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Serine/Threonine Protein Kinase SpkA in *Synechocystis* sp. Strain PCC 6803 Is a Regulator of Expression of Three Putative *pilA* Operons, Formation of Thick Pili, and Cell Motility▽

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Previous studies showed that a Ser/Thr protein kinase, SpkA, in *Synechocystis* sp. strain PCC 6803 is involved in cell motility. The present study, in which DNA microarray analysis and electron microscopy were used, demonstrated that SpkA regulates the expression of putative *pilA9-pilA10-pilA11*-slr2018, *pilA5-pilA6*, and *pilA1-pilA2* operons and is essential for the formation of thick pili.

Serine/threonine protein kinases (Ser/Thr kinases) are key components of signal-transducing systems in eukaryotic cells. Recent studies of prokaryotic genomes have revealed that Ser/Thr kinases, which are found in eukaryotic organisms, are also present in many bacterial species (11). The genome of the cyanobacterium *Anabaena* sp. strain PCC 7120 (9) contains 52 putative genes for Ser/Thr kinases (12). One of these genes encodes PknA, which is essential for normal cell growth and the differentiation of heterocysts (21). Another Ser/Thr kinase, Pkn22, is involved in the acclimation of *Anabaena* cells to iron starvation and oxidative stress, and this enzyme also regulates expression of the *isiA* gene for the CP43′ protein (19).

The genome of another cyanobacterium, *Synechocystis* sp. strain PCC 6803, contains 12 genes for Ser/Thr kinases (8, 10, 11). Kamei et al. (6) demonstrated that SpkA encoded by the *spkA* gene is essential for the motility of *Synechocystis* cells, and Bhaya et al. (1) showed that the *pilA10*, *pilA11*, and slr2018 genes are important for cell motility. Bhaya et al. (2) further demonstrated that the *pilA1* gene is essential for the formation of thick pili. However, the relationship among the action of SpkA, the expression of the *pilA* genes, the formation of thick pili, and cell motility remained to be clarified.

In the present study, we examined systematically the genome-wide expression of genes, the formation of pili, and cell motility in *spkA* mutant cells. Our results suggest that SpkA in *Synechocystis* regulates the expression of genes in the three putative operons, namely, *pilA1-pilA2*, *pilA5-pilA6*, and *pilA9-pilA10-pilA11*-slr2018, and, as a result, leads to the formation of thick pili and cell motility.

TABLE 1. Changes in gene expression induced by mutation of the *spkA* gene, as determined by DNA microarray analyses[a]

Open reading frame	Gene	Product	Ratio of transcript levels
Genes whose expression was enhanced by mutation of the *spkA* gene			
slr1928	*pilA5*	Type 4 pilin-like protein PilA5	2.39 (±0.24)
slr1929	*pilA6*	Type 4 pilin-like protein PilA6	2.32 (±0.28)
sll1694	*pilA1*	Pilin polypeptide PilA1	2.18 (±0.35)
sll1695	*pilA2*	Pilin polypeptide PilA2	2.14 (±0.18)
Genes whose expression was decreased by mutation of the *spkA* gene			
slr2016	*pilA10*	Type 4 pilin-like protein, essential for motility	0.12 (±0.01)
slr2015	*pilA9*	Type 4 pilin-like protein, essential for motility	0.15 (±0.04)
slr2017	*pilA11*	Type 4 pilin-like protein, essential for motility	0.15 (±0.03)
slr2018		Unknown protein	0.22 (±0.01)

[a] The gene and open reading frame designations correspond to those in CyanoBase (http://www.kazusa.or.jp/cyano/cyano.html). Two independent experiments were performed. Genes whose ratios of transcript levels were higher than 2.0 or lower than 0.5 are shown. The complete list of changes in gene expression can be accessed at http://www.genome.ad.jp/KEGG/expression/.

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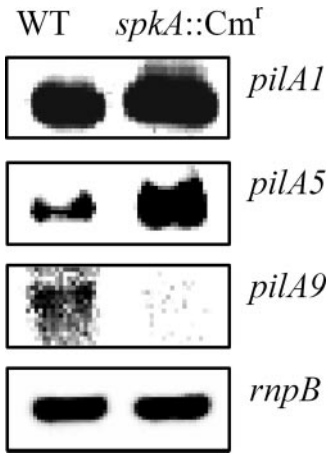

FIG. 1. Northern blot analysis of changes in expression of the *pilA1*, *pilA5*, and *pilA9* genes due to mutation of the *spkA* gene. Fragments of the *pilA1*, *pilA5*, *pilA9*, and *rnpB* genes were used as probes. WT, wild type.

Wild-type *Synechocystis* sp. strain PCC 6803 was obtained from the Pasteur Culture Collection. This strain has a functional *spkA* gene (sll1575) and can move on agar plates (6). The *spkA* gene was inactivated by insertion into the central region of the gene of a Cm^r cassette that included the *cat* gene. We first amplified a 1,384-bp fragment that contained part of the *spkA* gene by PCR using forward primer 5′-CCCGTCAACCCGTCACCGCCGTCTATTGG-3′ and reverse primer 5′-GCAACGGTAGCGGTCAAC-3′. We cloned the resultant fragment in pUC18 (20). The newly generated plasmid was digested with the restriction enzyme SmaI, and the linearized plasmid was ligated with the Cm^r cassette from plasmid pACYC184 (15), which contained the *cat* gene for chloramphenicol acetyltransferase. The resultant plasmid was used to transform *Synechocystis* cells as described previously (4). Transformed cells were selected on agar-solidified BG11 medium as described previously (4). Examination by PCR using DNA isolated from wild-type and *spkA*::Cm^r mutant cells as the template, forward primer 5′-TAAAATTCTCG ATTTTGGTATCGCC-3′, and reverse primer 5′-GACAAT TTCGCCTCAATTTTAGGTT-3′ revealed that replacement of wild-type copies of the chromosome was complete (data not shown).

Cyanobacterial cells were grown as described previously (14). Mutant cells were maintained on solidified or liquid medium in the presence of 20 $\mu g\ ml^{-1}$ chloramphenicol. No chloramphenicol was added to the final cultures that were used for experiments in order to eliminate possible alterations in phenotype due to the presence of the antibiotic.

We examined the effects of mutation of the *spkA* gene on the genome-wide expression of genes using DNA microarrays that covered 3,074 of the 3,168 genes in the *Synechocystis* genome, as described previously (5). Table 1 shows that mutation of the *spkA* gene decreased expression of the *pilA9*, *pilA10*, *pilA11*, and slr2018 genes and increased expression of the *pilA1*, *pilA2*,

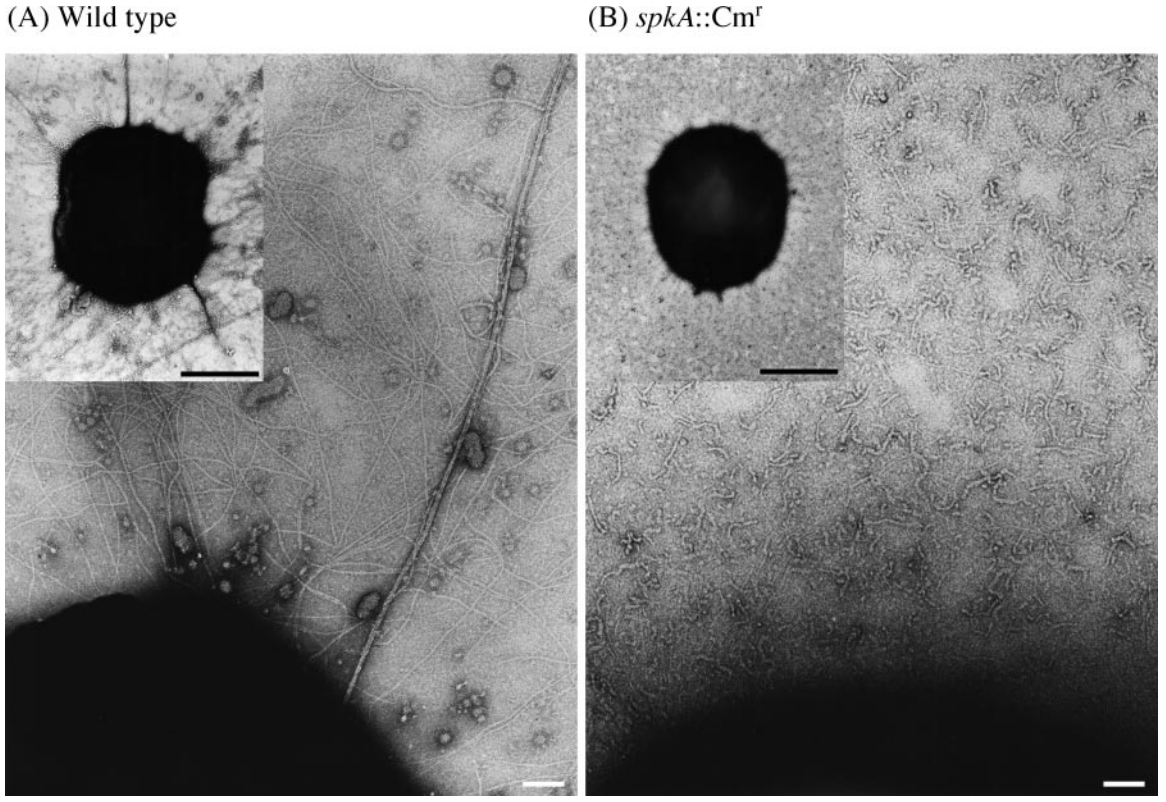

FIG. 2. Mutant *spkA*::Cm^r cells failed to form thick pili. Wild-type (A) and *spkA*::Cm^r (B) cells were processed by negative staining techniques and examined with an electron microscope. In *spkA*::Cm^r cells thick pili were not observed, and the thin pili were more fragmented than those of wild-type cells. White bars = 0.1 μm; black bars = 1 μm.

pilA5, and *pilA6* genes. These genes are organized in three putative operons, namely, *pilA9-pilA10-pilA11*-slr2018, *pilA1-pilA2*, and *pilA5-pilA6.* Our results suggest that under normal growth conditions, SpkA regulates the expression of these operons by producing a signal that enhances the expression of the putative *pilA9-pilA10-pilA11*-slr2018 operon and decreases the expression of the putative *pilA1-pilA2* and *pilA5-pilA6* operons.

To confirm the results obtained with microarrays, we performed a Northern blot analysis of total RNA from wild-type and *spkA*::Cm^r mutant cells, as described elsewhere (16). The probes for *pilA1*, *pilA5*, and *pilA9* mRNA were generated by performing PCR with primers 5′-AACTCCTCTCTCAACTCT CC-3′ and 5′-CTTCAGCACCACCACAATCA-3′, with primers 5′ATGTTCGAGGTGCTGATTGCCTTGA-3′ and 5′-GAACC TCGGTGTAAAGTGTTGCAAG-3′, and with primers 5′-CAA GTCTCCATTTTTCAAGCTCCGC-3′ and 5′-TCTCTTTTGC TTCTTTCTCGGCTCG-3′, respectively.

The results showed that the level of expression of the *pilA9* gene was reduced by mutation of the *spkA* gene, whereas the expression of the *pilA1* and *pilA5* genes was enhanced (Fig. 1). These observations confirmed the results of the DNA microarray analysis shown in Table 1.

Kamei et al. (6) reported that the *spkA* mutation did not affect pilus formation in *Synechocystis*. However, *pilA* genes are essential for formation of pili in *Pseudomonas aeruginosa* (13, 17), *Azoarcus* sp. strain BH72 (3) and *Eikenella corrodens* (18). Therefore, we examined wild-type and *spkA*::Cm^r mutant cells of *Synechocystis* by electron microscopy to determine whether mutation of the *spkA* gene affected the formation of pili. Wild-type and *spkA*::Cm^r mutant cells were negatively stained with 1% uranyl acetate for 1 min. The stained specimens were examined with an electron microscope (JEM 100 CX; JEOL, Tokyo, Japan) operated at 80 kV. Micrographs were originally taken at a magnification of ×50,000, were photographically magnified to obtain a magnification of ×170,000, and were digitized with a scanner (Es-2000; Epson, Tokyo, Japan), which was connected to a personal computer (Power Macintosh; NEC, Tokyo, Japan) using commercial software (Photoshop 6.0; Adobe Systems Inc., San Jose, CA).

Figure 2 shows that wild-type cells had two types of well-developed pili, namely, thick pili and thin pili. By contrast, *spkA*::Cm^r cells lacked thick pili but retained fragments of thin pili. We examined 50 wild-type and 50 *spkA*::Cm^r mutant cells and confirmed that the mutation of the *spkA* gene eliminated thick pili in all the *spkA* mutant cells.

As noted above, mutation of the *spkA* gene decreased the expression of the putative *pilA9-pilA10-pilA11*-slr2016 operon and enhanced the expression of the putative *pilA1-pilA2* and *pilA5-pilA6* operons. Thus, the expression of the putative *pilA9-pilA10-pilA11*-slr2018 operon might be involved in the formation of thick pili in wild-type cells, whereas the decrease in the expression of this operon in *spkA*::Cm^r mutant cells might be related to the disappearance of thick pili. Alternatively, it is possible that the expression of the putative *pilA1-pilA2* and/or *pilA5-pilA6* operons might have negatively regulated the formation of thick pili. Therefore, it also seems likely that the decreased expression of the putative *pilA1-pilA2* and/or *pilA5-pilA6* operon in wild-type cells resulted in the formation of thick pili, whereas the enhanced expression of these operons in *spkA*::Cm^r mutant cells did not result in the formation of thick pili.

We examined the effects of mutation of the s*pkA* gene on the motility of *Synechocystis* cells by monitoring the shapes of colonies that formed during cultivation on agar-solidified medium; the method used was similar to that used by Kamei et al. (7). The results showed that wild-type cells produced large diffuse colonies, whereas *spkA*::Cm^r mutant cells produced small distinct colonies (data not shown). These observations suggest that mutation of the *spkA* gene resulted in a defect in motility.

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