellow. The alignment was performed with ClustalW.

A pCB19 : 7019pb

NdeI ATG HpaI

psbA2up slr1964 psbA2down

499pb 524pb 493pb

Sp/Sm K7

2082pb

B pCB14 : 8045pb

NdeI ATG HpaI

psbA2up slr1963 slr1964 psbA2down

499pb 1523pb 493pb

Sp/Sm K7

2082pb

C

kb Over FRP WT

1 2 3

3

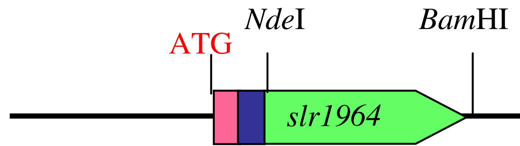
2

1.5

Fig. S2. Gene arrangement in the *psbA2* region of the mutant overexpressing the *frp* gene (*A*) and of the mutant overexpressing the putative *ocp-frp* operon (*B*). (*C*) Amplification of genomic *Synechocystis* DNA from the overexpressing N-terminal (1) and C-terminal (2) His-tagged FRP mutants and from the WT (3) using *psbA1* and *psbA2* as primers. The amplification gave an unique fragment of 3 kb in the mutants. No traces of 1.5 kb fragment (observed in WT) were detected indicating that the mutants were completely segregated. *psbA1*, 5′-ACGCCCTCTGTTTACCCATGGAA-3′; *psbA2*, 5′-CCAGGCCTCAACCCGGTACAGAG-3′.

Fig. S3. (*A*) Fluorescence spectra of WT and ΔSlr1964 mutant cells grown under low light conditions. The excitation was done at 430 nm (specially absorbed by chlorophyll) and at 600 nm (specially absorbed by PBs). The cells were diluted to 3 μg chlorophyll/mL and the spectra were done in the presence of 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The mutant cells had no or very small fluorescence quenching. In the figure, it is shown the maximal quenching observed. (*B*) Blue-green light induced fluorescence quenching and fluorescence recovery in the Δ*psbA2* mutant. Decrease of maximal fluorescence (Fm) during exposure of WT (red) and Δ*psbA2* mutant (blue) cells to 550 μmol m^{-2} s^{-1} of blue-green light (400–550 nm) and Increase of maximal fluorescence (Fm) during exposure of "quenched" WT (red) and Δ*psbA2* mutant (blue) cells to 80 μmol m^{-2} s^{-1} of blue-green light (400–550 nm). The cells were diluted to 3 μg chlorophyll/mL. In the Δ*psbA2* mutant the *psbA2* gene was replaced by a kanamycin-resistant cassette.

A



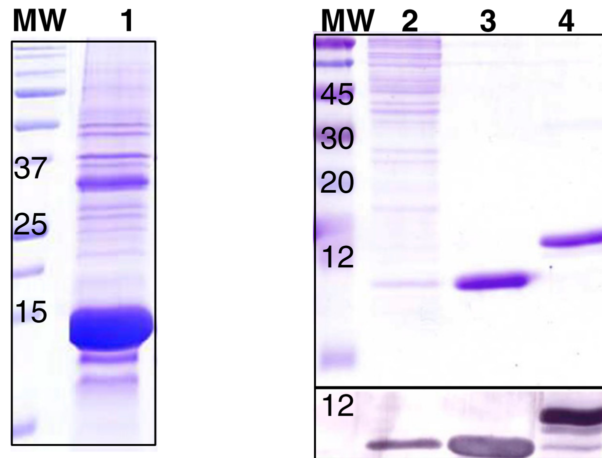
1 26

MGFSLQSRLTFFIMVMIITNQYHKLMLQTAEAPWSQAETQSAHALFRKAYQREL
DGLLATVQAQASQITQIDDLWKLHDFLSAKRHEIDGKYDDRQSVIIFVFAQLLKE
GLVQAEELTFLAADKQSKIKALARL

Long FRP: cloning from the first GTG encoding Met1; His-tag (rose square) and thrombin site (blue square) just after Met1

Short FRP : cloning from the fourth ATG encoding Met26; His-tag and thrombin site after Met26

B



C

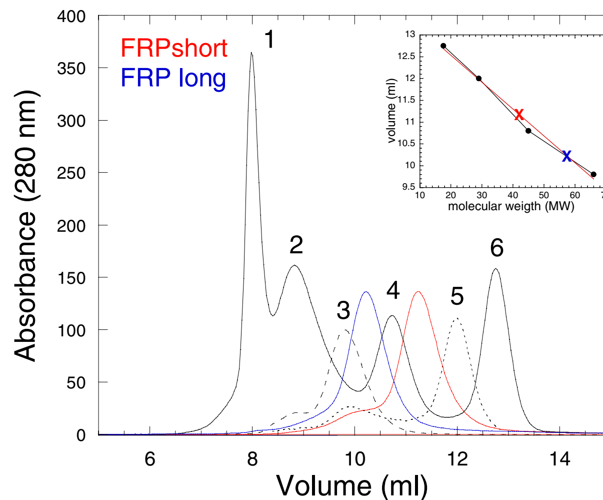


Fig. S4. FRP overexpression in *E. coli* and isolation of long and short FRP. (*A*) Gene arrangement of the *slr1964* gene in the pET-15b plasmid (Novagene) for overexpression of long (from Met-1) and short (from Met26) FRP. (*B*) Coomassie blue–stained gel electrophoresis of inclusion bodies containing the long FRP (1), of the supernatant containing the short FRP (2), and of isolated FRP after elution of the Ni column (3 and 4). Short FRP isolated from the soluble fraction (3), long FRP isolated from the inclusion bodies after solubilization with LiDS and renaturation (4). This partial purified long FRP was used to produce antibodies.

Legend continued on following page

The immunodetection of the FRP in *E. coli* supernatant (2) and the elution fraction of the Ni column (3 and 4) is also shown in the lower panel. (*C*) Size exclusion chromatograms of short (red) and long FRP (blue) and of standards labeled “1” for thyroglobulin (670 kDa), “2” for γ-globulin (158 kDa), “3” for bovine albumin (66 kDa), “4” for ovalbumin (45 kDa), “5” for carbonic anhydrase (29 kDa), and “6” for myoglobin (17.6 kDa). The theoretical mass of the short and long FRP monomers (both containing 6 His plus the site for thrombin cut) are 14.5 and 17.5 kDa, respectively. Short FRP was eluted at 11.2 mL, indicating a complex with a MW of approximately 40 to 42 kDa and long FRP was principally eluted at 10.2 mL, indicating a MW of approximately 56 to 58 kDa (*Inset*) indicating that FRP forms trimeric complexes in solution. No traces of monomers or dimers were detected. The trimers of short FRP seems to be more “compact” than those of long FRP.

Fig. S5. The anti-FRP antibodies are specific to the N-terminal His-tagged part of FRP. A gel stained with Coomassie blue (*Top*) and a Western blot anti-FRP (*Lower*) are shown. Wells contain: (1) cells and (2) membranes of the mutant overexpressing FRP with an N-terminal His tag, (3) membranes from the WT, (4) membranes from the mutant overexpressing FRP without tag, (5) membranes from the mutant with a C-terminal His tag, and (6) cells of the mutant overexpressing FRP with a C-terminal His tag. Each well contains 4 μg chlorophyll.

Fig. S6. Effect of *E. coli* soluble proteins, solubilized *E. coli* membrane proteins, and *E. coli* and *Synechocystis* membranes on OCP^{r}-to-OCP^{o} conversion. OCP (2.3 μM) alone or in the presence of other proteins or membranes was illuminated 5 min at 18 °C and then the OCP^{r}-to-OCP^{o} conversion occurring in darkness was followed. The figure shows the spectrum of the OCP^{r} (after 5 min illumination) and the spectrum of the OCP^{o} after 20 min of darkness: (*A*) OCP alone, (*B*) in the presence of soluble *E. coli* proteins (0.2 mg protein/mL) or (*C*) of solubilized membrane *E. coli* proteins or (*D*) *E. coli* membranes (nonexpressing *frp*) or (*E*) *Synechocystis* membranes (0.1 mg protein/mL). For the last two spectra, the membranes of *E. coli* and *Synechocystis*, respectively, were used as references to avoid the superposition of the OCP spectrum with those of chlorophyll, carotenoids, cytochromes, etc., present in the membranes. This has a minor effect on the OCP spectra. To solubilize the membrane proteins, the membranes of *E. coli* were treated as the FRP-containing inclusion bodies (*SI Materials and Methods*). The presence of soluble *E. coli* proteins (even at a concentration 10 times higher than the concentration of FRP used in Fig. 5 in the main text) did not affect dark OCP^{r}-to-OCP^{o} conversion whereas the presence of solubilized membrane *E. coli* proteins completely inhibited this conversion. The presence of untreated membranes of *E. coli* or *Synechocystis* did not stabilize the OCP^{r}. Moreover, the kinetics of OCP^{r}-to-OCP^{o} conversion was not affected by the presence of *E. coli* or *Synechocystis* membranes (*F*, OCP alone, red; OCP plus *Synechocystis* membranes, blue; OCP plus *E. coli* membranes, black).

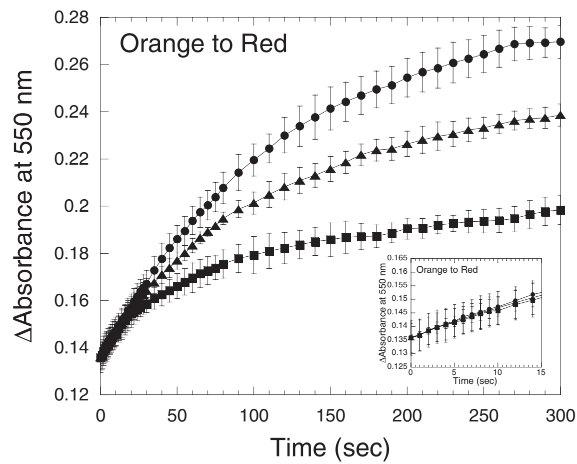


Fig. S7. The presence of FRP did not affect the initial rate of OCP^r accumulation. Light photoconvertion of OCP^o to OCP^r (2.3 μM) (increase of the absorbance at 550 nm) in the absence (circle) or in the presence of 2.3 μM FRP (square) or 1.15 μM FRP (triangle) at 18 °C. The curves in the presence of FRP are the average of three independent experiments. The control (OCP without FRP) was done three times in the presence of 0.2 mg/mL soluble *E. coli* proteins and three times in the absence of these proteins. The control curves shown in Fig. S7 and Fig. 5*D* are an average of these six experiments. (*Inset*) First 15 s of illumination. Whereas FRP did not affect the initial rates of the photoconversion, the steady-state concentration of OCP^r depended on the concentration of FRP as it accelerated the OCP^r-to-OCP^o conversion (Fig. 5*D*).

Table S1. Genes coding for Slr1964 homologues in cyanobacteria and identity of these proteins to the Slr1964 protein from *Synechocystis*

Strain	Gene name	Identity, %	Upstream *slr*1963
Synechocystis PCC 6803	Slr1964	100	Yes
Lyngbya sp. PCC 8106	L8106_29205	52	Yes
Microcystis aeruginosa NIES-843	MAE_18920	52	Yes
Microcystis aeruginosa PCC 7806	IPF_5685	52	Yes
Anabaena sp. PCC 7120	all3148	50	Yes
Anabaena variabilis ATCC 29413	Ava_3842	49	Yes
Synechococcus sp. PCC 7335	S7335_121 S7335_1726	45 44	Yes
Cyanothece sp. PCC 7424	PCC7424_4468	50	Yes
Microcoleus chthonoplastes PCC 7420	MC7420_3635	50	Yes
Arthrospira maxima CS-328	AmaxDRAFT_3432	48	Yes
Cyanothece sp. CCY 0110	CY0110_09682	42	Yes
Synechococcus PCC 7002	SYNPCC7002_A2808	44	Yes +K[3]
Cyanothece ATCC 51142	cce_1648	42	Yes
Synechococcus sp. WH 5701	WH5701_04000	42	Yes +K
Cyanobium sp. PCC 7001	CPCC7001_233	38	Yes +K
Synechococcus sp. RS9917	RS9917_00682	35	Yes +K
Synechococcus sp. CC9902	Syncc9902_0971	34	Yes +K
Synechococcus sp. CC9311	sync_1805	32	Yes +K
Synechococcus sp. BL107	BL107_14115	33	Yes +K
Synechococcus sp. WH8102	SYNW1369	32	Yes +K
Synechococcus sp. WH7805	WH7805_01192	31	Yes +K
Synechococcus sp. WH7803	SynWH7803_0927	31	Yes +K
Synechococcus sp. RCC307	SynRCC307_1994	26	Yes +K

The ranking of the strains (from the closest homologue of *Synechocystis slr1964*) has been performed by the program NCBI BLASTP 2.2.16 (March 25, 2007). +K, gene coding for a β-carotene ketolase is found between the *slr1963* and *slr1964* homologue genes.

Table S2. Primers used for RT-PCR and DNA amplification in Fig. 3

Name	Sequence 5′-3′
CarF	AAT TTT CCC TAA CAC CCT AGC
CarR	AGT AAT TCT TTG GGG GAA GCA
64F	TTG CAG AGC AGG CTC ACT TTT
64R	TTT AGA TTG CTT ATC GGC GGC
64–33R	AAA AGT GAG CCT GCT CTG CA
64–232R	GGA GTT TCC AAA GAT CAT CG
64–394R	CCA GGG CCT TAA TTT TAG ATT GC
64–136F	TTC CGT AAG GCC TAC CAA AGA
63–612F	CCT CAA TGC CAA TGA CTT C
63–744R	CAG GTT TTG GCA CTC TTC
63–334F	CGT TTA GGC GAA CTG ATG

The primers used to construct cDNA were 64–394R (lanes 1 and 4), CarR (lane 2), 63–744R (lane 3), 64–33R (lanes 5 and 6), 64–232R (lane 7) and 64–394R (lane 8). The primers used for PCR amplification on cDNA were 64–394R/64F (1), CarR/CarF (2), 63–744R/63–334 (3), 64–394R/64–136 (4), 64–33R/63–612F (5 and 6), 64–232R/63–612F (7) and 64–394R/63–612F (8).