RNA-seq Profiling Reveals Novel Target Genes of LexA in the Cyanobacterium Synechocystis sp. PCC 6803

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LexA is a well-established transcriptional repressor of SOS genes induced by DNA damage in Escherichia coli and other bacterial species. However, LexA in the cyanobacterium Synechocystis sp. PCC 6803 has been suggested not to be involved in SOS response. In this study, we performed RNA-seq analysis of the wild-type strain and the lexA-disrupted mutant to obtain the comprehensive view of LexA-regulated genes in Synechocystis. Disruption of lexA positively or negatively affected expression of genes related to various cellular functions such as phototactic motility, accumulation of the major compatible solute glucosylglycerol and subunits of bidirectional hydrogenase, photosystem I, and phycobilisome complexes. We also observed increase in the expression level of genes related to iron and manganese uptake in the mutant at the later stage of cultivation. However, none of the genes related to DNA metabolism were affected by disruption of lexA. DNA gel mobility shift assay using the recombinant LexA protein suggested that LexA binds to the upstream region of pilA7, pilA9, ggpS, and slr1670 to directly regulate their expression, but changes in the expression level of photosystem I genes by disruption of lexA is likely a secondary effect.

Keywords: cyanobacteria, LexA, RNA-seq, Synechocystis, transcriptome

INTRODUCTION

The LexA protein in Escherichia coli has been well-characterized as the key regulator of the SOS response induced by DNA damage (Butala et al., 2009). Under non-stress conditions, LexA binds to the promoter regions of more than 40 genes involved in the SOS response and represses their expression. When DNA is damaged, LexA undergoes autoproteolytic cleavage upon association with RecA protein activated through binding of single-stranded DNA fragments. As a consequence of auto-cleaveage of the Ala84-Gly85 peptide bond carried out by Ser119 and Lys156, LexA loses DNA binding activity, thereby inducing the SOS response.

Genes encoding LexA homologs are highly conserved in bacterial genomes and LexA–dependent transcriptional regulation of genes involved in DNA repair has been reported in various bacterial species (Erill et al., 2007; Butala et al., 2009), indicating that the regulation of SOS regulon by LexA might be a universal adaptation strategy of bacteria to DNA damage. However, LexA homologs
in several cyanobacterial species were suggested not to be involved in the typical *E. coli*-type SOS regulation. In *Anabaena* sp. PCC 7120, auto-cleavage of the Ala84-Gly85 bond of LexA does not occur at physiological pH even in the presence of activated RecA (Kumar et al., 2015). In the case of *Synechocystis* sp. PCC 6803 (S.6803), LexA lacks the conserved Ala-Gly auto-cleavage site and the serine of the Ser-Lys dyad required for auto-cleavage activity (Patterson-Fortin et al., 2006) and auto-cleavage of LexA in S.6803 has not been reported so far. DNA microarray analysis revealed that LexA depletion did not affect the expression level of genes involved in DNA metabolism (Domain et al., 2004).

The cellular processes regulated by LexA in S.6803 have been implied by studies reporting isolation of LexA as a binding factor to the promoter region of specific genes, such as the *hoxEFUYH* operon encoding bidirectional hydrogenase (Gutekunst et al., 2005; Oliveira and Lindblad, 2005), *crhR* encoding RNA helicase (Patterson-Fortin et al., 2006), and *sbtA* encoding sodium-dependent bicarbonate transporter (Lieman-Hurwitz et al., 2009). Domain et al. (2004) performed DNA microarray analysis of the LexA-depleted strain and found that most of genes affected were previously reported to be regulated by the availability of inorganic carbon (Wang et al., 2004). Kamei et al. (2001) reported that the *lexA*-disrupted mutant of the motile strain of S.6803 (denoted PCC strain) showed non-motile phenotype. DNA microarray analysis revealed that expression of the *pilA* genes encoding the subunits of the type IV pilus-like structure was lowered in the mutant. Although regulation of various cellular processes has been suggested, we currently have still a fragmentary understanding of the function of LexA in S.6803.

DNA microarray analysis has been the most popular methods of genome-wide transcriptome profiling. However, it has been supplanted by RNA-seq analysis in which isolated transcripts are converted into the complementary DNA (cDNA) followed by direct sequence in a massively parallel DNA sequencing-based approach. The advantages of RNA-seq over DNA microarray are its higher resolution and better dynamic range of detecting differential gene expression (Zhao et al., 2014). In order to obtain the comprehensive view of LexA-regulated genes in S.6803, here we performed RNA-seq analysis of the wild-type (WT) strain and the *lexA*-disrupted mutant. The results of RNA-seq analysis indicate that LexA in S.6803 regulates specific cellular functions such as phototactic motility, accumulation of the major compatible solute glucosyglycerol and subunits of bidirectional hydrogenase, and photosynthetic complexes, but not the SOS response. DNA gel mobility shift assay using the recombinant LexA protein suggested that LexA binds to the upstream region of *pilA7, pilA9, ggpS*, and *slr1670* to directly regulate their expression.

**MATERIALS AND METHODS**

**Strains and Culture Conditions**

A glucose-tolerant non-motile strain (GT strain) of *Synechocystis* sp. PCC 6803 was grown at 32°C in BG-11 medium containing 20 mM HEPES-NaOH, pH 7.0, under continuous illumination at 20 μmol photons m⁻² s⁻¹ with bubbling of air. The *lexA* (*sll1626*)-disrupted mutant (∆*lexA*) was grown under the same conditions, except that 20 μg mL⁻¹ kanamycin (Km) was added to the medium. Cell density was estimated by measuring OD₇₃₀ using a spectrophotometer (model UV-160A, Shimadzu).

**Generation of the lexA (sll1626)-Disrupted Mutant**

The coding region of *lexA* (612 bp, from nucleotide 1319330 to 1318719 according to numbering in CyanoBase) was disrupted by insertion of a kanamycin resistance (Km') cassette. The upstream and downstream fragments including the *lexA* coding sequence were amplified by PCR from the genomic DNA of the WT strain using the primer sets *lexA*-F and Km-lexA-R (for amplification of 404 bp upstream fragment, from nucleotide 1319525 to 1319122) and Km-lexA-F and LexA-R (for amplification of 394 bp downstream fragment, from nucleotide 1318996 to 1318603; Table S1). Km' cassette was PCR amplified from the pRL161 plasmid using the primer set Km'-F and Km'-R (Table S1). The amplified *lexA* fragments and Km' cassette were fused together by the fusion PCR method (Wang et al., 2002) using the primer set *lexA*-F and *lexA*-R. The WT strain was transformed with the fusion PCR product and transformants (∆*lexA* mutant) were selected in the presence of Km.

**RNA Gel Blot Analysis**

Isolation of total RNA by the hot phenol method and RNA gel blot analyses, using DIG RNA Labeling and Detection Kit (Roche), were performed as described previously (Muramatsu and Hihara, 2003). Template DNA fragments for *in vitro* transcription to generate RNA probes were prepared by PCR using the primers shown in Table S1.

**Immunoblot Analysis**

Total proteins were extracted from *Synechocystis* cells as described previously (Ishii and Hihara, 2008) and separated by 15% SDS-PAGE, followed by electroblotting onto PVDF membranes (Immobilon-P; Millipore). Immunodetection was done using a rabbit polyclonal antibody raised against His-LexA recombinant protein. Goat anti-rabbit IgG conjugated to alkaline phosphatase was used as a secondary antibody.

**Determination of Pigment Contents**

*In vivo* absorption spectra of whole cells suspended in BG-11 medium were measured at room temperature using a spectrophotometer (V-650 Spectrometer, JASCO) with ISV-722 integrating sphere. Chlorophyll and phycocyanin contents were calculated from the peak heights of absorption spectra using the equations described in Arnon et al. (1974).

**RNA-seq Analysis**

RNA-seq analysis was carried out using cultures at OD₇₃₀ = 0.5 and OD₇₃₀ = 1.0 with three biological replicates. WT and ∆*lexA* were inoculated into new media at OD₇₃₀ = 0.1 and incubated for 50 and 80 h, respectively, to be harvested at OD₇₃₀ = 0.5. Similarly, WT and ∆*lexA* were inoculated at OD₇₃₀ = 0.1 and incubated for 70 and 120 h, respectively, to be harvested at OD₇₃₀ = 1.0. Isolation of total RNA by the hot phenol method
was performed as described previously (Muramatsu and Hihara, 2003). To eliminate genomic DNA from total RNA samples, each sample was added with DNase I (TaKaRa) and incubated at 37°C for 3 h. Total RNA concentration was measured with Nanodrop 2000 (Thermo Fisher Scientific). The Ribo-Zero Magnetic Kit for Bacteria (Epicentre) was used to remove ribosomal RNA from each sample. Concentration and quality of mRNA samples were examined using an Agilent 2100 Bioanalyzer. TruSeq RNA Sample Prep Kit v2 (Illumina) was used for cDNA library construction, and the libraries were sequenced using the Illumina MiSeq system. 12 samples in total were analyzed using two cartridge of MiSeq Reagent Kit v3 (Illumina).

A total of 64 million reads data was obtained from 12 samples. To quantify expression level of each gene, nucleotide sequences of obtained reads were mapped to the genomic sequence of GT-I strain of S.6803 (Kanesaki et al., 2012) (NC_017038:1; http://www.ncbi.nlm.nih.gov/nuccore/NC_017038) using CLC Genomics Workbench 7.5.1 software (Qiagen). Raw read counts were divided by length of the transcripts and total number of million mapped reads in each sample to obtain reads per kilobase per million (RPKM) values (Mortazavi et al., 2008). TCC package of R software (Sun et al., 2013) was used to detect the differentially expressed genes between WT and ΔlexA. A false discovery rate of <0.01 was considered to be significant.

Overexpression and Purification of His-lexA
The coding region of the lexA gene was amplified by PCR using the primers lexA-NdeI-F and lexA-XhoI-R (Table S1), containing Ndel and Xhol sites at their 5′ end, respectively. The amplified lexA coding fragment was cloned into the pT7Blue T-vector (Novagen), digested with Ndel and Xhol and subcloned into the same restriction sites in pET28a vector (Novagen) to express the LexA protein with an N-terminal 6 × His-tag.

E. coli BL21(DE3) harboring the His-LexA expression construct was grown to an OD 600 = 0.6 in 250 mL of 2 × yeast extract-tryptone (YT) medium containing 20 μg mL⁻¹ Km at 37°C and induced with 0.013% of isopropyl β-D-thiogalactoside for 3 h. The cells were pelleted by centrifugation at 5800 g for 2 min, resuspended in 50 mM sodium phosphate buffer, pH 7.4, containing 0.5 M NaCl and 60 mM imidazole, and disrupted by three rounds of sonication with Sonifier 450 (Branson) for 2 min with interval of 1 min on ice. After the removal of whole cells and insoluble material by centrifugation, the soluble protein fraction was filtered through a 0.2 μm filter (DISMIC-25CS; ADVANTEC). His-LexA was purified by nickel-affinity column chromatography using a HisTrap FF crude (GE Healthcare). The soluble protein fraction was applied to the column equilibrated with 20 mM phosphate buffer, pH 7.4, containing 0.5 M NaCl and 60 mM imidazole, washed with 20 mM phosphate buffer, pH 7.4, containing 0.5 M NaCl and 80 mM imidazole, and eluted with 20 mM phosphate buffer, pH 7.4, containing 0.5 M NaCl and 300 mM imidazole. Purified His-LexA was desalted by a HiTrap Desalting column (GE Healthcare). Protein composition was examined by 15% (w/v) SDS-PAGE followed by staining with Coomassie Brilliant Blue R-250.

DNA Gel Mobility Shift Assay
Probes for DNA gel mobility shift assays were obtained by PCR amplification with primers shown in Table S1 using genomic DNA as a template. The 3′ end of the DNA fragment for each probe was labeled with digoxigenin (DIG)-ddUTP by using the terminal transferase method according to the manufacturer's instructions (DIG gel shift kit 2nd generation; Roche). Gel mobility shift assays were performed by using a DIG gel shift kit 2nd generation (Roche) according to the manufacturer's instruction except that 1 mM DTT was added to the reaction mixture.

RESULTS
Characterization of the lexA (ssl1626)-Disrupted Mutant
To reveal the function of LexA in GT strain of S.6803, we disrupted the lexA gene by inserting a Km′ cassette within the coding region (Figure 1A). Although a fully segregated mutant was not obtained (Figure 1B), RNA gel blot and immunoblot analyses revealed that both the lexA transcript (Figure 1C) and LexA protein (Figure 1D) levels were below the detection limit in the partially segregated mutant (ΔlexA) grown under normal growth conditions. Under the same conditions, lexA displayed several abnormal phenotypes. The doubling time of ΔlexA was longer (31.4 h) than that of WT (19.5 h) at log phase, whereas the difference in growth rate between strains became smaller at stationary phase (Figure 1E). Amounts of chlorophyll and phycocyanin in ΔlexA calculated from the peak heights of cellular absorption spectra were 93 and 80% of WT levels, respectively (Figure 1F). Microscopic observation revealed that cell size of ΔlexA was heterogeneous and tended to be larger than that of WT (Figure 1G).

RNA-seq Transcriptome Analysis
To investigate the difference in gene expression profile between WT and ΔlexA, total RNA was isolated from cultures incubated under normal growth conditions and RNA-seq analysis was performed. Figure 2 shows MA plots of the gene expression data obtained from cultures at OD 730 = 0.5 and OD 730 = 1.0. There were 1011 genes differentially expressed between strains at OD 730 = 0.5 and OD 730 = 1.0 as shown in magenta (Table S2). Among them, expression levels of 315 genes were more than two-fold higher and those of 21 genes were more than two-fold lower in ΔlexA than in WT (Table S2). In the case of WT and ΔlexA cells at OD 730 = 1.0, there were 447 genes differentially expressed between strains (Figure 2B). Among them, expression levels of 360 genes were more than two-fold higher and those of 21 genes were more than two-fold lower in ΔlexA than in WT (Table S3).

Table 1 shows the list of genes whose expression was affected by disruption of lexA. The higher resolution and better dynamic range of RNA-seq analysis compared to DNA microarray analysis enabled listing of small ORFs such as ssl1577, ggpR (ssl3076), ssl1251, ssl1473 and ssl3589, and genes with low expression level (low RPKM value) that cannot be detected by previous DNA microarray analyses. Differentially expressed genes can
be categorized into several groups according to related cellular functions as mentioned below.

**Motility-Related Genes**
The motile strain of S.6803 exhibits phototactic motility dependent on the type IV-like thick pilus structure (Brahamsha and Bhaya, 2014). In S.6803 genome, there are multiple genes homologous to the pilA gene encoding the subunit of the type IV pilus-like structure. Among them, pilA1 was shown to be responsible for the thick pilus structure, motility, and transformation efficiency (Bhaya et al., 1999; Yoshihara et al., 2001), whereas functions of other pilA-like genes are unknown. We observed that their expression is positively or negatively affected by disruption of the lexA gene. Expression of pilA7-pilA8 was largely enhanced whereas that of pilA9-pilA10-pilA11 and pilA1-pilA2 decreased. The observed decrease in expression level of pilA1 and pilA9-pilA10-pilA11 was consistent with the results of DNA microarray analysis of the ΔlexA mutant in the motile PCC strain (Kamei et al., 2001).

Furthermore, we observed that several genes other than pilA involved in motility were affected by disruption of lexA. Expression of pixG-pixH-pixJ1-pixJ2-pixL (sll0038-0043) encoding regulatory factors involved in positive phototaxis increased in the mutant (Tables S2). It has been reported that motility is controlled by cAMP level in S.6803 and inactivation of cya1 encoding adenylate cyclase or sycrp1 encoding cAMP receptor protein results in loss of motility (Terauchi and Ohmori, 1999; Yoshimura et al., 2002a). Although expression of cya1 and sycrp1 itself was not so much affected by disruption of lexA, decrease in expression levels of five genes, pilA9-pilA10-pilA11-slr2018 and cccS (slr1667), out of six genes reported to be decreased by disruption of sycrp1 (Yoshimura et al., 2002b) was observed (Table 1). cccS is also considered to be related to motility, since its disruption resulted in loss of the thick pili (Yoshimura et al., 2010). We observed expression level of sycrp2 is lower in ΔlexA (Table 1), although involvement of SYCRP2 in regulation of motility has not been reported.
TABLE 1 | Genes with altered expression in the lexA-disrupted mutant.

| Gene No. | Gene symbol | Definition | OD<sub>730</sub> = 0.5 | Average RPKM | Ratio | OD<sub>730</sub> = 1.0 | Average RPKM | Ratio |
|----------|-------------|------------|-----------------|--------------|-------|----------------|--------------|-------|
| sll1694  | pilA1       | Pilin polypeptide PilA1 | 7672.37 | 3764.79 | 0.49 | 9711.78 | 3875.35 | 0.40 |
| sll1695  | pilA2       | Pilin polypeptide PilA2 | 245.64 | 161.17 | 0.66 | 272.03 | 149.86 | 0.55 |
| sll1300  | pilA7       | Type 4 pilin-like protein | 110.72 | 1163.62 | 10.51 | 148.23 | 1231.28 | 8.31 |
| sll1301  | pilA8       | Type 4 pilin-like protein | 193.59 | 1186.22 | 6.13 | 226.51 | 1250.18 | 5.52 |
| sll1302  | pilA9       | Type 4 pilin-like protein | 68.30 | 16.96 | 0.25 | 55.96 | 19.50 | 0.35 |
| sll1303  | pilA10      | Type 4 pilin-like protein | 38.83 | 23.24 | 0.60 | 37.41 | 16.90 | 0.45 |
| sll1304  | pilA11      | Type 4 pilin-like protein | 72.06 | 31.47 | 0.44 | 73.43 | 35.82 | 0.49 |
| sll1285  | taxP2       | Two-component response regulator PatA subfamily | 185.08 | 63.22 | 0.34 | 203.50 | 86.70 | 0.43 |
| slr1670  | Unknown protein | | | | | | | |
| slr1671  | Unknown protein | | | | | | | |
| slr1672  | glpK        | Glycerol kinase | 52.30 | 299.46 | 5.73 | 43.84 | 227.47 | 5.19 |
| slr1673  | spoU        | Probable tRNA/rRNA methyltransferase | 38.42 | 179.60 | 4.67 | 42.98 | 144.32 | 3.36 |
| ssl3076  | ggpR        | Unknown protein | 2.03 | 16.39 | 8.07 | 0.00 | 0.87 | N.D |
| sll1566  | ggpS        | Glucosylglycerolphosphate synthase | 34.65 | 497.47 | 14.36 | 27.78 | 431.85 | 15.54 |
| sll1085  | glpD        | Glycerol-3-phosphate dehydrogenase | 32.37 | 191.71 | 5.92 | 36.05 | 215.11 | 5.97 |
| slr0529  | ggtB        | Glucosylglycerol transport system substrate-binding protein | 17.28 | 79.09 | 4.58 | 18.76 | 76.80 | 4.09 |
| slr0530  | ggtC        | Glucosylglycerol transport system permease protein | 21.55 | 102.21 | 4.74 | 23.34 | 88.37 | 3.79 |
| sll1220  | hoxE        | Diaphorase subunit of the bidirectional hydrogenase | 100.28 | 48.94 | 0.49 | 62.85 | 32.54 | 0.52 |
| sll1221  | hoxF        | Diaphorase subunit of the bidirectional hydrogenase | 64.58 | 31.77 | 0.49 | 49.47 | 31.90 | 0.64 |
| sll1223  | hoxU        | Diaphorase subunit of the bidirectional hydrogenase | 96.63 | 49.39 | 0.51 | 68.18 | 47.23 | 0.69 |
| sll1224  | hoxY        | Hydrogenase subunit of the bidirectional hydrogenase | 70.59 | 35.93 | 0.51 | 37.57 | 28.59 | 0.76 |
| sll2420  | Unknown protein | | | | | | | |
| slr1675  | hypA1       | Putative hydrogenase expression/formation protein HypA1 | 31.16 | 266.71 | 8.56 | 33.07 | 195.50 | 5.91 |
| slr0737  | psaD        | Photosystem I subunit II | 9924.04 | 5201.83 | 0.52 | 6914.54 | 4585.06 | 0.66 |
| slr1835  | psaB        | P700 apoprotein subunit lb | 3454.96 | 2284.72 | 0.66 | 4297.65 | 2504.56 | 0.58 |
| smr0004  | psaI        | Photosystem I subunit VIII | 3093.51 | 2356.63 | 0.76 | 290.74 | 157.92 | 0.54 |
| ssl3076  | psaC        | Photosystem I subunit VII | 10241.74 | 5794.74 | 0.57 | 5317.26 | 3374.82 | 0.63 |
| ssl3090  | psaK1       | Photosystem I subunit X | 2883.40 | 1628.37 | 0.56 | 1835.89 | 1152.40 | 0.70 |
| slr0120  | rbcS        | Rubisco small subunit | 3477.08 | 1936.92 | 0.56 | 4542.25 | 3204.02 | 0.71 |
| slr0111  | rbcX        | Possible Rubisco chaperonin | 3913.65 | 2224.26 | 0.57 | 5157.07 | 3765.52 | 0.73 |
| slr0247  | isiA        | Iron-stress chlorophyll-binding protein | 55.34 | 124.36 | 2.25 | 53.76 | 76.15 | 14.16 |
| slr0248  | isiB        | Flavodoxin | 10.09 | 35.32 | 3.50 | 5.91 | 121.62 | 20.59 |

(Continued)
| Gene No. | Gene symbol | Definition                                                                 | OD\textsubscript{730} = 0.5 | Ratio | Average RPKM | Ratio |
|---------|-------------|---------------------------------------------------------------------------|----------------------------|-------|--------------|-------|
| slr0506 | por         | Light-dependent NADPH-protochlorophyllide oxidoreductase                   | 247.75                     | 186.82 | 0.75         | 318.96 | 181.53 | 0.57 |
| slr0749 | chlL        | Light-independent protochlorophyllide reductase iron protein subunit ChlL | 489.76                     | 109.67 | 0.22         | 98.28  | 41.69  | 0.42 |
| slr0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         | 240.63 | 81.30  | 0.34 |
| sll0430 | htpG        | HtpG, heat shock protein 90                                               | 156.26                     | 589.81 | 3.77         | 115.48 | 607.00 | 5.26 |
| sll0559 | dnaJ        | DnaJ, heat shock protein 40                                                | 24.21                      | 216.45 | 8.94         | 22.63  | 304.56 | 13.48 |
| sll1514 | hspA        | 16.6 kDa small heat shock protein                                         | 100.08                     | 1082.85| 10.82        | 121.59 | 1307.29| 10.75 |
| sll0094 | hik37       | Two-component sensor histidine kinase                                      | 52.56                      | 33.12  | 0.63         | 74.37  | 39.50  | 0.53 |
| sll0777 | spkD        | Serine/threonine kinase                                                   | 16.14                      | 248.58 | 15.40        | 27.99  | 235.49 | 8.41 |
| sll0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         | 240.63 | 81.30  | 0.34 |

### CHAPERONES

| Gene No. | Gene symbol | Definition                                                                 | OD\textsubscript{730} = 0.5 | Ratio |
|---------|-------------|---------------------------------------------------------------------------|----------------------------|-------|
| sll0430 | htpG        | HtpG, heat shock protein 90                                               | 156.26                     | 589.81 | 3.77         |
| sll0559 | dnaJ        | DnaJ, heat shock protein 40                                                | 24.21                      | 216.45 | 8.94         |
| sll1514 | hspA        | 16.6 kDa small heat shock protein                                         | 100.08                     | 1082.85| 10.82        |
| sll0094 | hik37       | Two-component sensor histidine kinase                                      | 52.56                      | 33.12  | 0.63         |
| sll0777 | spkD        | Serine/threonine kinase                                                   | 16.14                      | 248.58 | 15.40        |
| sll0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         |
| sll0094 | hik37       | Two-component sensor histidine kinase                                      | 52.56                      | 33.12  | 0.63         |
| sll0777 | spkD        | Serine/threonine kinase                                                   | 16.14                      | 248.58 | 15.40        |
| sll0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         |

### REGULATORY FUNCTIONS

| Gene No. | Gene symbol | Definition                                                                 | OD\textsubscript{730} = 0.5 | Ratio |
|---------|-------------|---------------------------------------------------------------------------|----------------------------|-------|
| sll0430 | htpG        | HtpG, heat shock protein 90                                               | 156.26                     | 589.81 | 3.77         |
| sll0559 | dnaJ        | DnaJ, heat shock protein 40                                                | 24.21                      | 216.45 | 8.94         |
| sll1514 | hspA        | 16.6 kDa small heat shock protein                                         | 100.08                     | 1082.85| 10.82        |
| sll0094 | hik37       | Two-component sensor histidine kinase                                      | 52.56                      | 33.12  | 0.63         |
| sll0777 | spkD        | Serine/threonine kinase                                                   | 16.14                      | 248.58 | 15.40        |
| sll0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         |
| sll0094 | hik37       | Two-component sensor histidine kinase                                      | 52.56                      | 33.12  | 0.63         |
| sll0777 | spkD        | Serine/threonine kinase                                                   | 16.14                      | 248.58 | 15.40        |
| sll0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         |

### TRANSPORT AND BINDING PROTEINS

| Gene No. | Gene symbol | Definition                                                                 | OD\textsubscript{730} = 0.5 | Ratio |
|---------|-------------|---------------------------------------------------------------------------|----------------------------|-------|
| sll0430 | htpG        | HtpG, heat shock protein 90                                               | 156.26                     | 589.81 | 3.77         |
| sll0559 | dnaJ        | DnaJ, heat shock protein 40                                                | 24.21                      | 216.45 | 8.94         |
| sll1514 | hspA        | 16.6 kDa small heat shock protein                                         | 100.08                     | 1082.85| 10.82        |
| sll0094 | hik37       | Two-component sensor histidine kinase                                      | 52.56                      | 33.12  | 0.63         |
| sll0777 | spkD        | Serine/threonine kinase                                                   | 16.14                      | 248.58 | 15.40        |
| sll0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         |
| sll0094 | hik37       | Two-component sensor histidine kinase                                      | 52.56                      | 33.12  | 0.63         |
| sll0777 | spkD        | Serine/threonine kinase                                                   | 16.14                      | 248.58 | 15.40        |
| sll0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         |

### OTHER CATEGORIES

| Gene No. | Gene symbol | Definition                                                                 | OD\textsubscript{730} = 0.5 | Ratio |
|---------|-------------|---------------------------------------------------------------------------|----------------------------|-------|
| sll0430 | htpG        | HtpG, heat shock protein 90                                               | 156.26                     | 589.81 | 3.77         |
| sll0559 | dnaJ        | DnaJ, heat shock protein 40                                                | 24.21                      | 216.45 | 8.94         |
| sll1514 | hspA        | 16.6 kDa small heat shock protein                                         | 100.08                     | 1082.85| 10.82        |
| sll0094 | hik37       | Two-component sensor histidine kinase                                      | 52.56                      | 33.12  | 0.63         |
| sll0777 | spkD        | Serine/threonine kinase                                                   | 16.14                      | 248.58 | 15.40        |
| sll0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         |
| sll0094 | hik37       | Two-component sensor histidine kinase                                      | 52.56                      | 33.12  | 0.63         |
| sll0777 | spkD        | Serine/threonine kinase                                                   | 16.14                      | 248.58 | 15.40        |
| sll0750 | chlN        | Light-independent protochlorophyllide reductase subunit ChlN              | 149.90                     | 49.34  | 0.33         |

(Continued)
TABLE 1 | Continued

| Gene No. | Gene symbol | Definition | OD<sub>730 = 0.5</sub> | Average RPKM | Ratio | OD<sub>730 = 1.0</sub> | Average RPKM | Ratio |
|----------|-------------|-----------|-----------------|-------------|-------|-----------------|-------------|-------|
|          |             |           | WT   | ΔlexA | ΔlexA/WT | WT   | ΔlexA | ΔlexA/WT |
| sll0249  | Hypothetical protein | 10.39 | 23.76 | 2.29 | 6.55 | 84.74 | 12.95 |
| sll0327  | Unknown protein | 113.13 | 1929.23 | 17.05 | 157.08 | 1310.83 | 8.35 |
| sll0328  | Unknown protein | 47.79 | 859.88 | 17.99 | 48.45 | 595.60 | 12.29 |
| sll0443  | Unknown protein | 71.82 | 419.75 | 5.84 | 75.08 | 336.96 | 4.49 |
| sll0444  | Unknown protein | 116.90 | 563.08 | 4.82 | 97.11 | 410.52 | 4.23 |
| sll0445  | Unknown protein | 114.46 | 528.48 | 4.62 | 117.45 | 444.43 | 3.78 |
| sll0448  | Unknown protein | 10.17 | 49.14 | 4.83 | 7.21 | 42.81 | 5.94 |
| sll0543  | Hypothetical protein | 677.95 | 37.76 | 0.06 | 535.50 | 31.01 | 0.06 |
| sll0783  | Unknown protein | 84.80 | 38.09 | 0.45 | 86.35 | 68.05 | 0.79 |
| sll0846  | Hypothetical protein | 133.72 | 577.77 | 4.32 | 106.12 | 532.70 | 5.02 |
| sll0910  | Unknown protein | 29.09 | 170.08 | 5.85 | 16.73 | 124.75 | 7.46 |
| sll0911  | Unknown protein | 611.42 | 3545.01 | 5.80 | 926.18 | 3621.41 | 3.91 |
| sll1009  | Unknown protein | 109.22 | 577.77 | 4.32 | 106.12 | 532.70 | 5.02 |
| sll1019  | Hypothetical protein | 88.93 | 495.38 | 5.47 | 86.35 | 495.38 | 5.47 |
| sll1236  | Unknown protein | 30.41 | 472.85 | 15.55 | 27.60 | 136.10 | 4.93 |
| sll1239  | Unknown protein | 88.93 | 495.38 | 5.47 | 86.35 | 495.38 | 5.47 |
| sll1240  | Unknown protein | 23.31 | 240.78 | 10.33 | 18.06 | 205.72 | 11.39 |
| sll1241  | Unknown protein | 26.35 | 199.16 | 7.56 | 14.51 | 161.65 | 12.52 |
| sll1247  | Hypothetical protein | 137.85 | 61.68 | 0.45 | 194.48 | 104.16 | 0.54 |
| sll1359  | Unknown protein | 76.42 | 37.70 | 0.49 | 54.18 | 36.91 | 0.76 |
| sll1396  | Unknown protein | 18.55 | 130.15 | 7.01 | 13.41 | 45.98 | 3.43 |
| sll1472  | Unknown protein | 59.93 | 13.16 | 0.22 | 54.18 | 13.94 | 0.26 |
| sll1483  | Periplasmic protein | 57.32 | 302.45 | 5.28 | 46.76 | 183.74 | 3.93 |
| sll1549  | Salt-enhanced periplasmic protein | 232.67 | 121.52 | 0.52 | 18.94 | 215.48 | 11.37 |
| sll1660  | Hypothetical protein | 45.49 | 351.04 | 7.72 | 48.25 | 355.40 | 7.37 |
| sll1722  | Hypothetical protein | 18.55 | 130.15 | 7.01 | 13.41 | 45.98 | 3.43 |
| sll1723  | Probable glycosyltransferase | 11.63 | 67.76 | 5.83 | 7.87 | 24.45 | 3.11 |
| sll1851  | Unknown protein | 136.11 | 100.98 | 0.74 | 15.16 | 103.21 | 6.81 |
| sll1913  | Hypothetical protein | 24.83 | 103.47 | 4.17 | 18.85 | 108.19 | 5.74 |
| sll1921  | Hypothetical protein | 136.06 | 1118.71 | 8.22 | 152.96 | 1173.63 | 7.67 |
| sll1956  | Hypothetical protein | 68.61 | 42.42 | 0.62 | 60.44 | 37.54 | 0.62 |
| slr0105  | Unknown protein | 40.10 | 187.90 | 4.87 | 54.18 | 13.94 | 0.26 |
| slr0106  | Unknown protein | 38.61 | 187.90 | 4.87 | 54.18 | 13.94 | 0.26 |
| slr0179  | Hypothetical protein | 11.14 | 405.28 | 36.37 | 19.47 | 345.32 | 17.74 |
| slr0196  | Unknown protein | 38.61 | 187.90 | 4.87 | 14.43 | 111.92 | 7.75 |
| slr0317  | Hypothetical protein | 18.01 | 103.04 | 5.72 | 20.82 | 119.39 | 5.73 |
| slr0362  | Hypothetical protein | 48.52 | 240.11 | 4.95 | 55.46 | 204.71 | 3.69 |
| slr0364  | Hypothetical protein | 5.58 | 25.68 | 4.61 | 5.73 | 22.58 | 3.91 |
| slr0393  | Unknown protein | 17.69 | 35.91 | 2.03 | 7.26 | 38.90 | 5.36 |
| slr0442  | Unknown protein | 175.06 | 105.14 | 0.60 | 207.58 | 121.90 | 0.59 |
| slr0572  | Unknown protein | 350.05 | 18.16 | 0.05 | 194.88 | 16.91 | 0.09 |
| slr0573  | Unknown protein | 18.80 | 3.65 | 0.19 | 22.65 | 6.20 | 0.27 |
| slr0581  | Unknown protein | 79.35 | 334.01 | 4.21 | 60.26 | 159.45 | 2.65 |
| slr0617  | Unknown protein | 85.17 | 16.67 | 0.20 | 89.51 | 25.91 | 0.29 |
| slr0709  | Hypothetical protein | 88.58 | 76.13 | 0.86 | 96.00 | 56.95 | 0.59 |
| slr0868  | Unknown protein | 20.22 | 326.40 | 16.14 | 13.93 | 203.19 | 14.59 |

(Continued)
TABLE 1 | Continued

| Gene No. | Gene symbol | Definition | OD_{730} = 0.5 | OD_{730} = 1.0 | Average RPKM | Ratio | Average RPKM | Ratio |
|----------|-------------|------------|----------------|----------------|---------------|-------|---------------|-------|
| slr0869  | Hypothetical protein | 23.29 | 186.20 | 7.95 | 24.87 | 165.85 | 6.67 |
| slr0870  | Hypothetical protein | 31.36 | 196.83 | 6.28 | 14.82 | 110.90 | 7.48 |
| slr0871  | Unknown protein | 12.74 | 102.10 | 8.01 | 5.49 | 63.27 | 11.53 |
| slr1161  | Hypothetical protein | 306.59 | 134.80 | 0.44 | 251.76 | 84.91 | 0.34 |
| slr1162  | Unknown protein | 131.90 | 66.56 | 0.50 | 104.40 | 63.19 | 0.61 |
| slr1278  | Hypothetical protein YCF62 | 32.54 | 27.87 | 0.86 | 78.79 | 41.89 | 0.53 |
| slr1484  | Unknown protein | 48.58 | 129.12 | 2.66 | 29.37 | 273.35 | 9.31 |
| slr1485  | Salt-induced periplasmic protein | 12.48 | 53.60 | 4.29 | 14.24 | 110.54 | 7.76 |
| slr1704  | Hypothetical protein | 162.27 | 1747.93 | 10.77 | 179.40 | 617.74 | 3.44 |
| slr1767  | Hypothetical protein | 39.67 | 197.11 | 4.97 | 19.06 | 96.15 | 5.04 |
| slr1772  | Probable hydrolase, periplasmic protein | 49.33 | 225.97 | 4.58 | 49.08 | 245.26 | 5.00 |
| slr1788  | Unknown protein | 33.57 | 388.00 | 11.56 | 65.92 | 359.18 | 5.45 |
| slr1798  | Unknown protein | 16.30 | 152.34 | 9.36 | 29.57 | 152.66 | 5.16 |
| slr1820  | Unknown protein | 165.94 | 109.76 | 0.70 | 186.54 | 617.74 | 3.44 |
| slr1885  | Unknown protein | 12.48 | 53.60 | 4.29 | 14.24 | 110.54 | 7.76 |
| slr1920  | Unknown protein | 16.30 | 53.60 | 3.33 | 14.24 | 110.54 | 7.76 |
| slr2119  | Unknown protein | 39.67 | 197.11 | 4.97 | 19.06 | 96.15 | 5.04 |
| ssl0104  | Hypothetical protein | 573.18 | 1747.93 | 10.77 | 179.40 | 617.74 | 3.44 |
| ssl0137  | Hypothetical protein | 69.57 | 33.34 | 0.48 | 14.09 | 14.00 | 0.33 |
| ssl0577  | Hypothetical protein | 20.16 | 114.07 | 5.66 | 7.78 | 45.35 | 5.83 |
| ssr0332  | Hypothetical protein | 218.38 | 134.04 | 0.70 | 120.96 | 73.51 | 0.61 |
| ssr1155  | Hypothetical protein | 670.58 | 374.80 | 0.56 | 164.79 | 140.34 | 0.85 |
| ssr1251  | Hypothetical protein | 52.57 | 15.34 | 0.29 | 6.04 | 2.22 | 0.37 |
| ssr1473  | Hypothetical protein | 13.23 | 91.14 | 6.89 | 10.36 | 45.68 | 4.41 |
| ssr2194  | Unknown protein | 14.79 | 614.16 | 41.52 | 8.50 | 181.59 | 21.37 |
| ssr2615  | Hypothetical protein | 24.65 | 17.45 | 0.71 | 27.18 | 9.41 | 0.35 |
| ssr2962  | Hypothetical protein | 63.09 | 276.40 | 4.38 | 41.60 | 191.31 | 4.60 |
| ssr3570  | Unknown protein | 61.19 | 27.67 | 0.45 | 30.69 | 17.30 | 0.56 |
| ssr3589  | Hypothetical protein | 19.15 | 115.47 | 6.03 | 9.11 | 61.37 | 6.73 |

**Glucosylglycerol-Related Genes**

In S.6803, glucosylglycerol (GG) is a major compatible solute to adapt to high-salt or high-osmotic pressure conditions (Klähn and Hagemann, 2011). A set of genes related to GG biosynthesis (ggp, glp) and uptake (ggt) are organized into several gene clusters such as ggpBCD (sll0529-0531), ggpS-glpD (sll1566-sll1085), ggpP-ggtA (slr0746-0747), and slr1674-sll1085. This observation is consistent with the previous study reporting that LexA acts as a transcriptional activator for the hox operon (Gutekunst et al., 2005). On the other hand, the expression level of the hoxA1 gene involved in hydrogenase maturation increased in ΔlexA.

**Photosynthesis-Related Genes**

In S.6803, photosystem (PS) I complex is comprised of 11 subunits and genes encoding these subunits (psa) are dispersed throughout the genome (Kaneko et al., 1996). We found that the expression level of every PSI gene was lower in ΔlexA than WT (Table 1 and Table S2). Klähn et al. (2010) reported that a small ORF, ggpR (sll3076), exists overlapping with the transcription initiation site of ggpS and its promoter region. Expression of ggpR was also induced by disruption of lexA (Table 1).

**Hydrogenase-Related Genes**

Expression level of the hoxE-hoxF-hoxU-hoxY-hoxH operon encoding subunits of bidirectional NiFe-hydrogenase was lower in ΔlexA. This observation is consistent with the previous study...
hliB encoding high-light inducible proteins was higher in ΔlexA than WT.

**SOS-Response Related Genes**

Previous studies suggested that LexA in S.6803 is not involved in the SOS response. Neither lexA nor recA expression was induced upon UV-irradiation (Domain et al., 2004; Patterson-Fortin et al., 2006) and none of DNA metabolism-related genes was listed as genes induced or repressed by LexA depletion (Kamei et al., 2001; Domain et al., 2004). Similarly, induction or repression of DNA metabolism-related genes by disruption of lexA was not observed in our RNA-seq analysis.

**Genes Differentially Expressed in ΔlexA at the Later Stage of Growth**

Several genes expressed under iron-limiting conditions such as exbB-exbD-flhuA operon involved in inorganic iron uptake (Jiang et al., 2015), futA1 and futA2 encoding subunits of iron transporter (Katoh et al., 2001), and isiA-isiB operon encoding iron-stress inducible proteins (Vinnemeier et al., 1998) were highly induced in ΔlexA at OD\textsubscript{730} = 1.0 but not in OD\textsubscript{730} = 0.5. In the case of mntA and mntC encoding subunits of manganese transporter (Bartsevich and Pakrasi, 1995), their expression level was already higher in ΔlexA at OD\textsubscript{730} = 0.5 and showed further increase at OD\textsubscript{730} = 1.0.

**DNA Gel Mobility Shift Assay**

DNA gel mobility shift assay was performed to examine whether LexA directly regulates expression of putative target genes listed by RNA-seq analysis (Figure 3). We observed induction of the pilA7-pilA8 operon and repression of the pilA9-pilA10-pilA11 operon in ΔlexA (Table 1). Binding of His-LexA to the promoter regions of both operons (for the pilA7 operon from nucleotide 2222102 to 2222304 and for the pilA9 operon from nucleotide 755577 to 755778, according to numbering in Cyanobase) was observed, indicating that LexA directly activates or represses expression of these pilA operons. We also examined whether His-LexA binds to the upstream region of the two divergently transcribed operons, ggpS-glpD and slr1670-glpK-spoU-slr1674-hypA1, both of which are highly induced in ΔlexA. His-LexA bound to the promoter fragment of each operon (for the ggpS operon from nucleotide 1949371 to 1949186 and for the slr1670 operon from nucleotide 1949332 to 1949534). It is notable that LexA-binding site for the the ggpS operon is within the coding region of ggpR (nucleotide 1949372 to 1949100). Our results suggest that LexA binds to at least two binding site located in the intergenic region of the ggpS and slr1670 operons to repress their expression. Next, we examined the binding of LexA to the upstream region of PSI genes by using light-responsive promoter fragments containing the HLR1 sequence recognized by the response regulator RpaB (Seino et al., 2009). Binding of His-LexA to the promoter region of PSI genes was not observed (Figure 3) or much weaker than that to the pilA7, pilA9, ggpS, and slr1670 promoters and not reproducible. This indicates that decrease in expression levels of PSI genes in ΔlexA may be a secondary effect.

**DISCUSSION**

**Effects of Disruption of the lexA Gene in S.6803**

In this study, we created the gene-disrupted mutant of lexA in GT strain of S.6803 to obtain the comprehensive view of LexA regulon by RNA-seq analysis. Although Kamei et al. (2001) successfully obtained the fully-segregated lexA mutant from the motile PCC strain, in most cases the ΔlexA mutant invariably retained the WT copy of the lexA gene (Domain et al., 2004; Gutekunst et al., 2005) and we also could not obtain fully-segregated mutant (Figure 1B). The heterogeneous appearance of the ΔlexA mutant cells (Figure 1G) may be caused by difference in the extent of segregation. However, despite the existence of the WT copy of lexA, immunoblot analysis revealed that LexA protein level was below the detection limit in our mutant (Figure 1D).

To date, LexA in S.6803 has been reported to be involved in transcriptional regulation of genes related to various cellular functions. Our RNA-seq data are consistent with some of these...
reports, e.g., positive regulation of the hox operon reported by Gutkunst et al. (2005) and positive regulation of the pilA genes reported by Kamei et al. (2001). However, we could not observe the large effect of LexA depletion on carbon metabolism-related genes reported by Domain et al. (2004). Domain et al. isolated RNA for DNA microarray analysis from concentrated cultures incubated on plates for 2 h. The growth condition must be largely different from our liquid culture, which may cause the difference in gene expression profile. in vitro transcription/translation assay performed by Patterson-Fortin et al. (2006) showed that CrhR protein accumulation decreased in response to increasing LexA concentration. However, in our data, expression level of crhR was not affected by disruption of lexA.

RNA-seq data in this study suggested involvement of LexA in regulation of (1) phototactic motility, (2) accumulation of GG, (3) bidirectional hydrogenase, and (4) photosystem I and phycobilisome complexes. We also observed increase in expression level of genes related to iron and manganese uptake in ΔlexA at OD730 = 1.0. LexA may be involved in stage specific repression of these genes, but it is also possible that these genes were upregulated as a consequence of iron and manganese limitation in the mutant culture during prolonged incubation. We will discuss regulation of cellular processes (1)–(4) by LexA in the following sections.

Cellular Processes Regulated by LexA in S.6803

Phototactic Motility

Kamei et al. (2001) reported that disruption of the lexA gene in the motile PCC strain resulted in decrease in expression level of pilA genes and loss of thick pili and motility. Our RNA-seq analysis showed that expression levels of genes related to phototactic motility are largely affected by disruption of lexA also in the non-motile strain. In addition to the decrease in expression level of pilA1 and pilA9-pilA10-pilA11 reported in Kamei et al. (2001), we observed significant induction of pilA7-pilA8.

Furthermore, expression levels of several genes related to positive phototaxis and cAMP signaling were affected. Although many non-motile mutants were so far isolated from the PCC strain, information on the mechanism of transcriptional regulation of motility-related genes is limited. Bhaya et al. (1999) reported decrease in expression level of pilA1 and pilA2 by disruption of the sigF gene encoding an alternative sigma factor. Yoshimura et al. (2002b) and Dienst et al. (2008) reported decrease in expression level of pilA9-pilA10-pilA11-sl1381 and cccS-cccP by disruption of sycrP1 encoding cAMP receptor protein and hfq encoding RNA chaperone homolog, respectively. Panichkin et al. (2006) reported decrease in expression level of pilA7-pilA10-pilA11-sl1381 and increase in that of pilA5-pilA6 and pilA2 by disruption of spkA encoding Serine/threonine protein kinase. None of these reports showed the direct interaction of these regulatory factors with pilA genes and LexA in this study is the first report of binding of transcriptional regulator to their upstream region (Figure 3). Involvement of SYCRP1 in transcriptional regulation of pilA genes through the direct regulation of LexA is not likely, since no SYCRP1 binding sequence has been detected in the upstream region of the lexA gene (Omagari et al., 2008; Xu and Su, 2009). Further examination of relationship between LexA and previously identified regulatory factors which affect motility may be a key to understanding of signal transduction mechanism regulating phototactic motility.

Accumulation of GG

In order to acclimate to high-salt or high-osmotic pressure conditions, S.6803 accumulates the compatible solute GG. Upon a salt shock, genes related to both GG biosynthesis (ggp, glp) and uptake (gtr) are induced (Kanesaki et al., 2002; Marin et al., 2004). GG is synthesized by a two-step reaction in S.6803. First, condensation of ADP-glucose and glycerol 3-phosphate is catalyzed by GG-phosphate synthase (GgpS) and then the intermediate is dephosphorylated by GG-phosphate phosphatase (GgpP) (Hagemann and Erdmann, 1994). Glycerol-3-phosphate
dehydrogenase (GldD) and glycerol kinase (GldK) are involved in the metabolism of glycerol-3-phosphate, a precursor of GG. Uptake of GG from the environment is performed by ABC transporter consisting of an ATP-binding protein (GgtA), a substrate-binding protein (GgtB) and two integral membrane proteins (GgtC and GgdT) in S.6803 (Mikkat and Hagemann, 2000). All of these genes are induced by the disruption of lexA (Table 1 and Table S2). DNA gel mobility shift assay revealed that His-LexA protein binds to the upstream region of two divergently transcribed operons, ggpS-glpD and slr1670-glpK-spoU-slr1674-hypA1 (Figure 3). To date, sigma factors SigF (Marin et al., 2002) and SigB (Nikkenen et al., 2012), a small protein GgpR (Klahn et al., 2010) and a response regulator Slr1588 (Chen et al., 2014) were reported to be involved in transcriptional regulation of the ggpS-glpD operon. Our result suggests the existence of the additional regulatory mechanism, namely, repression of the divergent ggpS and slr1670 operons by LexA. Expression of ggpR may also be repressed by LexA, judging from the fact its expression was induced by the disruption of lexA (Table 1). Salt-stress inducible genes such as hliA, hliB, hspa, proR, degP, slt1723, slt0846, slt1483, slr1704, slr1236, slr0581, and ssr2194, reported in the previous DNA microarray studies (Kanesaki et al., 2002; Marin et al., 2004), were also induced by the disruption of lexA (Table 1). There is possibility that LexA acts as a repressor for multiple salt-stress inducible genes as well as the ggpS and slr1670 operons.

Bidirectional Hydogenase

Regulation of the hox operon by LexA has been extensively studied in S.6803 (Oliveira and Lindblad, 2009). LexA was shown to bind to two distinct regions of the hox promoter, −198 to −338 and −592 to −690, relative to the start codon of hoxE (Gutukn et al., 2005; Oliveira and Lindblad, 2005) and work for positive regulation of hydrogenase activity (Gutukn et al., 2005). Regulation of hydrogenase-related genes by LexA may be common among cyanobacterial species, judging from the reports on LexA homologs in Anabaena sp. PCC 7120 (Sjöholm et al., 2007) and Lyngbya majusa CCAP 1446/4 (Ferreira et al., 2007).

Photosystem I and Phycobilisome

In the ΔlexA mutant, chlorophyll and phyocyanin contents were lower than those in WT (Figure 1F). This may be caused by decreased expression level of genes encoding subunits of PSI (psa), subunits of phycobilisome (cpc, apc) and both light-dependent and -independent protoclorophyllide reductase (chlL, chlB, por). It is known that these photosynthesis-related genes show the quite similar response to the changing light environment (Muramatsu and Hihara, 2012). The response regulator RpaB regulates high-light response of photosynthesis-related genes by binding to their promoter regions under low-light conditions (Wilde and Hihara, 2016). PSI genes and hli genes are positively- and negatively-regulated target genes of RpaB, respectively (Kappell and van Waasbergen, 2007; Seki et al., 2007; Seino et al., 2009). Repression of PSI genes and induction of hli genes by disruption of LexA (Table 1) seem to suggest overlapping roles of RpaB and LexA in regulation of photosynthetic gene expression. However, clear and reproducible band shift was not observed when binding of His-LexA to the promoter regions of PSI genes was examined (Figure 3). It is possible that changes in expression levels of photosynthesis-related genes in ΔlexA are not the consequence of loss of regulation by LexA but a secondary effect.

Search for LexA Binding Sites in the Target Promoters

Our results of DNA gel mobility shift assay suggest that LexA binds to the upstream region of piA7, piA9, ggpS and slr1670 to directly regulate their expression (Figure 3). To date, several nucleotide sequences for LexA binding site have been identified by DNA gel mobility shift assay, for example, 5′-TTTATTTGACATTTTTTTTT-3′, 5′-TTTTTGTTTGCATTAAAATT-3′ (Oliveira and Lindblad, 2005), 5′-CTA-N9(AT-rich)-CTA-3′ (Patterson-Fortin and Owtrtm, 2008), and 5′-AGT AACTAGTCG-3′ (Gutukn et al., 2005) in S.6803 and 5′-TAGTACTAATGTGTTTCTA-3′ in A.7120. (Mazón et al., 2004). However, these LexA binding sequences could not be found in the promoter fragments to which His-LexA bound. Instead, we found that a 5′-TTTTTTG(A/T)TNAC-3′ sequence commonly exists in these promoter fragments (Figure S1). The sequence is located around the putative transcription start site in the case of the negatively-regulated target genes, ggpS, piA7, and slr1670, whereas it is located further upstream in the case of the positively-regulated piA9 gene. It has been reported that a certain global transcriptional regulator, such as NtcA and RpaB in S.6803, can act as both repressor and activator dependent on the location of the binding site (García-Dominguez et al., 2000; Seino et al., 2009). Binding of the transcriptional regulator causes repression when its binding site overlaps the RNA polymerase-binding site, whereas activating effect is observed when the binding site is located further upstream. The location of 5′-TTTTTTG(A/T)TNAC-3′ sequence in four LexA-target promoters seems consistent with the scheme.

Physiological Roles of Cyanobacterial LexA

Results of RNA-seq analysis (Table 1) together with DNA gel mobility shift assay (Figure 3) suggest LexA in S.6803 can positively or negatively regulate various cellular processes such as phototactic motility, GG accumulation and hydogenase activity. Regulation of such a wide range of cellular processes by LexA shows pleiotrophic phenotypes such as filamentous structure due to inhibition of cell division, decreased sporulation, decrease in swimming motility and increased biofilm formation (Walter et al., 2015). In this case, LexA acts as a regulator of DNA damage in addition to the above mentioned biological functions. In contrast, DNA microarray data from different research groups (Kamei et al., 2001; Domain et al., 2004) and our RNA-seq data suggest LexA in S.6803 is not involved in regulation of SOS genes. In S.6803, expression of lexA and recA was not induced upon UV-irradiation (Domain
et al., 2004; Patterson-Fortin et al., 2006). Similarly, in *Anabaena* sp. PCC 7120, expression of *lexA* was not induced upon UV-B exposure or treatment with a DNA damaging agent mitomycin C (Kumar et al., 2015). In these freshwater species, LexA-independent protection mechanism for DNA damage may have evolved and LexA may have become devoted to regulating other cellular processes. Then, what is the physiological meaning of the coordinated regulation of phototactic motility, GG accumulation, and hydrogenase activity by LexA in *S*.6803? We searched for environmental conditions where LexA-target genes are coordinately regulated using CyanoExpress gene expression database (http://cyanoexpress.sysbiolab.eu/) and found that salt stress causes induction of GG metabolism-related genes and repression of *hox* operon and *pidA* genes in WT (Shoumskaya et al., 2005; Dickson et al., 2012). The expression profile is similar to that observed by disruption of *lexA* (Table 1), indicating the possibility that transcriptional regulation by LexA is temporarily inactivated under salt stress conditions.

Recently, it has been suggested that the SOS response in the marine *Synechococcus* is regulated by LexA like *E. coli* (Blot et al., 2011; Tetu et al., 2013). Cyanobacterial LexA genes can be clustered into three groups, Clade A containing *Gloeobacter violaceus* PCC 7421, Clade C containing marine picocyanobacteria and Clade B containing most remaining species (Li et al., 2010). There may exist high degree of variation of LexA regulons among species belonging to these three clades. By examination of what kind of cellular processes LexA regulates, we will be able to know decision of each species about how to use the transcriptional regulator LexA for better adaptation to changing environment.

**AUTHOR CONTRIBUTIONS**

The study was conceived by AYK and YH, with design input from AKK. Experiments were performed by AYK and AKK. Data analysis and interpretation was done by all authors. The manuscript was prepared by AYK and YH, and reviewed by all authors.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2016.00193

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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