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Mutagenesis of Dimeric Plasmids by the Transposon γδ (Tn*1000*)

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The *Escherichia coli* F factor mediates conjugal transfer of a plasmid such as pBR322 primarily by replicative transposition of transposon γδ (Tn*1000*) from F to that plasmid to form a cointegrate intermediate. Although resolution of this cointegrate always yields a plasmid containing a single γδ insertion, the occasional recovery of transposon-free plasmids after conjugal transfer has led to alternative hypotheses for F mobilization. We show here that γδ-free plasmids are found after F-mediated conjugal transfer only when the donor plasmid is a dimer and the recipient is Rec$^+$.

Most widely used cloning vectors, such as pBR322 and its derivatives, are nonconjugative (1). The *Escherichia coli* F factor mediates their conjugal transfer by replicative transposition of γδ (Tn*1000*) from F to the plasmid to form a cointegrate. In the recipient, this cointegrate is resolved by site-specific recombination to yield a parental F factor plus the plasmid carrying one copy of γδ (Fig. 1). γδ is widely used as a mutagen to delimit cloned genes (3, 7, 8) and has also been used to provide mobile primer-binding sites for dideoxy sequencing of target DNA (3, 10; L. D. Strausbaugh, M. T. Bourke, M. D. Sommer, M. E. Coon, and C. M. Berg, submitted for publication). However, several reports describe frequent recovery of nonconjugative plasmids that lack γδ after F-mediated transfer (6, 9). Because the usefulness of γδ for genetic and molecular analyses of cloned DNA is compromised by recovery of γδ-free plasmids, we reexamined the basis of this phenomenon. The data presented here demonstrate that inadvertant use of dimeric starting plasmids can result in recovery of γδ-free plasmids in a *rec*$^+$ recipient and that their recovery is avoided by the use of starting plasmids that are monomers.

For descriptions of the *E. coli* K-12 strains used in this study, see the footnotes to Tables 1 and 2. Plasmid pBR325 is a 6.0-kilobase (kb) derivative of pBR322 containing the determinants for ampicillin resistance (*amp*), chloramphenicol resistance (*cam*), and tetracycline resistance (*tet*) (1). Plasmid pIF005 is an 8.6-kb derivative of pBR322 containing *amp* and 4.6 kb of the *E. coli avtA* region (13). Transposon γδ, a member of the Tn*3* family, is 6 kb long (3; R. Reed, unpublished data). γδ mutagenization was accomplished by selecting for conjugal transfer of the plasmid on Lennox complex medium plus thymine (0.079 mM), chloramphenicol (20 μg/ml), and streptomycin (200 μg/ml) for pBR325 or ampicillin (100 μg/ml) and kanamycin (30 μg/ml) for pIF005, as described previously (13).

In matings involving pBR325 (*amp*$^+$ *cam*$^+$ *tet*$^+$) monomers, more than half of the Cam^r exconjugants had an insertion in one of the other antibiotic resistance genes (Table 1). All plasmids were found to be 12 kb long (Table 2; Fig. 2, lane 2), indicating that they are composed of one copy of γδ inserted into one copy of pBR325.

When the matings were repeated with dimers, all of the Cam^r exconjugants tested were both Tet^r and Amp^r (Table 1). Plasmids recovered from the mating with the *recA* recipient were all 18 kb long (Fig. 2, lane 4), but plasmids from the mating with the *rec*$^+$ recipient were 6, 12, and 18 kb long (Fig. 2, lane 5; Table 2).

Plasmid size cannot be used to distinguish pBR325::γδ monomers from γδ-free pBR325 dimers, because γδ and pBR325 monomers are each about 6 kb long. Therefore, two further tests were used. (i) Total plasmid DNA from each of the four exconjugants that contained only 6- and 12-kb bands (Table 2) was digested with *Bam*HI, which cuts both pBR325 and γδ once (1, 7). Only a single 6-kb linear fragment was obtained, indicating that γδ was absent from these plasmids. (ii) The 12-kb band from one exconjugant that contained 6-, 12-, and 18-kb plasmids was transformed into CBK869 (*recA*), and the resultant plasmids were digested with *Eco*RI, which cuts pBR325 once (1) and γδ twice (7). A single 6-kb fragment was obtained from two transformants, and three fragments were obtained from two other transformants, indicating that the 12-kb band contained both pBR325::γδ monomers and γδ-free dimers. Thus, when the donor plasmid was a dimer, Rec$^+$ exconjugants contained a mixture of four plasmid types: monomers lacking γδ (6 kb), monomers containing γδ (12 kb), dimers lacking γδ (12 kb), and dimers containing one copy of γδ (18 kb).

To confirm that the plasmid types recovered in *rec*$^+$ cells were breakdown products of 18-kb dimeric pBR325::γδ plasmids, a stable 18-kb plasmid recovered from a mating between a dimeric donor strain and a *recA* recipient (Table 2, line 3; Fig. 2, lane 4) was transformed into a *rec*$^+$ strain. Plasmids from single colonies were found to contain 6-, 12-, and 18-kb bands (Fig. 2, lane 6). Thus, the variety of plasmid

TABLE 1. Phenotypes of cells following conjugal transfer of pBR325[a]

Plasmid in donor[b]	Recipient genotype[c]	No. (%) of Amp^s colonies	No. (%) of Tet^s colonies
Monomer	*recA*	192 (48.0)	36 (9.0)
Monomer	*rec*$^+$	188 (47.0)	26 (6.5)
Dimer	*recA*	0	0
Dimer	*rec*$^+$	0	0

[a] Cam^r Str^r exconjugants were selected, and 400 colonies were tested per mating.
[b] Donor strain: MG1063 (F$^+$ *recA56 thi*) (7).
[c] *rec*$^+$ strain: CBK861, *rpsL109* transductant of CBK699 [Δ(*pro-lac*) *thyA*] (14), using P1 grown on χ697 (4). *recA* strain: CBK869 [Δ(*srl-recA*)*306*::Tn*10* transductant of CBK861, using P1 grown on MC601, Δ(*srl-recA*)*306*::Tn*10 ara* Δ(*pro-lac*)XIII, obtained from Jeffrey Miller].

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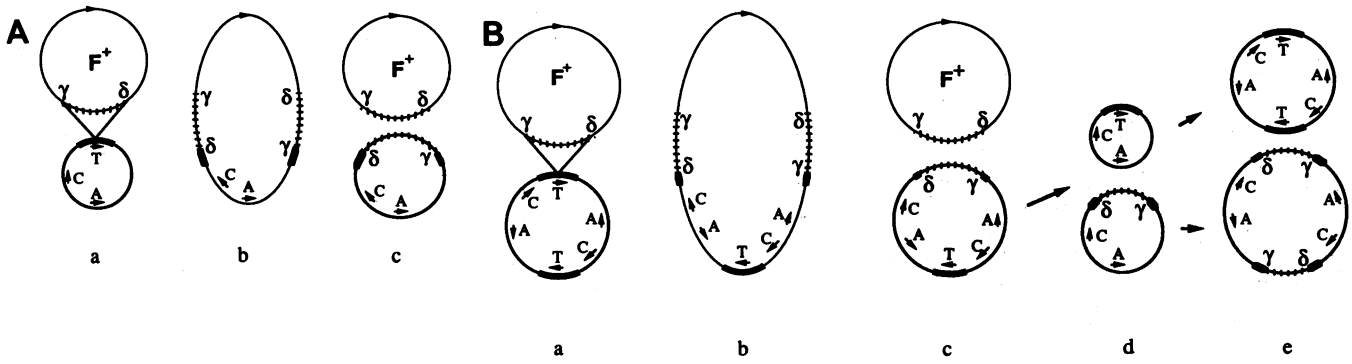

FIG. 1. Transposition of γδ from the F factor to pBR325, cointegrate resolution, and subsequent *rec*-mediated recombination. (A) Monomeric target plasmid. (a) γδ transposition to a site in the *tet* gene. (b) Cointegrate formed by replicative transposition of γδ from F to pBR325. (c) Resolution products: F factor and pBR325::γδ. (B) Dimeric target plasmid. (a) γδ transposition to a site in one copy of the *tet* gene. (b) Cointegrate formed by replicative transposition of γδ from F to a pBR325 dimer. (c) Resolution products: F factor and a heterodimeric pBR325::γδ plasmid. (d) *rec*-mediated breakdown products of heterodimeric pBR325::γδ. (e) Homodimers derived from breakdown products shown in diagram d. Arrowhead, *oriT* of F^+ factor. Tetracycline (T), chloramphenicol (C), and ampicillin (A) resistance genes of pBR325 are shown. Arrows, Direction of transcription of resistance genes; heavy lines, *tet* target gene; hatched lines, γδ; γ and δ, gamma and delta ends, respectively, of γδ.

sizes found following F-mediated transfer of a dimer to a rec^+ recipient resulted from *recA*-mediated homologous crossing over in the recipient, as diagrammed in Fig. 1B.

Because γδ and pBR325 are both 6 kb long; certain plasmid classes are distinguishable only by restriction analysis. Therefore, we tested γδ insertion into pIF005, which is 8.6 kb long. γδ mutagenization of monomers yielded exclusively 14.6-kb pIF005::γδ exconjugants that contain one copy of pIF005 and one copy of γδ (13), whereas mutagenization of dimers yielded exclusively 23-kb plasmids if the recipient was *recA* but 8.6-, 14.6-, 17-, and 23-kb plasmids if the recipient was rec^+ (Table 2, right). The 23-kb band found in both *recA* and rec^+ exconjugants corresponds to a heterodimer with one γδ insert, while the 14.6-kb band corresponds to a monomer with one γδ insert and the 8.6- and 17-kb bands correspond to pIF005 monomers and homodimers, respectively, that lack γδ (Fig. 1B).

pBR322-derived plasmids may occur as monomers, dimers, or higher oligomers in rec^+ cells of *E. coli* K-12 (2, 12). There is generally a correlation between plasmid size and the frequency of oligomers in the population, with most plasmids larger than 7 kb existing as oligomers (5). Consequently, unless care is taken to assure that the plasmid being used is a monomer, many plasmids, especially among those containing large inserts, will be oligomers. This is not important if the plasmid is cut with restriction enzymes, but as we show here, it can seriously interfere with the efficiency with which γδ-mutagenized plasmids can be recovered.

We have shown that when F catalyzes the transfer of a plasmid from a *recA* donor into a *recA* recipient after resolution, all exconjugants contain the parental plasmid with one γδ insert (Table 2; Fig. 1Ac and Bc). If a dimeric starting plasmid is used, this exconjugant plasmid contains two plasmid moieties, only one of which has a γδ insert (Fig. 1Bc). If the recipient is rec^+ or if this plasmid is transformed into a rec^+ cell, a variety of additional plasmid types are recovered (Table 2); homologous recombination results in both mutagenized and γδ-free monomers (Fig. 1Bd), and these can subsequently recombine to yield dimers that are indistinguishable from the parental unmutagenized dimer (Fig. 1Be, top). Dimers derived from γδ-carrying monomers (Fig. 1Be, bottom) (24 kb from pBR325::γδ and 29 kb from

TABLE 2. Plasmids isolated from exconjugants

Plasmid in donor[a]	Recipient genotype[b]	No. of colonies with pBR325 plasmids with sizes of[c]:				No. of colonies with pIF005 plasmids with sizes of[d]:				
		12 kb	18 kb	6 and 12 kb	6, 12, and 18 kb	14.6 kb	23 kb	8.6 and 17 kb	8.6, 14.6, and 17 kb	8.6, 14.6, 17, and 23 kb
Monomer	*recA*	16	0	0	0	72	0	0	0	0
Monomer	rec^+	16	0	0	0	49	0	0	0	0
Dimer	*recA*	0	48	0	0	0	31	0	0	0
Dimer	rec^+	0	0	4[e]	60[f]	0	0	2[g]	3[h]	60[i]

[a] Donor strain: MG1063 (see Table 1, footnote *b*).
[b] Matings involving pBR325: rec^+ strain, CBK861; *recA* strain, CBK869 (see Table 1, footnote *c*). Matings involving pIF005: rec^+ strain, CBK741 [Δ(*pro-lac*) *thyA ilvE12 avtA23*::Tn*5*] (15); *recA* strain, CBK870 [Δ(*srl-recA*)*306*::Tn*10* transductant of CBK741 using P1 grown on MC601] (see Table 1, footnote *c*).
[c] Cam^r Str^r exconjugants were selected. Plasmids 6, 12, and 18 kb long were monomers, either monomer::γδ or dimers, and dimer::γδ, respectively. Colonies containing only 6-kb-long plasmids were not detected.
[d] Amp^r Kan^r exconjugants were selected. Plasmids 8.6, 14.6, 17, and 23 kb long were monomers, monomer::γδ, dimers, and dimer::γδ, respectively. Colonies containing only 8.6- and 17-kb-long plasmids were not detected.
[e] The major band was 12 kb.
[f] The major band was 18 kb.
[g] The major band was 8.6 kb.
[h] The major band was 14.6 kb.
[i] The major band was 23 kb.

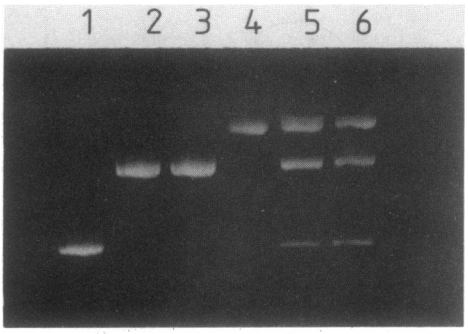

FIG. 2. Supercoiled pBR325 and pBR325::γδ plasmids analyzed by standard methods (11). Lanes: 1, monomer from MG1063 (*recA*); 2, dimer from MG1063; 3, monomeric pBR325::γδ plasmid following conjugal transfer of monomer from MG1063 (lane 1) to CBK869 (*recA*); 4, heterodimeric pBR325::γδ plasmid following conjugal transfer of dimer from MG1063 (lane 2) to CBK869 (*recA*); 5, heterodimeric pBR325::γδ plasmid and breakdown products following conjugal transfer of dimer from MG1063 (lane 2) to CBK861 (rec^+); 6, heterodimeric pBR325::γδ plasmid and breakdown products after transformation of CBK861 (rec^+) with DNA from lane 4.

pIF005::γδ) were not detected, possibly because such dimers would be converted to their component γδ-containing monomers by the resolution reaction.

The data presented here show that γδ transpositional cointegrates are the only significant participants in F-mediated transfer of pBR322-related plasmids and thus that special hypotheses to explain γδ-independent F-mediated transfer of nonconjugative plasmids (6, 9) need not be entertained.

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