Plasmid-to-Plasmid Recombination in *Haemophilus influenzae*

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No recombination between plasmids was observed after conjugal transfer of a plasmid into a cell carrying another plasmid. Two types of such recombination took place after transformation, one type being Rec* dependent and suggesting a preferred site of recombination. The other much rarer type was at least partially Rec* independent.

In *Haemophilus influenzae*, plasmids containing cloned segments homologous to the chromosome interact with the chromosome only after transformation, and no interaction is observed after conjugation (1). The interaction after transformation results in the transfer of a genetic marker on the chromosomal segment from the plasmid to the chromosome or transfer of a marker on the chromosome to the plasmid (13). This interaction has been considered to result from the special processing of DNA after it enters the cell (2, 9); this processing does not take place after conjugal transfer (1).

To investigate plasmid-plasmid recombination, we constructed a plasmid, pMB5, conferring chloramphenicol resistance and containing part of a cloned gene conferring novobiocin resistance. The recipient cell contained a stable resident plasmid, pNov1s, coding for ampicillin resistance and novobiocin sensitivity from a larger fragment of the *H. influenzae* chromosome (12). Since a plasmid, pNov1, carrying the larger fragment but with the novobiocin resistance allele, complements a temperature-sensitive mutant of the gyrase B subunit gene of *Escherichia coli* (11), the *H. influenzae* gene must be around 3 kilobase pairs (kbp), like that of *E. coli* (8). Thus, although the cloned portion of pMB5 could transform for novobiocin resistance, it was only 1.7 kbp, which is too small to contain the entire gene for the gyrase B subunit.

The parent plasmid for pMB5 was the 10.4-kbp pMB1, a derivative of the cloning vector pDM2 (5) carrying a chromosomal insert. pMB1 conferred resistance to ampicillin (5 μg/ml) and chloramphenicol (4 μg/ml) and was able to transform *H. influenzae* to resistance to novobiocin (25 μg/ml) although it could not itself confer this resistance (1). The ampicillin gene was inactivated by digestion with *Pvu*I, and S1 nuclease digestion of the linear plasmid removed the cohesive ends. Religation was with T4 ligase at 12°C for 4 h and then at 4°C overnight. The resultant mixture was used to transform (14) competent rec-1 cells (10) to chloramphenicol resistance, and one clone was picked that was sensitive to ampicillin.

Neither pMB5 nor pNov1s could confer novobiocin resistance, but these two together could recombine to form a plasmid that does. Some of the results of triparental matings of the recombination-defective rec-2(pHD147), rec-1(pMB5), and wild-type BC200(pNov1s) are shown in Table 1. Transconjugants occurred at a frequency of ca. 5 × 10⁻⁴ when the mobilizing plasmid pHD147 was present. The lack of clones resistant to streptomycin, ampicillin, and novobiocin together indicated that there were among the streptomycin-resistant BC200 recipients no detectable cells carrying plasmids that conferred novobiocin resistance. Similar results were obtained with competent recipient cells. Gel electrophoresis of six transconjugant plasmid DNAs also showed no evidence of recombination. Thus, there was no detectable recombination between pMB5 and pNov1s after conjugation, despite the two regions of homology between them. One region is the 1.7-kbp piece of chromosomal DNA bounded by *EcoR*I sites, and the other is the whole of the RSF0885 portion of the plasmids (5.7 kbp), with the exception of a small deletion in the beta-lactamase gene of pMB5 (Fig. 1).

Table 1. Recombinant plasmids from crossing pMB5 and pNov1s

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Chloramphenicol</th>
<th>Novobiocin</th>
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</thead>
<tbody>
<tr>
<td>pMB5</td>
<td>25 μg/ml</td>
<td>25 μg/ml</td>
</tr>
<tr>
<td>pNov1s</td>
<td>25 μg/ml</td>
<td>25 μg/ml</td>
</tr>
<tr>
<td>pMB5</td>
<td>25 μg/ml</td>
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Results of transformation of three strains carrying pNov1s by pMB5 DNA are shown in Table 2. The recombination-proficient BC200 strain (3) was transformed to chloramphenicol resistance more than two orders of magnitude more efficiently than were the Rec⁻ strains. Furthermore, whereas in BC200(pNov1s) the frequency of ampicillin novobiocin double transformants was only slightly less than two orders of magnitude lower than the frequency of chloramphenicol transformants, this ratio was about four orders of magnitude lower in the Rec⁻ strains. Transformation of novobiocin resistance alone into BC200(pNov1s) was several orders of magnitude higher than transformation to ampicillin and novobiocin resistance together. These single transformants undoubtedly involved recombination of part of the novobiocin resistance gene of pMB5 into the recipient chromosome, as was earlier shown to occur with high frequency in the case of the plasmid pNov1 (13). These transformants were also chloramphenicol sensitive and thus did not carry pMB5.

The double resistance in BC200(pNov1s) could have arisen either because the partial novobiocin resistance gene from pMB5 was recombined into the chromosome while pNov1s conferred the ampicillin resistance or because this partial gene was recombined with the sensitive allele on pNov1s. To distinguish between these possibilities, cleared lysates (7) were made of four of the doubly resistant clones and used to transform rec-1, with selection for both markers. Since the rec-1 strain transforms at a very low frequency (10), the probability of finding double transformants resulting from transformation with one marker on the contaminating chromosomal DNA in the cleared lysate and another on the plasmid would be expected to be vanishingly small. Since such double transformants were found, we conclude that both markers were on a recombinant plasmid in these lysates. Twelve of the BC200(pNov1s) transformants resistant to ampicillin and novobiocin were also tested for chloramphenicol resistance, and all were sensitive, indicating that the clones did not contain any intact pMB5. Analysis of the cleared lysates of the four doubly resistant BC200(pNov1s)

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[Table 1 and Table 2 are not provided in the text, but are likely contained in the original document.]
TABLE 1. Resistance of cells to antibiotics after a triparental mating

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Kanamycin</th>
<th>Chloramphenicol</th>
<th>Streptomycin</th>
<th>Streptomycin plus chloramphenicol</th>
<th>Streptomycin plus ampicillin plus novobiocin</th>
</tr>
</thead>
<tbody>
<tr>
<td>rec-2(pHD147)</td>
<td>$7 \times 10^8$</td>
<td>$5.2 \times 10^8$</td>
<td>$1.6 \times 10^8$</td>
<td>$8.5 \times 10^8$</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>$6.5 \times 10^8$</td>
<td>$2.1 \times 10^8$</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

* Methods were described previously (1). DNase (125 µg/ml) was added to the mating mixture. Mating was done on a filter for 2 h.

* rec-2 (10) carried pMB5.

* rec-2/pMB5 (10) recipient carried pNov1s.

* Less than $2 \times 10^{-5}$ times the number of transconjugants (cells resistant to streptomycin plus chloramphenicol).

* Less than $2 \times 10^{-7}$ times the number of recipients.

Transformants on a gel (6) showed that all these clones contained plasmids the same size as pNov1 and pNov1s. Thus, at least the majority of the ampicillin-novobiocin-resistant transformants of BC200(pNov1s) shown in Table 2 resulted from recombination between pMB5 and pNov1s to produce pNov1.

Transformation of BC200(pNov1s) to chloramphenicol resistance took place at the highest frequency (Table 2) and could have arisen from establishment of pMB5. However, 10 of 12 chloramphenicol transformants analyzed on a gel carried only one plasmid, which was larger than either pMB5 or pNov1, was stable upon subculture even without selective pressure by chloramphenicol, and appeared to be the same size from all 10 transformants. The other two were the same size as pMB5. Partially purified preparations of all 10 large plasmids were subjected to digestion by BamHI, and they all showed the same gel pattern. Highly purified plasmid DNA was prepared from one of the clones and further analyzed by

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FIG. 1. Plasmids pNov1s and pMB5 and the hybrid plasmid pMB5-novs resulting from recombination between them. The drawings are to scale. Novs and novr, Regions carrying information for sensitivity and resistance to novobiocin, respectively. Heavy lines represent cloned chromosomal DNA. Lighter lines represent RSF0885 DNA. Dotted lines represent DNA of the plasmid p2265, used to construct the vector pDM2 (5), the parent plasmid of pMB5 (1).
restriction enzymes. Gel patterns of the digested large plasmid were obtained in comparison with those of pNovl5s and pMB5. Sizing in fragments was by way of mobility with those of DpnII and HpaI fragments of T7 DNA, kindly donated by S. A. Lacks and F. W. Studier. Digestion was with nuclease combinations PvuII-BamHI, BglII-BamHI, BstEII-PstI, and XhoI-EcoRI, as well as single-enzyme digestions with all of these plus PvuI and SstII. The data indicated that the 9.0-kbp chromosomal insert of pNovls had been inserted at or near the PvuII site of pMB5 (Fig. 1) and that all the rest of pNovls was lost, rendering the cells ampicillin sensitive. We called the plasmid pMB5-novs.

Further evidence for the structure of pMB5-novs was obtained by transforming strain BC200 and strain BC200 that was resistant to novobiocin (25 μg/ml) with the purified plasmid. Results (Table 3) demonstrate that both the novobiocin resistance and novobiocin sensitivity information was present on the plasmid, although the cells harboring the plasmid were not themselves novobiocin resistant. The frequency of transformation to novobiocin resistance was considerably lower than that for novobiocin sensitivity, in accord with our previous observation that the novobiocin transformation from plasmids increases markedly with increasing length of the chromosomal insert carrying the novobiocin marker (1). Here, the chromosomal inserts were 1.7 kbp in the case of the resistance marker and 9.0 kbp for the sensitivity marker (Fig. 1).

The fact that all 10 of the large plasmids were alike in their BamHI restriction pattern, although they arose from independent transformation events, suggests that there was a strong preference for a homologous region at or near the PvuII sites of pMB5 and pNovls for the interaction. Furthermore, the interaction of this type was much more probable than the recombination resulting in formation of pNov1 (Table 2), since the majority of chloramphenicol transformants contained this plasmid.

The formation of pMB5-novs appeared to be Rec+ dependent. Four chloramphenicol-resistant transformants of BC200 rec-1 (pNovls) by pMB5 and five of rec-2(pNovls) were analyzed on a gel. The plasmids were all the size of pMB5 and thus could not be recombinants of the type found to be prevalent among the BC200(pNovls) transformants to chloramphenicol resistance. However, the formation of pNov1 took place in the rec-1 strain. The only novobiocin-ampicillin resistant transformants of BC200rec-1(pNovls) by pMB5 were all six chloramphenicol sensitive, and gel analysis showed that all the plasmids were the same size as pNovls and pNov1. Thus, although the fraction of these transformants was very low (Table 2), it appeared that they were recombinant plasmids. The BC200rec-1 recombinants occurred at only 10⁻⁴ of the frequency that wild-type BC200 recombinants did. This is several orders of magnitude higher than chromosomal recombination in rec-1 strains, and phage recombination is undetectable in these strains (10). Thus, it appears that the type of interaction resulting in pNovl formation was not the usual Rec⁺-dependent recombination. The data suggested that the mechanisms of the two types of recombination, one resulting in pMB5-novs and the other in pNov1, were very different.

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**LITERATURE CITED**


11. Setlow, J. K., E. Cabrera-Juárez, W. L. Albrighton, D. Spikes,

