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Functional Analysis of *lexA*-like gene, *sll1626* in *Synechocystis* sp. PCC 6803 using DNA microarray

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Introduction

It is widely known that UV irradiation and reactive oxygen species cause damages of DNA molecule, which are reverted by various repair mechanisms. Due to unavoidable stresses in oxygenic photosynthesis, plants and cyanobacteria are supposed to develop the various repair systems, although they have been poorly understood at the molecular level. In this study, the role of *lexA*-like gene (*sll1626*) in *Synechocystis* sp. PCC 6803 was analyzed by DNA microarray, since *lexA* is one of the key components of SOS responses in *E. coli* and other heterotrophic bacteria.

Materials and Method

The unicellular cyanobacterium *Synechocystis* sp. PCC 6803 was obtained from Pasteur Culture Collection. Solid medium was supplemented with 0.8 % (w/v) agar and 0.3 % (w/v) sodium thiosulfate and used for examination of motility by colony morphology. *sll1626* was insertionally disrupted with a kanamycin-resistant readthrough cassette and segregation was confirmed by PCR. To maintain gene-disrupted mutants, 20 μg/ml kanamycin was added, while antibiotics were not included for characterization of the mutant phenotype. DNA microarry analysis and northern hybridization was performed according to Hihara et al (2001). In brief, total

RNA was extracted and converted to cDNA labeled with Cy3 or Cy5 (Pharmasia). CyanoCHIP (Takara, version 0.8) was used in this study. For northern analysis, the probe DNA of *pilA1* was labeled with horse radish peroxidase and hybridization was detected by chemiluminescence. Pili structure on the cell surface was observed by electron microscopy after staining with phosphotungstic acid according to Yoshihara et al (2001).

Results and Discussion

Phenotype of the sll1626-disrupted mutant

We could recognize a single ORF homologous to bacterial *lexA* in the cyanobacterial genomes such as *Synechocystis* sp. PCC 6803, *Anabaena* sp. PCC 7120, *Nostoc punctiforeme* ATCC 29133, *Prochlorococcus marinus* MED4, and *Synechococcus* sp. WH8102. Key amino acid residues for the autocleavage of LexA are conserved in the cyanobacterial *lexA*-like ORF, whereas DNA binding helix-turn-helix motif is poorly conserved between the heterotrophic bacteria and cyanobacteria. Thus, we use the gene ID number *sll1626* hereafter for the *lexA*-like ORF in *Synechocystis* sp. PCC 6803. We disrupted *sll1626* in the motile wild type. After complete segregation, it was found that the *sll1626*-disrupted mutant formed domed colonies of round-shape on agar plates, while the motile wild type formed flat sheet-like colonies, indicative of loss of motility in the mutant (Fig. 1). This result suggests that *sll1626* is involved in motility. To confirm whether *sll1626* is involved in SOS response of *Synechocystis* or not, DNA microarray was analysis performed.

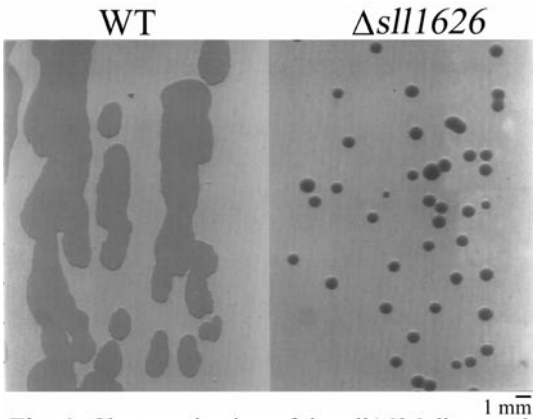

Fig. 1 Characterization of the *sll1626*-disrupted mutant.
Colony morphology of PCC and mutant cells grown at 20 $\mu E^{-2}s^{-1}$ on 0.8 % agar-BG11 medium plate for 3 days at 31ºC.

DNA microarray experiment

Unexpectedly, expression of several genes including *recA*, *umuC* and *sulA*, which are involved in DNA repair and strictly controlled at transcription level by LexA in *E. coli,* was hardly affected in the *sll1626*-disruptant of *Synechocystis*. By contrast, mRNA level of *sll1694*, *slr2015*, *slr2016*, *slr2017*, *slr2018*, *slr1667*, and *slr1668* was

suppressed (Table 1). *sll1694* (*pilA1*), which encodes a major prepilin subunit, is known to be essential for motility (Yoshihara et al. 2001, Bhaya et al. 2000). *slr2015* (*pilA9*), *slr2016* (*pilA10*), and *slr2017* (*pilA11*), which are arranged tandemly on the genome, are also predicted to code for prepilin subunits of the type IV pilus-like structure. *slr1667* and *slr1668* form an operon-like structure. According to Cyano2Dbase (http://www.kazusa.or.jp/cyano/cyano2D/index.html), N-terminal fifty amino acid residues are cleaved off from Slr1668 and the mature protein is present in the soluble fraction, while the function of Slr1667 and Slr1668 is still unknown. On the other hand, expression of *sll1626* itself and several other genes including *sll1009*, *slr0179* and *sll1765* was 5-8 fold enhanced in the mutant (Table 2). At the moment, physiological role or function of these genes is yet to be identified. To confirm reliability of the microarray data, we examined the mRNA level of *sll1694* (*pilA1*) by northern hybridization. The relative transcript levels in northern hybridization correlated well with those obtained by the DNA microarray analysis. These results suggest that Sll1626 plays critical roles in transcriptional regulation as both repressor and activator. We surveyed a potential binding site of *sll1626* at the upstream region of those genes, but could not find any conserved motif.

Table 1 List of genes whose transcript level were much lower in Δ*sll1626* mutant than in wild type strain

ORFNo.	gene name	annotation	expression level
slr2015	pilA9	Type IV-pilus like structure	0.19 ± 0.03
slr1668			0.24 ± 0.14
slr2018			0.27 ± 0.09
slr1667			0.28 ± 0.14
slr2016	pilA10	Type IV-pilus like structure	0.31 ± 0.07
slr2017	pilA11	Type IV-pilus like structure	0.42 ± 0.18
sll1694	pilA1	Type IV-pilus like structure	0.47 ± 0.13
sll0541	des9	acyl-CoA desaturase 1	0.50 ± 0.14
slr1259		hypothetical protein	0.59 ± 0.34

Table 2 List of genes whose transcript level were much higher in Δ*s111626* mutant than in wild type strain

ORFNo.	gene name	annotation	expression level
slr0179			8.12 ± 5.01
sll1626	lexA	SOS function regulatory protein	5.92 ± 1.85
sll1009	frpC	iron-regulated protein	5.43 ± 1.40
slr0530		membrane bound sugar transport protein	4.85 ± 3.46
sll1765			4.40 ± 1.71
slr1929			3.68 ± 1.72
slr0031		hypothetical protein	3.59 ± 1.61
slr1150			3.52 ± 1.89
slr0106			3.33 ± 1.86
slr1246			3.23 ± 1.38
slr2010		transposase	3.04 ± 1.42
sll1763			3.03 ± 1.24
sll0444			3.01 ± 1.28
slr1583			3.00 ± 1.56
slr1895			2.96 ± 0.69
sll0986			2.89 ± 1.56
slr0864		ABC transporter	2.55 ± 0.51

Provided that Sll1626 is the transcriptional factor like LexA of *E. coli*, many genes whose expression was elevated on depressed in the mutant may be regulated indirectly by a far upstream factor, *sll1626*.

Pilus structure on the cell surface

Irrespective of direct or indirect effects, *sll1626* was crucial for expression of various prepilin subunits. We examined the pilus structure on the cell surface by electron microscopy. Motile wild-type cells strain had two types of pili, thick and thin pili, the latter of which is known to be essential for motility (Yoshihara et al. 2001). On the other hand, the thick pili were clearly absent in the *sll1626*-disrupted mutant, while the thin pili were present such as wild type. This observation supports the non-motile phenotype of the *sll1626*-disruptant and the DNA microarray data. We also identified the protein kinase and phosphatase that are involved in the motility, previously (Kamei et al. 2001). It is possible that the signal transduction pathway, in which protein kinase and phosphatase take part, regulates the function of *sll1626*.

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