Stable Transformation of the Cyanobacterium *Synechocystis* sp. PCC 6803 Induced by UV Irradiation

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Irradiation of the photoheterotrophic cyanobacterium *Synechocystis* sp. PCC 6803 with low levels of UV light allows for stable, integrative transformation of these cells by heterologous DNA. In this system, transformation does not rely on an autonomously replicating plasmid and is independent of homologous recombination. Cells treated with UV light in the absence of DNA and cells given DNA but not exposed to UV do not yield antibiotic-resistant colonies in platings of up to $2 \times 10^4$ cells. Optimal conditions for this UV-induced transformation are described. Analysis of the transformants indicates that (i) only a segment of the introduced plasmid is found in the DNA of the transformed cells; (ii) in independently isolated clones, DNA insertion apparently occurs at different sites in the chromosome; and (iii) hybridization data suggest that insertion in one of the transformants may have occurred into a region of the chromosome that is repeated or that integration of plasmid DNA may have been accompanied by a rearrangement or duplication of DNA sequences near the insertion site. DNA isolated from the primary transformants as well as a cloned fragment containing the UV-inserted plasmid sequence and flanking cyanobacterial DNA transform wild-type cells at a high frequency ($5.0 \times 10^{-4}$ and $1.5 \times 10^{-5}$, respectively). Possible mechanisms of this transformation system are discussed, as are the potential uses of this system as an integrative cloning-complementation vector and as a mutagenic agent in which the genetic lesion is already tagged with a selectable marker.

The cyanobacteria are oxygen-evolving photosynthetic procaryotes which have a number of features ideally suited to the study of the molecular biology of photosynthesis. These organisms perform plantlike photosynthesis and, being procaryotes, they are in principle more amenable to genetic manipulation. It has also been demonstrated that several cyanobacterial species transform with purified DNA. These transformation systems, however, have generally relied on one of two approaches. Either endogenous plasmids have been isolated from these species and used to construct shuttle vectors capable of replication in both *Escherichia coli* and the cyanobacterium (3, 7–9, 17, 32), or DNA has been purified from cyanobacterial cells of a defined phenotype and used to transform wild-type cells of the same species (presumably by homologous recombination) (5, 10, 23, 25, 28, 30). It would, however, be advantageous to have a system for introducing heterologous DNA into these cells directly. In this report, we describe a transformation system for the cyanobacterium *Synechocystis* sp. PCC 6803 which does not require an autonomously replicating plasmid and in which transformation is independent of homologous recombination.

For these experiments we have chosen the photoheterotrophic cyanobacterium, *Synechocystis* sp. strain 6803 (24). This species is known to be transformable (10), and defined protosynthetic mutants of this strain have been isolated (2). The transformation procedure used in these experiments follows standard cyanobacterial protocols (17, 25) except in one important step. Prior to the addition of DNA to a suspension of cyanobacteria, the cells are exposed to low-level doses of UV irradiation. This approach was chosen for several reasons. In response to UV irradiation, *E. coli* and other procaryotic cells induce various repair and other processes collectively known as the SOS response (see references 11 and 29 for reviews). Among the phenomena associated with SOS repair is the temporary alleviation of host-controlled restriction (20). This phenomenon allows, for example, phage that are normally restricted for growth on the host to successfully propagate (4). The occurrence of restriction endonucleases in certain cyanobacteria has been well documented (12). We therefore reasoned that irradiation of the host cells with UV light might allow any DNA that is introduced into the cell to escape degradation by host restriction endonucleases. In addition, UV irradiation can lead to the introduction of single-strand nicks and double-strand breaks in DNA molecules (31). However, during SOS repair, these breaks are resealed. It is possible that during this time these cells, which also have elevated levels of proteins associated with recombination, may become "recombinogenic," and any exogenous DNA found in the cell may at a low frequency become integrated into the chromosome. In this article we report on the usefulness of this approach and define several parameters of this transformation protocol.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Axenic cultures of *Synechocystis* sp. strain 6803 (24) were obtained from the American Type Culture Collection (ATCC 27184) and were grown at 28°C in 80 ml of a modified medium C of Kratz and Myers (16). In place of KNO$_3$ and Fe$_3$(SO$_4$)$_3$ $\cdot$6H$_2$O, 1.5 g of NaNO$_3$ and 0.012 g of ferric ammonium citrate per liter, respectively, were added. The medium was also supplemented with 0.04 g of Na$_2$CO$_3$ per liter. The cells were illuminated with approximately 3,500 lx from a bank of four fluorescent cool white bulbs and were kept in suspension by agitation on rotary shakers. *Synechocystis* sp. strain 6803 derivative 6803V, 6803,789, and 6803V-T and their isolation are described in this report.
Plasmids pVAD1 (Ap' Cm') (6), pBR328 (Cm' Tc' Ap') (26), and pMH1 (Km' Ap') (14) have been described previously; plasmid pIBP1 and its construction are described below. Plasmids pBRMH20 (Ap' Km' Tc') and pBRMH6 (Ap' Km' Cm') both contain the kanamycin resistance marker derived from Tn5 inserted into pBR328. In the first plasmid, a HindIII-SmaI fragment of pMH1 (containing the kanamycin resistance gene) was filled in by using the Klenow fragment of *E. coli* DNA polymerase I and inserted into the PvuII site of pBR328. In the construction of pBRMH6, the same HindIII-SmaI fragment of pMH1 was inserted between the EcoRV and HindIII sites of pBR328. In the first plasmid, the chloramphenicol resistance gene is disrupted, while in the latter it is intact.

**Genetic procedures and recombinant DNA techniques.** Restriction endonucleases and other enzymes were obtained from commercial sources and were used according to the instructions of the manufacturer. Ligation conditions, transformation of *E. coli* HB101 cells, preparation of plasmid DNA, and other standard protocols were carried out as described elsewhere (21). The insert-to-vector ratios used in ligation reactions were those suggested by Lagers and Robberson (19).

**Transformation of Synechocystis sp. strain 6803 cells.** Transformations were performed as follows. Three flasks of strain 6803 cells were grown to optical densities at 730 nm of approximately 0.8, 1.0, and 1.2 (i.e., for mid- to late-log phase of growth). The cultures were mixed, collected by centrifugation at room temperature, suspended in one-quarter volume of growth medium, and recentrifuged. The pellet was suspended in growth medium at 1/10 the original volume, and 1-ml samples (approximately 2 \times 10^8 cells per ml) were pipetted into 1.5-ml clear microfuge tubes containing 1.0 to 3.0 \mu g of the DNA sample. When the cells were pretreated with UV light, the following protocol was used. Samples (5 ml) of the concentrated cells were pipetted into sterile petri dishes (60 by 15 mm) and then exposed (with the lid removed) to UV light as described in the Results section. Immediately after irradiation, 1-ml samples were pipetted into 1.5-ml clear microfuge tubes to which 1.0 to 3.0 \mu g of the DNA sample had been added. For control tubes, no DNA was present. Additional details of the UV treatment are presented below. In experiments involving the effects of photoreactivation, all manipulations after UV exposure were performed in darkness.

The UV-light-irradiated cells and control samples were illuminated for 6 h at 3,000 lx, with occasional shaking at 30°C, and were then plated in top agar (modified BG-11 [6, 24] plus 0.8% agar) onto BG-11T-containing plates (BG-11, 1.5% agar, and 5 mM sodium thiosulfate). Plates were illuminated at 30°C for 14 h, and then 1 ml of a chloramphenicol (final concentration of 10 \mu g/ml) or a kanamycin (final concentration of 20 \mu g/ml) solution was added under the agar of each plate, and the plates were maintained under constant illumination for 10 days. The number of resistant colonies was scored at this time, and individual colonies were selected at random for further analysis.

The UV light source used in these experiments was a standard 15-W germicidal bulb (General Electric Co.). UV-light intensities were measured with a Blak Ray UV meter (model no. J225; UltraViolet Products Inc.).

**DNA isolation.** DNA was prepared as described previously for *Anacystis nidulans* (6) with several modifications noted below. Strain 6803 cells containing the Cm' or Km' marker were grown in 80 ml of growth medium supplemented with 10 \mu g of chloramphenicol or 20 \mu g of Kanamycin per ml, respectively. Wild-type cells were grown in the absence of antibiotics. Cells were harvested in late-log phase (3 to 4 days of growth) and washed once in growth medium, and the pellet was frozen in liquid nitrogen. The pellets were thawed on ice and suspended in 3 ml of 50 mM Tris hydrochloride (pH 7.8)-40 mM disodium EDTA, and 100 \mu l of 20% sodium dodecyl sulfate and 50 \mu l of 3 M sodium acetate were added. The mixture was then ground in liquid nitrogen with a prechilled mortar and pestle. The final powder was thawed at 37°C and extracted with phenol-CHCl3 three times. The nucleic acid was precipitated from the final aqueous phase with 2.5 volumes of ethanol at -20°C and, after centrifugation, suspended in TE (10 mM Tris hydrochloride [pH 7.8]-1 mM disodium EDTA). Prior to restriction enzyme digestion, the DNA samples were passed through a 1-ml spin-column containing Sephadex G-50 equilibrated in TE (21).

**Nucleic acid hybridizations.** Restriction enzyme digests of DNA obtained from wild-type and transformed strain 6803 cells were fractionated on 0.8% agarose gels and transferred to nitrocellulose filters by the method of Southern (27). Hybridization conditions were as described previously (15, 22). DNA probes used in DNA blot analyses were labeled with 32P by nick translation (21). DNA fragments used for labeling were eluted from 1.0% low-melting-point agarose gels by the method of Langridge et al. (18); plasmid probes were labeled directly.

## RESULTS

**UV-induced transformation.** Several plasmids that were initially tested for their ability to transform strain 6803 cells independently of UV irradiation failed to yield antibiotic-resistant colonies from 2 \times 10^8 cells on plates, but each yielded antibiotic-resistant colonies when cells preexposed to UV light were used as DNA recipients (Table 1). In this experiment, the cells were prepared as described in Materials and Methods, and some were irradiated with UV light at 32 J/m2 (approximately 20 s at 10 cm from the light source) in an open petri dish. The irradiated cells were immediately divided into samples and put into 1.5-ml microfuge tubes containing the DNA sample and further treated as described above. Cells not given DNA, as well as those exposed to UV in the absence of DNA, do not give any antibiotic-resistant colonies in platings of up to 2 \times 10^8 cells (Table 1). The

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Selection</th>
<th>No. of colonies after UV irradiation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>No DNA</td>
<td>Cm</td>
<td>—</td>
<td>&lt;5 \times 10^{-9}</td>
</tr>
<tr>
<td>No DNA</td>
<td>Km</td>
<td>—</td>
<td>&lt;5 \times 10^{-9}</td>
</tr>
<tr>
<td>pVAD1</td>
<td>Cm</td>
<td>44</td>
<td>2.2 \times 10^{-7}</td>
</tr>
<tr>
<td>pBR328</td>
<td>Cm</td>
<td>23</td>
<td>1.2 \times 10^{-7}</td>
</tr>
<tr>
<td>pMH1</td>
<td>Km</td>
<td>110</td>
<td>5.5 \times 10^{-7}</td>
</tr>
<tr>
<td>pBRMH20</td>
<td>Km</td>
<td>24</td>
<td>1.2 \times 10^{-7}</td>
</tr>
<tr>
<td>pBRMH6</td>
<td>Km</td>
<td>15</td>
<td>7.5 \times 10^{-8} *</td>
</tr>
<tr>
<td>pBRMH6</td>
<td>Cm</td>
<td>4</td>
<td>2.0 \times 10^{-7}</td>
</tr>
</tbody>
</table>

* In each instance, 1.5 \mu g of plasmid DNA was added to the cells. Other details of transformation procedures are given in the text.

* UV dosage was at 32 J/m2 (approximately 20 s at 10 cm from the UV source). The values indicate the number of chloramphenicol- or kanamycin-resistant 6803 colonies obtained per plate after transformation with the indicated plasmid. Generally, if cells were not irradiated with UV light, no colonies were detected in platings of 2 \times 10^8 cells.

* Frequencies are given in number of resistant colonies per recipient cell. In each trial, 2 \times 10^8 cells were used.

* —, No colonies detected in platings of 2 \times 10^8 cells.
plasmids tested (pVAD1, pMH1, pBR328, pBRMH20, and pBRMH6) were chosen for this study because they possess either the Cm' or Km' marker. It has been our experience that these antibiotics and their cognate resistance markers are the most effective and reliable for the detection of transformed cyanobacterial cells.

Although each plasmid tested gives antibiotic-resistant colonies when the cells are pretreated with UV light, the frequency of transformation varies with the particular plasmid examined. The basis of this phenomenon is not clear. However, there does not appear to be a correlation between the type of marker (chloramphenicol or kanamycin resistance) and the number of colonies obtained. In addition, we have observed that the transformation frequency can vary by as much as threefold between individual experiments. This variation may reflect subtle differences in the growth and treatment of the cells.

**Analysis of transformed cells.** Several of the UV-induced transformants were propagated under selection in liquid culture and further analyzed. In particular we wished to determine the following. (i) Is the introduced plasmid present intact and replicating autonomously, or has it (or segments of it) integrated into the host chromosome? (ii) Are the transformants phenotypically stable? (iii) Is the marker found in single or multiple copies? (iv) If integration of plasmid DNA has occurred, what is the organization of the cyanobacterial and plasmid sequences at the site of integration?

Two transformants have been examined in detail. One clone, designated strain 6803V, was obtained under chloramphenicol selection after transformation with pVAD1; the other clone, strain 6803,789, was obtained under chloramphenicol selection after transformation with pBR328.

Figure 1 shows the results of Southern blot analysis of DNA derived from the strain 6803V clone. The blot was probed with a 1.3-kilobase-pair (kbp) radiolabeled EcoRI fragment of pVAD1 containing the chloramphenicol acetyltransferase (CAT) gene. For the first three lanes of Fig. 1, wild-type strain 6803 DNA was digested with HindIII, BamHI, and PstI, respectively. No background cross-hybridization is observed. Lanes 4 through 6 show strain 6803V DNA digested with the same three enzymes, and lanes 7 through 9 show pVAD1 (the introduced plasmid), again digested with HindIII, BamHI, and PstI, respectively. Whereas lanes 1 through 3 show no cross-hybridization between the CAT probe and wild-type strain 6803 DNA, lanes 4 through 6 indicate that CAT DNA sequences are present in 6803V. In addition, the CAT gene is present as a single band in each lane and is located on different-sized restriction fragments than in pVAD1. Judging from the sizes of DNA fragments in the hybridizing bands, the CAT gene is apparently no longer in the intact pVAD1 plasmid and has integrated in a single copy into the host DNA. This possibility is supported by the results of similar blots probed with the entire introduced plasmid. pVAD1 hybridizes to two bands in PstI-EcoRI (Fig. 2, lane 3; a doublet), EcoRI (lane 4), HindIII-EcoRI (lane 5), and HindIII (lane 6) digests of 6803V DNA and to a single fragment in a BamHI-PstI digest (lane 2). Again, no cross-hybridization between the probe and wild-type 6803 DNA is seen (lane 1).

From the above and other hybridization data (not shown), we have been able to construct a restriction map (Fig. 3) of the integrated CAT gene and the DNA surrounding this site. A comparison of this mapped region with a restriction map of the corresponding region of pVAD1 (Fig. 3) indicates that approximately 1.0 to 2.1 kbp of pVAD1 has become inserted into the 6803 chromosome. This section comprises all of the CAT gene plus 200 to 1,300 base pairs of the flanking DNA sequences. This comparison confirms the conclusion that only a fragment of the introduced plasmid has integrated into host DNA.

The strain 6803,789 clone (obtained by transformation with pBR328 and chloramphenicol selection) has been likewise characterized. Figure 4 shows the results of a Southern blot of 6803,789 DNA digested with BamHI, PvuII, and EcoRI (lanes 2 through 4, respectively), pBR328 linearized with EcoRI (lane 5), and wild-type strain 6803 DNA digested with EcoRI (lane 1). The blot was probed with radiolabeled pBR328. As is found with 6803V, strain 6803,789 contains DNA sequences derived from the introduced plasmid which are now located on restriction fragments of sizes different from that on which they were introduced. (The plasmid pBR328 contains unique BamHI, PvuII, and EcoRI sites.) The probe shows no cross-hybridization with wild-type 6803 DNA (lane 1).

The strain 6803,789 clone differs in several respects from the 6803V clone. (i) From Southern blots of strain 6803,789 DNA probed with pBR328 (Fig. 4 and data not shown), it appears that the amount of plasmid DNA transferred into the 6803 chromosome is greater in 6803,789 (3.0 to 4.0 kilobases [kbp]) than it is in 6803V (1.0 to 2.1 kbp). Included are several
UV-INDUCED TRANSFORMATION OF A CYANOBACTERIUM

1. UV-induced transformation of strain 6803V DNA to the $^{32}$P-labeled pVAD1 DNA. 6803V DNA was digested with BamHI-PstI (lane 2), PstI-EcoRI (lane 3), EcoRI (lane 4), HindIII-EcoRI (lane 5), and HindIII (lane 6). Lane 1 contains wild-type 6803 DNA digested with HindIII. Size standards are indicated on the left.

2. Southern blot hybridization of strain 6803V DNA to $^{32}$P-labeled pVAD1 DNA. DNA isolated from 6803,789 (obtained by transformation with pBR328 and UV light) was digested with BamHI, PvuII, and EcoRI (lanes 2 through 4, respectively). Lane 1 contains wild-type 6803 DNA digested with EcoRI, and lane 5 contains pBR328 linearized with EcoRI. The filter was probed with $^{32}$P-labeled pBR328. Size standards are indicated on the left.

3. Restriction endonuclease map of the integrated CAT gene and cyanobacterial DNA surrounding this site in strain 6803V (top) and restriction map of the corresponding region of pVAD1 (bottom). Solid lines represent cyanobacterial sequences, and open lines represent pVAD1 DNA.

4. Results of Southern blot analysis of strain 6803,789 DNA. DNA isolated from 6803,789 (obtained by transformation with pBR328 and UV) was digested with BamHI-PstI, and HindIII (lanes 2 through 4, respectively). Lane 1 contains wild-type 6803 DNA digested with EcoRI, and lane 5 contains pBR328 linearized with EcoRI. The filter was probed with $^{32}$P-labeled pBR328. Size standards are indicated on the left.

FIG. 2. Southern blot hybridization of strain 6803V DNA to $^{32}$P-labeled pVAD1 DNA. 6803V DNA was digested with BamHI-PstI (lane 2), PstI-EcoRI (lane 3), EcoRI (lane 4), HindIII-EcoRI (lane 5), and HindIII (lane 6). Lane 1 contains wild-type 6803 DNA digested with HindIII. Size standards are indicated on the left.

FIG. 4. Results of Southern blot analysis of strain 6803,789 DNA. DNA isolated from 6803,789 (obtained by transformation with pBR328 and UV light) was digested with BamHI, PvuII, and EcoRI (lanes 2 through 4, respectively). Lane 1 contains wild-type 6803 DNA digested with EcoRI, and lane 5 contains pBR328 linearized with EcoRI. The filter was probed with $^{32}$P-labeled pBR328. Size standards are indicated on the left.
indicates that the slower growth rate is not the result of a mutation but rather is an effect related to the presence of the antibiotic which may not be completely inactivated. Although the coding regions of the CAT genes in pVAD1 and pBR328 are identical, the genes differ in the origin of their respective promoters. The CAT promoter from pVAD1 in 6803V is derived from a chloroplast gene (6), while that in 6803,789 from pBR328 is taken from the bacteriophage P1 (26). It is possible, therefore, that the slower growth of strain 6803,789 in chloramphenicol-containing media is a reflection of the relative strengths in 6803 of the chloroplast- and phage-derived promoters. Alternatively, cyanobacterial DNA sequences into which the CAT gene is integrated may have an effect on the expression of the gene, or the slower growth may be due to changes in the coding or promoter regions of the CAT gene.

Stability of transformed phenotype. We wished to determine whether the inserted DNA sequences are maintained in the isolated clones in the absence of chloramphenicol selection. Cultures of strains 6803V and 6803,789 were grown to late-log phase in medium lacking the antibiotic, and 5-ml samples were transferred from the flasks to fresh medium. After a total of three such transfers, the cells were challenged with 10.0 μg of chloramphenicol per ml. Both cultures resumed growth in the presence of the drug without any lag phase. Southern blots of DNA isolated from these cells indicate that the CAT gene is found on restriction fragments of the same size as in the original transformants (data not shown). From this we infer that even under nonselective growth conditions, both 6803V and 6803,789 are able to retain the introduced CAT gene.

Secondary transformation. We have also determined whether DNAs purified from the two primary transformants (6803V and 6803,789) are able to transform wild-type cells of strain 6803. The results are reported in Table 2. DNA isolated from each of these cell lines is able to transform wild-type 6803 cells at a high frequency of nearly 5.5 × 10⁻⁴ per recipient cell. Nearly equal numbers of colonies are obtained regardless of whether the cells are pretreated with UV light or not. DNA of one of these secondary transformants, obtained by transformation with DNA isolated from 6803V (designated 6803V-T), has been studied by Southern analysis. The CAT gene in 6803V-T is found on identical-sized HindIII, EcoRI, and BamHI-PstI restriction fragments as in the primary transformant (Fig. 5; compare lanes 1 and 2, 3 and 4, and 5 and 6). This fact strongly suggests that the transformed phenotype is achieved by homologous recombination between the DNA presented and the recipient chromosome. Strain 6803V DNA, when digested with various restriction enzymes with recognition sequences not found in the CAT gene, transforms wild-type cells at a 5- to 100-fold-lower frequency than does undigested DNA (Table 2). Digestion of the DNA with enzymes that cut within the coding or promoter regions of the CAT gene (EcoRI, HindIII, and PvuII) destroys the ability of the DNA to transform cells to chloramphenicol resistance (Table 2).

Optimization of UV-induced transformation of strain 6803. Several experiments have been performed to study the relationship between the intensity of UV exposure and the frequency of transformation. The intensity of the UV exposure has been varied from 8 to 192 J/m², and approximately 32 J/m² has been found to be optimal (Table 3). At this level of UV exposure, 90% of the irradiated cells survive. Longer exposures produce fewer transformants without a compensating decline in cell viability (Table 3).

In many cases, the mutational effects of UV light can be in part reversed by illuminating the cells after irradiation. This type of photoreactivation repair has been shown to occur in several cyanobacteria (12). To determine whether photoreactivation repair has any effect on the transformation frequency, UV-irradiated cells were maintained in total darkness with pVAD1 DNA for 6 h before being plated. Control samples were treated normally. Utilizing this protocol, a small but reproducible increase in the transformation frequency was observed with cells maintained in darkness (Table 3). The difference may be attributable to the absence of light-induced repair or may alternatively be related to the cessation of photosynthetic activity.

Molecular cloning of the integrated CAT gene and flanking

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TABLE 2. Transformation of wild-type strain 6803 cells with DNA isolated from strain 6803V and 6803,789 cells

<table>
<thead>
<tr>
<th>Source of donor DNA</th>
<th>DNA type</th>
<th>UV</th>
<th>No. of transformants</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>6803V</td>
<td>Not digested</td>
<td>–</td>
<td>1.1 × 10⁴d</td>
<td>6.1 × 10⁻⁴</td>
</tr>
<tr>
<td>6803,789</td>
<td>Not digested</td>
<td>–</td>
<td>9.6 × 10⁴d</td>
<td>5.3 × 10⁻⁴</td>
</tr>
<tr>
<td>6803V</td>
<td>Not digested</td>
<td>+</td>
<td>9.8 × 10⁴d</td>
<td>5.4 × 10⁻⁴</td>
</tr>
<tr>
<td>6803,789</td>
<td>Not digested</td>
<td>+</td>
<td>8.7 × 10⁴d</td>
<td>4.8 × 10⁻⁴</td>
</tr>
<tr>
<td>6803V</td>
<td>BamHI digest</td>
<td>–</td>
<td>2.0 × 10⁴d</td>
<td>1.1 × 10⁻⁴</td>
</tr>
<tr>
<td>6803V</td>
<td>PstI digest</td>
<td>–</td>
<td>1.1 × 10⁹</td>
<td>6.1 × 10⁻⁶</td>
</tr>
<tr>
<td>6803V</td>
<td>EcoRI digest</td>
<td>–</td>
<td>30</td>
<td>1.7 × 10⁻⁷</td>
</tr>
<tr>
<td>6803V</td>
<td>HindIII digest</td>
<td>–</td>
<td>22</td>
<td>1.2 × 10⁻⁷</td>
</tr>
<tr>
<td>6803V</td>
<td>PvuII digest</td>
<td>–</td>
<td>35</td>
<td>1.9 × 10⁻⁷</td>
</tr>
</tbody>
</table>

* See Table 1, footnote b, and text for experimental details.

* In these experiments, 4.6 μg of 6803V and 4.8 μg of 6803,789 DNA were used.

* Restriction enzyme digests were found to be complete as judged by agarose gel electrophoresis of samples from each digest.

* Estimate.
TABLE 3. Optimization of UV dosage and effects of light and dark treatment on transformationa

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>UV dosage (J/m²)</th>
<th>Treatmentb</th>
<th>Viable cell density</th>
<th>No. of transformants</th>
</tr>
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<tbody>
<tr>
<td>pVAD1</td>
<td>0</td>
<td>Light</td>
<td>2.2 (cells/ml) × 10⁶</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Light</td>
<td>2.1 (cells/ml) × 10⁶</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Light</td>
<td>2.0 (cells/ml) × 10⁶</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Light</td>
<td>2.0 (cells/ml) × 10⁶</td>
<td>38</td>
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<tr>
<td></td>
<td>48</td>
<td>Light</td>
<td>ND</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>Light</td>
<td>1.0 (cells/ml) × 10⁶</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>192</td>
<td>Light</td>
<td>0.3 (cells/ml) × 10⁶</td>
<td>—</td>
</tr>
<tr>
<td>pVAD1</td>
<td>0</td>
<td>Dark</td>
<td>2.2 (cells/ml) × 10⁶</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>16</td>
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<td>2.2 (cells/ml) × 10⁶</td>
<td>33</td>
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<td>48</td>
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<td>96</td>
<td>Light</td>
<td>1.0 (cells/ml) × 10⁶</td>
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</tr>
</tbody>
</table>

a See Table 1, footnote a, and text for experimental details.
b Light treatment samples were treated normally; dark treatment samples were maintained in total darkness with plasmid DNA for 6 h, plated, and placed in light.

DNA sequences. From a complete BamHI-PstI digest of strain 6803V DNA, a 5.7-kbp fragment containing the integrated CAT gene, flanking plasmid DNA, and adjacent host sequences has been cloned into the corresponding sites of pBR322 (Fig. 2, lane 2, and Fig. 3). The resulting plasmid, p1BP1, contains the 1.0- to 2.1-kbp insert and 3.6 to 4.7 kbp of the adjacent host sequence. All of the restriction sites deduced from Southern mapping lie within this BamHI-PstI fragment (Fig. 3) have been mapped to the cloned fragment as well.

To study the organization of the integration site in the wild-type and pVAD1-transformed cells, the isolated plasmid (p1BP1) was hybridized to HindIII, EcoRI, and BamHI-PstI digests of strain 6803 and 6803V DNA (Fig. 6). In the BamHI-PstI digest of 6803V DNA, the expected 5.7-kbp fragment corresponding to the cloned fragment itself is detected (Fig. 6, lane 2; compare with lane 3). In addition, another band of 10.5 kbp is observed. This latter fragment with no additional bands is seen in the wild-type DNA (lane 1). From this fact we infer that a sequence found at the integration site is duplicated in the 6803V chromosome. Alternatively, during integration, a rearrangement or duplication of a portion of the genome may have occurred at the insertion site, thus retaining the wild-type chromosome arrangement.

The HindIII and EcoRI digests confirm this observation (Fig. 6). In lane 7, HindIII-generated restriction fragments of 0.7, 1.9, 2.8, and 9.5 kbp are detected in the wild-type DNA by the p1BP1 probe. Each of these bands is also present in 6803V DNA (lane 8). In addition, two bands of 1.3 and 3.0 kbp are present. These and only these fragment, which are found in 6803V but not in 6803 DNA, are detected in a similar blot of HindIII-digested 6803V DNA that is probed with pVAD1 (Fig. 6, lane 9). Analogous results are obtained in Southern blots of EcoRI-digested wild-type and transformed DNA (Fig. 6). Each band detected in wild-type DNA (lane 4) is also found in 6803V DNA (lane 5), and the additional bands detected in the 6803V digest correspond to those detected by the pVAD1 probe in EcoRI-digested 6803V DNA (Fig. 6, lane 6). These results support the conclusion that the host DNA near the integration site is found repeated in 6803V or that integration is accompanied by a rearrangement or duplication of a part of the chromosome. This possibility is discussed further below.

The fact that p1BP1 contains the integrated CAT gene and the immediate flanking cyanobacterial DNA (i.e., that the clone does not contain multiple inserts) was verified by restriction mapping of the clone. This analysis showed that all of the recognition sites deduced from Southern mapping to lie within and surrounding the integration site are located on the cloned fragment. We do not believe that these results are due to contamination of 6803V cultures with untransformed cells because (i) the culture was maintained under constant selection at over twice the lethal dosage of chloramphenicol; (ii) the culture was begun from a single colony; (iii) the individual signals seen in Southern blots are of equivalent intensity, indicating that the detected fragments are present in nearly equimolar concentrations; and (iv) a culture begun from a single colony obtained from a liquid culture of 6803V, grown under chloramphenicol selection and similarly analyzed, gives identical results.

Transformation with cloned cyanobacterial and CAT sequences. The cloned fragment of strain 6803V DNA containing the inserted pVAD1 sequence and flanking cyanobacterial DNA (p1BP1) has been used to transform wild-type 6803 cells. A 1.8-μg volume of intact p1BP1 DNA yields over 4,000 transformants per plate (Table 4). Digestion of an equal quantity of the plasmid with BamHI and PstI (releasing the cloned insert) gives fivefold fewer transformants. This result may indicate that a supercoiled or circular DNA template is more effectively incorporated into host DNA than is the linearized fragment. It is also interesting to note that no significant change in the transformation frequency is observed when UV-irradiated cells are transformed with the intact or digested plasmid (Table 4). This observation indicates that, at least in this instance, strain 6803 DNA pas-

![Fig. 6. Duplicated DNA sequences in strain 6803V. Lanes 1 through 3 contain BamHI-PstI double digests of wild-type 6803 (lane 1) and 6803V (lanes 2 and 3) DNAs; lanes 4 through 6 similarly contain EcoRI digests of wild-type (lane 4) and 6803V (lanes 5 and 6) DNAs; and lanes 7 through 9 contain HindIII digests of wild-type (lane 7) and 6803V (lanes 8 and 9) DNAs. The first two lanes in each of the three sets (lanes 1, 2, 4, 5, 7, and 8) were hybridized with 32P-labeled p1BP1. Lanes 3, 6, and 9 were hybridized to 32P-labeled pVAD1. Size standards are indicated on the left.](http://jb.asm.org/Downloadedfrom)
Table 4. Transformation of strain 6803 cells with cloned cyanobacterial and CAT sequences (p1BP1) and with 6803V DNA

<table>
<thead>
<tr>
<th>Source of donor DNA*</th>
<th>DNA type</th>
<th>UV</th>
<th>No. of transformants (Cm')</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>6803V Undigested</td>
<td>-</td>
<td>1.2 x 10^6</td>
<td>4.1 x 10^-4</td>
<td></td>
</tr>
<tr>
<td>6803V Undigested</td>
<td>+</td>
<td>1.2 x 10^6</td>
<td>4.0 x 10^-4</td>
<td></td>
</tr>
<tr>
<td>p1BP1 Undigested</td>
<td>-</td>
<td>4.5 x 10^3</td>
<td>1.3 x 10^-5</td>
<td></td>
</tr>
<tr>
<td>p1BP1 Undigested</td>
<td>+</td>
<td>4.2 x 10^3</td>
<td>1.4 x 10^-5</td>
<td></td>
</tr>
<tr>
<td>6803V BamHI-PstI digest</td>
<td>-</td>
<td>2.6 x 10^1</td>
<td>8.5 x 10^-6</td>
<td></td>
</tr>
<tr>
<td>6803V BamHI-PstI digest</td>
<td>+</td>
<td>2.3 x 10^3</td>
<td>7.8 x 10^-6</td>
<td></td>
</tr>
<tr>
<td>p1BP1 BamHI-PstI digest</td>
<td>-</td>
<td>880</td>
<td>2.9 x 10^-6</td>
<td></td>
</tr>
<tr>
<td>p1BP1 BamHI-PstI digest</td>
<td>+</td>
<td>440</td>
<td>1.5 x 10^-6</td>
<td></td>
</tr>
</tbody>
</table>

*See Table, footnote b, Table 2, footnote c, and text for experimental details.

A volume of 5.2 μg of 6803V or 1.8 μg of p1BP1 DNA was presented to the cells.

Estimate.

saged through *E. coli* is able to transform UV-irradiated and nonirradiated cells at equal frequencies. As with the clone 6803V-T, transformation with the plasmid p1BP1 appears to occur by homologous recombination (unpublished observations). Analogous plasmids for the directed integration of DNA into cells of the cyanobacterium *A. nidulans* have been constructed by Williams and Szalay (30).

The results obtained with p1BP1 may be compared with results from similar experiments in which intact and digested 6803V DNA was used to transform wild-type cells. Transformation with 5.2 μg of undigested 6803V DNA occurs very efficiently at an estimated frequency of 4 x 10^-4 (Table 4). Whereas digestion of p1BP1 with BamHI and PstI lowers the transformation frequency by approximately fivefold, digestion of 6803V DNA with these enzymes lowers the frequency approximately 50-fold. This result may indicate that a longer stretch of flanking cyanobacterial sequences increases the probability that homologous recombination with the recipient chromosome will occur, resulting in a higher transformation frequency. As was observed with p1BP1, UV irradiation of the recipient cells had no effect on the transformation frequency with 6803V DNA (Table 4).

Based on the estimated genome size of strain 6803 of 2.75 x 10^9 base pairs (13), a quantitative comparison may be made between transformation with p1BP1 and 6803V DNA. The 5.7-kb BamHI-PstI fragment from 6803V containing the pVAD1 sequence represents approximately 0.21% of the 6803 genome. Therefore, of the 5.2 μg of 6803V DNA used to transform wild-type cells, 1.08 x 10^-2 μg is the 5.7-kb BamHI-PstI piece. Likewise, in transformation experiments with p1BP1, 1.15 μg of the fragment was present. Although in the latter experiments more of the cloned fragment was presented to the cells, fewer transformants were obtained (Table 4). Per microgram of fragment, this result corresponds to 760 transformants for the cloned fragment compared with 2.37 x 10^3 transformants for the identical fragment derived directly from 6803V DNA. Apparently, DNA obtained directly from the transformed cyanobacterium is able to transform wild-type cells at a 300-fold-higher frequency than the identical fragment when it is propagated in *E. coli* as a plasmid.

**DISCUSSION**

**UV-induced transformation.** In this report we have described a novel system for transforming cyanobacteria with DNA from heterologous sources which does not require the construction of biphasic cloning vectors and which does not rely on homologous recombination to achieve transformation. As was shown with the transformants 6803V and 6803V-T, plasmid DNA sequences can be directly incorporated into chromosomal DNA without the need for introducing a particular fragment into a shuttle or integrative cloning vector. This fact allows, for example, the direct introduction of heterologous DNA sequences into cyanobacterial cells to complement genetic lesions, analyze promoter sequences, and express specific gene products.

**Mechanism of transformation.** The cyanobacterial strain used in these experiments, *Synechocystis* sp. strain 6803, has been shown by Grigorieva and Shestakov to be transformable with DNA isolated from erythromycin- and ethionine-resistant 6803 cells (10). Therefore, the effect of UV on transformation documented here probably occurs at a step after the uptake of DNA into the cells. Two possible explanations may be put forth to explain the ability of UV-treated cells to be transformed with DNA of non-6803 origin. It is possible that UV pretreatment of strain 6803 induces an array of biochemical processes similar to those of the UV-induced SOS response of *E. coli* (29). Among the phenomena associated with the SOS response is the temporary alleviation of host-controlled restriction (4, 20). Similar events may occur in UV-irradiated 6803 cells, allowing any introduced DNA fragments to escape degradation by host restriction endonucleases. Although the presence of a restriction system has yet to be demonstrated in strain 6803, such systems are known to occur in several other cyanobacteria (12). Alternatively, the induction of an SOS-like response may lead to the increased synthesis of various proteins involved in recombination (such as the equivalent of the recA gene product) (20). It is conceivable that any foreign DNA (or fragments thereof) present in the cell at the same time as these proteins are active may become illegitimately incorporated into host DNA. The above models can be tested experimentally.

**Duplicated DNA sequences in strain 6803V.** A DNA sequence located at the insertion site in 6803V is found duplicated in the transformant genome. Specifically, hybridization of a cloned fragment containing the integrated plasmid sequence and flanking cyanobacterial DNA (p1BP1) to digests of 6803V DNA detects the expected bands as determined from mapping of the insertion site with pVAD1 as well as the three fragments detected by p1BP1 in digests of wild-type 6803V DNA. These observations indicate that a sequence found at the insertion site is found in more than one copy in 6803V DNA. This duplication may account for the viability of these cells.

Several interpretations of these data are possible. If the region into which the CAT gene has integrated exists in duplicate copies in wild-type 6803 DNA, then the CAT gene may have become inserted into one of these sites while the other site retained its normal configuration. A second explanation is that integration of plasmid DNA in this clone was accompanied by a duplication of DNA sequences near the insertion site, thus retaining the wild-type chromosome arrangement. A partial duplication of the DNA sequence at the integration site may also account for the observation that the CAT gene in strain 6803V is located on a smaller BamHI-PstI fragment than that which is detected by p1BP1 when hybridized to wild-type DNA digested with BamHI-PstI. A third possibility is that the initial UV irradiation of the cells may have induced a rearrangement and duplication of part of the chromosome, yielding the type of transformant...
studied here. Such UV-induced duplications have been observed in other bacteria (see reference 1 for a review).

Potential applications. The ability of strain 6803 to grow photoheterotrophically makes this cyanobacterium a particularly appealing strain in which to obtain and analyze photosynthetically transformed DNA. Astier et al. (2) have already exploited this potential and have partially characterized several such mutants. In addition, the ability of 6803 to be transformed (10) makes possible the complementation of such genetic lesions and also facilitates the isolation of the affected gene(s). If insertion of plasmid DNA into the chromosome of UV-irradiated cells occurs randomly (as is suggested by the observation that the introduced DNA is inserted into different sites in 6803V and 6803,789), then by using the proper selection scheme, one would expect to obtain auxotrophic and photosynthetic mutants in which the genetic lesion is tagged with a selectable marker. Alternatively, mutants obtained by this and other methods could be complemented by creating a library of wild-type 6803 DNA in a vector (such as pBR315) which, by homologous recombination, will insert at a specific site of the chromosome. In addition, the UV-induced transformation system described here can be utilized to introduce DNA sequences directly into 6803 cells without reconfiguring the fragment. This procedure allows, as mentioned, the introduction of DNA sequences into 6803 cells for the purposes of complementation of genetic lesions, analysis of promoter sequences, and expression of specific gene products.

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