Cyanobacteria such as Synechocystis sp. strain PCC 6803 exhibit surface-dependent phototactic motility which requires type IV pili (2, 4). To identify structural and regulatory components important for motility, we screened for mutants with aberrant phototactic movement (i.e., nonmotile or negatively phototactic under conditions in which wild-type cells show positive phototaxis).

A Synechocystis genomic library was constructed that contained approximately 10^6 clones with an average insert size of 6 to 8 kbp (3). The library of clones was used for in vitro transposon mutagenesis, and mutagenized clones were used for transformation of wild-type Synechocystis cells. Following transformation, cells were spread on 0.6% agar plates (10 μg of kanamycin/ml) and placed under a directional light source. Colonies that were nonmotile or exhibited an inverted response were picked, rescreened, and maintained for further analysis. Approximately 300 independent mutants (90% were nonmotile and 10% exhibited negative phototaxis) from a population of 8,000 transformants were identified. This screen required robust cell growth, thus eliminating mutants that might have appeared nonmotile because of growth defects. To identify the site of transposon insertion, we used plasmid rescue or direct sequencing of genomic DNA using primers that anneal to the ends of the transposon-kanamycin resistance cassette. Of the 50 or so mutants characterized so far, several contained transposons in genes encoding chemotaxis-related proteins (3). We also identified transposons in several novel genes, described in this report, that provide us with clues concerning functional and possibly architectural features that are important for motility.

Although we have not yet confirmed the phenotypic results by insertionally inactivating all of the genes found to be disrupted by transposons in the mutant strains, a number of findings strongly argue that the insertion of the transposon cassette is responsible for the mutant phenotype. First, based on Southern blot hybridizations, all of the mutants examined appeared to have a single transposon insertion (data not shown). Second, in several cases, independent transposition events led to the insertion of the transposon into different positions within the same gene or genetic locus; in all cases, the insertions always yielded the same phenotype. Since the entire Synechocystis genome has been sequenced, the positions of the inserted transposon can be easily mapped, and the phenotypes of all of the mutants generated can be readily confirmed by targeted inactivations. The third and most convincing argument is that in all cases in which we have specifically inactivated a target gene identified by the transposon mutagenesis (10 cases to date), the targeted and transposon-generated mutants yielded identical phenotypes (2, 3, 4).

Table 1 shows the phenotypes of various mutants isolated from our screen (it excludes the chemotaxis-related genes which are described in Bhaya et al. [3]) and gives the positions of the independent transposon insertions (relative to the open reading frame), the size of the disrupted open reading frames (in amino acids and nucleotides), the gene designation (as given in Cyanobase), and the putative function of the gene product. Three nonmotile mutants were shown to harbor transposons in the pilC genes, which are located adjacent to each other on the genome. We have recently demonstrated that disruption of either of these genes results in a nonmotile phenotype (2). This provides evidence that the method of mutant generation is robust and can be used to isolate novel motility mutants. Four independent transposon insertions led to the disruption of a gene encoding a putative DnaK-like chaperone (sll0058). There are three DnaK-like genes on the Synechocystis genome, of which sll0170 and sll1932 are likely to be the major chaperones (based on theoretical predictions), while sll0058 may be a minor chaperone dedicated to particular aspects of pilus biogenesis (10). In Myxococcus xanthus it has been shown that a nonessential DnaK-like chaperone is required for social motility and the production of fibrils (15), although its exact role in these processes has yet to be established.

We identified a nonmotile mutant disrupted for an ABC transporter gene (sll0415), and the phenotype of the mutant strain was confirmed by specific targeted inactivation. Interestingly, the mutant strain appears to have a normal complement of thin and thick pili (P. Shahi, D. Bhaya, and A. R. Grossman, 2001).
unpublished results). Of the approximately 42 putative ABC transporters of *Synechocystis* (http://www.biology.ucsd.edu/~jpausen/transport/), the one encoded by slr0415 has the highest similarity to an ABC transporter (PilU) required for motility in *M. xanthus* (38% identity and 60% similarity), although the role of the *M. xanthus* protein in motility is not known (16).

Three transposition events have been mapped to slr0564, whose gene product shows low but significant homology (25% identity and 36% similarity) to a methytransferase of the ubiqiunone methyltransferase type which uses 3-adenosylmethionine as a methyl donor. The putative polypeptide encoded by slr0564 does not resemble the CheR polypeptide, which is a methytransferase involved in methylolation of the methyl-accepting chemoreceptor proteins (11). Therefore, we cannot assign a function to this putative polypeptide without detailed biochemical analyses. It is also notable that a transposon which mapped to the gene (sll0415) downstream of the putative methyltransferase also produced a nonmotile phenotype, raising the possibility that both genes are important for motility. Kamei et al. have also demonstrated that sll1575 is required for motility; however, the precise function of this kinase in motility is unclear (8). It is interesting that a eukaryotic-type kinase is involved in this process; this suggests that signal transduction events that govern phototaxis may involve complex interactions.

A novel locus containing five genes (slr2015 to slr2019) is interesting because transposons mapped to three of the genes (slr2016, slr2017, and slr2018). The polypeptide encoded by slr2016 shows weak but significant homology to the family of PilA-like polypeptides (4), while the slr2017 and slr2018 gene products do not show obvious homologies to other polypeptides in the databases, nor do they contain obvious functional motifs. We are currently examining the function of this locus

<table>
<thead>
<tr>
<th>Gene</th>
<th>Residues</th>
<th>Size (nucleotides)</th>
<th>Insertion position(s)</th>
<th>Amino acid composition (% E/% Q/% P)</th>
<th>Signal peptide</th>
<th>Coils</th>
<th>Transmembrane helices</th>
<th>PFAM designation(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>slr0358</td>
<td>374</td>
<td>1,122</td>
<td>437</td>
<td>13/4.3/10.4</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No hits</td>
</tr>
<tr>
<td>sll0565</td>
<td>133</td>
<td>399</td>
<td>270</td>
<td>10.5/11.3/0.8</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No hits</td>
</tr>
<tr>
<td>slr1964</td>
<td>134</td>
<td>402</td>
<td>147</td>
<td>5.2/10.4/0.75</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No hits</td>
</tr>
<tr>
<td>slr1301</td>
<td>535</td>
<td>1,605</td>
<td>3, 443, 1057</td>
<td>13.6/13.6/1.9</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No hits</td>
</tr>
<tr>
<td>sll0183</td>
<td>259</td>
<td>777</td>
<td>64, 348, 502</td>
<td>5.4/6.3/1.1</td>
<td>Yes (18/19)</td>
<td>No</td>
<td>Yes</td>
<td>00515, 00805</td>
</tr>
<tr>
<td>sll0414</td>
<td>286</td>
<td>858</td>
<td>342</td>
<td>4.5/9.1/3.5</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>00805</td>
</tr>
<tr>
<td>sll0301</td>
<td>169</td>
<td>507</td>
<td>97</td>
<td>3.2/4.1/1.2</td>
<td>Yes (32/33)</td>
<td>No</td>
<td>Yes</td>
<td>00805</td>
</tr>
</tbody>
</table>

* Numbers in bold represent putative amino acid percentages higher than 10% (usual levels are 3 to 5%).

* Numbers in parentheses represent putative signal peptide cleavage sites.
by generating specific, nonpolar gene disruptions in each open reading frame and analyzing the effect of the disruptions on pilus morphology and biogenesis.

Table 2 lists seven genes encoding putative novel polypeptides that we have identified as being required for motility, based on phenotypes of strains mutated in these genes. These polypeptides fall into two distinct categories. The first category, represented by slr0358, sll0565, slr1964, and slr1301, are polypeptides that are relatively rich (defined here as being higher than 10%, usual levels being between 3 and 5%) in glutamic acid (E) or glutamine (Q) residues and that do not appear to have any recognizable motifs (based on analysis by Pfam [http://pfam.wustl.edu/index.html] or COG [http://www.ncbi.nlm.nih.gov/COG/]) (9). Furthermore, all of these polypeptides except slr0358, which is proline (P) rich, have the possibility of forming coiled-coil structures, based on the predictions of Coils (http://www.ch.embnet.org/software/COILS_form.html) (9). The coiled-coil configuration of the putative slr1301 polypeptide may be especially extensive (data not shown). It has recently been demonstrated that FrzS of Synechocystis is an extensive coiled-coil structure and an FrzS mutant is incapable of social motility (14). The results of the mutant screen highlight two intriguing findings. First, there appear to be many polypeptides involved in cyanobacterial motility that have specific biochemical functions (based on homologies with other proteins), but their role in motility is relatively unexplored. We have evidence that two loci (tax1 and tax3) which contain several che-like genes are involved in pilus-mediated phototaxis (3). These loci include histidine kinases as well as a chemoreceptor with a putative phytochrome-like chromophore-binding domain. We postulate that these proteins are involved in a complex signal transduction pathway that regulates phototaxis (3). In some cases analogous proteins have been found to be required for motility in other organisms, such as M. xanthus. Second, groups of polypeptides with distinct structural characteristics are required for motility, and although their role in motility is not known, they may serve to facilitate interactions among proteins in large complexes or be part of an intracellular matrix that is required to assemble and/or anchor the pilus-associated motor complex.

FIG. 1. Comparison of sll0183, sll0301, and sll0414, with black boxes indicating identical or conserved residues in all polypeptides and gray boxes indicating residues conserved in two polypeptides. The region with pentapeptide repeats is underlined; the tetratricopeptide motif of sll0183 is indicated by black circles.

The work presented in this manuscript was supported in part by National Science Foundation grant MCB 9727836.

REFERENCES


