Physical and Genetic Mapping of the SPO2 Prophage on the Chromosome of Bacillus subtilis 168

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It has been found by density transfer and genetic mapping experiments that prophage SPO2 is linked to the antibiotic resistance marker ery-1 in Bacillus subtilis 168.

A site for the attachment of the prophage bacteriophage SPO2 (4) has been found on the chromosome of Bacillus subtilis 168. From transduction studies with phage PBS1 (7), the site was found to be closely linked to the erythromycin locus, ery-1 (3).

An approximate physical localization of the SPO2 prophage on the genetic map was first obtained by performing density transfer experiments. These experiments were carried out by transferring light spores of B. subtilis 168 thyA thyB, requiring thymine and lysogenized with SPO2, to a deuterated medium and allowing them to germinate in the manner described by Smith et al. (6). At 30-min intervals, beginning 90 min after the density transfer, samples of germinating cells were exposed to \(^{3}H\)-thymidine for 30 min. Deoxyribonucleic acid (DNA) was isolated from each labeled sample and fractionated by preparative CsCl density gradient centrifugation (6).

If the prophage were integrated into the bacterial chromosome, the above procedure might be expected to generate a newly replicated chromosomal fraction enriched with respect to SPO2 DNA sequences and labeled with respect to radioactivity and density. The peak radioactive fraction from each CsCl preparative centrifugation was examined for its ability to transform various genetically marked strains and to hybridize specifically with mature, denatured SPO2 phage DNA immobilized on a nitrocellulose membrane filter (2). The hybridization experiments showed that the labeled DNA replicating between 120 and 150 min, which represented portions of the chromosome close to the origin of replication, contained the highest percentage of SPO2 DNA.

Specific base sequences. When the same fractions were used to transform suitably marked recipient strains, those DNA fractions which showed maximal hybridizing specificity with SPO2 DNA corresponded to a position on the map between the replication of markers cysA14 and argC4, although much closer to cysA14. [For additional information concerning strains and their map location, see Dubnau et al. (3)].

The map position of prophage SPO2 was defined more precisely by PBS1-mediated transduction and transformation experiments. Defective lysogens incapable of liberating SPO2 spontaneously were constructed either by lysogenizing B. subtilis 168 with temperature-sensitive mutants of SPO2 incapable of replicating at a restrictive temperature or by mutagenizing lysogens with nitrosoguanidine (1). PBS1 lysates, obtained from erythromycin (ery-1)-resistant donor cells lysogenized with wild-type SPO2, were used to transduce suitably marked recipient cells lysogenized with defective SPO2. Transductants, selected for markers located across the B. subtilis chromosome, were assayed for their ability to liberate SPO2 spontaneously, i.e., to produce a clear zone when stabbed into a lawn seeded with sensitive cells. PBS1-mediated transduction established that the linkage of SPO2 prophage to ery-1 was between 60 and 65%. In similar experiments, linkage of the prophage to cysA14 was 20%, whereas no linkage was found to purB6, leu-8, hisA1, trp-2, or metB5. When PBS1 lysates, prepared on non-lysogenized B. subtilis 168 ery-1 cells, were employed to transduce lysogenized cysA14 purA16 recipients, the transductants were "cured" of prophage as anticipated from the above linkage results. Curing occurred among 80 to 90% of the ery-1 transductants, 50% of the cysA14 transductants, and approximately 10% of the purA16 transductants.

To ascertain whether SPO2 was integrated on
the side of ery-1 proximal or distal to the origin of replication of the B. subtilis 168 chromosome, crosses involving ery-1, cysA14 sul [sulfanilamide resistant marker in strain SB3029-1 of Polsinelli (5)], and the prophage were carried out. A four-factor cross, mediated by PBS1 (Table 1), can best be interpreted by the order sul - cysA14 - ery-1 - SP02. The sul marker would be the one closest to the origin of replication, since Dubnau et al. (3) showed that cysA14 is closer than ery-1 to the origin of replication.

Table 1. Analysis of a four-factor transduction cross involving the ery, cysA14, SPO2, and sul markers

<table>
<thead>
<tr>
<th>sul</th>
<th>cysA14</th>
<th>ery-1</th>
<th>SPO2</th>
<th>No. of recombinants</th>
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</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>1</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
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<td>2</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>254&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The PBS1 lysate used in the transduction experiment was grown on B. subtilis 168 (SPO2) ery-1 sul cys<sup>+</sup>. The recipient strain was B. subtilis 168 (SP02d) cysA14, lysogenic for a nitrosoguanidine-induced defective SPO2 phage mutant which does not induce spontaneously and which is sensitive to erythromycin and sulfanilamide. The selected phenotype was sulfanilamide resistance.

<sup>b</sup> Donor and recipient phenotypes are denoted by ‘1’ and ‘0’, respectively.

<sup>c</sup> Total number of recombinants.

Attempts to prepare specialized transducing lysates of SPO2 by induction of genetically marked lysogens have thus far been unsuccessful.

The finding that phage SPO2 integrates close to ery-1 locates the prophage on the B. subtilis 168 chromosome fairly close to the origin of replication and next to, or within, the majority of the cistrons coding for ribosomal and transfer ribonucleic acid (6). Advantage could be taken of this finding to study the genetics and physiology associated with this region of the chromosome.

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LITERATURE CITED


