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Transcriptomic analysis of *Synechocystis* sp. PCC6803 under low-temperature stress*

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Abstract In this study, cDNA microarrays were developed from 3569 mRNA reads to analyze the expression profiles of the transcriptomes of *Synechocystis* sp. PCC6803 under low temperature (LT) stress. Among the genes on the cDNA microarrays, 899 LT-affected genes exhibited a 1.5-fold (or greater) difference in expression compared with the genes from normal unstressed *Synechocystis* sp. PCC6803. Of the differentially expressed genes, 353 were up-regulated and 246 were down-regulated. The results showed that genes involved in photosynthesis were activated at LT (10°C), including genes for photosystem I, photosystem II, photosynthetic electron transport, and cytochrome b6/f complex. Moreover, *desB*, one of four genes that encode the fatty acid desaturases, was also induced by LT. However, the LT conditions to some degree enhanced the transcription of some genes. In addition, LT (10°C) may reduce cellular motility by regulating the transcription of *spkA* (*sll1575*), a serine/threonine protein kinase. The results reported in this study may contribute to a better understanding of the responses of the *Synechocystis* cell to LT, including pathways involved in photosynthesis and repair.

Keyword: *Synechocystis* sp. PCC6803; cyanobacteria; cDNA microarray; transcriptomics; low temperature stress

Abbreviation: Hik: histidine kinase; STK: serine/threonine protein kinase; Rre: response regulator; LT: low-temperature stress; PSI: photosystem I; PSII: photosystem II; Cyt.b6: cytochrome B6/f complex; PET: photosynthetic electron transport

1 INTRODUCTION

Low temperature (LT) is an important abiotic factor for all living organisms. LT induces the expression of specific subsets of genes involved in acclimation to a downward shift in temperature (Thieringer et al., 1998; Browse and Xin, 2001). For example, cold-shock genes (*csp*) in *Escherichia coli* (Jones et al., 1987) and *Bacillus subtilis* (Willimsky et al., 1992), *cor* and *cas* genes in plants (Gilmour et al., 1992; Wolfraim et al., 1993), and genes that encode fatty acid desaturases in cyanobacteria (Wada et al., 1990; Gibson et al., 1994; Murata and Wada, 1995), plants (Gibson et al., 1994), protozoan (Nakashima et al., 1996), dimorphic fungus (Laoteng et al., 1999), and fish (Tiku et al., 1996), have been shown to be essential for cell acclimation to LT. However, the mechanisms that organisms use for the perception and transduction of LT signals are not clearly understood. Importantly, cyanobacteria possess features of both

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bacteria and plants, which makes them attractive organisms for studies into their signal transduction systems. The unicellular cyanobacterium *Synechocystis* has become a model organism for studying the biochemistry and molecular biology of stress responses in photosynthetic organisms.

Changes in membrane fluidity, lipid composition, and physiological activity induced by LT have been studied in several strains of cyanobacteria, including *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) (Murata, 1989). Previous studies have found that LT stresses imposed on *Synechocystis* appear to be perceived first via changes in membrane lipids (Vigh et al., 1993; Los et al., 1997; Murata and Los, 1997). Moreover, the DesK/DesR system was found to sense and transduce the LT signal, and then control the transcription of the fatty acid desaturase (*des*) gene for delta 5 desaturase, uniquely and stringently in *B. subtilis* (Mansilla and de Mendoza, 2005).

It has been proposed that LT stimuli may be perceived by histidine kinases (Hiks) and/or serine/threonine protein kinases (STKs), which are localized in the cytoplasmic membrane or in the cytosol of some prokaryotes (Appleby et al., 1996; Mizuno et al., 1996; Zhang et al., 1998), yeast (Maeda et al., 1994), and plants (Chang et al., 1993; Kakimoto, 1996). In other words, temperature-inducible changes in membrane fluidity may be mediated by transmembrane Hik(s) or STK(s). In 1996, the complete nucleotide sequence of the *Synechocystis* genome was determined (Kaneko et al., 1996), and, in 2003, the plasmid sequences (including 397 genes) were reported (Kato et al., 2003). The plasmid genes were not commonly included in DNA microarrays. Forty-four and three putative genes encoding Hiks have been predicted in the *Synechocystis* genome (Hik1–Hik44) (Kaneko et al., 1996; Mizuno et al., 1996) and plasmids (Kaneko et al., 2003), respectively, In addition, 42 and three putative genes encoding the response regulators (Rres) have been predicted in the genome and plasmids, respectively. Moreover, among the 12 putative genes that encode the STKs in *Synechocystis*, seven encode proteins that belong to the PKN2 subfamily of STKs, namely, *spkA* (*sll1574–1575*), *spkB* (*slr1697*), *spkC* (*slr0599*), *spkD* (*sll0776*), *spkE* (*slr1443*), *spkF* (*slr1225*), and *spkG* (*slr0152*) (Kamei et al., 2003) (CyanoBase; http://genome.microbedb.jp/cyanobase/Synechocystis). Five genes (*spkH*, *spkI*, *spkJ*, *spkK*, and *spkL*) that encode proteins of the ABC1 subfamily of STKs (Leonard et al., 1998; Shi et al., 1998). SpkA, SpkB, SpkC, SpkD, and SpkF were found to autophosphorylate or phosphorylate their substrate proteins (Kamei et al., 2001, 2002, 2003), whereas SpkE showed no protein kinase activity in vitro (Kamei et al., 2002). SpkA was reported to be essential for cellular motility via phosphorylation of membrane proteins (Kamei et al., 2001), and was found to be involved in the formation of thick pili (Panichkin et al., 2006). SpkB was also shown to be vital to cellular motility (Kamei et al., 2003). Although SpkC was neither expressive nor inactive under normal conditions, the Δ*SpkC* mutant strain was found to be sensitive to high-temperature stress (Zhang X W, personal communication). Furthermore, SpkC was reported to regulate nitrogen metabolism (Galkin et al., 2003). SpkD was reported to be essential for survival and, as such, cannot be removed completely (Kamei et al., 2002). SpkE may be involved in nitrogen metabolism and the post-translational modification of pilin (Galkin et al., 2003; Kim et al., 2004). SpkG can sense high-salt stress signals directly (Liang et al., 2011), and *spkG* was found to be highly down-regulated in iron deficiency under peroxide stress (Singh et al., 2004). In addition, SpkC, SpkF, and SpkK work one after the other to finally phosphorylate the co-chaperonin GroES (Zorina et al., 2011).

In this study, we aimed to identify more details of the mechanisms of the perception and transduction of LT signals in *Synechocystis* by transcriptomic analysis of cold-stressed cells using newly developed cDNA microarrays.

2 MATERIAL AND METHOD

2.1 Cells and culture conditions

The motile strain of *Synechocystis* sp. PCC 6803 was obtained from the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences. Wild-type cells were grown photoautotrophically in BG-11 medium (Rippka et al., 1979) at 28°C and 30 μE/(m^2·s) under white light from incandescent lamps. The cells were grown to the mid-logarithmic growth phase (optical density at 730 nm of 0.3–0.6) before stress treatment, and then treated separately at 4°C, 10°C, 28°C (control) and 35°C for 30 min, 1 h, and 2 h (Suzuki et al., 2001). After treatment, samples were collected and kept in liquid nitrogen.

2.2 Isolation of total RNA

Total RNA was isolated from 30 mL cultures of

wild-type and LT-inducible cells. The cells were collected by centrifugation (4 000×*g* for 10 min), and the cell pellets were cooled in liquid nitrogen. The cooled cells were ground in liquid nitrogen, and resuspended in 1-mL Biozol reagent and chloroform (ratio 1:5). An equal volume of isopropyl alcohol was added to the aqueous phase after centrifuged at 12 000×*g* for 15 min at 4°C, and the solution was incubated for 30 min at -20°C. Precipitated RNA was collected by centrifugation at 12 000×*g* for 10 min at 4°C, washed with 70% ethanol, and resuspended in DEPC-H_2O. The total RNA was treated with RNase-free DNase I (Promega) at 37°C for 1 h to remove DNA. Total RNA from each sample was quantified using a NanoDrop ND-1000 and RNA integrity was assessed by standard denaturing agarose gel electrophoresis.

2.3 Reverse transcription

Reverse transcription reactions were performed with the random hexamer primers using M-MLV Reverse Transcriptase (Promega). The reaction system consisted of 2 μL 5×PrimeScript™ buffer, 0.5 μL PrimeScript™ reverse transcriptase, 0.5 μL dNTP mixture, 0.5 μL six random primers, RNA template, and RNase free dH2O up to 10 μL. The resulting cDNAs were used as the templates for the microarray.

2.4 cDNA microarray analysis

The *Synechocystis* 8×15K Gene Expression Array was manufactured by Agilent of Shanghai KangChen Bio-tech. Sample labeling and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technologies). Briefly, 1 μg of total cRNA from each sample was linearly amplified and labeled with Cy3-dCTP using an Agilent Quick Amp Labeling Kit.

The labeling reaction procedure was as follows: (i) 1 μg total RNA, 1.2 μL T7 promoter primer, and nuclease-free water (to a total reaction volume to 11.5 μL) were added to a 1.5-mL microcentrifuge tube, which was then incubated at 65°C in a circulating water bath for 10 min, and on ice for a further 5 min; (ii) 8.5 μL cDNA master mix (4 μL 5×first strand buffer, 2 μL 0.1 mol/L DTT, 1 μL 10 mmol/L dNTP mix, 1 μL MMLV-RT, and 0.5 μL RNaseOut) was added to each sample tube and mixed by pipetting up and down, then the samples were incubated in a circulating water bath at 40°C for 2 h, at 65°C for 15 min and on ice for a further 5 min; and (iii) 60 μL transcription master mix was added to each sample tube and mixed by pipetting, then the samples were incubated in a circulating water bath at 40°C for 2 h. The 60 μL transcription master mix consisted of 20 μL 4×transcription buffer, 6 μL 0.1 mol/L DTT, 8 μL NTP mix, 6.4 μL 50% PEG, 2.4 μL cyanine-3-CTP, 0.5 μL RNaseOUT, 0.6 μL inorganic pyrophosphatase, 0.8 μL T7 RNA polymerase, and 15.3 μL nuclease-free water.

The labeled cRNAs were purified using an RNAeasy mini kit (Qiagen). The concentration and specific activity of the labeled cRNAs (pmol Cy3/μg cRNA) were measured using a NanoDrop ND-1000. Then, 1 μg of each labeled cRNA was fragmented by adding 11 μL 10×blocking agent and 2.2 μL 25×fragmentation buffer, followed by heating at 60°C for 30 min. Finally 55 μL 2×GE hybridization buffer was added to dilute the labeled cRNA. For hybridization, 100 μL hybridization solution was added to the gasket slide, which was then assembled with the microarray slide. The slides were incubated for 17 h at 65°C in an Agilent hybridization oven. The hybridized arrays were washed, fixed, and scanned using the Agilent DNA microarray scanner (Part Number G2505B).

2.5 Data analysis

Agilent feature extraction software (version 10.7.3.1) was used to analyze the acquired array images. Quantile normalization and subsequent data processing were performed using the GeneSpring GX v11.5.1 software package (Agilent Technologies). After quantile normalization of the raw data, genes for which at least eight out of eight samples had flags in present or marginal ("All Targets Value") were chosen for further data analysis. Differentially expressed genes were identified by fold change filtering. Hierarchical clustering was performed using Agilent GeneSpring GX software (Version 11.5.1). Pathway analysis was performed using a standard enrichment computation method.

2.6 Real-time reverse-transcription PCR analysis

Real-time reverse-transcription PCR (qRT-PCR) was performed as follows. The primers were designed and checked using OMIGA2 software. The RNAse P (*rnp*) gene was used as a reference (Table 1). The qRT-PCR was performed using a SYBR® *Premix Ex Taq*™ II kit and the ABI 7500 fast real-time PCR system. The reaction system consisted of 0.4 μL forward primer (10 μmol/L), 0.4 μL reverse primer

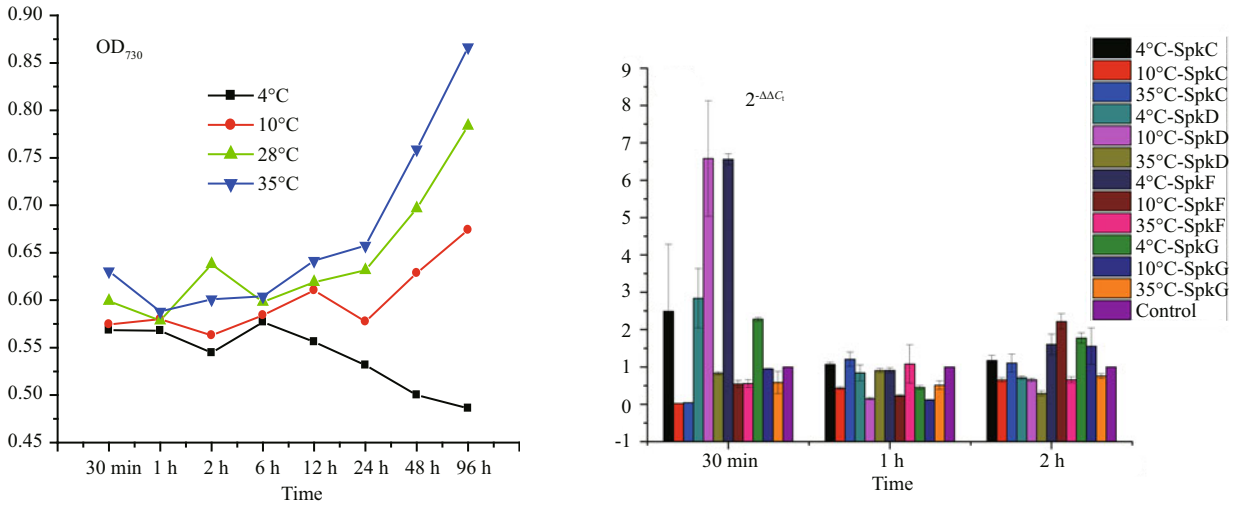

Fig.1 Growth curves and gene expression levels for *Synechocystis* cells under temperature stress

a. Growth curves under different temperature conditions. 28°C was taken as the normal condition. b. Expression levels of some selected genes under different temperature conditions. The $2^{-\Delta\Delta Ct}$ method was used to calculate the fold change in gene expression levels between cells under stress and under normal conditions.

Table 1 Primers used for the qRT-PCR experiment

Genes	Primer sequences
spkC (*slr0599*)	CAGTTTGGGACTAACGGC
	TAAACCTTGGTGGCTTGG
spkD (*sll0776*)	CACTAGGGGATTTATGG
	TTGGTGGAACTTCTCGT
spkE (*slr1443*)	TTTCTCCCCAACATTTCA
	TCAAACTTTCCAACATCC
hik34 (*slr1285*)	CATTCGCACCCTAACCAAGT
	AGCTTGGAACACCGCTTCTA
rnp (*slr1469*)	GGACTACCCAAAACACTGC
	CAATAATCCCAGCTTGGCT

(10 μmol/L), 0.4-μL ROX reference dye II, 6 μL cDNA template, 10 μL SYBR® Premix Ex Taq™II (2×), and 2.8 μL ddWater to a total volume of 20 μL. A two-step PCR reaction program was used. First the mixture was incubated at 50°C for 20 s and 95°C for 15 min; and then at 95°C for 15 s and at 60°C for 60 s, for 40 cycles.

3 RESULT AND DISCUSSION

3.1 Cell growth

Synechocystis cells were grown at various temperatures under a continuous light intensity of 30 μE/(m²·s). After 30 min, 1 h, and 2 h, cells were harvested separately for use in the qRT-PCR experiments.

The cells grown at 4°C became feeble and died after 6 h (Fig.1a). The growth curve of the cells at 10°C was significantly different from the growth curve of the cells grown at the other temperatures (Fig.1a), and the expression levels of the genes in the cells grown at 10°C were different at the different times (Fig.1b). After 30 min at 10°C, the transcriptional expression of *SpkC*, which has been linked to high-temperature stress, was clearly down-regulated (Fig.1b). *SpkD* was clearly up-regulated in cells after 30 min at 10°C (Fig.1b).

Furthermore, after 2 h, the cells grown at 10°C were greener than the cells grown under the other temperature conditions, perhaps because of the degradation of phycobilisomes. Therefore, we chose the cells grown at 10°C for 30 min for the transcriptomic analysis of *Synechocystis* using the cDNA microarrays.

3.2 Evaluation of the genome-wide expression of LT-stress genes

For the microarray analysis, the cDNA from both the LT-treated and untreated (control) cells were labeled with Cy3-dye (Fig.2a). To measure the quality control of the microarray data, a scatter-plot was used to visualize and assess variations in the gene expression levels (or reproducibility) between two repeat microarrays or groups (Fig.2b).

Among the 3 569 genes represented on the cDNA microarrays, 589 genes were differentially expressed by more than 1.5-fold between the LT-stressed cells and the normal controls; 353 genes were up-regulated

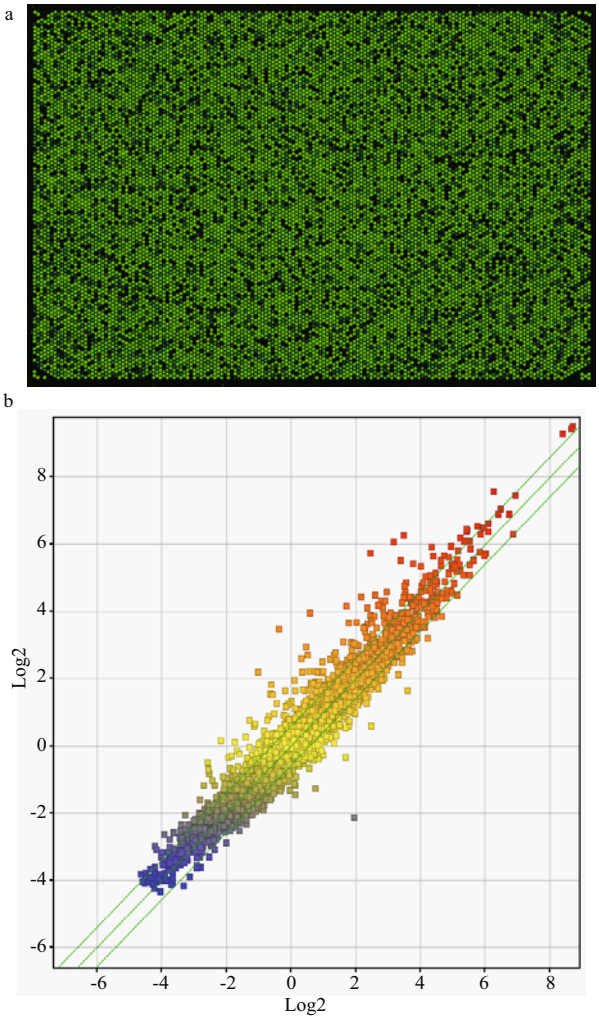

Fig.2 Evaluation of the microarray analysis of gene expression levels in *Synechocystis* cells under LT stress

a. Overlay images of the microarray hybridized with labeled cDNAs from cells grown at normal temperature (28°C, control) and cells treated at 10°C for 30 min. The up-regulated genes are shown in green and the down-regulated genes are in blank color; b. Scatter-plot of variations in the gene expression levels between two matched microarray experiments. The numbers on the *X* and *Y* axes are the normalized signal values of the samples (log2 scaled). The values that fall above the top green line and below the bottom green line indicate a more than 1.5 fold change in transcription levels (increase in upper part and decrease in lower part) between the two compared samples.

and 246 genes were down-regulated. We analyzed the differentially expressed genes based on their biological functions. The up-regulated genes were related mainly to aminoacyl-tRNA biosynthesis, photosynthesis, and ribosome (Table 2). Among the down-regulated genes, most were related to signal transduction (Table 2). There were many hypothetical genes among both the up- and down-regulated genes (Table 2).

3.2.1 Up-regulated genes related to photosynthesis

The genes involved in photosynthesis tended to be up-regulated at the transcriptional level in the LT-induced cells. Enzyme activity most often decreases at low temperatures; therefore, an increased quantity of photosynthetic enzymes may help the cells to survive under LT stress. In *Synechocystis*, the photosynthetic system is composed mainly of photosystem I (PSI), photosystem II (PSII), photosynthetic electron transport (PET) and cytochrome b6/f complex (Cyt.b6). In the LT-stressed cells, the up-regulated genes related to PSI were *PsaC* (*ssl0563*, PSI subunit VII), *PsaE* (*ssr2831*, PSI reaction center subunit IV), *PsaK* (*ssr0390*, PSI reaction center subunit X) and *PsaM* (*smr0005*, PSI reaction center subunit XII). The *psbA2* (*slr1311*) and *psbA3* (*sll1867*) genes that encode the PSII D1 subunits of PSII core complexes were up-regulated in cells stressed at 10°C. Other up-regulated genes related to PSII were *psbM* (*sml0003*), *psbH* (*ssl2598*), *psbI* (*sml0001*) for PSII reaction center proteins, *psbT* (*smr0001*), *psbX* (*sml0002*), *psbY* (*sml0007*), and *psb28-2* (*slr1739*) for the PSII proteins, and *psbO* (*sll0427*) for the PSII manganese-stabilizing polypeptide. Some up-regulated genes in *Synechocystis* under LT stress were related to Cyt.b6; namely, *petG* (*smr0010*, Cyt.b6 subunit), *petL* (*sll1994a*, Cyt.b6 subunit), *petN* (*sml0004*, Cyt.b6 subunit) and two *petC* genes (*sll1316* and *sll1182*, Cyt.b6 iron-sulfur subunit). Up-regulated genes related to PET were *PetE*, *sll0199*; plastocyanin (PC), *PetF*, *slr1828*; ferredoxin (Fd), and *PetJ*, *sll1796*; cytochrome C553 (cyt c6). The *AtpC* (*sml0004*, F0F1 ATP synthase subunit epsilon) gene, which encodes an F-type ATPase, was also up-regulated in the LT-treated cells. Manganese-depleted PSII core complexes of *Synechocystis* illuminated at very LT (77 K; i.e. -196°C), displayed the reversible photooxidation of carotenoids (Vrettos et al., 1999). Therefore, under extreme LTs, *Synechocystis* strains may invoke a photosynthesis mechanism that can supply chemical energy for cell's activities, or it is very probable that nothing was activated at 10°C, except *rbps* and a few other genes, and the observed increase in mRNA expression might be because of its stabilization.

3.2.2 Genes related to signal transduction

Organisms can acclimate to natural habitats because of their ability to sense changes in the ambient

Table 2 Differentially expressed genes between treated (10°C) and untreated (control) *Synechocystis* cells

Category	Up-regulated genes		Category	Down-regulated genes	
	Number of genes	Proportion (%)		Number of genes	Proportion (%)
Photosynthesis	23	5.62	Signal transduction	14	5.69
Aminoacyl-tRNA biosynthesis	36	8.80	Carotenoid biosynthesis	3	1.22
Ribosome	18	4.40	Butanoate metabolism	3	1.22
Repair	7	1.71	Pantothenate and CoA biosynthesis	3	1.22
Carbon fixation in photosynthetic organisms	7	1.71	Valine, leucine and isoleucine biosynthesis	3	1.22
Signal transduction	4	0.98	Pyruvate metabolism	4	1.63
Fatty acid	2	0.49	Fatty acid	3	1.22
Others	103	25.19	Others	72	29.27
Hypothetical proteins	209	51.10	Hypothetical proteins	142	57.72
	409	100.00		246	100.00

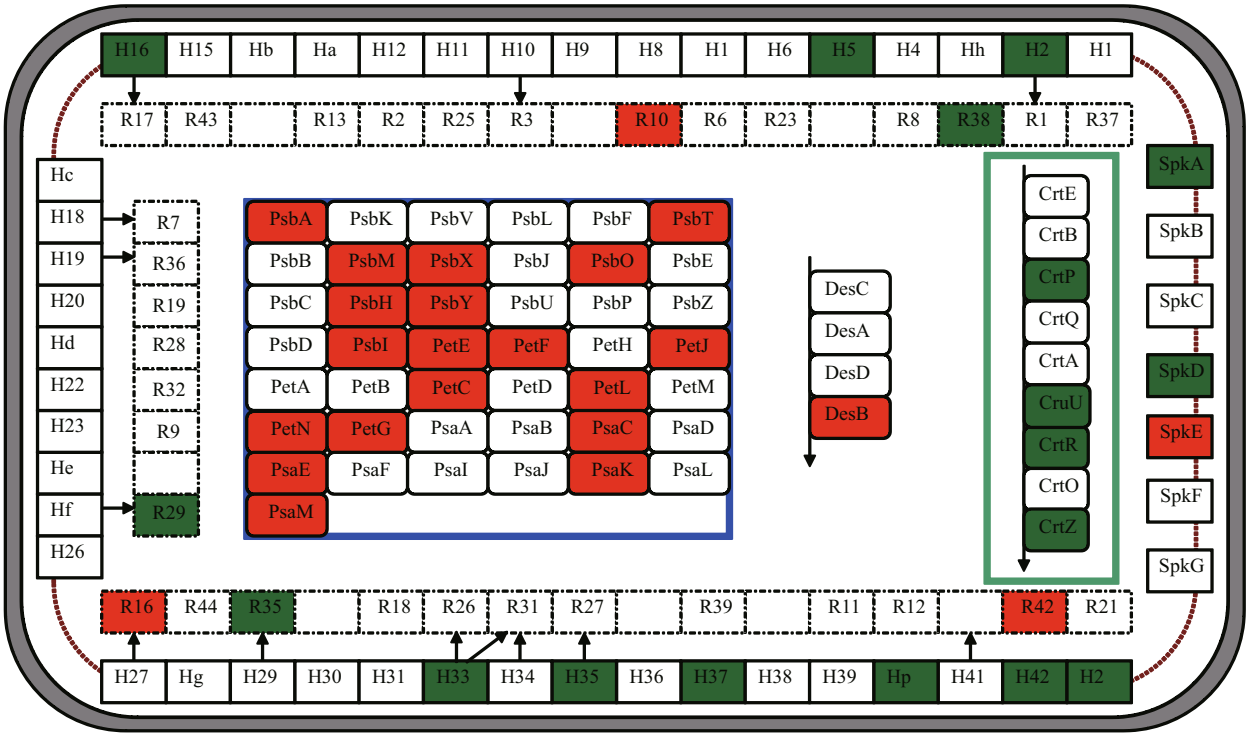

Fig.3 General scheme of the proposed network for the response of *Synechocystis* cells to LT stress

Red: up-regulated genes; green: down-regulated genes. H: short for histidine kinase; R: short for response regulator.

environment and to express a large number of genes in response. Thus, organisms can synthesize specific proteins and metabolites for protection against LT (Kaye and Guy, 1995; Murata and Los, 1997, 2006; Thieringer et al., 1998; Vigh et al., 1998) (Fig.3). Hik33 was shown to be an important participant in LT-signal transduction in *Synechocystis*, depending on the membrane rigidity (Suzuki et al., 2000). LT signals were found to be transferred directly from Hik33 to Rre26 to regulate the expression of 21 genes, including *ndhD2*, *hliA*, *hliB*, *hliC*, *feoB*, *crp*, and genes for proteins of unknown function (Suzuki et al., 2000; Murata and Los, 2006). Moreover, two other LT sensors that are homologues of DesK and DesR in *B. subtilis* were reported; one depended on membrane rigidness, and the other functioned independently in the saturation of membrane lipids (Inaba et al., 2003; Los and Murata, 2004; Mansilla and de Mendoza,

2005). In addition, the *hik13* (*sll1003*) (Kaneko et al., 1995; Kaneko and Mizuno, 1996), and *hik15* (*sll1353*) genes might be essential for growth under LT (Suzuki et al., 2000). Mikami et al. (2003) reported that DNA microarray data (unpublished results) indicated that Hik2 might be a second LT sensor, while 15 other LT-inducible genes were not regulated by Hik33, implying that there were some other, yet to be identified, sensors and Rres involved in signal transduction under LT conditions.

Our cDNA microarrays results suggested that *desB* was up-regulated in the LT-stressed cells. However, *Hik33* (*sll0698*) expression was down-regulated, which may be abnormal. The expression levels of *Hik2* (*slr1147*), *Hik5* (*sll1888*), *Hik16* (*slr1805*), *Hik24* (*slr1969*), *Hik35* (*sll0473*), *Hik37* (*sll0094*), *Hik42* (*sll1555*), and *Hikp* (*slr6041*) were all down-regulated in the LT-stressed cells. Some response regulators, such as *Rre29* (*slr0081*), *Rre35* (*sll0039*) and *Rre38* (*slr1584*), were also down-regulated in the LT-stressed cells (Table 4). In addition, *SpkE* (*slr1443*), which shows no protein kinase activity, was up-regulated, while the transcriptions of *spkA* (*sll1575*), essential for cellular motility, and of *spkD* were down-regulated. A response regulator related to cell division, *Rre42* (*slr2041*) (Galperin et al., 2001) was up-regulated in the LT-stressed cells. Some of the up-regulated genes had unknown functions in signal transduction systems; for example, *rre10* (*slr1037*, CheY family protein) and *rre16* (*slr1837*, OmpR subfamily). In addition, the transcription of three genes that encode the high-light inducible proteins Ssl1633 (HliC), Ssr2595 (HliB), and Ssl2542 (HliA) were also up-regulated in the LT-stressed cells. We propose that extreme LT conditions may trigger the evolution of strains that need more light. Further studies are needed into the mechanisms involved in LT-signal transduction.

3.2.3 Up-regulated genes related to fatty acids

Cold inducibility of gene expression in *Synechocystis* was reported to be enhanced by the rigidification of membrane lipids and the proportion of unsaturated fatty acids among the membrane phospholipids (Inaba et al., 2003). Moreover, for many years, it has been believed that the increased rigidity of membranes after a downward shift in temperature may be the primary signal of a decrease in ambient temperature (Murata and Los, 1997; Los and Murata, 2000). In *Synechocystis*, there are four genes that encode fatty acid desaturases, namely, genes *desA* (*slr1350*), *desB* (*sll1441*), *desC* (*sll0541*) and *desD* (*sll0262*) for the Δ12, Δ15, Δ9 and Δ6 fatty acid desaturases, respectively (Wada et al., 1990; Sakamoto et al., 1994; Sakamoto and Murata, 2002). The *desB* gene for ω3 fatty acid desaturase (Sakamoto et al., 1994) introduces double bonds into fatty-acyl chains at the omega 3 position forming trienoic fatty acids (Murata and Wada, 1995). The *desA* was found to be induced by the rigidification of plasma membrane as a result of the Pd-catalyzed hydrogenation of membrane lipids (Vigh et al., 1993). The *desA* gene was found to be crucial for the maintenance of the membrane fluidity, while the *desB* had much less effect on the fluidity. Furthermore, while *desA* and *desD* encode proteins that synthesize polyunsaturated fatty acids and modulate the fatty acid composition of the membrane lipids under cold stress, mutants that lack either of the two genes were still able to adjust the membrane fluidity (Mironov et al., 2012). In other words, other mechanisms, apart from changes in the fatty acid composition of membrane lipids, seem to be used to regulate membrane fluidity.

However, in the cDNA microarrays, only two genes, *desB* and *slr1609* (long-chain-fatty-acid CoA ligase), appeared to be significantly up-regulated in the cells grown at 10°C. Thus, *slr1609* may be crucial for fatty acid activation and the biosynthesis of alkanes (Gao et al., 2012), while *desB* may play an essential role in changes in fatty acid composition of membrane lipids, which may be crucial for the terrifically cold resistance of *Synechocystis*.

3.2.4 Up-regulated genes related to ribosome and aminoacyl-tRNA biosynthesis

Ribosomal RNAs in *Synechocystis* have 5S rRNA, 16S rRNA and 23S rRNA. All the genes that encode these rRNAs were up-regulated or stabilized in the cells under LT stress. Furthermore, the transcription of some genes for the large subunits (L2, *RplB*, Sll1802; L4, RplD, Sll1800; L7/L12, RplL, Sll1746; L10, RplJ, Sll1745; L17, RplQ, Sll1819; L19, RplS, Sll1740; L20, RplT, Sll0767; L23, RplW, Sll1801; L27, RpmA, Ssr2799; L28, RpmB, Ssr1604; and L34, RpmH, Ssr0011), for the 50S ribosomes and small subunits (S2, RpsB, Sll1260; S10, RpsJ, Sll1101; S14, RpsN, Slr0628; and S19, RpsS, Ssl3432), and for the 30S ribosomes was also up-regulated in the cells under LT stress (Table 3). Two other genes were up-regulated in the cells under LT stress, *rpoC1* (*slr1265*) for the subunit of the RNA polymerase and *gyrB* (*sll2005*) for the protein responsible for negative

Table 3 Up-regulated genes of *Synechocystis* related to various biological functions in cells under LT stress compared with cells under normal conditions

Protein_id	Gene	Locus_tag	Product	Fold change
			Photosynthesis	
NP_441966.1	*psaC*	*ssl0563*	Photosystem I subunit VII	1.8407861
NP_441703.1	*psaE*	*ssr2831*	Photosystem I reaction center subunit IV	2.3936045
NP_440039.1	*psaK*	*ssr0390*	Photosystem I reaction center subunit X	1.8613524
NP_440325.1	*psaM*	*smr0005*	Photosystem I reaction center subunit XII	2.4283636
NP_439906.1	*psbA2*	*slr1311*	Photosystem II D1 protein	1.9500653
NP_441550.1	*psbA3*	*sll1867*	Photosystem II D1 protein	1.963303
NP_440949.1	*psbH*	*ssl2598*	Photosystem II reaction center protein H	2.822149
NP_442015.1	*psbI*	*sml0001*	Photosystem II reaction center I protein I	2.2614248
NP_440028.1	*psbM*	*sml0003*	Photosystem II reaction center protein M	3.630347
NP_441796.1	*psbO*	*sll0427*	Photosystem II manganese-stabilizing polypeptide	1.5307822
NP_442063.1	*psbT*	*smr0001*	Photosystem II reaction center protein T	2.4433758
NP_442241.1	*psbX*	*sml0002*	Photosystem II protein PsbX	2.0402317
NP_441042.1	*psbY*	*sml0007*	Photosystem II protein PsbY	2.382206
NP_441099.1	*Psb28-2*	*slr1739*	Photosystem II protein	1.515849
NP_440948.1	*petC*	*sll1316*	Cytochrome B6-f complex iron-sulfur subunit	1.6417295
NP_441583.1	*petC*	*sll1182*	Cytochrome B6/f complex iron-sulfur subunit	1.5772114
NP_442157.1	*petE*	*sll0199*	Plastocyanin	1.5058625
NP_440748.1	*petF*	*slr1828*	Ferredoxin	2.1043668
NP_441556.1	*petG*	*smr0010*	Cytochrome B6-f complex subunit PetG	2.4847147
NP_440674.1	*petJ*	*sll1796*	Cytochrome C553	5.530795
NP_001035872.1	*petL*	*sll1994a*	Cytochrome B6f complex subunit PetL	1.8637409
NP_440044.1	*ycf6*	*sml0004*	Cytochrome B6-f complex subunit PetN	1.8057883
NP_441408.2	*atpC*	*slr1330*	F0F1 ATP synthase subunit epsilon	1.7710309
			Aminoacyl-tRNA	
		6803t32	tRNA-Ala	2.7152689
		6803t06	tRNA-Arg	2.9374962
		6803t33	tRNA-Arg	4.172092
		6803t08	tRNA-Arg	5.742981
		6803t24	tRNA-Asn	4.3037295
		6803t13	tRNA-Asp	1.8763491
		6803t35	tRNA-Cys	2.9889767
		6803t23	tRNA-Gln	4.657067
		6803t14	tRNA-Glu	7.6358933
		6803t17	tRNA-Gly	3.3794053
		6803t36	tRNA-Gly	2.5291226
		6803t30	tRNA-Gly	3.693971
		6803t22	tRNA-His	3.382573
		6803t29	tRNA-Ile	4.417949
		6803t41	tRNA-Leu	3.7745998
		6803t07	tRNA-Leu	3.6744406

To be continued

Table 3 Continued

Protein_id	Gene	Locus_tag	Product	Fold change
		6803t12	tRNA-Leu	14.837352
		6803t10	tRNA-Leu	9.476376
		6803t39	tRNA-Leu	4.8880386
		6803t27	tRNA-Lys	4.9500713
		6803t18	tRNA-Met	3.5158145
		6803t11	tRNA-Met	2.3305147
		6803t15	tRNA-Pro	5.089357
		6803t01	tRNA-Pro	5.4028554
		6803t04	tRNA-Pro	2.1356065
		6803t02	tRNA-Ser	9.784478
		6803t21	tRNA-Ser	6.974377
		6803t20	tRNA-Ser	4.302078
		6803t31	tRNA-Ser	10.416905
		6803t03	tRNA-Thr	2.3783782
		6803t38	tRNA-Thr	3.36025
		6803t25	tRNA-Thr	4.631181
		6803t09	tRNA-Trp	3.6057217
		6803t37	tRNA-Tyr	3.7333024
		6803t42	tRNA-Val	3.630308
		6803t05	tRNA-Val	2.7371922
			Ribosome	
NP_440666.1	*rplB*	*sll1802*	50S ribosomal protein L2	1.876755
NP_440668.1	*rplD*	*sll1800*	50S ribosomal protein L4	1.8015074
NP_440737.1	*rplJ*	*sll1745*	50S ribosomal protein L10	1.7290424
NP_440736.1	*rplL*	*sll1746*	50S ribosomal protein L7/L12	2.4353411
NP_440644.1	*rplQ*	*sll1819*	50S ribosomal protein L17	1.7167804
NP_440742.1	*rplS*	*sll1740*	50S ribosomal protein L19	1.7200234
NP_442051.1	*rplT*	*sll0767*	50S ribosomal protein L20	1.6531644
NP_440667.1	*rplW*	*sll1801*	50S ribosomal protein L23	1.7551737
NP_441681.1	*rpmA*	*ssr2799*	50S ribosomal protein L27	2.0218675
NP_440186.1	*rpmB*	*ssr1604*	50S ribosomal protein L28	2.414365
NP_441561.1	*rpmH*	*smr0011*	50S ribosomal protein L34	1.6212666
NP_441467.1	*rpsB*	*sll1260*	30S ribosomal protein S2	1.6079607
NP_441640.1	*rpsJ*	*sll1101*	30S ribosomal protein S10	1.587542
NP_442274.1	*rpsN*	*slr0628*	30S ribosomal protein S14	1.5602026
NP_440665.1	*rpsS*	*ssl3432*	30S ribosomal protein S19	1.5036769
	rrn16Sa	*6803r03*	16S ribosomal RNA	1.7202241
	rrn23Sa	*6803r02*	23S ribosomal RNA	1.7961024
	rrn5Sa	*6803r01*	5S ribosomal RNA	1.8599547
NP_441040.1	*gyrB*	*sll2005*	DNA gyrase B subunit	1.523912
NP_441586.1	*rpoC1*	*slr1265*	DNA-directed RNA polymerase subunit gamma	1.7419988

To be continued

Table 3 Continued

Protein_id	Gene	Locus_tag	Product	Fold change
			Signal transduction	
NP_440496.1	*rre10*	*slr1037*	CheY family protein	1.7065983
NP_440764.1	*rre16*	*slr1837*	OmpR subfamily	1.9730282
NP_440423.1	*divK*	*slr2041*	Cell division response regulator; DivK	1.5388181
NP_440875.1	*spkE*	*slr1443*	Protein kinase	1.5049236
			Carbon fixation	
NP_442114.1	*cbbA*	*sll0018*	Fructose-1,6-bisphosphate aldolase	2.6146588
NP_441308.1	*glpX*	*slr2094*	Fructose 1,6-bisphosphatase II	1.7083902
NP_442343.1	*rpiA*	*slr0194*	Ribose-5-phosphate isomerase A	≤1.5
NP_441738.1	*fbp*	*slr0952*	Fructose-1,6-bisphosphatase	≤1.5
NP_442114.1	*cbbA*	*sll0018*	Fructose-1,6-bisphosphate aldolase	2.6146588
NP_441843.2	*pgk*	*slr0394*	Phosphoglycerate kinase	1.7886404
NP_442120.1	*rbcL*	*slr0009*	Ribulose bisophosphate carboxylase	≤1.5
			High-light inducible proteins	
	hliC	*ssl1633*	High light inducible protein	
	hliB	*ssr2595*	High light inducible protein	
	hliA	*ssl2542*	High light-inducible protein	
			Repair	
NP_440778.1		*sll1712*	DNA binding protein HU	2.464211
NP_442053.1	*radC*	*sll0766*	DNA repair protein RadC	1.9226356
NP_441790.1	*recN*	*sll1520*	DNA repair protein RecN	1.7318777
NP_442000.2	*recO*	*slr0181*	DNA repair protein RecO	1.7317327
NP_441184.1	*topA*	*slr2058*	DNA topoisomerase I	1.502423
			Fatty acid metabolism	
NP_441622.1	*desB*	*sll1441*	Delta 15 desaturase	2.4022195
NP_440344.1		*slr1609*	Long-chain-fatty-acid CoA ligase	2.1193707

DNA supercoiling. Only the tRNA-Phe (*6803t16*) had no significance; all the others were up-regulated in *Synechocystis* under LT stress (Table 3). The above data show that extremely LT signals may have an important effect on *Synechocystis* cells at the translation level, especially on the 36 genes that are involved in aminoacyl-tRNA biosynthesis, which may be related to rapid and efficient signal transductions.

3.2.5 Other up-regulated genes

A number of other up-regulated genes related to some biological pathways between cells grown under LT stress and cells grown under normal conditions. Some of these genes were found to be related to DNA repair systems; for example, *radC* (*sll0766*), *recN* (*sll1520*), *recO* (*slr0181*), *topA* (*slr2058*), and *sll1712*, all of which were up-regulated or stabilized at the mRNA level (Table 3). In addition, genes that encode Pds (Slr1254, phytoene desaturase), Bhy (Sll1468, β-carotene hydroxylase), and isorenieratene synthase (Sll0254) related to carotenoid biosynthesis were down-regulated in treated cells compared with untreated cells. All the cellular processes were probably arrested at 10°C, which is an extremely LT for *Synechocystis* (Table 4).

3.3 qRT- PCR validation

We validated the expression of the spkC, *spkE* and *spkE* genes obtained by our microarray analysis by qRT-PCR (Fig.4). Because the Δ*SpkC* mutant strain was found to be sensitive to high-temperature stress (Zhang X W, personal communication), we speculated that the *spkC* gene will be down-regulated in cells

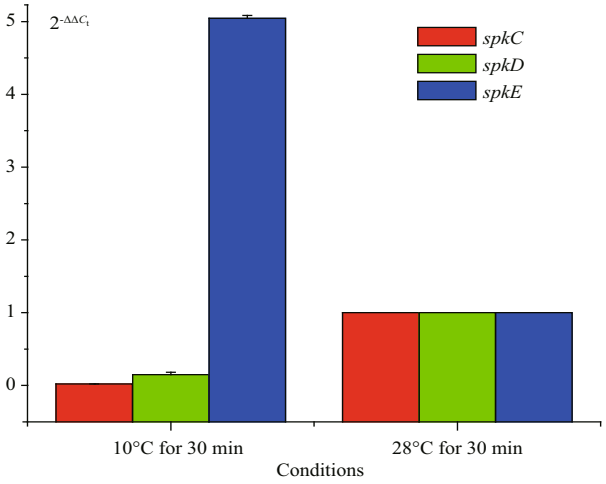

Fig.4 Expression of *spkC*, *spkD*, and *spkE* in LT-stressed and normal *Synechocystis* cells measured by qRT-PCR

The $2^{-\Delta\Delta Ct}$ method was used to calculate the fold change in gene expression levels between cells under stress and under normal conditions. 28°C for 30min was taken as the normal condition.

grown at 10°C. The qRT-PCR results show that the expression of *spkE* was up-regulated and the expression of *spkE* was down-regulated in the LT-stressed cells, which is in line with the microarray data (Fig.4). However, while the qRT-PCR results showed that the *spkC* was down-regulated at 10°C, *spkC* was not detected in the cDNA microarray data in this study. Therefore, further studies are needed to test our speculation.

4 CONCLUSION

We used cDNA microarray technology to analyze gene expression in *Synechocystis* under LT stress. A large number of differentially expressed genes were found. An important finding is that the genes that were up-regulated at the transcriptional level in cells under LT stress compared with cells under normal conditions included most of the genes that encode components of the photosynthetic system. The

Table 4 Down-regulated genes of *Synechocystis* related to various biological functions in cells under LT stress compared with cells under normal conditions

Protein_id	Gene	Locus_tag	Product	Fold change
			Signal transduction	
NP_440624.1	*hik2*	*slr1147*	Sensory transduction histidine kinase	-1.5425982
NP_441521.1	*hik5*	*sll1888*	Sensory transduction histidine kinase	-1.8129581
NP_440365.1	*hik13*	*sll1003*	Sensory transduction histidine kinase	-1.5486623
NP_441053.1	*hik16*	*slr1805*	Sensory transduction histidine kinase	-2.0885978
NP_441517.1	*hik24*	*slr1969*	Hybrid sensory kinase	-1.6010792
NP_440007.1	*hik33*	*sll0698*	Drug sensory protein A	-1.5136964
NP_442577.1	*hik37*	*sll0094*	Sensory transduction histidine kinase	-1.5801467
NP_441702.1	*hik42*	*sll1555*	Hybrid sensory kinase	-1.5844272
NP_942484.1	*hikm*	*slr6041*	Two-component sensor histidine kinase	-1.7874668
NP_440555.1	*spkA*	*sll1575*	Serine/threonine protein kinase	-1.7022479
NP_442655.1	*spkD*	*sll0776*	Serine/threonine protein kinase	-1.8333734
NP_442484.1	*rre29*	*slr0081*	OmpR subfamily	-1.5930618
NP_442718.1	*rre35*	*sll0039*	CheY family protein	-1.8846409
NP_442923.1	*rre38*	*slr1584*	OmpR subfamily	-1.7764021
			Carotenoid biosynthesis	
NP_440788.1	*bhy/crtZ*	*sll1468*	β-carotene hydroxylase	-1.5127941
NP_441254.1	*crtU*	*sll0254*	Isorenieratene synthase	-2.3443117
NP_441167.1	*pds/crtP*	*slr1254*	Phytoene desaturase	-1.6156873
			Pyruvate metabolism	
NP_441027.1	*pta*	*slr2132*	Phosphate acetyltransferase	-1.7396748
NP_441683.1	leuA	sll1564	Alpha-isopropylmalate/homocitrate synthase family transferase	-1.7055957
NP_442022.1	*accD*	*sll0336*	Acetyl-CoA carboxylase subunit beta	-1.599048
NP_442597.1	*ppsA*	*slr0301*	Phosphoenolpyruvate synthase	-1.5075309

To be continued

Table 4 Continued

Protein_id	Gene	Locus_tag	Product	Fold change
			Butanoate metabolism	
NP_441304.1	*ilvB*	*sll1981*	Acetolactate synthase	-1.5532244
NP_440839.1	*sdhA*	*slr1233*	Succinate dehydrogenase flavoprotein subunit	-1.5117372
NP_440750.1	*phbC*	*slr1830*	Poly(3-hydroxyalkanoate) synthase	-1.563616
			Valine, leucine and isoleucine biosynthesis	
NP_441304.1	*ilvB*	*sll1981*	Acetolactate synthase	-1.5532244
NP_442721.1	*ilvE*	*slr0032*	Branched-chain amino acid aminotransferase	-1.6124098
NP_441683.1	*leuA*	*sll1564*	Alpha-isopropylmalate/homocitrate synthase family transferase	-1.7055957
			Transposase	
NP_443008.1		*slr0462*	Transposase	-1.6912371
NP_942290.1		*sll7002*	Transposase	-1.6664679
NP_441693.1		*slr1683*	Transposase	-1.5729775
NP_441354.1		*slr1522*	Transposase	-1.9166062
NP_441299.1		*sll1985*	Transposase	-1.822878
NP_441118.1		*slr0857*	Transposase	-1.6183379
NP_441694.1		*slr1684*	Transposase	-1.7607863
NP_942436.1		*sll8042*	Transposase	-1.6369566
NP_442584.1		*ssl0172*	Transposase	-1.7723324
NP_442560.1		*slr0099*	Transposase	-1.5619414
NP_440891.1		*slr1357*	Transposase	-1.5267483
NP_442094.1		*slr0352*	Transposase	-1.5239737
NP_441355.1		*slr1523*	Transposase	-1.906241
NP_442679.1		*sll0668*	Transposase	-1.9163983
NP_439940.1		*sll1397*	Transposase	-1.599705
NP_440005.1		*sll0700*	Transposase	-1.525472
NP_443007.1		*ssr0817*	Transposase	-1.6080397
NP_441419.1		*ssr2227*	Transposase	-1.9786897
NP_442585.1		*sll0092*	Transposase	-1.6198834
			Others	
NP_442891.1	*rpoF*	*slr1564*	RNA polymerase sigma factor SigF	-1.9298439
NP_440117.1	*bvdR*	*slr1784*	Biliverdin reductase	-1.6996859
NP_441350.1	*menA*	*slr1518*	1,4-dihydroxy-2-naphthoate octaprenyltransferase	-1.526862
NP_443064.1		*slr0609*	47 kD protein	-1.512096
NP_440614.1		*sll1081*	ABC transporter membrane protein	-2.0568955
NP_440937.1		*slr0889*	ABC1-like	-2.1852384
NP_442136.1		*sll0005*	ABC1-like	-1.5963937
NP_440638.2	*purA*	*sll1823*	Adenylosuccinate synthetase	-1.6858243
NP_440618.1	*speB*	*sll1077*	Agmatine ureohydrolase	-1.5067617
NP_441451.2	*lnt*	*slr0819*	Apolipoprotein N-acyltransferase	-1.6708694
NP_442954.1		*sll0720*	ApxIC gene product hemolysin activation protein	-1.5292122
NP_442762.1	*argS*	*sll0502*	Arginyl-tRNA synthetase	-1.6080518

To be continued

Table 4 Continued

Protein_id	Gene	Locus_tag	Product	Fold change
NP_442399.1	*panD*	*sll0892*	Aspartate alpha-decarboxylase	-1.8848246
NP_442135.1	*aspC*	*sll0006*	Aspartate aminotransferase	-2.0371778
NP_440558.1	*aspA*	*slr1705*	Aspartoacylase	-2.0660949
NP_441629.1	*gatB*	*sll1435*	Aspartyl/glutamyl-tRNA amidotransferase subunit B	-1.6340806
NP_443038.1	*rfbB*	*sll0575*	ATP-binding protein	-1.6006606
NP_443041.1	*arcC*	*sll0573*	Carbamate kinase	-1.8178267
NP_442183.1	*ccmK*	*slr0436*	Carbon dioxide concentrating mechanism protein CcmK	-1.5782174
NP_442507.1	*cobW*	*slr0502*	CobW protein	-1.6048905
NP_442547.1	*cbiP*	*slr0618*	Cobyric acid synthase	-1.5467187
NP_441538.1	*hemN*	*sll1876*	Coproporphyrinogen III oxidase	-4.0097356
NP_442426.1	*cyp*	*slr0574*	Cytochrome P450	-1.6143006
NP_441861.1	*pyrC*	*slr0406*	Dihydroorotase	-2.0390232
NP_442419.1		*sll0544*	DNA polymerase III subunit delta	-1.7347811
NP_440291.1		*sll0644*	Esterase	-1.5326122
NP_441180.1	*suhB*	*sll1959*	Extragenic suppressor	-1.6110706
NP_441567.1	*suhB*	*sll1383*	Extragenic suppressor SuhB	-1.7025776
NP_440484.1		*sll0990*	Formaldehyde dehydrogenase (glutathione)	-1.5098546
NP_442130.1	*fumC*	*slr0018*	Fumarate hydratase	-1.7996609
NP_440244.1	*yefA*	*slr1072*	GDP-D-mannose dehydratase	-1.814762
NP_440268.1	*obgE*	*slr1090*	GTPase ObgE	-1.6336955
NP_440544.1	*gumB*	*sll1581*	GumB protein	-1.5111989
NP_441539.1	*ho2*	*sll1875*	Heme oxygenase	-3.6761715
NP_440269.1	*hliA*	*ssr1789*	High light inducible protein	-1.529347
NP_441181.1	*hisC*	*sll1958*	Histidinol phosphate aminotransferase	-1.5352646
NP_441417.1	*hoxF*	*sll1221*	Hydrogenase subunit	-1.5090066
NP_441577.1	*melB*	*sll1374*	Melibiose carrier protein	-1.9115357
NP_441449.1	*entC*	*slr0817*	Menaquinone-specific isochorismate synthase	-1.746671
NP_440941.1	*amiA*	*slr0891*	N-acetylmuramoyl-L-alanine amidase	-1.5475873
NP_440808.1	*narB*	*sll1454*	Nitrate reductase	-1.7130357
NP_440613.1	*nrtD*	*sll1082*	Nitrate transport protein NrtD	-2.2711887
NP_440516.1	*pobA*	*sll1297*	Phenoxybenzoate dioxygenase	-1.7330594
NP_442529.1	*purL*	*slr0520*	Phosphoribosyl formylglycinamidine synthase	-1.6109879
NP_440548.1	*cpcD*	*ssl3093*	Phycocyanin associated linker protein	-1.7311424
NP_440294.1	*pleD*	*slr0687*	PleD gene product	-2.378852
NP_440724.1	*pmgA*	*sll1968*	PmgA	-1.9903225
NP_441363.1	*kpsT*	*slr2108*	Polysialic acid transport ATP-binding protein KpsT	-2.1367464
NP_442636.1	*ziaA*	*slr0798*	P-type ATPase	-1.5178888
NP_439987.1	*llaI.2*	*sll0709*	Restriction enzyme LlaI protein	-1.81438
NP_442087.1	*rnd*	*sll0320*	Ribonuclease D	-1.5848689
NP_442349.1	*rpoD*	*sll0184*	RNA polymerase sigma factor SigC	-1.6865757
NP_441666.1		*sll1087*	Sodium-coupled permease	-1.8265866

To be continued

Table 4 Continued

Protein_id	Gene	Locus_tag	Product	Fold change
NP_440118.1		*sll1678*	Spore maturation protein A	-2.000186
NP_441770.1	*smpB*	*slr1639*	SsrA-binding protein	-1.5850962
NP_440777.1	*hisC*	*sll1713*	Threonine-phosphate decarboxylase	-1.7819513
NP_942400.1		*sll8006*	Type I restriction-modification system S subunit	-1.8833483
NP_440239.1	*galE*	*slr1067*	UDP-glucose-4-epimerase	-1.6093016
NP_442823.1	*murB*	*slr1424*	UDP-N-acetylenolpyruvoylglucosamine reductase	-1.5154201
NP_442822.1	*murC*	*slr1423*	UDP-N-acetylmuramate--L-alanine ligase	-1.5469677
NP_440822.1	*ureE*	*slr1219*	Urease accessory protein E	-1.9285307

transcription of almost the genes that encode the components of PSI, PSII, PET, and Cyt.b6 was increased. It is possible that these up-regulated mRNAs are translated in the cells grown at 10°C to produce the proteins that support photosynthetic activity. We also found that *desB* may play a crucial role in the resistance of *Synechocystis* to an extremely LT (10°C). In addition, the results showed that *slr1609* may be crucial for fatty acid activation and for the biosynthesis of alkanes at 10°C. However, we found that at LT (10°C) cellular motility may be reduced by decreasing the transcription of *SpkA*. The abundance of mRNAs that encode the components of signal perception-transduction pathways may not be important when the proteins operate at the post-translational level, for example in protein phosphorylation. Further investigation of gene expression profiles under various temperature conditions with time-coursed profiling are needed to clarify all of the biological processes involved in the response of *Synechocystis* LT stress.

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