The Cyanobacterium Synechocystis sp. Strain PCC 6803 Expresses a DNA Methyltransferase Specific for the Recognition Sequence of the Restriction Endonuclease PvuI

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By use of restriction endonucleases, the DNA of the cyanobacterium Synechocystis sp. strain PCC 6803 was analyzed for DNA-specific methylation. Three different recognition sites of methyltransferases, a dam-like site including N6-methyladenosine and two other sites with methylcytosine, were identified, whereas no activities of restriction endonucleases could be detected in this strain. slr0214, a Synechocystis gene encoding a putative methyltransferase that shows significant similarities to C5-methylcytosine-synthesizing enzymes, was amplified by PCR and cloned for further characterization. Mutations in slr0214 were generated by the insertion of an aphII gene cassette. Analyses of chromosomal DNAs of such mutants demonstrated that the methylation pattern was changed. The recognition sequence of the methyltransferase was identified as 5'-CGATCG-3', corresponding to the recognition sequence of PvuI. The specific methyltransferase activity was significantly reduced in protein extracts obtained from mutant cells. Mutation of slr0214 also led to changed growth characteristics of the cells compared to wild-type cells. These alterations led to the conclusion that the methyltransferase Slr0214 might play a regulatory role in Synechocystis. The Slr0214 protein was also overexpressed in Escherichia coli, and the purified protein demonstrated methyltransferase activity and specificity for PvuI recognition sequences in vitro. We propose the designation SynMI (Synechocystis methyltransferase I) for the slr0214-encoded enzyme.

DNA of prokaryotic and eukaryotic cells is usually modified by methylation. This modification is carried out by DNA-specific methyltransferases, which transfer methyl groups from the universal substrate S-adenosylmethionine (AdoMet) to specific target sequences in the host DNA. On the basis of different chemical reactions, methyltransferases can be divided into three different groups leading to the appearance of N6-methyladenosine, C5-methylcytosine, or C4-methylcytosine. Amino acid sequence alignments of enzymes from these groups revealed conserved motifs characteristic of each methyltransferase family (25). In the enteric bacterium Escherichia coli, three methyltransferases are present: (i) Dam (DNA adenine methyltransferase), creating N6-methyladenosine in the specific target sequence 5'-CCGG-3'; (ii) Dcm (DNA cytosine methyltransferase), leading to an internal C5-methylcytosine in the specific target sequence 5'-CGATCG-3'; and (iii) methyltransferase M·EcoK, modifying the second adenine in the sequence A(m)AC(N)GTGC, which is specifically recognized as an unmethylated sequence by restriction endonuclease EcoK, which is part of the host-specific restriction-modification (R-M) system (for a review, see reference 22). Methylation of cytosine in CG dinucleotides dominates in cells of higher eukaryotes. In these cells, the degree of methylation is tightly controlled and plays an important role in the regulation of gene expression, organization of chromatin, and developmental and DNA repair processes (4).

Methyltransferases involved in R-M systems have been found in all taxonomic groups of eubacteria examined so far. R-M systems typically comprise two enzymatic activities, a restriction endonuclease and a methyltransferase. The genes of both enzymes are often organized in one operon. By extensive screening, more than 2,750 type II restriction endonucleases with a total of 211 different specificities have been found (27). Cyanobacteria represent a rich source of restriction enzymes. In several strains, two or three different enzymes are present; among them, isoschizomers of the enzyme AvoII have been often found (10). Six restriction endonucleases with different specificities were detected in the cyanobacterium Dactylococcus salina (19). The cognate methyltransferases have been investigated less. The genes encoding AvoI and AquI methyltransferases were cloned and sequenced from Anabaena variabilis and Agmenellum quadruplicatum, respectively. The amino acid sequences of both proteins show significant similarities to those of functionally related enzymes from heterotrophic bacteria (13, 30).

Genetic tools are well developed only for a few cyanobacterial model strains. A general problem in establishing such systems for other strains is the barriers made by endogenous restriction systems that prevent stable access of foreign DNA into the cells. As has been found before in many instances, in vitro methylation of DNA also improves the efficiency of DNA transfer into cyanobacterial strains (35). The cyanobacterium Synechocystis sp. strain PCC 6803 (hereafter referred to as Synechocystis) belongs to the group of naturally competent strains which are transformable by free exogenous DNA (6). A Ca2+-dependent nuclease located in the cytoplasmic membrane is involved in DNA uptake and is assumed to convert double-stranded DNA into the single-stranded form (3). Recently, the entire genome of Synechocystis was sequenced, making this strain the favored cyanobacterium for genetic studies (11).

In this work, we examined the chromosomal DNA of Synechocystis for modifications by methylation. Sequence-specific methylations were detectable, whereas no restriction endonu-
TABLE 1. Plasmids and mutants of Synechocystis used and constructed in this study (see also Fig. 2)

<table>
<thead>
<tr>
<th>Designation</th>
<th>Size (kb)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pGGM02</td>
<td>4.32</td>
<td>pGEM7 containing a 1.3-kb BamHI/EcoRI fragment, the coding sequence of slr0214 amplified by PCR</td>
</tr>
<tr>
<td>pUC19GM</td>
<td>4.0</td>
<td>pUC19 containing a 1.3-kb BamHI/EcoRI fragment, the coding sequence of slr0214 amplified by PCR</td>
</tr>
<tr>
<td>pGGMK29−</td>
<td>5.6</td>
<td>pGEM7 containing inactivated slr0214 (aphII gene inserted at the Hincll site opposite to the direction of transcription of slr0214)</td>
</tr>
<tr>
<td>pGGML05</td>
<td>6.3</td>
<td>pGEM7 containing a 3.3-kb BamHUPol fragment, the coding sequence of slr0214 with an additional 1 kb upstream and 1 kb downstream amplified by PCR</td>
</tr>
<tr>
<td>pGGMDE12−</td>
<td>6.3</td>
<td>pGMM02 containing partially deleted slr0214 (aphII gene inserted, after deletion of the internal BsrI fragment, opposite to the direction of transcription of slr0214)</td>
</tr>
<tr>
<td>pGEXGM</td>
<td>6.2</td>
<td>pGEX4T3 containing a 1.3-kb BamHI/EcoRI fragment, the coding sequence of slr0214 fused to GST</td>
</tr>
<tr>
<td>slr0214 mutant</td>
<td></td>
<td>Synechocystis mutant obtained after transformation of the WT with pGGM29−</td>
</tr>
<tr>
<td>slr0214A mutant</td>
<td></td>
<td>Synechocystis mutant obtained after transformation of the WT with pGGMDE12−</td>
</tr>
</tbody>
</table>

**RESULTS**

The occurrence of sequence-specific DNA methylation was examined by incubation of chromosomal DNA isolated from *Synechocystis* with different restriction enzymes to test whether or not their activities are specifically influenced by methylation of their recognition sequences. In these experiments, it was found that the DNA was resistant to the action of *MboI*, *ApaI*, *EaeI*, *HaeIII*, *PstI*, and *SfiI*, while other restriction endonucleases were able to cut (Table 2). Different sensitivities of the DNA to different restriction endonucleases indicated that at least three methylation activities seemed to be present. One represented a Dam-like methyltransferase, which was demonstrated by the inhibition of the restriction enzyme *MboI* and the activities of *DpnI* and *Sau3A* (Table 2). The activities of these enzymes were differentially influenced by methylation of the adenine in their recognition sequence. The *MboI* could not cleave Dam-modified DNA; *DpnI* could cleave only Dam-modified DNA; and *Sau3A* was not influenced by Dam modification, since its cognate methyltransferase modifies the
TABLE 2. Restriction enzymes used for identification of methyltransfer sites on chromosomal DNA of *Synechocystis* 

<table>
<thead>
<tr>
<th>Restriction enzyme</th>
<th>Recognition sequence</th>
<th>Cleavage of chromosomal DNA</th>
<th>Frequency (in bp) of occurrence in: Chromosomal DNA</th>
<th>Random sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pvu</em>&lt;sup&gt;III&lt;/sup&gt;</td>
<td>G C A T G G C</td>
<td>No&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1/698</td>
<td>1/4,096</td>
</tr>
<tr>
<td><em>Sgf</em>&lt;sup&gt;I&lt;/sup&gt;</td>
<td>G G C A T G G C</td>
<td>No&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1/1,131</td>
<td>1/65,536</td>
</tr>
<tr>
<td><em>Apa</em></td>
<td>G C A T G G C C</td>
<td>No</td>
<td>1/3,788</td>
<td>1/4,096</td>
</tr>
<tr>
<td><em>Eae</em>&lt;sup&gt;III&lt;/sup&gt;</td>
<td>(C/T) G C A T G G C (G/A)</td>
<td>No</td>
<td>1/544</td>
<td>1/2,048</td>
</tr>
<tr>
<td><em>Hae</em>&lt;sup&gt;II&lt;/sup&gt;</td>
<td>G G C A T G G C</td>
<td>No&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1/186</td>
<td>1/2,56</td>
</tr>
<tr>
<td><em>Dpn</em>&lt;sup&gt;I&lt;/sup&gt;</td>
<td>A T G C</td>
<td>Yes&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1/239</td>
<td>1/2,56</td>
</tr>
<tr>
<td><em>Mbo</em>&lt;sup&gt;II&lt;/sup&gt;</td>
<td>A C</td>
<td>No&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1/239</td>
<td>1/2,56</td>
</tr>
<tr>
<td><em>Sau</em>&lt;sup&gt;3AI&lt;/sup&gt;</td>
<td>G A C</td>
<td>Yes&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1/239</td>
<td>1/2,56</td>
</tr>
</tbody>
</table>

<sup>a</sup> The following enzymes were additionally tested and found able to cut *Synechocystis* DNA: *AflIII*, *AflIV*, *AlaI*, *AhaII*, *AhuI*, *AluI*, *AvuI*, *BamHI*, *BglII*, *BsuRI*, *CotI*, *Clal*, *DdeI*, *DpnI*, *EcoRV*, *EcoRI*, *EcoRV*, *EspI*, *Fnu4II*, *HindIII*, *HpaI*, *HpaII*, *MboI*, *MnlI*, *PstI*, *PvuII*, *SacI*, *SmaI*, *XbaI*, and and *XhoI*. For enzymes in boldface, see footnote <sup>e</sup>. 

<sup>b</sup> Underlining indicates that methylation inhibited restriction. 

<sup>c</sup> Methylation of the first cytosine probably inhibited restriction (see Results). 

<sup>d</sup> Restriction was controlled additionally to gel electrophoresis by Southern hybridization experiments.

cytosine (24). Comparison of the recognition sequences of the other inhibited restriction endonucleases showed that at least two different cytosine-specific methyltransferases were involved. The activities of *Apa*I, *Eae*<sup>III</sup>, and *Hae*<sup>II</sup> were most probably affected by one enzyme recognizing the sequence 5'-GGCC-3'. *Pvu* and *Sgf*<sup>I</sup> should have been inhibited by a cytosine-specific methyltransferase modifying one of the two cytosines within the sequence 5'-CGATCG-3', which includes the Dam recognition site. The complete digestion of chromosomal DNA by *Sau*<sup>3AI</sup> excluded methylation of the second cytosine. Thus, this cytosine-specific methyltransferase most probably modified the first cytosine of a *Pvu* site (Table 2). Statistical analyses showed that the frequency of occurrence of *Pvu* and *Sgf*<sup>I</sup> recognition sequences was increased severalfold in the chromosomal DNA of *Synechocystis* compared to their theoretical frequency in a random sequence. The frequencies of the recognition sequences of *Eae*<sup>III</sup> and *Hae*<sup>II</sup> were also increased but to a lesser extent, whereas the occurrence of the *MboII* site corresponded to its frequency in a random sequence (Table 2).

In order to test if these methyltransferase activities were part of a strain-specific R-M system, the restriction endonuclease activity of *Synechocystis* was examined. This was done by incubating unmethylated DNA of phage λ (MBI Fermentas) with protein extracts (150 μg) in the presence of the basic reaction buffers (React 1 to 4; Life Technologies) recommended for most restriction endonucleases at 37°C overnight (19). These experiments clearly showed that there was no restriction endonuclease activity, since the λ DNA was not cut into defined fragments after incubation with the large amounts of proteins used over a long time under different reaction conditions (data not shown).

On the genome of *Synechocystis*, there is one ORE (sh0214) encoding a putative cytosine-specific methyltransferase (11). The amino acid sequence of Sh0214 was aligned with those of other methyltransferases. The proteins showing the best alignments all belonged to the group of C<sup>5</sup>-cytosine methyltransferases (Fig. 1). The highest amino acid identity (48.2%) over the whole sequence was found with the *XorII* methyltrans-

FIG. 1. Amino acid sequence comparison between the *Sh*0214 protein (SynM) (11) and the C<sup>5</sup>-methylcytosine-synthesizing methyltransferases *XorIII* (5), *DdeM* (34), *NgoII* (33), and *HphIM* (21). Uppercase letters and a shaded background indicate amino acids identical to those in SynM. The conserved motifs characteristic for C<sup>5</sup>-cytosine methyltransferases (26) are indicated by Roman numerals.
cles under the arrows indicating the protein-encoding regions in panel C. (D) The binding sites of the primers are represented by triangles (conferring kanamycin resistance) whose transcription (resulting in plasmid pGGM02) (Table 1), an aphII gene cartridge (resulting in plasmid pGGM05) (data not shown) could be cleaved under the selected reaction conditions. In contrast to the WT DNA, the DNA of the slr0214 mutant (Fig. 4) as well as the DNA of the slr0214Δ mutant (data not shown) became accessible to restriction by PvuII and SgfI. The other methyltransfer activity, the Dam-like methylation and the second cytosine-specific methylation, remained intact, because the DNA of both mutants was still resistant to MboI as well as HaeIII and was digested by DpnI (Fig. 4; compare to Table 2). The ability of PvuII and SgfI to cut mutant DNA was confirmed by Southern hybridization experiments. With PvuII- and SgfI-cut DNAs from the WT and the mutants, three and two fragments (PvuII and SgfI, respectively) (Fig. 2) of the expected sizes appeared after application of the slr0214 gene probe, whereas with the WT DNA only hybridization to the uncut high-molecular-weight DNA was visible (data not shown). Therefore, the methyltransferase encoded by slr0214 is specifically directed to the recognition sequence of PvuII or its isoschizomer XorII. Furthermore, the methylation is directed to a cytosine within the recognition sequence, since the internal adenosine remained modified, as shown by the inhibition of MboI.

The lesion in the DNA of these mutants was characterized by Southern hybridization experiments (Fig. 3; compare to Fig. 2). In NcoI-digested DNA of the slr0214 mutant, one fragment of 4.5 kb was found with the slr0214 gene probe. This fragment was 1.2 kb larger than the signal detected in the WT DNA, consistent with the integration of the 1.2-kb aphII gene cartridge (Fig. 3A). One fragment of only 2.4 kb became visible by hybridization with the slr0214Δ mutant DNA. This size exactly matched the size of the fragment expected for the deletion mutant (Fig. 3A). After cutting of chromosomal DNA with HindIII, in the WT only one fragment of 2.5 kb was detectable with the slr0214 gene probe, while in the mutants two fragments (the aphII gene contains one HindIII site) of the expected sizes (2.2 and 1.7 kb for the slr0214 mutant and 2.0 and 1.0 kb for the slr0214Δ mutant [with the slr0214 gene probe, the 1.0-kb fragment of the deletion mutant was detectable only as a very faint band, since it shared only 30 bp with the DNA probe]) became visible (Fig. 3A). The same pattern was obtained for the DNA of the mutants when the aphII gene was used as a probe (Fig. 3B). Again, after digestion with NcoI, fragments of 4.5 and 2.4 kb were detected. After cutting with HindIII, the aphII gene probe hybridized with a 2.2-kb fragment and a 1.7-kb fragment of the slr0214 mutant DNA, whereas a 2.0-kb band and a 1.0-kb band were clearly visible with the slr0214Δ mutant DNA. No specific signal could be detected by hybridization with the WT DNA. These hybridization patterns indicated that the plasmid constructs used for the transformation of Synechocystis to obtain the mutants were correctly integrated by double crossing over and replaced completely the WT copy of the slr0214 gene.

In order to analyze changes in the methylation pattern, chromosomal DNA isolated from the mutants was incubated with different restriction enzymes which were found to be inhibited by the WT DNA (Table 2). Digestion with NcoI was used as a control reaction in order to show that the DNA preparations could be cleaved under the selected reaction conditions. In contrast to the WT DNA, the DNA of the slr0214 mutant (Fig. 4) as well as the DNA of the slr0214Δ mutant (data not shown) became accessible to restriction by PvuII and SgfI. The other methyltransfer activity, the Dam-like methylation and the second cytosine-specific methylation, remained intact, because the DNA of both mutants was still resistant to MboI as well as HaeIII and was digested by DpnI (Fig. 4; compare to Table 2). The ability of PvuII and SgfI to cut mutant DNA was confirmed by Southern hybridization experiments. With PvuII- and SgfI-cut DNAs from the WT and the mutants, three and two fragments (PvuII and SgfI, respectively) (Fig. 2) of the expected sizes appeared after application of the slr0214 gene probe, whereas with the WT DNA only hybridization to the uncut high-molecular-weight DNA was visible (data not shown). Therefore, the methyltransferase encoded by slr0214 is specifically directed to the recognition sequence of PvuII or its isoschizomer XorII. Furthermore, the methylation is directed to a cytosine within the recognition sequence, since the internal adenosine remained modified, as shown by the inhibition of MboI.

FIG. 2. Schematic drawing showing the genetic organization, restriction map, and protein-encoding regions of the chromosomal region encoding slr0214 (SynMI) in Synechocystis (11). (A and B) The insertion of the aphII gene in selected sites to generate mutants is shown. (A) BalI was used to obtain the slr0214 mutant. (B) HincII was used to obtain the slr0214 Δ mutant. (C) Protein-encoding region. (D) The binding sites of the primers are represented by triangles under the arrows indicating the protein-encoding regions in panel C.
We propose the designation SynMI (Synechocystis methyltransferase I) for the slr0214-encoded enzyme. Beside this enzyme, a second cytosine-specific methyltransferase which is responsible for the modification of the HaeIII recognition sequence must exist in Synechocystis.

In further experiments, slr0214 and slr0214A mutants of Synechocystis were characterized with regard to changes in their physiology. During cultivation on agar plates, no obvious differences between mutant and WT cells were observed. Surprisingly, the growth of the slr0214 mutant changed dramatically in CO2-enriched cultures when the medium of Allen and Arnon (1) was used. It became very difficult or impossible to cultivate the mutant under the growth conditions described for the WT. It was possible to grow these cells only at higher densities or, alternatively, at lower light intensities. However, even at lower light intensities, the growth of the mutant was significantly reduced in comparison to that of the WT (Fig. 5). These changes in the growth characteristics implied that methylation SynMI (Slr0214) seems to fulfill an important function in Synechocystis. Additionally, the total DNA-specific methyltransferase activity was measured in protein extracts obtained from cells of WT Synechocystis and the slr0214 mutant. The specific methyltransferase activity of this mutant was reduced to about 30% the WT activity (Fig. 5).

The biochemical reaction of the SynMI (Slr0214) protein was confirmed by an in vitro assay. The slr0214 gene was cloned in pGEX4T3 and overexpressed in E. coli. From IPTG-induced E. coli cultures proteins were isolated and purified with a glutathione-Sepharose column. From the affinity matrix a protein of the expected size of 43 kDa was purified to homogeneity after cleavage by thrombin (Fig. 6A). The purified protein showed significant methyltransferase activity in an in vitro enzyme assay (Fig. 6B). The methyltransferase activity was detected only in the low-salt buffer; increased salt content in the buffer led to complete inhibition of the activity of SynMI. Furthermore, the methylation specificity of the purified SynMI protein was tested in vitro, confirming the PvuI recognition sequence as its target. A 1.8-kb internal fragment of the clpC gene of Synechocystis, which contains two PvuI sites, was amplified by PCR, methylated in vitro, and treated with restriction enzymes (Fig. 6C). While the unmethylated PCR fragment was completely cut by PvuI (fragments of 1.07 and 0.73 kb appeared), the methylated fragment was found to be fully resistant to the PvuI treatment. Clal digestion was used as a control in order to show that the methylated fragment could be cut by enzymes with different specificities.

**DISCUSSION**

Extensive restriction analyses revealed that the chromosomal DNA of the cyanobacterium Synechocystis is modified by methylation. One DNA-specific methyltransferase activity was indicated by the resistance of chromosomal DNA to the restriction activity of PvuI and SgfI, which are known to be influenced by C5-cytosine methylation of their recognition sequences in CG dinucleotides (12, 32). The ORF slr0214 (11) was identified as the gene encoding the methyltransferase responsible for this modification. Three lines of evidence led to this conclusion. (i) By comparison of the Slr0214 amino acid sequence to related sequences, the highest degree of similarity was found to C5-cytosine methyltransferases. The N- and C-terminal parts are especially well conserved, which is characteristic for this group of enzymes, while the central part is variable and determines their sequence specificities (14). Among the related sequences, that of the XorII (an isoschizomer of PvuI) methyltransferase showed the highest degree of homology. (ii) After overexpression of the slr0214 gene in E. coli, it was possible to demonstrate methyltransferase activity in vitro. The methylation of PvuI recognition sequences was directly shown by use of methylated and unmethylated PCR fragments for restriction analysis. (iii) The final proof was obtained by analyzing the features of mutants impaired in slr0214; the insertion and deletion mutants showed the same alterations in phenotype. In both mutant types, the chromosomal DNA could be digested by PvuI as well as by SgfI, while in the WT, these sites were completely blocked. The PvuI site was identified as the recognition sequence, since in Southern blot experiments it became obvious that PvuI sites which were not part of an SgfI site were cut in the mutant DNA, while such PvuI sites were not cut in the WT DNA. Furthermore, the specific activity of methyltransferases was significantly decreased in extracts obtained from the mutants. Nevertheless, there was a significant residual activity, which can be explained by the occurrence of the other methyltransferases. The identity of the methylated base produced by SynMI in the PvuI recognition sequence has not yet been experimentally determined. However, it is likely...
that the first cytosine represents the target for methylation by SynMI, a conclusion which could be drawn from the data obtained for the activity and inhibition of different restriction enzymes affecting the PvuI site and its internal sequence, 5'-GATC-3', by use of DNAs from the WT and the Slr0214 mutant (Table 2 and Fig. 4).

At least a second type of methyltransferase activity is present in Synechocystis; this activity resembles the Dam modification characterized in E. coli, in which the adenine sequence 5'-GATC-3' is modified. This finding was clearly shown by the inhibition of MboI, which is affected by the adenine methylation, and by the activity of DpnI, which cuts only if the adenine is methylated (24). In E. coli, Dam is involved in multiple functions: (i) mismatch repair, (ii) regulation of initiation of chromosome replication, and (iii) modification of expression functions: (i) mismatch repair, (ii) regulation of initiation of chromosome replication, and (iii) modification of expression.

In many bacteria, DNA methylation is related to a strain-specific R-M system. However, in Synechocystis, no restriction endonuclease activity was demonstrated by incubation of lambda DNA with large amounts of cellular protein. The capacity of this strain for natural transformation (6, 35) is a further indication for the absence of such enzymes; in addition, on the entire chromosome no ORF showed homologies to genes for known restriction endonucleases (11). Therefore, Synechocystis expresses methyltransferases which are not part of a host-specific R-M system. Nevertheless, at least the enzyme SynMI (Slr0214) seems to have an important function, since the mutants showed altered growth characteristics.

The recognition sequence of the methyltransferase SynMI is part of the HIP1 sequence (highly iterated palindrome) (8), which is identical to the SgfI site. HIP1 is very abundant in the DNA of several but not all cyanobacteria, where it occurs in coding and intergenic regions (28). The complete genome sequence of Synechocystis (11) allowed us to estimate their frequencies. SynMI or PvuI sites were found every 698 bp and SgfI or HIP1 sequences were found every 1,131 bp in the DNA (Table 2). In Synechocystis sp. strain PCC 6301, HIP1 was found to be extremely frequent (every 320 bp). However, in this strain, HIP1 is not methylated at all or by another methylation as in Synechocystis, since its chromosomal DNA was completely digested by PvuI (28). The HIP1 sequence seems to be involved in excision and gene rearrangement (29).

In Synechocystis, the target sequences of the SynMI methyltransferase, PvuI, including the HIP1 sequence, are highly over-represented and are completely methylated in the WT. Hypothetically, these methylated sequences could serve as binding sites for chromatin-like proteins. The same function was suggested for another repetitive sequence found in filamentous cyanobacterial strains (23). The proteins associated with the DNA might have an influence on the rate of gene expression, as was found for histones in eukaryotic cells. Interestingly, the sequences of some highly expressed genes in the genome of Synechocystis (e.g., genes encoding for rRNAs and for subunits of photosystem I [psaF, -D, and -L] and of photosystem II [psbA1 to psbA3, psbCD]) (11) do not contain any PvuI site and are therefore also not methylated in WT cells. The complete absence of methylation in the slr0214 mutants of Synechocystis could disturb the balanced gene expression program, leading to problems in growth at higher light intensities in CO₂-enriched cultures. In future experiments, we will analyze the physiological function of the SynMI methyltransferase in more detail by growing the WT and the mutants under different conditions.
conditions and by comparing the expression of genes with or without PvuI sites in both the WT and the mutants.

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REFERENCES

AUTHORS’ CORRECTIONS

The Highly Conserved, Coregulated SNO and SNZ Gene Families in *Saccharomyces cerevisiae* Respond to Nutrient Limitation

PAMELA A. PADILLA, EDWINA K. FUGE, MATTHEW E. CRAWFORD, ALLISON ERRETT, AND MARGARET WERNER-WASHBURNE

Volume 180, no. 21, p. 5718–5726, 1998. Page 5725, Acknowledgments, second paragraph, lines 3–5: “and by MBRS grant 5SO6GM52576-03 from the National Science Foundation and a Patricia Roberts Harris Fellowship to P.A.P.” should read “and by MBRS and Patricia Roberts Harris grants from NIH to P.A.P.”

The Cyanobacterium *Synechocystis* sp. Strain PCC 6803 Expresses a DNA Methyltransferase Specific for the Recognition Sequence of the Restriction Endonuclease *Pvu*I

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Volume 180, no. 16, p. 4116–4122, 1998. Pages 4116–4122: The DNA methyltransferase from *Synechocystis* sp. strain PCC 6803 named SynMI should have been named *M*S*6*803*I in accordance with the usual nomenclature for DNA methyltransferases.