The role of an alternative sigma factor in motility and pilus formation in the cyanobacterium *Synechocystis* sp. strain PCC6803

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ABSTRACT Disruption of a gene for an alternative sigma factor, designated *sigF*, in the freshwater, unicellular cyanobacterium *Synechocystis* sp. strain PCC6803 resulted in a pleiotropic phenotype. Most notably, this mutant lost phototactic movement with a concomitant loss of pili, which are abundant on the surface of wild-type cells. The *sigF* mutant also secreted both high levels of yellow–brown and UV-absorbing pigments and a polypeptide that is similar to a large family of extracellular proteins that includes the hemolysins. Furthermore, the *sigF* mutant had a dramatically reduced level of the transcript from two tandemly arranged *pilA* genes (*sll1694* and *sll1695*), which encode major structural components of type IV pili. Inactivation of these *pilA* genes eliminated phototactic movement, though some pili were still present in this strain. Together, these results demonstrate that SigF plays a critical role in motility via the control of pili formation and is also likely to regulate other features of the cell surface. Furthermore, the data provide evidence that type IV pili are required for phototactic movement in certain cyanobacteria and suggest that different populations of pili present on the *Synechocystis* cell surface may perform different functions.

The evolutionarily ancient cyanobacteria may have been one of the first groups of organisms to evolve directional motility. While motility has been extensively studied in some microorganisms, the nature of motility in cyanobacteria has been considered enigmatic because neither the mechanism nor the environmental factors that control movement has been elucidated.

Several species of both filamentous and unicellular cyanobacteria are motile (1, 2). The filamentous cyanobacteria show gliding motility, which requires contact with a solid surface and occurs in a direction parallel to the long axis of the filament. Interestingly, nonmotile *Phormidium uncinatum* cells are deficient in components of the cell surface, including extracellular slime and helically arrayed fibrils that are comprised of a rod-shaped glycoprotein called oscillin (3, 4). Oscillin has been proposed to play a passive role in motility with propulsion resulting from shear forces generated between the oscillin fibrils on the cell surface and slime as it is exuded from cells ("surface mucilage" hypothesis). In certain marine unicellular cyanobacteria, motility in an aqueous environment has been termed "swimming" (5). In *Synechococcus* WH8102 the generation of thrust for swimming requires SwmA, which is an extracellular protein that exhibits similarity to oscillin and is associated with the outer membrane (6). Directional motility in both the unicellular and filamentous cyanobacteria appears to depend on Ca^{2+}, although the exact role of this cation in motility is not clear (1, 2, 7).

Neither *P. uncinatum* nor the marine *Synechoccus* species that have been examined have obvious cell appendages, such as flagella or pili (3–5), that have been shown to facilitate movement in other microbes. However, pili-like structures have been observed on the surface of certain unicellular and filamentous cyanobacteria (8–11). No function has been demonstrated for these appendages, and there is no understanding of the cellular mechanisms or environmental factors that control their biosynthesis. In this study we have inactivated a gene encoding an alternative sigma factor (gene number *slr1564* in the Cyanobase), which we have designated *sigF* because of its similarity to the group III sigma factors SigF of *Bacillus coagulans* and SigB of *Bacillus subtilis*. We demonstrate that it is required (either directly or indirectly) for the biosynthesis of pili and that specific *pilA* genes (*sll1694* and *sll1695*) are needed for directional motility of the cyanobacterium *Synechocystis* sp. strain PCC6803 (henceforth referred to as *Synechocystis*).

METHODS

Culture and Growth Conditions. The motile strain of *Synechocystis* sp. strain PCC 6803 was originally from the Pasteur Culture Collection but was obtained from Young Mok Park (Korean Basic Science Institute, Teajon, Korea). Cells were grown in BG-11 medium in moderate light (70 μmol photons·m^{-2}·s^{-1}). When appropriate, antibiotics were included in the medium at a final concentration of 2 μg/ml of ampicillin, 25 μg/ml of spectinomycin, or 10 μg/ml of kanamycin. Whole-cell spectra from 350 to 800 nm were measured with a Beckman DU70 spectrophotometer (Fullerton, CA).

Motility Assays. Five to 10 microliters of log-phase cells were streaked as an approximately 1-mm thick line onto BG-11 solid medium containing 0.4% agar and supplemented with 15 mM glucose. The cells, maintained at 30°C, were exposed to unidirectional incandescent light of 40 μmol photons·m^{-2}·s^{-1}.

DNA Manipulation, RNA Isolation, and Northern Blot Hybridization Analysis. Molecular techniques were performed according to standard procedures (12). DNA was isolated from *Synechocystis* according to the method of Tandeau de Marsac *et al.* (13). RNA was isolated from pelleted cells frozen at -80°C by using a slight modification of the method of de Saizieu *et al.* (14) in which 100 mg of glass beads (0.1 μm average diameter) were added to the cells after the addition of the acid phenol and the suspension was vortexed for 1 min. This procedure yielded between 150 and 250 μg of RNA from a typical 50-ml culture (cell density approximately 5×10^8 cells).

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Gene Inactivation. The *sigF* gene (*slr1564* and originally designated *rpoF* in the Cyanobase) was identified by examination of the Cyanobase (http://zearth.kazusa.or.jp/cyanobase/). It was isolated by PCR amplification using the forward primer 5′-*GG***TCTAGA**ATGACCAATGCC-3′ (GG clamp shown in italics, *Xba*I site in bold followed by the first 12 bases of the *sigF* gene encoding amino acids 1–4) and the reverse primer 5′-*GG***ATCGAT**TTAAAAAATTTC-3′ (GG clamp shown in italics, *Cla*I site in bold, followed by the last 12 bases of the *sigF* gene encoding the stop codon and amino acids 258–256). The PCR product was cloned into the *Xba*I/*Cla*I site of Bluescript KS⁻. The *sigF* gene was interrupted at a unique *Pst*I site (base 463) with the *aphII* gene cassette that confers kanamycin resistance to *Synechocystis* cells. The Bluescript KS⁻ vector containing the interrupted *sigF* gene was used to transform *Synechocystis,* and kanamycin-resistant transformants that were ampicillin sensitive were selected to ensure replacement of the wild-type gene by a double homologous recombination event. DNA was isolated from individual transformants, and PCR analysis was used to check that all copies of the chromosome in the cell carried the interrupted *sigF* gene. To ensure that the phenotype observed in the *sigF* mutant was not the result of a polar effect, we inactivated the gene downstream of *sigF*, designated *slr1565* in the Cyanobase. The strain carrying this inactivated gene exhibited a wild-type phenotype (data not shown). Both the mutant and wild-type cells were grown in BG-11 medium in moderate light (70 μmol photons·m^{-2}·s^{-1}).

To inactivate the tandemly arranged genes encoding PilA1 and PilA2 (*sll1694* and *sll1695*), a single 1.1-kbp PCR product containing these genes was generated by using the forward primer 5′-ATGGCTAGTAATTTTAAA-3′ (encoding the first six amino acid residues of *sll1694*) and the reverse primer 5′-TCAAAAAAGCTTAAT-3′ (encoding the last five amino acid residues and stop codon of *sll1695*). The PCR product was cloned into pGEM T vector (Promega). A 0.38-kbp fragment (excised by *Hpa*I and *Kpn*I) was replaced with a 2-kbp fragment that contains the gene conferring spectinomycin resistance (which is flanked by short inverted repeats carrying transcription and translation termination signals) (15), resulting in a vector construct that had both genes disrupted. This vector was used to transform *Synechocystis,* and the same strategy as described above was used to ensure that all copies of the chromosome in the cell carried the interrupted *pilA* genes.

Protein Isolation and SDS/PAGE. Cells were grown for 6 days in 50 ml of BG-11 medium in moderate light and pelleted by centrifugation at 3,600 × *g* for 5 min at 4°C. The centrifugation step was repeated on the growth medium to ensure complete removal of whole cells and cellular debris. The absorbance of the growth medium was measured, and proteins in the medium were precipitated by adding trichloroacetic acid to a final concentration of 10%, incubating on ice for 2 h, and then centrifuging for 15 min at 3,600 × *g*, 4°C. The protein pellet was washed twice with ice-cold 90% acetone, air-dried, and resuspended in 1× loading buffer (125 mM Tris·HCl, pH 6.8/1% SDS/5% glycerol/10 mM DTT/0.005% bromophenol blue). Ten or 20 μl of solubilized sample was analyzed by 8% SDS/PAGE in Laemmli buffer (16) and stained either with Coomassie brilliant blue (R-250) or by periodic acid Schiff staining (17) for the detection of glycoproteins.

Amino Terminal Sequencing. The 200-kDa polypeptide, present in the medium in which the *sigF* mutant was grown, was resolved by SDS/PAGE, transferred to poly(vinylidene difluoride) membranes and stained with amido black. The N-terminal amino acid sequence was determined by the Protein and Nucleic Acid facility, Stanford University Medical School. The first 15 aa of the polypeptide [LSPNV(I/A)AALQIMYT] exactly matched the deduced N-terminal encoded by *hlyA* (*sll1951*).

Electron Microscopy. Log-phase cells were negatively stained with 1% phosphotungstic acid and examined by using a Phillips CM12 microscope.
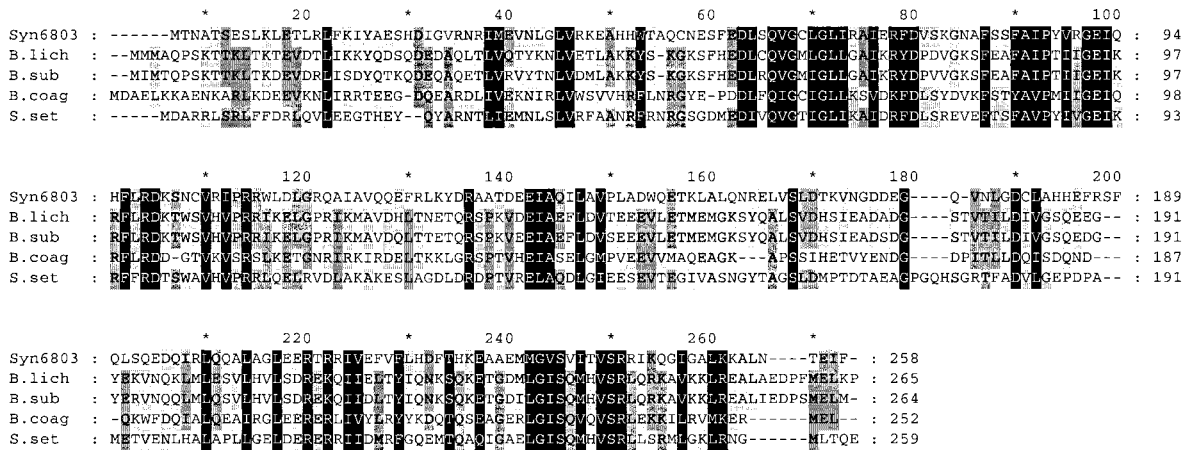
RESULTS

As shown in Fig. 1*A*, the *Synechocystis* SigF polypeptide is most similar to SigB (also known as RpoF or Sigma 37) of *B. subtilis* and *Bacillus licheniformis* (31% identical and 54% similar to both). It is also similar to the sporulation-specific sigma factor F of *Bacillus coagulans* and to a sigma factor from *Streptomyces setonii* that is involved in controlling carotenoid biosynthesis, as well as more distantly to RpoS from various organisms (data not shown). SigB of *B. subtilis* has been designated a regulator of "general stress" responses and is involved in activation of more than 40 genes. The activity of this sigma factor is modulated by a signal transduction network composed of *rsb* gene products. This network responds to stress signals generated by (*a*) reduced energy supplies resulting from nutrient limitation or entry into stationary phase and (*b*) environmental stresses such as heat shock (18–20). Genes with homology to the *rsb* genes of *B. subtilis* are present on the *Synechocystis* genome, which has been completely sequenced (21).

We analyzed the phenotype of a mutant of *Synechocystis* in which the *sigF* gene was disrupted. This mutant produced yellowish-brown and UV-absorbing (absorbance maxima at 397, 363, and 335 nm) pigments that were released from the cells and accumulated in the growth medium (Fig. 2*A,* traces a, c, and e). This pigmentation was barely detectable in cultures of wild-type cells (Fig. 2*A*, traces b, d, and f). UV-absorbing pigments have been associated with the cyanobacterial cell sheath and often increase when cells are exposed to elevated levels of UV light (22, 23). In addition, the medium in which the *sigF* mutant was grown accumulated a glycoprotein (based on Schiff staining, data not shown) of molecular mass of approximately 200 kDa (and less abundant lower molecular mass polypeptides) (Fig. 2*B*, lane 3). This protein is not readily detectable in the culture medium from wild-type cells (Fig. 2*B*, lane 2). The N-terminal sequence of the 200-kDa polypeptide established its identity as the 1,741-aa polypeptide designated HlyA (sll1951). The C-terminal half of HlyA (the last 1,000 aa) is similar to a diverse family of cell surface/extracellular Ca^{2+} binding proteins that includes the pore-forming cytolysins typified by the hemolysins of *Escherichia coli* (24). This protein class is characterized by tandem arrays of a 9-aa motif rich in glycine, aspartic acid, and asparagine [LXGGXG(N/D)DX], which is involved in Ca^{2+} binding. The *Synechocystis* HlyA protein also shows similarity to the motility-related glycoproteins oscillin (30% identity and 60% similarity), and SwmA (25% identity and 50% similarity), both of which contain several repeats of the 9-aa motif and are cell-surface associated (3, 4, 6). The elevated levels of extracellular pigments and HlyA polypeptide in the growth medium of cultures of the *sigF* mutant suggested that this strain had a defect in structuring or synthesizing components of its extracellular surface layers.

Because the hemolysin-like SwmA and oscillin polypeptides have been linked to motility in cyanobacteria, we examined the response of wild-type cells and the *sigF* mutant to a unidirectional light source. Exposure of wild-type cells to unidirectional incandescent light of 40 μmol photons·m^{-2}·s^{-1} resulted in a phototactic response. Finger-like projections of cells migrating toward the direction of the light source were visible within 1–2 days of streaking the cells onto agar plates (Fig. 3*A,* WT). Under these conditions the rate of movement was calculated to be approximately 70 μm/h. Photophobic movement was observed when the cells were exposed to light intensities of above 50 μmol photons·m^{-2}·s^{-1} (D.B. and A.R.G., unpublished data). In striking contrast, the *sigF* mutant showed no directional motility whatsoever (Fig. 3*A, sigF*). Furthermore, wild-type cells when grown under a uniform

A

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               *        20         *        40         *        60         *        80         *       100
Syn6803 : ------MTNATSESLKLETLRLFKIYAESHDIGVRNRIMEVNLGLVRKEAHHYTAQCNESFRDLSQVGCLGLIRAIERFDVSKGNAFSSFAIFYVRGEIQ :  94
B.lich  : --MMMAQPSKTTKLTKTEVDTLIKKYQDSQDEDAQLTLVQTYKNLVETILAKKYS-KGKSFHEDLCQVGMLGLLGAIKRYDPDVGKSFEAPAIPIIIGEIK :  97
B.sub   : ---MIMTQPSKTTKLTKDEVDRLISDYQTKQDEQAQETLVRVYTNLVDMLAKKYS-KGKSFHEDLRQVGMIGLLGAIKRYDPVVGKSFEAPAIPTIIGEIK :  97
B.coag  : MDAELKKAENKARLKDEEVKNLIRRTEEG-DQEARDLIVEKNIRLVWSVVHRELNRGYE-PDDLFQIGCIGLLKSVDKFDLSYDVKFSTYAVPMIIGEIQ :  98
S.set   : -----MDARRLSRLFFDRLQVLEEGTHEY--QYARNTLIEMNLSLVRFAANRIRRNRGSGDMEDIVQVGTIGLIKAIDRFDLSREVEFTSFAVFYIVGEIK :  93

               *       120         *       140         *       160         *       180         *       200
Syn6803 : HELRDKSNCVRIPRRWLDLGRQAIAVQQEFRLKYDRAATDEEIAQILAVPLADWQETKLALQNRELVSLDTKVNGDDEC----Q-VNLGDCLAHHEFRSF : 189
B.lich  : RFLRDKTWSVHVPRRIKELGPRIKMAVDHLTNETQRSPKVDEIAEFLDVTEEEVLETMEMGKSYQALSVDHSIEADADG----STVTILDIVGSQEEG-- : 191
B.sub   : RFLRDKTWSVHVPRRIKELGPRIKMAVDQLTTETQRSPKVEEIAEFLDVSEEEVLETMEMGKSYQALSVDHSIEADSDG----STVTILDIVGSQEDG-- : 191
B.coag  : RFLRDD-GTVKVSRSLKETGNRIRKIRDELTKKLGRSPTVREIAQDLGIPVEEVVMAQEAGK---APSSIHETVYENDG----DPITLLDQISDQND--- : 187
S.set   : RFFRDTSWAVHVPRRLQELRVDLAKAKESLAGDLDRDRPTVRELAQDLGIEESEVTEGIVASNGYTAGSLDMPTDTAEACPGQHSCRTFADVLGEPDPA-- : 191

               *       220         *       240         *       260         *
Syn6803 : QLSQEDQIRLQQALAGLEERTRRIVEFVLHDFTHKEAAEMMGVSVITVSRRIKQGFCGALKKALN----TRIF- : 258
B.lich  : YEKVNQKLMLESVLHVLSDREKQIIELTYIQNKSQKETGDMLGISQMHVSRLQRKAVKKLREALAEDPFMELKP : 265
B.sub   : YERVNQQLMLQSVLHVLSDREKQIIDLTYIQNKSQKETGDMLGISQMHVSRLQRKAVKKLREALIEDPSMELM- : 264
B.coag  : -QKWFDQIALEQEAIRGLEEREPLIVYTEYYKDQTQSEAGERLGISQVQVSRLEEKEILRVMKER------MEI- : 252
S.set   : METVENLHALAPLEGELDERERRIIDMREGQEMTQAQIGAELGISQMHVSRLLSRMEGKLRNG------MLTQE : 259
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B

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               *        20      ↓ *        40         *        60
Myxo   : ----------MRVSRFNPR--NR----GFTLIELMIVVALIGILAAIAIPNFIKFQARSK :  44
Pseu   : ---------------MTAQK-------GFTLIELMIVVAIIGILAAVALPAYQDYTIRAR :  38
sll1694 : --MAS-NFKFKLLSQLSKKRAEG----GFTLIELLVVVIITGVLAAIALPNLLGQVGKAR :  53
sll1695 : --MKA---QFSIGSSSNCRHFNGTRQRGFTLIELLVVVIITGVLGAMTLPNLFSQIGKAR :  55
slr1929 : MVMKLPNPTFLLKLVFNPHPSTSSQ--GWTLIEIGVVTVIVGILAAMAFPSLAGIQARNQ :  58
slr1930 : MAYKN-ELIKALKLCLFTQKSTS----GWTLIEIGVVTTIVGILAASVAFPSLMGIKAKMD :  55
slr1456 : --MNSSKTAFRGHNVFLLRHALACS-TGFTLIEILVVVIVIGILGAIAVPNLLAQVDKAR :  57
slr2016 : ------MSAKQLWKIFNPRPMKG----GFTLTENLVSLIVLSITLTAMLPAFMNFGLQNA :  50
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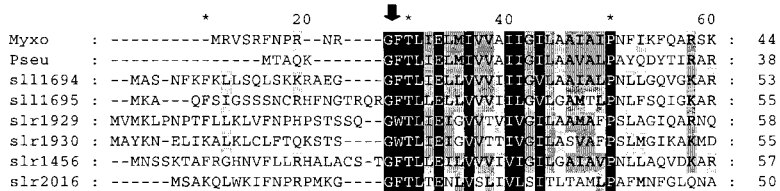
Fig. 1. (*A*) Alignment of SigF of *Synechocystis* (Syn6803) with SigB of *Bacillus licheniformis* (B.lich) and *B. subtilis* (B.sub), SigF of *Bacillus coagulans* (B.coag), and a sigma factor from *Streptomyces setonii* (S.set). Black boxes indicate identical or conserved residues at the same position in all five sequences while dark gray and light gray boxes indicate identical or conserved residues in four of the five and three of the five sequences, respectively. The GenBank accession nos. for the *B. licheniformis*, *B. subtilis, B. cogulans* and *S. setonii* sequences are AF034567, M14508, Z44161, and D17466, respectively. (*B*) Alignment of the conserved N-termini of six putative PilA-like sequences (encoded by sll1694, sll1695, slr1929, slr1930, slr1456, and slr2016) from *Synechocystis* with PilA polypeptides of *M. xanthus* (Myxo) and *P. aeruginosa* (Pseu). The GenBank accession nos. for the *M. xanthus* and *P. aeruginosa pilA* genes are L39904 and L37109, respectively. The arrow indicates the putative cleavage site of the precursor with phenylalanine (F) being the first amino acid of the mature protein. Only the leader sequence and the highly conserved 32 aa at the amino terminus of the mature protein are shown. Black boxes indicate identical or conserved residues at the same position in all eight sequences, while dark gray and light gray boxes indicate identical or conserved residues in at least six of eight and five of eight sequences, respectively. Comparisons were performed by using CLUSTAL or the gapped BLAST search available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Alignments were plotted by using the GENEDOC program.

light source formed spreading colonies (indicative of motility) whereas the *sigF* mutant formed tight colonies that did not spread (data not shown). Thus the *sigF* mutant appears unable to move under a uniform light source, which suggests that the motility process itself is impaired; as a consequence there is no phototactic movement.

To determine whether inactivation of the *sigF* gene resulted in gross cell surface changes, we viewed the cyanobacterial cells by transmission electron microscopy. Wild-type *Synechocystis* had numerous, peritrichously arranged pili or fimbriae that were in bundles or aggregates that extended from the cell surface (Fig. 3*B*, WT, *Upper Left*). Some of the very long pili (between 1 and 3 μm) linked individual cells (Fig. 3*B*, WT, *Lower Left*). In contrast, no pili were observed on the surface of the *sigF* mutant (Fig. 3*B*, *sigF*), although some short knob-like structures were sometimes visible. These results suggested that the *sigF* mutant was either unable to make or assemble components of the pilus.

In a number of Gram-negative bacteria, twitching and gliding motility have been associated with the presence of type IV pili. Several genes required for pilus formation, assembly, and for coupling energy to pili movement have been identified in *Myxococcus xanthus* and pathogenic bacteria (25, 26). Type IV pili are composed primarily of 500–1,000 units of a single pilin polypeptide (between 145 and 170 aa) encoded by the *pilA* gene (*pilE* in *Neisseria gonorrhoeae*). Pilins typically are synthesized as precursors with unique, short, basic, amino-terminal leader peptides, show high conservation of their amino terminal 32 aa and have an unusual *N*-methylphenyl-

alanine at the amino terminus. Analysis of the *Synechocystis* genome revealed several genes encoding proteins with homology to those required for the biogenesis of type IV pili in other microorganisms, including multiple genes encoding putative PilA-like polypeptides. Of the six genes (*sll1694*, *sll1695*, *slr1929*, *sll1930*, *slr1456*, and *slr2016*) encoding putative PilA prepilins, the tandemly repeated genes *sll1694* (*pilA1*) and *sll1695* (*pilA2*) encode proteins with the greatest overall similarity to PilA of *Pseudomonas* (Pseu) and *Myxococcus* (Myxo) (Fig. 1*B*).

We isolated RNA from both wild-type cells and the *sigF* mutant and examined the level of transcripts from *pilA1A2* by Northern blot hybridization using the *pilA1* gene as a probe. Wild-type cells accumulated high levels of a 1.1-kbp transcript during growth in moderate light (Fig. 4*B*, lane 1), suggesting that the two *pilA* genes, each with a coding region of approximately 500 nt, are transcribed as a single operon. In contrast, the *sigF* mutant had no detectable *pilA1A2* mRNA (Fig. 4*B*, lane 3). These results demonstrate that *sigF* is essential for high-level *pilA1A2* transcript accumulation and suggest that the release of cell surface pigments and proteins in cultures of the *sigF* mutant may be the consequence of the inability of this strain to form pili and/or other cell surface structures.

To determine whether the *pilA1A2* gene products were required for motility, we constructed a strain in which both genes were disrupted by insertion of a spectinomycin cassette at a position 300 bp downstream of the *pilA1* translation start site (Fig. 4*A*). The gene disruption was confirmed by Northern blot hybridization, which showed that the *pilA1A2* transcript
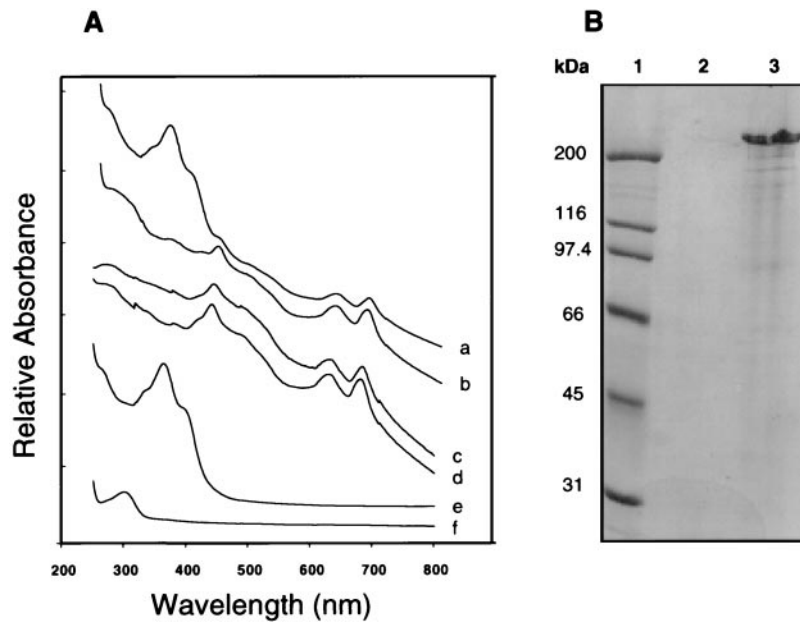
Fig. 2.   (*A*) Spectra of wild-type (WT) and *sigB* cultures. Spectra (250–800 nm) were measured from 6-day-old cultures of the *sigF* mutant (a) and WT (b), the cell pellet of the *sigF* mutant (c) and WT (d), and the growth media of the *sigF* mutant (e) and WT (f). Spectra are offset along the ordinate and normalized to cell density as reflected by absorbance at 750 nm. (*B*) SDS/PAGE of polypeptides precipitated from the growth media from WT (lane 2) and *sigF* mutant (lane 3) cultures and stained with Coomassie blue. Molecular mass markers (in kDa) are shown in lane 1.

was truncated in the mutant (Fig. 4*B*, lane 2). The size of the truncated transcript, estimated to be about 0.7 kbp, suggests that transcription might initiate about 150 bp upstream of *pilA1*, continue through the truncated *pilA1* gene, and terminate at the terminator positioned upstream of the spectinomycin casssette. The *pilA1A2* mutant, like the *sigF* mutant, exhibited no phototactic movement (Fig. 3A, *pilA1A2*). However, as revealed by transmission electron microscopy, peritrichously arranged pili were still present on the surface of the cell (Fig. 3B, *pilA1A2*), although they were generally not as abundant as in wild-type cells (based on observations of several different fields). These results suggest that PilA1 and PilA2 are

involved in motility, probably forming motility-specialized pili on the cell surface. The pili that remain in the *pilA1A2* mutant may be encoded by the other *pilA*-like genes (Fig. 1*B*), and we speculate that they are involved in processes such as conjugation and adhesion, both of which have been observed in cyanobacteria (27, 28).

DISCUSSION

The results presented here allow us to make four important conclusions. First, the *sigF* mutant of *Synechocystis* has a pleiotropic phenotype that includes the accumulation of ex-
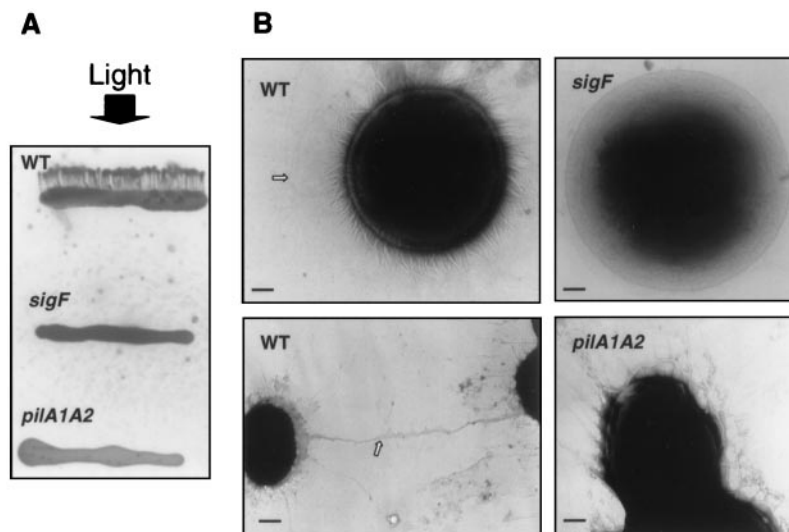

Fig. 3.   (*A*) Directional motility assay. Five to 10 microliters of logarithmically growing wild-type cells (WT) and the *sigF* and *pilA1A2* mutants were streaked as an approximately 1-mm thick line onto solid (0.4% agar) BG-11 medium containing 15 mM glucose. The cells were subjected to unidirectional light (indicated by an arrow) of 40 μmol photons·m^{-2}·s^{-1} for 48 h. The temperature during the incubation was 30°C. Note the finger-like projections emerging from the WT streak. (*B*) Transmission electron microscopy of whole cells. Logarithmically growing WT (*Left*), *sigF* mutant (*Upper Right*) and *pilA1A2* mutant (*Lower Right*) cells. The cells were negatively stained with 1% phosphotungstic acid and examined by using a Phillips CM12 microscope. The arrow in WT (*Upper Left*) points to a very long pilus while the arrow in WT (*Lower Left*) points to pili that appear to connect neighboring cells. The bars represent 0.28 μm (*Upper Left*), 0.24 μm (*Upper Right*), 0.74 μm (*Lower Left*), and 0.31 μm (*Lower Right*).
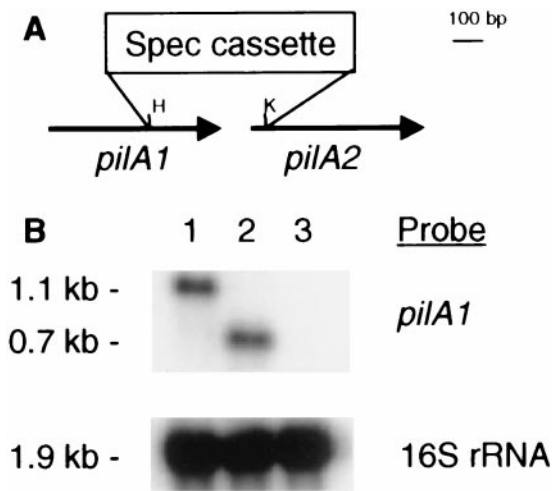
Fig. 4.   (*A*) The tandemly arranged *pilA1* (*sll1694*) and *pilA2* (*sll1695*) genes showing insertion of the spectinomycin cassette into the region between the *Hpa*I (H) and *Kpn*I (K) sites. (*B*) Hybridization of RNA from wild-type cells (lane 1) and the *pilA1A2* (lane 2) and *sigF* (lane 3) mutants to probes for the *pilA1* gene (*Upper*) or 16S rRNA (*Lower*), which was used as a loading control.

tracellular pigments and proteins, the loss of pili, and a marked reduction in the level of *pilA1A2* mRNA, which encodes the structural subunits of the pili. Hence, SigF either directly regulates expression of *pilA1A2* genes or is involved in controlling some aspect of the architecture of the cell surface, which, in turn, results in decreased accumulation of *pilA1A2* mRNA. The latter scenario could occur if the PilA1 and PilA2 polypeptides were not transported out of the cell in the *sigF* mutant and the unassembled monomeric PilA in the cytoplasm repressed transcription of the *pilA1A2* operon.

Second, we have demonstrated that directional motility required for phototaxis in *Synechocystis* depends on the activity of type IV pili. Type IV pili also are required for social motility in *M. xanthus* during fruiting body formation and twitching motility characteristic of *N. gonorrhoeae, Pseudomonas aeruginosa,* and pathogenic strains of *E. coli* (29–33). Furthermore, pili appear to be required for bacterial pathogens to invade the tissue of their host, which makes elucidation of the structure and function of pili and the delineation of environmental factors that regulate their synthesis critical for developing strategies to treat diseases caused by bacterial pathogens (34–37).

Third, the *sigF* mutant has no apparent surface appendages, whereas the *pilA1A2* mutant still has pili, suggesting that elimination of SigF from the cell has a global effect on the cell surface and that there are different pili types in cyanobacteria. It should be possible in future studies to distinguish the different classes of pili by using specific antibodies and other genetic tools. In pathogenic bacteria there are several different kinds of pili or fimbriae with varied functions such as adhesion and competence (38). The conclusion that different types of pili are present on the surface of *Synechocystis* is supported by the observation that the genome of this organism contains several different genes encoding PilA-like polypeptides. We currently are investigating the functions of the four other PilA-like polypeptides encoded on the *Synechocystis* genome. However, expression patterns for these other genes are not as clear as that of *pilA1A2*; the transcript levels of the other *pilA* genes are not very abundant, and distinct mRNA species are not resolved by Northern blot analysis (data not shown). It also is becoming increasingly apparent that there are several common components and homologues between the type II secretion or general secretory pathway and the type IV pili biogenesis pathway (39). For instance, the processing of the leader

peptide of prepilin and the subsequent *N*-methylation is carried out by the bifunctional peptidase PilD, which also is used by the general secretory pathway. Furthermore, there are several PilA homologues that are required for the assembly of pili and the functioning of the general secretory pathway (26, 39). Thus, at this point we cannot exclude the possibility that some of the putative PilA-like polypeptides encoded on the genome of *Synechocystis* may play a role in type II secretion or in pilus assembly rather than being structural subunits of pili.

Fourth, the control of *pilA* genes by SigF is thus far unique to cyanobacteria. In other organisms that have been examined, which includes *Vibrio* species, *P. aeruginosa*, *N. gonorrhoeae,* and *M. xanthus,* the alternative sigma factor RpoN has been shown to be essential for motility (40–43). Mutants in *P. aeruginosa* that are null for *rpoN* expression cannot make pili, which reflects the inability of these strains to transcribe the gene encoding the pilin subunit. This sigma factor works in conjunction with PilR and PilS, which are members of a two-component regulatory system (44–47). However, it is not known which environmental cues modulate the phosphorylation state of these regulatory molecules and if these regulators control processes in addition to the production of pili. It is notable that our attempts to identify genes encoding possible homologues of RpoN, PilR, and PilS in *Synechocystis*, based on the whole genome sequence, were unsuccessful. On the other hand, the similarity of the *Synechocystis* SigF to the SigB of *B. subtilis* (which plays a crucial role in acclimation to a variety of stresses) and the presence of *rsb*-like genes on the *Synechocystis* genome raises the possibility that SigF participates in the acclimation of cyanobacteria to environmental stresses via its control of cell surface characteristics and/or pili-dependent motility.

In conclusion, we have shown that a SigF mutant in *Synechocystis* lacks pili and appears to have other cell-surface related abberations. As a result of the loss of pili these mutants lack motility and exhibit no phototaxis. Our findings provide an indication that type IV pili are involved in phototaxis. These mutants also may have other cell surface defects, including aberrations of the inner and outer cell membranes and alterations in the polysaccharide sheath. Furthermore, recent evidence suggests that type IV pili play a role in the formation of biofilms (48); these biofilms may provide an important growth habit for bacteria under specific environmental conditions. It is still unclear whether SigF directly regulates *pilA1A2* gene expression or whether the marked reduction of the *pilA1A2* transcript is the outcome of a global effect of SigF on cell surface architecture. Analysis of this regulation may reveal how environmental factors impact on morphological and functional characteristics of the bacterial cell surface.

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