Effects of a type-II RNA-binding protein on fatty acid composition in *Synechocystis* sp. PCC 6803

TANG Qing, TAN XiaoMing & XU XuDong[*]

State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

In the cyanobacterium *Synechocystis* 6803, *rbp3*, a type-II RNA-binding protein gene, is slightly induced by temperature downshift. An *rbp3* mutant shows significant reduction in total polyunsaturated fatty acids (PUFA) in membrane lipids. However, the reduction in PUFA has not attained the extent that would significantly affect the growth of the mutant at low temperature. Transcripts of fatty acid desaturase genes *desA*, *desB* and *desD*, and *ccr-1*, a gene required for growth at 15°C, are significantly reduced in the mutant relative to the wild type, while transcripts of *rbp1* (RNA-binding protein 1) and *crhR* (RNA helicase Light) are not affected. Rbp3 may directly or indirectly affect mRNA levels of certain genes.

Rbp3, fatty acid composition, *des* genes, *Synechocystis*

RRM (RNA-recognition motif)-type RNA-binding proteins (Rbps) are involved in many post-transcriptional regulation processes [1]. In cyanobacteria, Rbps contain a single RRM with (type I) or without (type II) a short C-terminal glycine-rich domain [2]. Genes encoding type-I Rbps are rapidly induced by cold stress, while genes encoding type-II Rbps are usually not or slightly inducible [2,3]. An *rbpA* mutant of *Anabaena variabilis* shows induction of early heterocyst differentiation by a shift from 38 to 22°C, indicating the role of the RNA-binding protein in maintaining a correct gene expression pattern [4]. An *rbp1* mutant of *Synechococcus* sp. strain PCC 7942 shows poor growth at a low temperature relative to the wild type [5]. *Anabaena* RbpA and *Synechococcus* Rbp1 both belong to type-I RNA-binding proteins. Hitherto, no function of type-II Rbps in cyanobacteria has been reported.

There are 4 types of fatty acid desaturase genes in cyanobacteria, *desA*, *desB*, *desC* (including *desC1* and *desC2* in certain species) and *desD* [6,7]. In *Synechocystis* sp. PCC 6803 (hereafter referred to as *Synechocystis* 6803), *desA*,

desB and *desD* are induced by exposure to low temperature in the presence of light [8]. The 4 fatty acid desaturases introduce double bonds respectively at $\Delta 6$, $\Delta 9$, $\Delta 12$ and $\omega 3$ positions of C18 fatty acids, and the increased expression of fatty acid desaturase genes leads to resumption of membrane fluidity at the low temperature [6,9]. Inactivation of *desA* rather than *desB* or *desD* significantly reduces the growth of *Synechocystis* 6803 at 20°C [10]. The growth at low temperature appears to depend upon the total percentage of C18:2, C18:3 and C18:4 fatty acids in membrane lipids [10].

In this report, we present evidence that a type-II RNA-binding protein Rbp3 is required for maintaining the high degree of fatty acid unsaturation in *Synechocystis* 6803. Consistently, the mRNA levels of *desA*, *desB* and *desD* are significantly reduced in an *rbp3* mutant of the cyanobacterium.

1 Materials and methods

1.1 Strains, culture conditions, measurement of fatty acid composition and luciferase activity

Synechocystis 6803 from ZHAO JinDong at Institute of Hydrobiology (CAS) was grown in BG11 on a rotary shaker

*Corresponding author (email: xux@ihb.ac.cn)

at 30°C in the light of 30 $\mu E\ m^{-2}\ s^{-1}$. For mixotrophic growth, 5 mmol/L glucose was supplemented to the BG11 medium. For transformants, erythromycin, kanamycin or spectinomycin was added to the medium at 5, 20 or 10 $\mu g\ mL^{-1}$. Measurements of fatty acid composition and assays of luciferase activities were performed as previously described [11]. Data presented are means of measurements from 3 independent cultures with deviations.

1.2 Construction of plasmids and mutants

Molecular manipulations were performed according to standard protocols. Molecular tool enzymes were used according to instructions provided by the manufacturers. PCR clones were confirmed by sequencing. Primers are listed in Table 1.

To express Rbp1 in *Escherichia coli*, we constructed plasmid pHB2540. The PCR fragment containing *rbp3* was amplified with primers slr0193-a3 and slr0193-a4, cloned into the T-vector pMD18-T (TaKaRa), resulting in pHB2494. The *Nde* I-*Xho* I fragment containing *rbp3* was excised from pHB2494 and cloned into pET21b cut with *Nde* I and *Xho* I, resulting in pHB2540.

To inactivate *rbp3* in *Synechocystis* 6803, we constructed pHB2547 and pHB3175. The PCR fragment overlapping the 5′-end of *rbp3* was generated with primers slr0193-k1 and slr0193-k2 and cloned into pMD18-T, resulting in pHB2495. A *Spr* gene was excised with *Xba* I from pHB576 [12] and cloned into the *Xba* I site of pHB2495, resulting in pHB2534. On the other hand, The PCR fragment overlapping the 3′-end of *rbp3* was generated with primers slr0193-k3 and slr0193-k4 and cloned into pMD18-T, resulting in pHB2496. The fragment containing the up- stream sequence of *rbp3* and the *Sp*^{r} gene was excised with PvuII from pHB2534, ligated with SmaI-cut pHB2496, resulting in pHB2547, in which

Table 1 Cyanobacterial strains and primers[a]

Strains, primers	Derivation, relevant characteristics or sequences (5′→3′)	References, source or gene
Cyanobacterial strains		
Synechocystis sp. PCC 6803	Wild type	ZHAO, Peking University/Institute of Hydrobiology, CAS
DRHB2263*	Sp^r, *desB*: *luxAB*-Sp^r, *Synechocystis* 6803 transformed with pHB2263	This study
DRHB2547	Sp^r, Δ*rbp3*/Sp^r, *Synechocystis* 6803 transformed with pHB2547	This study
DRHB2547/DRHB2830	Cm^{r}Em^{r}Sp^r, Δ*rbp3*/Sp^r complemented with the wild type *rbp3*, DRHB2547 transformed with pHB2830	This study
DRHB3175	Km^r, Δ*rbp3*/Km^r, *Synechocystis* 6803 transformed with pHB3175	This study
DRHB3175/DRHB2263	Km^{r}Sp^r, Δ*rbp3*/Km^r *desB*: *luxAB*-Sp^r, DRHB3175 transformed with pHB2263	This study
Primers		
crhR-1[†]	CTTTGCCCTACCTTTGATG	*crhR*
crhR-2[†]	GGCTTCCTTGAGTTGTTCC	
desA-1[†]	CAACGGTAACGCCCAGCA	*desA*
desA-2[†]	CAAACAGGAAGACAACGGCA	
desB-1[†]	CGACACCGACGAAAGTTGG	
desB-2[†]	CTTGATGGCTTCAGTCGCC	*desB*
sll1141-1	CTTGATGGCTTCAGTCGCC	
sll1141-2	CTGCTCAAGGGTTTGGATGA	
sll1242-1[†]	CTAGTGGGTCGGAAGGTTT	*ccr-1*
sll1242-2[†]	CTGAGTAACTGGGCTGGTTC	
desD-1[†]	AACCAACGGGTGGATGCCT	*desD*
desD-2[†]	AGAGCCAGAGGTAAGCCGA	
sll0517-1[†]	TCTCATATGTCAATTTATGTAGGCAACCTGTCC	*rbp1*
sll0517-2[†]	TTTCTCGAGTGGTGGAACGACGGCGAA	
slr0193-a3	TTTCATATGTCCATTCGTCTCTACGTCGGTAA	
slr0193-a4	AAACTCGAGAGGGAAAACCTGTGGACCTACT	
slr0193-k1	CGGACTTCACCACTATTTACGA	
slr0193-k2	GAGACGAATGGACATGATCTAAATACCT	
slr0193-k3	CAGTAGGTCCACAGGTTTTCC	*rbp3*
slr0193-k4	AACTCCGCTGGGTTGGTAA	
slr0193-c	CTGGCTCTGATGTTGGCT	
slr0193-4	AAACTCGAGAGGGAAAACCTGTGGACCTACT	
rnpB-1[†]	GTTAGGGAGGGAGTTGCGG	*rnpB*
rnpB-2[†]	AAGAGAGTTAGTCGTAAGCCG	

a) Ap, ampicillin; Cm, chloramphenicol; Em, erythromycin; Km, kanamycin; Sp, spectinomycin. *, DRHB (number) refers to a product of double homologous recombination between plasmid pHB (number) and the *Synechocystis* sp. genome. †, Primers used for PCR to generate probes for Northern blot hybridizations.

the upstream and downstream sequences of *rbp3* oriented as in the genome sand wich the *Sp*[r] gene. The *Km*[r] gene excised with *Xba* I from pRL446 [13] was cloned into pHB2547 replacing the *Sp*[r] gene, resulting in pHB3175.

To complement the *rbp3* mutant DRHB2547, we constructed pHB2830. The PCR fragment containing *rbp3* was amplified with primers slr0193-c and slr0193-4, cloned into pMD18-T, resulting in pHB2822. The C.CE2 cassette excised with *Xba* I from pHB598 [13] was inserted into the *Xba* I site downstream of *rbp3* in pHB2822, resulting in pHB2825. The *rbp3*-C.CE2 was excised with *Kpn* I and *Hin*d III from pHB2825, blunted with T4 DNA polymerase, and inserted into *Eco*R I-cut and T4 DNA polymerase-blunted pKW1188 [14], resulting in pHB2830.

To detect the promoter activity of *desB*, we constructed pHB2263. The PCR fragment containing *desB* was amplified with primers sll1141-1 and sll1141-2, and cloned into pMD18-T, resulting in pHB512. The *luxAB*-Spr fragment excised with *Sma* I from pRL58 [15] was cloned into the *Bal* I site within *desB* in pHB512, resulting in pHB2263.

Synechocystis mutants were generated by transformation of the cyanobacterium with the described plasmids as listed in Table 1. Transformation of *Synechocystis* 6803 was performed according to Williams [14]. Segregation of the *rbp3* mutant was confirmed by PCR.

1.3　Northern blot hybridization

Total RNA was extracted from *Synechocystis* 6803 using TRIzol reagent (Invitrogen, USA), treated with RNase-free DNase I (Takara, Japan) to eliminate the contaminating chromosomal DNA. Gene-specific PCR products were labeled by incorporation of digoxigenin-dUTP and used in Northern blot analyses. PCR primers used to generate the probes are listed in Table 1. Hybridization and immunological detection were performed with a DIG High Prime DNA Labeling and Detection Starter Kit I (Roche, USA) according to the manufacturer's recommendation. The gene encoding the RNA subunit of ribonuclease P was used as the control of constitutive transcription [16]. The intensity of *rbp3* hybridization band was quantified using the software Image J 1.42q by Wayne Rasband (National Institute of Health, USA) from results of 3 independent experiments.

1.4　RNA-binding activity assay

Synechocystis Rbp3 was produced in *E. coli* BL21 (DE3) harboring pHB2540 (Table 1). Soluble proteins extracted from *E. coli* were applied onto a HiTrap heparin column (Amersham, USA) equilibrated with 10 mmol/L sodium phosphate (pH 7.0). After an extensive wash of the column with the phosphate buffer, Rbp3 was eluted with the same buffer containing 0.2 mol/L NaCl. The gel-filtration assay of RNA-binding activity was performed according to Sato [17]. Proteins were separated with 12% SDS-PAGE and visualized with CBB (Coomassie Brilliant Blue R-250)-staining.

2　Results

2.1　Rbp3 is a type-II RNA-binding protein

In *Synechocystis* 6803, *rbp3* is predicted to encode an RNA-binding protein without any C-terminal glycine-rich domain. Northern blot hybridization showed that the size of its transcript is about 600 nt (Figure1(a)). Quantitative analysis of Northern blot hybridization bands of 3 independent experiments indicated a slight increase in the mRNA level of *rbp3* in *Synechocystis* 6803 transferred from 30 to 15°C (Figure 1(b)).

To test the RNA-binding activity of the encoded protein Rbp3, we produced it in *E. coli*, and purified it with a heparin column. The purified protein was assayed for RNA-binding activity by gel-filtration (Figure 1(c)). Under the experimental conditions, Rbp3, or Rbp3 combined with double-stranded DNA, was retained in the Sephadex G-50 column, while mixed with total RNA from *Synechocystis* 6803, a certain amount of Rbp3 was eluted from the column. Therefore, Rbp3 is indeed able to bind RNA. These results indicated that Rbp3 in this cyanobacterium is a type-II RNA-binding protein.

2.2　Effects of Rbp3 on fatty acid composition

To investigate the Rbp3 function, we replaced its encoding gene with a spectinomycin-resistance gene in *Synechocystis* 6803, resulting in strain DRHB2547 (Δ*rbp3*/Sp[r]) (Figure 2,
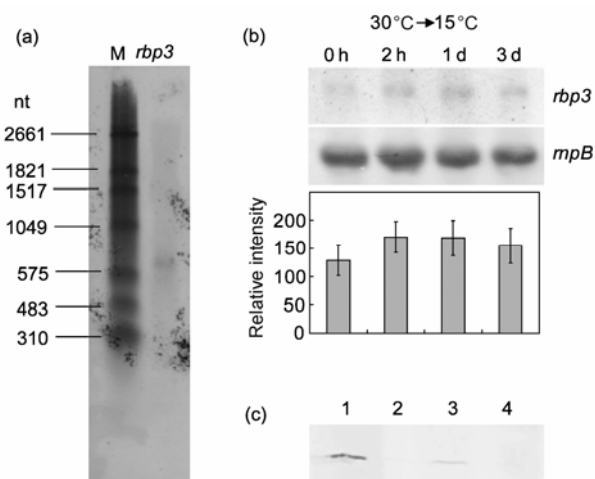

Figure 1　Northern blot analysis of *rbp3* mRNA and an assay of the RNA-binding activity of its encoded product. (a) Northern blot analysis showing the size of *rbp3* mRNA. M, RNA markers. (b) The change of the mRNA level of *rbp3* in *Synechocystis* 6803 transferred from 30 to 15°C as shown with Northern blot hybridization. The intensities of hybridization bands were quantified using the software Image J 1.42q from the results of 3 independent experiments. The transcription of *rnpB* (RNase P subunit B) [16] was used as the internal control. (c) SDS-PAGE examination of elutes resulted from gel-filtration assays for the RNA-binding activity of Rbp3. Lane 1, Rbp3 (5 μg); lanes 2–4, elutes from the Sephadex G-50 column loaded with Rbp3 (5 μg), Rbp3 (5 μg) + RNA (15 μg), Rbp3 (5 μg) + DNA (15 μg). Total RNA extracted from *Synechocystis* 6803 and the lambda DNA digested by *Hin*dIII were used in the binding assays.
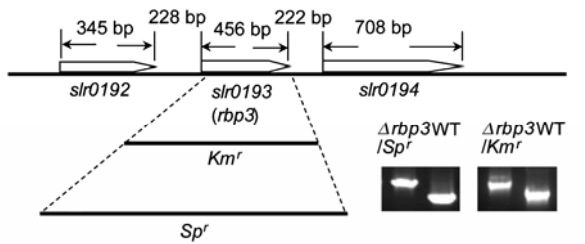
Figure 2   Replacement of *rbp3* with a kanamycin-resistance or a spectinomycin-resistance gene in *Synechocystis* 6803. Complete segregation of the resulting mutants DRHB2547 (Δ*rbp3*/Sp^r) and DRHB3175 (Δ*rbp3*/Km^r) was confirmed with PCR using primers slr0193-k1 and slr0193-k4 (Table 1). WT, wild type.

Table 1). The *rbp3* mutant showed the same growth rates ((1.33±0.06) and (0.45±0.02) doublings d^{-1}, respectively) as that of the wild-type strain ((1.33±0.20) and (0.45±0.04) doublings d^{-1}, respectively) at 30 and 15°C. However, C18 polyunsaturated fatty acids (PUFA, including 18:2, 18:3 and 18:4) were reduced by 11.74% in the mutant relative to the wild type at 15°C, and reduced by 14.11% in the mutant at 30°C (Table 2). Compared to the increase of PUFA in the wild type transferred from 30°C to 15°C (5.34%), the decrease of PUFA in the mutant was significant.

To confirm the role of Rbp3 in maintaining fatty acid composition in the cyanobacterium, we introduced *rbp3*-C.CE2 into a neutral integrative platform [14,18,19] in the genome of the *rbp3* mutant, resulting in strain DRHB2547 DRHB2830 (Table 1). The complemented strain showed almost no change in growth ((1.45±0.06) doublings d^{-1} at 30°C and (0.44±0.03) doublings d^{-1} at 15°C) compared to the wild type and the mutant. However, relative to the *rbp3* mutant, the percentage of PUFA in C18 fatty acids was increased by 7.3 at 30°C and by 12.81 at 15°C (Table 2). Also, the complemented strain showed differences in fatty acid composition from that of the wild-type strain. Especially, C18:4 was increased by 2.6 folds relative to the wild-type level at 15°C. The integrated *rbp3* partially complemented the mutation, but fully restored the percentage of total PUFA in the mutant at 15°C (Table 2).

2.3   Effects of Rbp3 on mRNA levels of *desA*, *desB* and *desD*

In *Synechocystis* 6803 transferred to 15°C, *desA*, *desB* and *desD* are induced to express as shown with Northern blot analysis. However, in the *rbp3* mutant, these transcripts are not or almost not detectable under the same conditions (Figure 3). Cells grown autotrophically and mixotrophically produced similar results. By contrast, *rbp1* (RNA-binding protein 1) and *crhR* (RNA helicase Light), both induced in *Synechocystis* 6803 exposed to 15°C, were not affected by *rbp3* (Figure 3). *rbp1*, *crhR* and *des* genes are induced at an early stage of cold acclimation, while *ccr-1*, recently identified by our lab [11], is induced at a rather late stage. Apparently, there are multiple mRNA bands for *ccr-1*. After transfer to 15°C for 6 d, the *rbp3* mutant also showed reduction in the level of *ccr-1* mRNAs relative to the wild type. Rbp3 appears to selectively affect the abundance of mRNA of some genes.

Regulation of mRNA levels could be based on promoter activity or mRNA stability or both. RNA-binding proteins are supposed to affect mRNA levels by the mRNA stability. To exclude the effect of Rbp3 on promoter activity, we tested the transcription of *desB* with bacterial luciferase reporter genes *luxAB*. The *luxAB*-Spr cassette [15] was introduced into the BalI site of *desB* in the wild type and another *rbp3* mutant DRHB3175 (Δ*rbp3*/Kmr, Table 1). In DRHB3175, *rbp3* was replaced with a kanamycin-resistance cassette. Insertion of *luxAB*-Spr into *desB* did not affect the growth of *Synechocystis* strains at 15°C. Expression of the bacterial luciferase activity indicated that *desB* was more actively transcribed in the mutant than in the wild type upon transfer from 30 to 15°C (Figure 4). In other words, inactivation of *rbp3* did not reduce the promoter activity of *desB*.

3   Discussion

In this paper, we report the role of Rbp3 in maintaining fatty

Table 2   Fatty acid composition of membrane lipids from *Synechocystis* 6803 strains (mole %)[a)]

Fatty acid	WT		*rbp3* mutant		Complemented strain	
	30°C	15°C	30°C	15°C	30°C	15°C
16:0	52.99±2.24	49.89±0.63	53.26±0.71	51.67±0.81	47.82±4.17	46.41±1.59
16:1 (9)	2.83±0.51	3.87±0.31	2.01±0.25	3.93±0.59	2.07±0.27	5.39±0.16
18:0	0.95±0.15	0.81±0.13	1.32±0.07	0.66±0.13	3.08±0.93	2.74±0.43
18:1 (9)	7.88±0.49	4.69±1.78	22.12±1.66	14.74±1.27	18.44±2.51	3.65±0.42
18:2 (9,12)	18.06±0.49	11.00±0.49	8.70±0.12	5.69±1.16	9.05±0.26	4.38±0.53
18:3 (6,9,12)	15.57±1.01	20.29±0.89	11.02±0.50	15.45±0.37	17.67±1.25	23.95±0.40
18:3 (9,12,15)	1.11±0.05	6.56±0.50	0.9±0.03	4.79±1.44	1.12±1.36	3.06±0.16
18:4 (6,9,12,15)	0.61±0.05	2.89±0.56	0.68±0.1	3.06±1.16	0.75±0.13	10.41±0.06
C18 PUFA	35.40±1.49	40.74±1.22*	21.29±0.69	29.00±1.34	28.59±1.44	41.81±0.88*

a) Values represent means ± standard deviation of measurements from 3 independent cultures. Except the two denoted with *, all PUFA values at the same temperature are significantly different from each other (Duncan's multiple range test, *P* = 0.05).
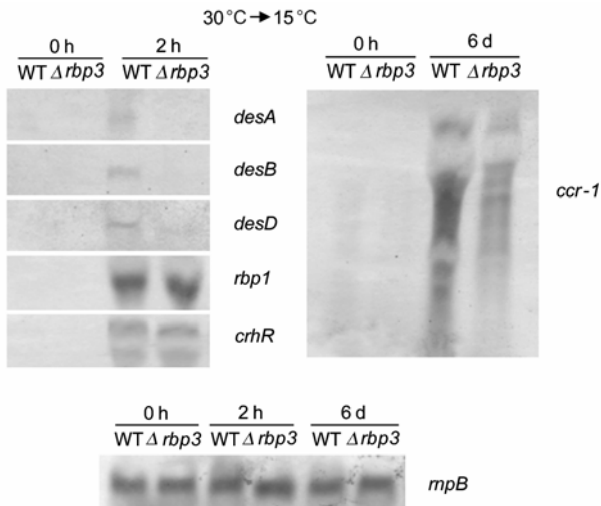
Figure 3 Effects of Rbp3 on the mRNA levels of fatty acid desaturase genes, *rbp1*, *crhR* and *ccr-1* as shown with Northern blot analyses. *desA*, *desB*, *desD*, *rbp1* and *crhR* mRNAs were detected in the wild type and Δ*rbp3* strains of *Synechocystis* 6803 transferred from 30 to 15°C for 2 h, while *ccr-1* mRNA was detected after exposure to 15°C for 6 d. WT, wild type.
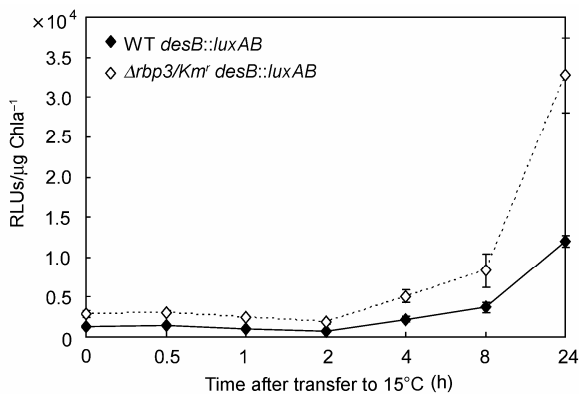

Figure 4 Expression of the bacterial luciferase genes *luxAB* from the *desB* promoter in the wild type and the Δ*rbp3*/Km^r strain of *Synechocystis* 6803. RLU, relative luminescence unit. WT, wild type.

acid composition in the cyanobacterium *Synechocystis* 6803. Rbp3 does not possess the C-terminal glycine-rich domain, and is only slightly induced by temperature downshift, therefore, it is a typical type II RNA-binding protein. Because *rbp3* is sized 456 bp, its downstream gene *slr0194* is sized 708 bp, and between them is an interval of 228 bp, the 600 nt-long mRNA of *rbp3* should not be a co-transcript with the downstream gene.

In the *Synechocystis rbp3*: Sp^r mutant, the percentage of total polyunsaturated fatty acids (PUFA) was significantly reduced relative to the wild type. The complementation by *rbp3* fully restored the percentage of PUFA in the mutant at 15°C to the wild-type level, which further confirmed the role of *rbp3* in maintaining fatty acid composition. The differences between the complemented strain and the wild type

could be due to the difference in expression of the supplemented *rbp3* at the integrative platform from that in the wild type strain. Because *rbp3* is only slightly regulated by temperature, it may exert similar effects on cell activities at 30 and 15°C. Consistent with this hypothesis, an *rbp3* mutant showed reduced content of C18 PUFA at both temperatures. The effects of Rbp3 on the fatty acid composition may be valuable for certain applied purposes.

Although the PUFA was significantly reduced in the *rbp3* mutant, it showed the same growth rates as the wild type at both 30 and 15°C. It is noteworthy that a greater reduction of total PUFA in a *desA* mutant led to poor growth of the cyanobacterium at low temperature [10]. Our explanation is that the percentage of PUFA is high enough in *Synechocystis* 6803 at 30°C, even with the reduction of PUFA in the *rbp3* mutant, the degree of fatty acid unsaturation is still sufficient to allow its growth at 15°C.

The mRNA levels of fatty acid desaturase genes *desA*, *desB* and *desD*, and *ccr-1*, a gene required for the growth at 15°C, were greatly reduced in the *rbp3* mutant relative to the wild type, while transcripts of *rbp1* and *crhR* were not affected. These results suggest that Rbp3 directly or indirectly affects the maintenance of mRNA levels of certain genes, especially *desA*, *desB* and *desD*, and the degree of fatty acid unsaturation in membrane lipids. The expression of *luxAB* from the *desB* promoter indicated that the reduced RNA level was not due to an effect on the promoter. One possibility is that Rbp3 may affect the stability of certain mRNAs. Because mRNAs of *des* genes were reduced to very low levels in the *rbp3* mutant, it was not feasible to measure the life time based on Northern blot hybridization. In the future, tests of ectopically expressed *des* genes and *ccr-1* in the mutant and the wild type may reveal the differences in life time of their trancripts.

1   Albà M M, Pagès M. Plant proteins containing the RNA-recognition motif. Trends Plant Sci, 1998, 3: 15–21

2   Maruyama K, Sato N, Ohta N. Conservation of structure and cold-regulation of RNA-binding proteins in cyanobacteria: Probable convergent evolution with eukaryotic glycine-rich RNA-binding proteins. Nucleic Acids Res, 1999, 27: 2029–2036

3   Sugita M, Sugiura M. The existence of eukaryotic ribonucleoprotein consensus sequence-type RNA-binding proteins in a prokaryote, *Synechococcus* 6301. Nucleic Acids Res, 1994, 22: 25–31

4   Sato N, Wada A. Disruption analysis of the gene for a cold-regulated RNA-binding protein, *rbpA1*, in *Anabaena*: Cold-induced initiation of the heterocyst differentiation pathway. Plant Cell Physiol, 1996, 37: 1150–1160

5   Sugita C, Mutsuda M, Sugiura M, et al. Targeted deletion of genes for eukaryotic RNA-binding proteins, Rbp1 and Rbp2, in the cyanobacterium *Synechocuccus* sp. strain PCC 7942: Rbp1 is indispensable for cell growth at low temperatures. FEMS Microbiol Lett, 1999, 176: 155–161

6   Murata N, Wada H. Acyl-lipid desaturases and their importance in the tolerance and acclimation to cold of cyanobacteria. Biochem J, 1995, 308: 1–8

7   Sakamoto T, Murata N. Regulation of desaturation of fatty acids and its role in tolerance to cold and salt stress. Curr Opin Microbiol, 2002, 5: 208–210

8   Kis M, Zsiros O, Farkas T, et al. Light-induced expression of fatty acid desaturase genes. Proc Natl Acad Sci USA, 1998, 95: 4209–4214

9   Chintalapati S K, Prakash J S S, Gupta P, et al. A novel Delta9 acyl-lipid desaturase, DesC2, from cyanobacteria acts on fatty acids esterified to the *sn*-2 position of glycerolipids. Biochem J, 2006, 398: 207–214

10   Tasaka Y, Gombos Z, Nishiyama Y, et al. Targeted mutagenesis of acyl-lipid desaturases in *Synechocystis*: Evidence for the important roles of polyunsaturated membrane lipids in growth, respiration and photosynthesis. EMBO J, 1996, 15: 6416–6425

11   Yin C, Li W, Du Y, et al. Identification of a gene, *ccr-1* (*sll1242*), required for chill-light tolerance and growth at 15°C in *Synechocystis* sp. PCC 6803. Microbiology, 2007, 153: 1261–1267

12   Zhang W, Du Y, Khudyakov I, et al. A gene cluster that regulates both heterocyst differentiation and pattern formation in *Anabaena* sp. strain PCC 7120. Mol Microbiol, 2007, 66: 1429–1443

13   Elhai J, Wolk C P. A versatile class of positive-selection vectors based on the nonviability of palindrome-containing plasmids that allows the cloning into long polylinkers. Gene, 1988, 68: 119–138

14   Williams J G K. Construction of specific mutantions in photosystem II photosynthetic reaction center by engineering methods in *Synechocystis* 6803. Methods Enzymol, 1988, 167: 766–778

15   Black T A, Wolk C P. Spatial expression and autoregulation of *hetR*, a gene involved in the control of heterocyst development in *Anabaena*. Mol Microbiol, 1993, 9: 77–84

16   Vioque A. Analysis of the gene encoding the RNA subunit of ribonuclease P from cyanobacteria. Nucleic Acids Res, 1992, 20: 6331–6337

17   Sato N. A family of cold-regulated RNA-binding protein genes in cyanobacterium *Anabaena variabilis* M3. Nucleic Acids Res, 1995, 23: 2161–2167

18   Kunert A, Hagemann M, Erdmann N. Construction of promoter probe vectors for *Synechocystis* sp. PCC 6803 using the light-emitting reporter systems Gfp and LuxAB. J Microbiol Methods, 2000, 41: 185–194

19   Gao H, Xu X. Depletion of Vipp1 in *Synechocystis* sp. PCC 6803 affects photosynthetic activity prior to the loss of thylakoid membranes. FEMS Microbiol Lett, 2009, 292: 63–70