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The role of a methyl-accepting chemotaxis protein in gliding motility of the cyanobacterium *Synechocystis* sp. PCC 6803

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Introduction

Many motile photosynthetic microorganisms respond to the alterations of the quality and quantity of light and search actively for optimal environments for survival and growth. We reported previously that the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (henceforth referred to as Syn6803) could display phototactic and photokinetic gliding motility (Choi et al. 1999). There have been only a few reports dealing with the mechanism of cyanobacterial photomovement. It was shown that an extracellular glycoprotein, oscillin, was responsible for the gliding movement in *Phormidium uncinatum*. In addition, the marine unicellular cyanobacterium, *Synechococcus* sp. WH8102 can propel swimming motility by the outer membrane glycoprotein, SwmA, which possesses a similar feature to oscillin. Recently, it was addressed that type IV pili were required for phototactic gliding motility in Syn6803 and the synthesis of pili was controlled by an alternative sigma factor, SigF (Bhaya et al. 1999). In *P. aeruginosa* and *M. xanthus*, the pil gene clusters have been shown to be required for pilus biogenesis (Wall and Kaiser 1999). Possible homologous genes corresponding to pil genes of *P. aeruginosa* and *M. xanthus* were inactivated in Syn6803 to confirm which genes were required for type IV pilus biogenesis (Bhaya et al. 2000).
Here we isolated a transposon-generated mutant of Syn6803, S1-105, which did not show phototactic gliding motility. We identified that the S1-105 had an insertion in the ORF of *slr1044* (Cyanobase, http://www.kazusa.or.jp/cyano/) by an inverse PCR method (Ochman et al. 1988). The *slr1044* gene, renamed *ctr1* (cyanobacterial transducer), was required for the normal expression of *pilA1* and the biogenesis of thick pili.

Materials and methods

Transposon mutagenesis of Syn6803 was performed as the previously described method in *Synechococcus* sp. PCC 7942 with minor modification (Katayama et al. 1999). In brief, Tn*5* transposon-bearing plasmid, pAM1037, which is a derivative of pRL1058, was introduced into wild-type motile Syn6803 cells by triparental conjugal transfer from *E. coli* strains, AM1452 and AM1460. Transconjugants were plated on BG11 agar (1.5%) plates containing 5% LB (vol/vol) and incubated under low intensity light (3 μmol/m^2/s) for 2 days at 28°C without antibiotic. Kanamycin was

then underlaid beneath the plates to a final concentration of 50 μg/ml. The plates were incubated at a fluence rate of 15 μmol/m^2/s. Kanamycin-resistant colonies appeared after 4 weeks of incubation. Inverse PCR was performed as described previously in *E. coli* (Ochman et al. 1988) with minor modifications. Genomic DNA was completely digested by *Taq*I restriction enzyme. The digested DNA products were self-ligated using T4 DNA ligase. PCR was performed for 36 cycles at 96°C for 30 sec, 58°C for 30 sec, 72°C for 2 min and extended at 72°C for an additional 15 min using a sense primer (5'-GCACGATGAAGAGCAGAAGT-3') and an antisense primer (5'-GGATAAATCCCGCGGATGG-3') with the self-ligated DNA products as template. The *ctr1* gene was amplified by PCR from the genomic DNA of wild-type Syn6803 using the primers 5'-TAAAACCGACTGAGGAAACC-3' and 5'-CAGTGTATTTCCCCCAGCCT-3'. The resulting 2.7 kbp product, containing the entire ORF of *ctr1* gene, was cloned into pGEM-T easy vector. The *ctr1* gene was disrupted by excising an internal 1.7 kbp *Kpn*I/*Sma*I fragment from coding region of the gene and replacing it with a cassette conferring spectinomycin resistance to the cells. Total RNA isolated from Syn6803 was transferred to a nylon membrane after agarose gel electrophoresis and was then hybridized to the ^{32}P-labeled *pilA1* DNA fragment using ULTRAhyb (Ambion Co., USA) as a hybridization buffer. Syn6803 cells were negatively stained with 0.2% phosphotungstic acid for 1 min and examined using Carl Zeiss EM912-omega microscope at Korea Basic Science Institute.

Results and discussions

In an attempt to identify genes involved in cyanobacterial phototactic gliding motility, mutants of Syn6803, which showed altered gliding motility on agar plates, were isolated by Tn*5* transposon mutagenesis. 35 mutants that had nonmotile phenotype were isolated among about 2000 kanamycin-resistant colonies generated by Tn*5* transposon mutagenesis. One nonmotile mutant, S1-105, was chosen for further study. Wild-type Syn6803 cells displayed positive phototaxis on the surface of 0.4% soft agar plate upon exposure of lateral incident light from a fluorescent lamp at 10 μmol/m^2/s. In contrast, none of S1-105 colonies showed any traces of motility on agar plate under the same condition.

We performed inverse PCR (Ochman et al. 1988) to determine the Tn*5* insertion site in S1-105. The PCR product was directly sequenced. DNA sequence analysis by Blast search in Cyanobase on the web (http://www.kazusa.or.jp/cyano/) showed that the Tn*5* transposon was inserted into the 1956th bp of the *slr1044* gene of Syn6803. PSI-blast search revealed that the *slr1044* gene product was homologous to a large group of bacterial proteins known as methyl-accepting chemotaxis proteins (MCPs) (Dahl et al. 1989), such as Tsr of *E. coli* (Boyd et al. 1983) and PilJ of *P. aeruginosa* (Darzins 1994). Thus, we designated the *slr1044* gene as *ctr1* (*c*yanobacterial *tr*ansducer 1). Ctr1 has significant similarity to the C-terminal half of enteric and *P. aeruginosa* MCPs. Ctr1 has 20% identity and 41% similarity with *E. coli* Tsr, and 31% identity and 53% similarity with *P. aeruginosa* PilJ.

The Ctr1 has several important characteristics as a MCP: First, the region of amino acids 694 to 755 of Ctr1 corresponds to the highly conserved domain (HCD), which is known to be important for the interaction between *E. coli* Tsr and CheW as well as CheA. Second, the predicted amino acid sequence of Ctr1 also has two potential consensus methylation sites (K1 and R1) such as those seen in the enteric MCPs (Fig. 1). Third, hydrophobicity plot of Ctr1 shows the presence of two transmembrane domains (TM1 and TM2) at the central region (Fig. 1). Fourth, Ctr1 has two HAMP

domains, followed by TM2 domain in the central region (Fig. 1). The periplasmic domain between TM1 and TM2 in most *E. coli* MCPs except Aer was generally composed of more than 150 amino acid residues. Whereas the putative periplasmic domain of Ctr1 was relatively short, only 52 amino acid residues, and had no significant homology to any known proteins. The periplasmic domain of 150 amino acid residues in *E. coli* MCP was known to be responsible for receiving external chemical signals as a chemoreceptor. It is likely that the short external 52 amino acid residues of the Ctr1 do not have adequate periplasmic ligand binding domain and therefore are not involved in chemoreception of extracellular signal in Syn6803. Therefore Ctr1 may recognize intracellular signal(s) or extracellular signal(s) in a different way from enteric bacteria. Specially, Ctr1 has a long N-terminal cytoplasmic tail, composed of about 380 amino acid residues, while most enteric MCPs have only several amino acid residues. This N-terminal cytoplasmic tail in Ctr1 might have roles in recognizing intracellular signal(s), like Aer or FrzCD.

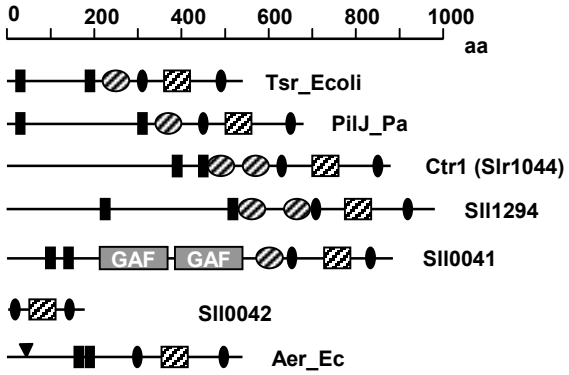

Figure 1. Comparison of domain structure of Ctr1 with MCP-like proteins. Tsr_Ecoli, chemotaxis receptor from *E. coli*; PilJ_Pa, methyl-accepting chemotaxis protein from *P. aeruginosa*; Sll1294, Sll0041, and Sll0042, cyanobacterial methyl-accepting chemotaxis protein from Syn6803. Dark vertical box indicates transmembrane region. Hatched circle means HAMP domain. Black circle is K1/R1 methylation region. Hatched box indicates highly conserved domain (HCD). Gray box means GAF domain, which has the known tetrapyrolle chromophore-binding site in eubacterial photoreceptor.

In order to find out that the Tn*5* insertion in *ctr1* gene is responsible for nongliding phenotype, in S1-105 mutant strain, we adopted an interposon mutagenesis to create knockout mutation in *ctr1* gene. The interposon mutagenized *ctr1* gene was used to mutate the *ctr1* gene in the chromosome of Syn6803 by transforming wild-type cells. All the gene knockout mutants displayed nonmotile phenotype on the surface of 0.4% soft agar plates to lateral light stimulus with the intensity of 10 μmol/m^2/s. One of the mutants was chosen for further characterization and named CKN5. The mutant CKN5 grew as compact colonies with well-defined edges like the previously described Tn*5* nonmotile mutant, S1-105. It was thus confirmed that the *ctr1* gene was involved in gliding movement in Syn6803.

In order to test whether the *ctr1* gene is participated in the production of Syn6803 pili, we, as a first step, observed the cyanobacterial cells by transmission electron microscope. Wild type Syn6803 had numerous, peritrichously arranged thick pili that were distributed entire cell surface (Fig. 2A). In contrast, no thick pili were observed on the surfaces of the *ctr1* interposon mutant, CKN5, although some thin pili were visible (Fig. 2B). This results suggested that *ctr1* mutant was either unable to make or assemble components of the thick pilus.

To determine whether *ctr1* gene affects the biosynthesis of Type IV pilin that is a structural subunit of thick pilus in Syn6803, we examined the expression of the *pilA1* gene coding for pilin subunit of thick pilus in Syn6803. Wild-type cells accumulated high level of *pilA1* transcript during growth under the white light with the intensity of 15 μmol/m^2/s. In contrast, the level of *pilA1* transcript in the CKN5 mutant was less

than 20% of that in the wild-type Syn6803 (Fig. 2C). These results suggest that *ctr1* gene is essential for the normal expression of *pilA1*.
In conclusion, our results imply that *ctr1* could be responsible for regulating pilus biogenesis and gliding movement in Syn6803. Ctr1 protein may act as a transducer whose function is to receive intracellular signal(s) and transduce these signal(s) to the pilus production machinery thus regulating the expression of the *pilA1* gene.

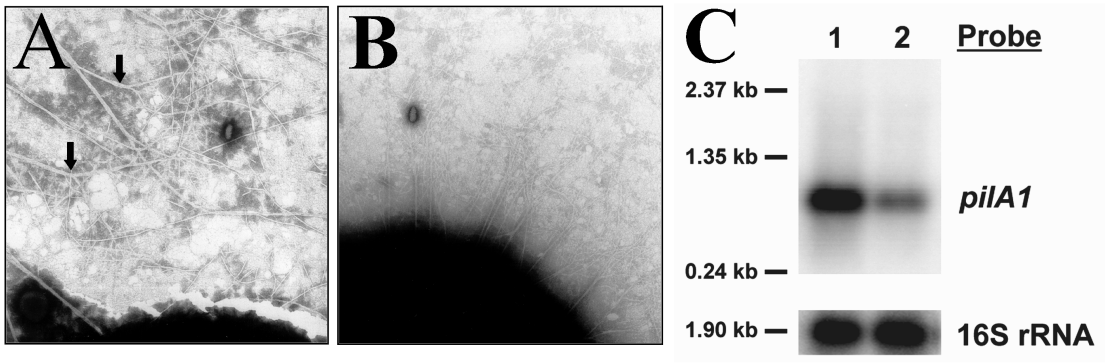

Figure 2. Morphology and gene expression. Transmission electron microscopy of wild type (A) and *ctr1* interposon mutant, CKN5 (B). The bars represent 0.27 μm (A, B). The arrows in wild type (A) point to a thick pilus. Expression of *pilA1* gene in Syn6803 cells (C). Total RNA from wild-type cells (lane 1) and, *ctr1* interposon mutant, CKN5, (lane 2) were hybridized with probes for *pilA1* gene (Upper) or 16S rRNA (Lower), which was used as a loading control.

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