Analysis of Mobilization Elements in Plasmids from Shigella flexneri

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The mobilization properties of three plasmids were examined after cotransfer from Shigella flexneri to Escherichia coli. The largest plasmid, pCN1, was shown to be a conjugative R factor that could promote its own transfer and allow cotransfer of a 4.1-kilobase plasmid, pCN3; mobilization of the third plasmid, pCN2 (6.3 kilobases), required the presence of both pCN1 and pCN3. Sequences from pCN2 and pCN3 homologous to the bom (basis of mobilization) sites of ColE1 and pBR322 were localized by analysis of site-specific deletion derivatives generated in vivo during the transfer of composite plasmids and were characterized by DNA sequencing.

The intercellular transfer of bacterial plasmids plays an important role in the spread of antibiotic resistance. For nonconjugative plasmids, two essential elements that allow their comobilization in the presence of a conjugative plasmid have been genetically defined (reviewed in references 4 and 13). One of these is a DNA sequence referred to as a bom (basis of mobilization) site (7, 12). It has been postulated that this sequence is specifically recognized and nicked by a plasmid-encoded mobilization protein, referred to as mob, to prime the plasmid for mobilization by a single-strand transfer mechanism (12). Many of the molecular details of this process remain to be characterized.

We have examined the mobilization characteristics of a set of three plasmids, pCN1, pCN2, and pCN3, that were originally transferred to Escherichia coli K-12 by conjugation from a multiply-resistant clinical isolate of Shigella flexneri 2a. Trimethoprim and streptomycin-spectinomycin resistance genes on pCN1 and streptomycin and sulfanilamide resistance genes on pCN2 were used as selective markers in plasmid transformations (6) to construct strains containing either single plasmids or various combinations of plasmids. Conjugal transfer of plasmids from these strains to recipient cells was then examined. Plasmid pCN1, which has a size of ca. 45 kilobases (kb), was found to be self-mobilizable; the transfer frequency was not significantly affected by the presence of pCN2 (6.3 kb) or pCN3 (4.1 kb) in the donor strain. Cotransfer of pCN3, which was monitored by a minilysate plasmid-screening method (2) because no resistance phenotype could be assigned to the plasmid, was found to occur at a high frequency (ca. 50%) from strains carrying pCN1 and pCN3.

However, when the mobilization behavior of pCN2 was examined, the results shown in Table 1 were obtained. In these experiments, the transfer of streptomycin or sulfanilamide resistance or both to recipient cells was measured; the former selection identified transconjugants that received either pCN1 or pCN2, whereas the Su' phenotype was specific for pCN2. When the donor cells carried pCN1, pCN2, and pCN3, the frequency of detection of Su' transconjugants was about 15% of that found for Sm' transconjugants. On the other hand, when pCN3 was not present in the donor, the transfer of pCN2 (Su') was reduced by a factor of at least 10^3. Thus, mobilization of pCN2 during pCN1-mediated conjugation is clearly dependent on the presence of pCN3. Thus, on the basis of these experiments, pCN1 can be classified as self-mobilizable, pCN3 as comobilizable, and pCN2 as conditionally comobilizable, requiring the presence of pCN3 (as well as pCN1).

Several lines of evidence indicate that the mobilization of pCN2 does not involve or require a recombination event between pCN2 and pCN3. First, gel electrophoretic analysis of DNA from randomly selected transconjugants from the first mating in Table 1 showed only the monomeric forms of pCN3 or pCN2 or both and not recombinant molecules (data not shown). Furthermore, transconjugants that acquired pCN2 did not always contain pCN3. For example, of 10 transconjugants selected with sulfanilamide plus streptomycin, all contained pCN1 and pCN2, but only five also carried pCN3. Finally, it was found that the relative frequency of pCN2 mobilization (10 to 15% of that of pCN1-containing Sm' colonies) from a recA donor strain was equivalent to that found from recA^+ strains. Thus, we conclude that the transfer of both pCN2 and pCN3 is dependent on a trans-acting mobilization protein encoded by the latter plasmid.

It has been shown that the transfer of plasmids with two bom regions frequently results in sequence-specific recombination events involving these regions; these results can be explained by models involving the transfer of single-stranded DNA after nicking (3, 10, 11). Chimeric plasmids were constructed and used in mobilization assays in an attempt to further define the functional bom sites of pCN3 and pCN2. Recombinant plasmids were constructed by ligating BglII-linearized pCN3 DNA into pBR322 cut at the unique BamHI site (located within the Tc^r gene) and selecting for Ap^T Tc^r transformants (8). Restriction mapping was used to identify recombinants carrying the input sequences in both relative orientations. These recombinants were designated as pCN3-6a and pCN3-6b; the relevant features of these constructs are summarized in Fig. 1. Each of the recombinants includes a functional mobilization protein (mob) region and two bom sites. These plasmids were introduced by transformation into cells carrying the conjugative plasmid pCN1 and subsequently transferred to recipient cells in mating experiments.

Plasmid DNA prepared from 10 transconjugants from each of the two matings was digested with AvaI and analyzed by agarose gel electrophoresis (Fig. 2). The restriction

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fragment patterns of all 10 plasmids from transconjugants from the mating with cells containing pCN3-6b (panel A) were identical to that of the parental plasmid shown in lane C. However, as shown in panel B, 80% of the transconjugants from the mating with cells containing pCN3-6a carried plasmids with a consistent alteration in the restriction fragment pattern; rather than the normal pattern of 3.6-, 3.5-, and 1.4-kb fragments, the transferred plasmids had only two fragments of 4.6 and 1.4 kb. Within the limits of subsequent restriction mapping analysis, this appeared to be the result of a precise deletion of all sequences between the AccI site within the bom region of pBR322 and the unique AccI site in the pCN3 insert (Fig. 2). A single AccI site remained in this derivative plasmid (pCN3-6c), and subsequent experiments showed that it still contained a functional bom sequence and could be transferred intact to recipient cells during conjugation. Digestion of pCN3-6a with AccI, followed by religation under dilute conditions, led to the isolation of plasmid pCN3-6d (Fig. 1), with properties identical to those of the in vivo-generated pCN3-6c. A similar set of experiments was conducted with recombinants constructed by fusing pCN2 and pBR322 at their unique EcoRI sites in both relative orientations and, again, orientation-dependent deletions occurred between the AccI site within the bom region of pBR322 and sequences near the unique AccI site in pCN2 to generate a derivative designated pCN2-6c.

To examine the degree of sequence conservation in the bom regions, we determined the DNA sequences of pCN2 and pCN3 in both directions from their unique AccI sites by the method of Maxam and Gilbert (9) and compared them with the published sequences for the analogous regions of pBR322 (5) and ColEl (1). Perfect sequence homology between pCN3 bom and pBR322 bom extended from 51 base pairs (bp) 5' of the AccI cleavage site to 13 bp 3' of the AccI cleavage site (Fig. 3). Similarly, identical pCN2 bom and pBR322 bom sequences extended 11 bp 5' and 11 bp 3' of this site. Outside these regions, the extent of sequence divergence increased rapidly.

An analysis of the DNA sequence of the hybrid bom site in pCN3-6c, which was created in vivo by a conjugation-dependent deletion event (Fig. 1), indicated that the recombination event must have occurred within the 64-bp stretch of perfect homology, because 5' of this region the sequence was identical to that of pCN3, and on the 3' side of this block the derivative matched pBR322 (Fig. 3). Similarly, the endpoints of the deletion in the derivative pCN2-6c could be localized within the 22-bp homologous region that includes the nick site of pBR322. These results are consistent with a model in which nicking at both bom sites within the original chimeric plasmids results in the transfer of only the selectable DNA sequences between the two sites and imply that the nick sites for pCN2 and pCN3 must be at the same

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**TABLE 1. Mobilization of pCN2 from donor cells carrying pCN3**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Plasmids</th>
<th>No. of transconjugants per 10⁶ recipients</th>
<th>Sm'</th>
<th>Su'</th>
<th>Sm' Su'</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHB55</td>
<td>pCN1, pCN2, and pCN3</td>
<td>17,000</td>
<td>2,500</td>
<td>1,800</td>
<td></td>
</tr>
<tr>
<td>HHB89</td>
<td>pCN1, pCN2, and pCN3</td>
<td>9,400</td>
<td>1,200</td>
<td>1,400</td>
<td></td>
</tr>
<tr>
<td>HHB90</td>
<td>pCN1 and pCN2</td>
<td>19,000</td>
<td>&lt;0.07</td>
<td>&lt;0.07</td>
<td></td>
</tr>
<tr>
<td>HHB91</td>
<td>pCN1 and pCN2</td>
<td>27,000</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

* Mobilization was monitored by selecting for transconjugants exhibiting the appropriate antibiotic resistance phenotypes after overnight mating at 37°C of plasmid-containing donor cells (derivatives of wild-type W3110) and nalidixic acid-resistant recipient cells at a 1:5 ratio in L broth without aeration. Drug concentrations used in selective agar plates were 50 μg/ml for nalidixic acid, 200 μg/ml for streptomycin, and 1 mg/ml for sulfanilamide.

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**FIG. 2.** *AvaI* restriction fragment patterns of plasmids from transconjugants after matings with donor cells containing pCN3-6b (A) or pCN3-6a (B). DNA from colony-purified transconjugants was isolated by the alkaline extraction method (2), digested with *AvaI*, and subjected to electrophoresis on 1% agarose slab gels. The numbers on the sides represent sizes in kilobase pairs of fragments derived from the parental plasmids or from in vivo-generated derivatives. Lanes C show the normal patterns for the parental plasmids, and lanes M contain size markers. Other fragments visible in the gel are derived from the coresident conjugative plasmid pCN1 and small amounts of chromosomal DNA present in the preparations.
location as that for pBR322, because no bases in these regions were added or deleted as a result of the resolution event.

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LITERATURE CITED