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A novel gene involved in phototactic motility of the unicellular cyanobacterium *Synechcocystis* sp. PCC 6803.

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Introduction

Many cyanobacteria show unique gliding or twitching motility without flagella and move themselves toward optimal conditions for photosynthesis or survival (Häder 1987). Recently, many genes responsible for the motility were identified by mutational analysis in *Synechocystis* sp. PCC 6803. They are *pilA1*, *pilB1*, *pilM*, *pilN*, *pilO*, *pilQ*, *pilD*, *pilC* and *pilT1* (Bhaya et al. 2000, Yoshihara et al. 2001). They show similarity to the *pil* genes that are involved in biogenesis of type IV pili in diverse Gram-negative bacteria. Type IV pili are filamentous appendages that are responsible for twitching motility in *Pseudomonas* and *Neisseria* and for natural transformation in *Neisseria* (Mattick et al. 1996, Fussenegger et al. 1997). On the other hand, *Synechocystis* has two types of pili, the thick pili and the thin pili on the cell surface. Disruption of the above-mentioned *pil* genes in *Synechocystis* mostly resulted in simultaneous loss of the thick pili on the cell surface, transformation competency and motility on agar plates, while the thin pili remained (Yoshihara et al. 2001). These results suggested that the type IV-like thick pili are the machinery for both motility and natural transformation in *Synechocystis*.

In addition to motility, *Synechocystis* show positive or negative phototaxis via cell motility towards or away from the light source. By gene disruption, we have demonstrated that a series of genes are specifically required for the positive phototaxis. We designated these genes *pixG* (*sll0038*), *pixH* (*sll0039*), *pixI* (*sll0040*), *pixJ1* (*sll0041*), *pixJ2* (*sll0042*) and *pixL* (*sll0043*) (positive phototaxis, formerly *pis* in Yoshihara et al. (2000). The predicted products of *pixG*, *pixH*, *pixI*, *pixJ1*, *pixJ2* and *pixL* show similarities to the regulatory components for bacterial chemotaxis, CheY, CheY, CheW, MCP (methyl-accepting chemotaxis protein) and CheA, respectively. Furthermore, the predicted *pixJ1* product harbors a region carrying a phytochrome-like chromophore-binding motif. It is suggested that the positive phototactic movement of *Synechocystis* cells is mediated by the phytochrome-like photoreceptor (PixJ1) and two-component signal transduction system (PixG, PixH, PixL). On the other hand, those *pix* mutants still retained ability to show directional movement away from the light source. It is, thus, assumed that *Synechocystis* has both pix system for positive phototaxis and nix system for negative phototaxis.

To uncover more components involved in regulation of phototactic motility of *Synechocystis*, we studied gene clusters that are homologous to the *pixG* cluster.

Materials and Methods

The motile strain of the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 was obtained from the Pasteur Culture Collection. Cells were grown in liquid BG11 medium bubbled with air containing 1 % (v/v) CO_2 at 31 °C under illumination with a white fluorescent lamp at a fluence rate of 10-50 μE m^{-2} s^{-1}. Kanamycin or spectinomycin was included at 20 μg/ml when mutants were screened and maintained. Phototactic movement was examined on 0.8 % (w/v) agar-solidified BG11 (Bacto-Agar, Difco, Detroit, USA) supplemented with 0.3 % (w/v) sodium thiosulfate under lateral illumination with a white fluorescent lamp at 10 μE m^{-2} s^{-1} for 2 days. Pili structure on the cell surface was observed by electron microscopy after negative staining. Natural transformation competency of wild type and mutants was estimated as recombination of spectinomycin resistance into a specific site in the genome according to Yoshihara et al. (2001). The protein database derived from the genome of *Synechocystis* sp. PCC 6803 was searched for homology using the BLAST program (Altschul et al. 1997). Insertion mutagenesis with the kanamycin-resistant cassette for *slr1041*, *slr1042*, *slr1044* and *slr0322* or with the spectinomycin-resistant cassette for *slr1043* was carried out as previously described (Yoshihara et al. 2001).

Results and Discussion

Phototactic movement regulation clusters

In the whole genome of *Synechocystis* sp. PCC 6803, there are two clusters that are homologous to the *pixG* cluster (Fig. 1). Gene arrangement of these clusters are conserved to each other except the absence of *cheA*-like ORF in the *slr1041* cluster. Their arrangement is also similar to that of the *pilG*(*cheY*-like)-*pilH*(*cheY*-like)-*pilI*(*cheW*-like)-*pilJ*(*mcp*)-*pilK*-*pilL*(*cheA*-like) in the pathogenic bacterium *Pseudomonas aeruginosa* (Darzins 1993, 1994). To examine whether the other two clusters are involved in the phototactic motility, we disrupted all ORFs in their clusters.

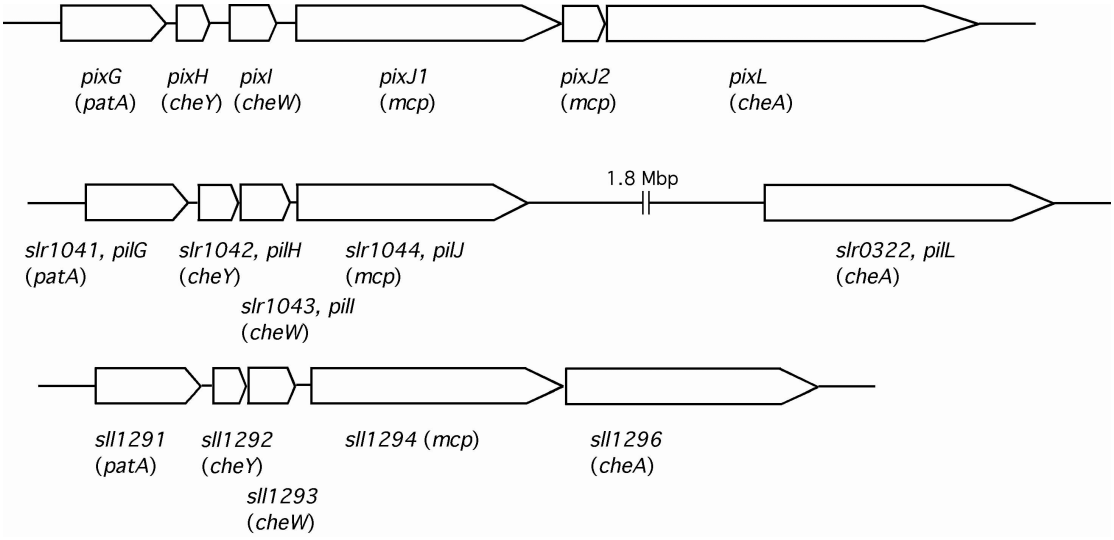

Fig.1 Gene organization of the *Synechocystis pixG, slr1041* (*pilG*) and *sll1294* cluster. (): homologous regulatory components for the chemotactic flagellar switching in enteric bacteria. The direction of arrows indicates relative transcriptional orientation.

Motility of the mutants

Phototactic movement of *Synechocystis* cells was examined by colony morphology under lateral illumination. Wild type cells moved towards the light source and formed flat sheet-like colonies showing irregular shapes due to motility of individual cells. *slr1042, slr1043* and *slr1044* mutants remained at the original position indicative of non-motile phenotype. *slr1041* retained positive phototactic motility, although ability to move was reduced. On the other hand, disruption of ORFs in the *sll1291* cluster did not give any discernible defect in the phototactic motility.

As mentioned above, there is no *cheA*-like ORF in the *slr1041* cluster. When we searched *cheA* homolog in the *Synechocystis* genome, *slr0322* and *slr0073* were detected in addition to *pixL* and *sll1296.* We disrupted them and found that disruption of *slr0322* but not *slr0073* abolished the motility completely. Taking the similarity to the *pilG* cluster in *P.aeruginosa* into account, we designated here after *slr1041, slr1042, slr1043, slr1044* and *slr0322* as *pil* (pili-related) *G, pilH, pilI, pilJ* and *pilL*, respectively. It is of note that these genes including *pilL* are arranged in a single cluster in the *Anabaena* genome.

pilus structure on the cell surface

The pilus structure on the cell surface of the mutants as well as the wild type strain was examined by electron microscopy after negatively staining with phosphotungstic acid (Fig.2). On the cell surface of wild type, two types of pili are observed, the thick pili and the thin pili (Fig.2A). Our previous study demonstrated that many *pil* genes

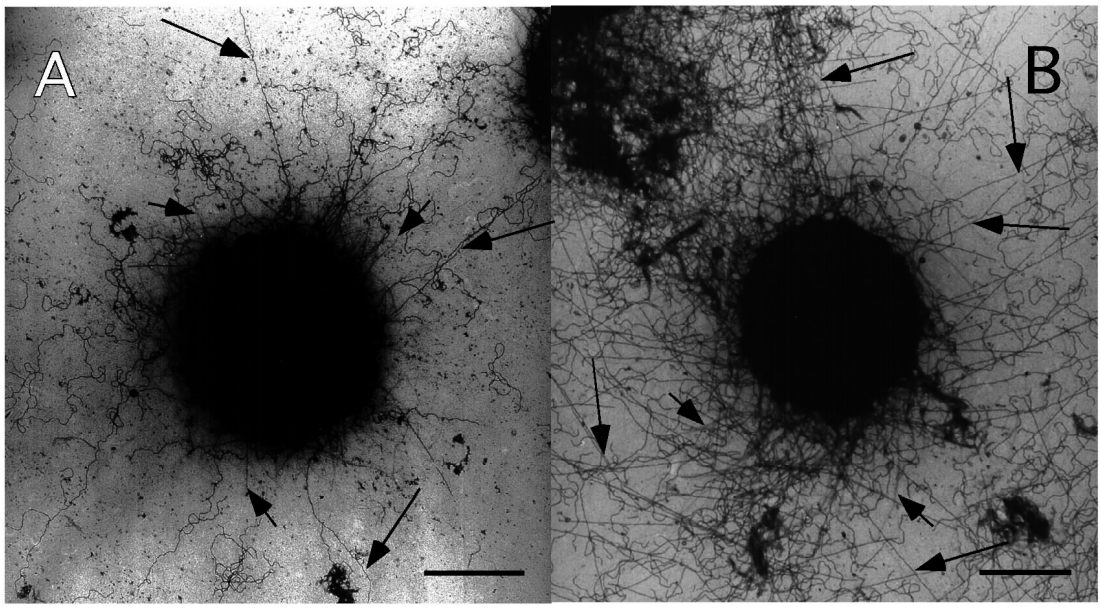

Fig.2 Electron micrographs of negatively stained images of wild type and *pilH* mutant cells. Panel A, wild, B, Δ*pilH*. Bars show 1 μm. Long arrows and short arrows indicate the thick pili and the thin pili, respectively.

are essential for biogenesis of the thick pili as well as cell motility. In the non-motile mutants of *pilJ* and *pilL*, the thick pili were almost completely absent, while non-motile *pilI* mutant displayed the pili in much reduced number. On the other hand, non-motile *pilH* mutant clearly displayed the thick pili that were larger in length and more in number than wild type, while thin pili were seemingly unaffected (Fig. 2B). Such phenotype was described about *pilT1* mutant previously (Okamoto and Ohmori 1999, Bhaya et al. 2000). PilT protein having ATPase activity is essential for motility

and transformation competency but its disruptant shows hyper-piliation in contrast with no piliation phenotype of the other *pil* gene mutants. It could be speculated that PilH directly interacts with PilT1, leading to normal motility via retraction of the thick pili.

Transformation competency

We examined transformation competency of the *pil* mutants to correlate with assembly of the thick pili and motility. Under the experimental conditions employed here, wild type gave about 0.42 x 10^{-4} competency. *pilJ* and *pilL* mutants, which were non-motile and non-piliated, retained slight competency although degree of competency was lower than wild type almost by two orders. Competency of *pilH* mutant, which lost motility but assembled more thick pili, was about one-third of wild type. Motile and piliated *pilG* mutant showed normal transformation competency. These results are in contrast with the complete absence of transformation competency in the other *pil* mutants such as *pilM* mutant. Probably, defect in assembly of the thick pili is much less in the *pilH*, *pilJ* and *pilL* mutants than the *pilM* mutant. This suggests that genes in the *pilG* cluster are regulatory but not involved directly in assembly of the thick pili. Based on analogy with *pixJ1*, we could expect that N-terminal half of *pilJ* encodes a sensor domain for regulation of motility or thick pili assembly. Judging from the fact that PilJ protein has two putative transmembrane-spanning segments at interface between the N-terminal domain and C-terminal MCP domain, the N-terminal hydrophilic domain seems to reside in the cytoplasm. It could be speculated that PilJ responds to a regulatory signal molecule such as cyclic AMP in the cytoplasm instead of extracellular sensing like in typical chemotaxis.

Table 1 Transformation efficiencies of wild type and mutants.

Strain	Transformation efficiency (%)*
wild type	100
Δ*slr1041* (*pilG*)	97.6
Δ*slr1042* (*pilH*)	28.6
Δ*slr1043* (*pilI*)	ND
Δ*slr1044* (*pilJ*)	1.5
Δ*slr0322* (*pilL*)	1.5

* Value (%) represents the frequency of transformation relative to wild type.

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