NOTES

Fertility Inhibition of RP1 by IncN Plasmid pKM101

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IncN plasmids, including pKM101, strongly inhibit the conjugal transfer of cohabiting IncP plasmids. We localized the pKM101 DNA sufficient for this phenomenon to a 1.1-kilobase region (denoted fip). Two fip-deficient Tn5 insertion derivatives of pKM101 were isolated; neither affected other pKM101-mediated functions. fip did not inhibit either the synthesis of the IncP plasmid’s sex pilus or its ability to mediate entry exclusion against other IncP plasmids.

A number of instances have been reported of an inhibition of conjugal transfer of one plasmid by a second plasmid cohabiting the same cell. Six distinguishable types of fertility inhibition of F by various plasmids have been described (11). Most, but not all, of these systems of transfer inhibition seem to be mediated at the level of transcription. The best studied of these is mediated by the F-like plasmid R100, which represses the transcription of the entire tra operon of F, as well as its own transfer operon (3, 4).

pKM101 is a 35.4-kilobase (kb) self-transmissible plasmid of the N incompatibility group which has received considerable attention due to its ability to enhance the susceptibility of its host to mutagenesis by a wide range of mutagenic agents (8, 10). We have also studied a plasmid-encoded endonuclease (12), the plasmid’s system of conjugal transfer and entry exclusion, and other plasmid-mediated phenomena (13–15). Although Olsen and Shipley (7) reported results to the contrary, Willetts (personal communication) found that pKM101 inhibits the conjugal transfer of coresident IncP plasmids. We have confirmed Willetts’ observation, using plasmid RP1-amp1 (an Ap’ derivative of RP1 obtained from S. Levy).

Overnight cultures of AB1157(RP1-amp1) and AB1157 (RP1-amp1)(pKM101), were spread with a toothpick onto a lawn of NG624 (a trp’ strain) that had been freshly spread on an LB plate; AB1157 is a multiple auxotroph (12). After overnight growth, this plate was replicated onto an minimal glucose plate containing tryptophan and 20 μg of tetracycline per ml. AB1157(RP1-amp1)(pKM101) yielded no transconjugants on such plates, whereas AB1157(RP1-amp1) gave rise to a confluent patch of transconjugants.

To assay this phenotype quantitatively, 200 μl of mid-log-phase donor cultures was added to 4 ml of log-phase cultures of NG624, collected onto membrane filters (Millipore Corp.), and placed onto prewarmed LB plates. After 1-h matings cells were suspended in saline and plated on minimal media containing tryptophan and tetracycline. RP1-amp1 transferred approximately 10,000-fold less efficiently in the presence of pKM101 than in its absence. Even this may be a conservative estimate, since a small fraction of such donor cells may lack pKM101.

In an effort to localize this locus (denoted fip, for fertility inhibition of IncP plasmids), we tested a number of insertion and deletion derivatives of pKM101 as well as plasmids containing cloned fragments of pKM101 DNA. These tests included two plasmids shown in Fig. 1: (i) pGW1502, a derivative of pBR322 containing pKM101 DNA from the SalI-2 site to the point of insertion of 11035::Tn5 (12); and (ii) pGW1550, a derivative of pKM101 having a deletion of the DNA from the SalI-1 site and the point of insertion of Ω630::Tn5. Both of these plasmids were Fip’, indicating that the gene responsible must lie within the 3.0-kb region between Ω630::Tn5 and Ω1035::Tn5.

Libraries of random Tn5 insertion derivatives of both pKM101 and pGW1502 were made by using a procedure described elsewhere (12), and these derivatives were screened for Fip deficiency by the patch mating assay described above. Two Fip’ derivatives of each plasmid were isolated. All four of these insertions (fip-1204::Tn5, fip-1206::Tn5, fip-1212::Tn5, and fip-1215::Tn5) lie within this 3.0-kb region. We assayed the Fip phenotype of these four insertion derivatives quantitatively as described above and found that none of them contained any demonstrable residual levels of fertility inhibition. The region containing these insertions is flanked by the insertions nuc-1210::Tn5 and Ω1246::Tn9 (Fig. 1). We found that both of these insertions were Fip’, and we therefore believe that the fip gene(s) lies in the 1.1-kb interval which separates them. Both pKM101 fip-1204::Tn5 and pKM101 fip-1206::Tn5 were found to be fully proficient in both conjugal transfer and nuc-encoded endonuclease activity (data not shown).

A subset of the RP1 genes necessary for conjugal transfer are also required for sensitivity to the donor-specific phage PRD1 (1), and we have found that fip does not alter the sensitivity of the host to this phage. Such an experiment is complicated by the fact that PRD1 infects cells harboring not only IncP plasmids, but also cells containing IncN plasmids (including pKM101) or IncW plasmids (2). We have found that the pKM101 DNA required for sensitivity to PRD1 is limited to traA, traB, traC, traD, traE, traF, and traG (Fig. 1) (13). We therefore used pGW1550 for this test, since these genes have been deleted in this plasmid. Strain AB1157(pGW1550)(RP1-amp1) was fully sensitive to PRD1, indicating that fip does not affect the synthesis of the IncP sex pilus.

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IncP plasmids have been found to render their hosts poor conjugal recipients with genetically distinguishable IncP plasmids (6). We have found that this property is not affected by a cohabiting IncN plasmid. This was determined by measuring the efficiency of transfer of R751 (a trimethoprim-resistant IncP plasmid, provided by J. Shapiro) into (i) AB1157, (ii) AB1157(RP1-amp1), (iii) AB1157(RP1-amp1) (pKM101), and (iv) AB1157(pKM101). The data shown in Table 1 indicate that the effects of pKM101 and RP1 on the efficiency of transfer of R751 are roughly multiplicative, which would not be expected if fip inhibited the entry exclusion genes of RP1-amp1.

The finding that fip does not affect either pilus synthesis or entry exclusion of a cohabiting IncP plasmid suggests that fip acts in a different way from R100-mediated inhibition of F transfer. Since R100 represses the entire F transfer operon, it also abolishes both the host sensitivity to male-specific phage and the activity of the entry exclusion determinants traS and traT (12).

Recently it was reported that F also inhibits the fertility of RP1 (9). The locus responsible for this effect does not alter the sensitivity of the host to phage PRD1, nor does it alter RP1-mediated entry exclusion of IncP plasmids. In these respects, the F-mediated system therefore appears similar to that of pKM101.

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**LITERATURE CITED**


**TABLE 1. Entry exclusion of an IncP plasmid mediated by RP1-amp1 and pKM101**

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Transconjugants per donor-h</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1157</td>
<td>1.5</td>
</tr>
<tr>
<td>AB1157(RP1-amp1)</td>
<td>2.5 x 10^{-4}</td>
</tr>
<tr>
<td>AB1157(RP1-amp1)(pKM101)</td>
<td>3.8 x 10^{-5}</td>
</tr>
<tr>
<td>AB1157(pKM101)</td>
<td>2.6 x 10^{-1}</td>
</tr>
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*Recipient strains were mixed with E5689(R751) in a ratio of 100:1, deposited on Millipore filters, incubated for 1 h at 37°C, suspended, and plated on media selective for transfer of trimethoprim resistance into AB1157.