Plasmid Fusions Mediated by One End of TnA

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We have observed plasmid fusions in a *recA* background mediated by a single end of TnA. These occur when transposase is provided either in *cis* or in *trans*. Insertions of the plasmid carrying the TnA inverted repeat sequence occur at many sites in the target plasmid. The point of fusion on the plasmid carrying TnA sequences always appears to be located in the region which carries the TnA inverted repeat sequence. In contrast to the transposition of an intact TnA element, plasmid fusions mediated by one end of TnA are very rare events. The implications of our results for models of transposition are discussed.

INTRODUCTION

Transposition of the TnA family of transposons involves the duplication of a discrete piece of DNA bounded by short, perfect or near-perfect inverted repeat sequences (IR sequences), and its insertion, apparently at random, into a non-homologous site in a target replicon, resulting in the formation of a transposition cointegrate (Gill *et al.*, 1978). The reaction requires the presence of both of the IR sequences, since these define the limits of the element, and also requires a transposon-encoded diffusible product, transposase (Heffron *et al.*, 1977). Resolution of cointegrate structures to generate transposition products requires a second transposon-encoded diffusible product, resolvase, which mediates a site-specific recombination between the duplicate copies of the *res* site of the transposon (Arthur & Sherratt, 1979).

Recently it has been reported that derivatives of the TnA-related elements Tn21 (Avila *et al.*, 1984) and Tn1721 (Motsch & Schmitt, 1984) that possess only one end of the particular transposon are capable of mediating the formation of plasmid fusions in *recA* strains provided that the appropriate transposase is also present. We now report *recA*-independent plasmid fusions mediated by a single end of TnA, and we compare products of this type of recombination with the transposition of an intact TnA element.

METHODS

Bacterial strains and plasmids used. The *Escherichia coli* strains used were UB5201 (Pro⁻ Met⁻ RecA⁻ Nal^R) (Bennett & Richmond, 1976), JC6310 (His⁻ Lys⁻ Trp⁻ RecA⁻ Str^R) (Bennett & Richmond, 1976) and UB2272 (His⁻ Lys⁻ Trp⁻ PolA⁻ Str^R Rif^R) (Jenkins & Bennett, 1978). The plasmids used are shown in Table 1.

Construction of plasmids pUB3032 and pUB3033. Plasmids pUB3032 and pUB3033 were generated from a pACYC184 derivative, pUB2992 (H. M. Dodd, unpublished results), a plasmid that carries an analogue of the TnA *res* site originating on the resistance plasmid R46 (Dodd & Bennett, 1983), by transposition of TnA to pUB2992 and subsequent site-specific recombination between the TnA *res* site and the R46 *res* site analogue. In these particular plasmids the recombination resulted in deletion of all TnA sequences from the *res* site to the right-hand IR sequence, in addition to plasmid sequences between the point of TnA insertion and the R46 *res* site analogue. Hence each plasmid carries the TnA transposase gene *tnpA*, and the adjacent IR sequence IR(l)

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Abbreviation: IR, inverted repeat.

Table 1. *Plasmids*

The abbreviations used for plasmid nomenclature are those recommended by Novick *et al.* (1976).

Plasmid	Phenotype conferred	Reference or source
R388	Tp Su Tra ⁺ IncW	Datta & Hedges (1972)
pUB307	Km Tc Tra ⁺ IncP	Bennett <i>et al.</i> (1977)
pUB501	Tp Su Tra ⁺ IncW	Robinson <i>et al.</i> (1977)
pBR322	Ap Tc Tra ⁻	Bolivar <i>et al.</i> (1977)
RSF1596	Ap Tra ⁻	Heffron <i>et al.</i> (1977)
pUB2280	Tp Tra ⁻ IncW	This paper (Fig. 1)
pUB2284	Tp Tra ⁻ IncW	This paper (Fig. 1)
pUB3032	Cm Tra ⁻	H. M. Dodd* (Fig. 1)
pUB3033	Cm Tra ⁻	H. M. Dodd* (Fig. 1)
pUB3078	Tp Tra ⁻ IncW	This paper

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(Fig. 1), as well as the *cat* gene of pACYC184. In all cases the expression of the *tnpA* gene is assumed to be derepressed due to deletion of the *tnpR* gene (Chou *et al.*, 1979). The plasmids pUB3032 and pUB3033 were used as a source of transposase throughout these experiments.

Construction of pUB2280. Plasmid pUB2280 was generated *in vitro* from plasmid pUB1605, a *Pst*I-generated deletion derivative of the R388::Tn802 recombinant plasmid pUB1604 (Wallace *et al.*, 1981), by circularizing the smaller of the two *Bgl*II fragments. The deletion removed the genes needed for self-mediated transfer. Plasmid pUB2280 is 8 kb long, encodes trimethoprim resistance and carries 1000 bp, including the IR sequence, from the left-hand end of TnA (Fig. 1).

Construction of pUB2284. Plasmid pUB2263 was isolated following transposition of a copy of Tn802 onto pUB501 (R388::Tn802 *bla*) (Heritage & Bennett, 1984). This transposition event resulted in a deletion which removed most, if not all, of the transfer functions encoded by pUB501, and regenerated, by transposon-encoded site-specific recombination, a functional copy of Tn802 at the site of the mutant transposon carried on pUB501. Plasmid pUB2284 was generated *in vitro* by circularizing the largest *Pst*I fragment derived from pUB2263. Plasmid pUB2284 is 16 kb long, encodes trimethoprim resistance and carries 450 bp, including the IR sequence, from the right-hand end of TnA (Fig. 1).

Construction of pUB3078. Plasmid pUB3078, which was generated *in vitro* by circularizing the smaller of the two *Bgl*II fragments derived from R388, is 8 kb long and encodes trimethoprim resistance.

Antibiotic selection. Minimal salts agar supplemented with the appropriate growth requirements and 0.4% glucose was used as the solid growth medium into which antibiotics were incorporated (Bennett & Richmond, 1976). Antibiotics were used at the following concentrations: carbenicillin, 500 µg ml⁻¹; chloramphenicol, 25 µg ml⁻¹; kanamycin, 30 µg ml⁻¹; nalidixic acid, 25 µg ml⁻¹; streptomycin, 100 µg ml⁻¹; sulphonamide, 500 µg ml⁻¹; tetracycline, as achromycin, 20 µg ml⁻¹; and trimethoprim, 25 µg ml⁻¹.

Conjugal transfer of plasmids. Plasmids were transferred in liquid matings as described by Bennett & Richmond (1976), or on solid medium as described by Avila *et al.* (1984).

Tests for genetic linkage. Tests for genetic linkage of drug resistance determinants were made by toothpicking clones onto minimal salts agar, appropriately supplemented and containing one of the set of antibiotics to be tested.

Isolation of DNA. Isolation of plasmid DNA was by the method of Birnboim & Doly (1979) or by the method of Wallace *et al.* (1981), as was convenient.

Restriction endonuclease analysis. Commercially available enzymes were used from a variety of sources. Digestions and agarose gel electrophoresis were performed as described by Grinsted *et al.* (1978).

Transformation. Transformation was by the method of Cohen *et al.* (1972), using the modifications of Humphries *et al.* (1976).

RESULTS

Demonstration of TnA tnpA gene on pUB3032 and pUB3033

Plasmids pUB3032 and pUB3033 were introduced by transformation into *E. coli* UB5201(RSF1596). RSF1596 (TnpA⁻ Res⁻ TnpR⁻) is a plasmid which carries a deleted TnA element but which retains a functional β -lactamase gene and both IR sequences. The deleted TnA element on this plasmid must be complemented for transposase if it is to transpose.

Fig. 1. Maps of TnA derivatives used in this study, and of plasmids on which they reside. L and R denote the left- and right-hand IR sequences IR(l) and IR(r) respectively. On plasmids pUB3032 and pUB3033, the thin line denotes pACYC184 sequences, and the double lines denote sequences originally derived from plasmid R46. TnA sequences are shown as bold lines. B, *Bam*HI; BII, *Bgl*II; E, *Eco*RI; H, *Hind*III; P, *Pst*I.

Transposition generates cointegrate end products (Heffron *et al.*, 1977). The IncP plasmid pUB307 was introduced by conjugation into *E. coli* UB5201(RSF1596, pUB3032) and UB5201(RSF1596, pUB3033). After allowing transposition products to accumulate (Wallace *et al.*, 1981), *E. coli* UB5201(RSF1596, pUB3032, pUB307) and UB5201(RSF1596, pUB3033, pUB307) were each mated with *E. coli* UB2272, a *PolA*⁻ strain in which RSF1596, pUB3032 and pUB3033 cannot replicate. In both experiments plasmid pUB307 transferred to *E. coli* UB2272 with a frequency of about 10^{-1} , while the frequency of transfer of carbenicillin resistance was 2×10^{-4} , and that of chloramphenicol resistance was 4.5×10^{-6} . Genetic analysis of the transposition products revealed that the carbenicillin resistant transconjugants contained pUB307::RSF1596 cointegrates, as expected, conferring kanamycin, tetracycline and carbenicillin resistance but not chloramphenicol resistance. Transconjugants selected on chloramphenicol, however, always displayed carbenicillin resistance in addition to kanamycin and tetracycline resistance, indicating that the recombinant plasmids in these cells had arisen by two independent transposition events involving all three replicons in the donor cell, creating cointegrates which possess three replication origins, and three copies of Tn596 originating on RSF1596. These recombinants have proved to be unstable, and after a period of time, chloramphenicol resistance is lost if no selection is maintained. In no case was chloramphenicol resistance found linked to kanamycin or tetracycline resistance independently of cabenicillin resistance.

Recombination between pUB307 and either pUB3032 or pUB3033

Having established the ability of pUB3032 and pUB3033 to express their *tnpA* genes, the ability of pUB3032 and pUB3033 to mediate plasmid fusions was tested. The IncP plasmid pUB307 was introduced by conjugation into *E. coli* UB5201(pUB3032) and UB5201(pUB3033). The double plasmid strains were stored for 3 weeks on Dorset egg slopes to allow accumulation of fusion products, and were then mated with *E. coli* UB2272. Plasmid pUB307 transferred to *E.*

Table 2. *Transfer frequencies of pBR322, pUB3032 and pUB3033 markers mobilized by R388*

Mobilization frequencies were calculated as the ratio of the frequency of inheritance of the appropriate non-conjugative marker to the total frequency of inheritance of trimethoprim, the selection imposed to measure the transfer of R388. The plasmid host strain was *E. coli* UB5201.

Plasmids in donor strain	Relative transfer frequency	
	Cm/Tp	Cb/Tp
R388, pBR322, pUB3032	6×10^{-8}	2×10^{-7}
R388, pBR322, pUB3032	$< 1 \times 10^{-8}$	4×10^{-7}
R388, pBR322, pUB3033	3×10^{-7}	3×10^{-7}
R388, pBR322, pUB3033	2×10^{-7}	4×10^{-7}

coli UB2272 at a frequency of about 10^{-1} as was found in the crosses described above, while chloramphenicol resistance was transferred at a frequency of 2.9×10^{-7} when the donor plasmid was pUB3032 and 5.5×10^{-7} when it was pUB3033. These results indicated linkage of the pUB3032 and pUB3033 chloramphenicol resistance genes to pUB307 since neither donor plasmid can replicate in *E. coli* UB2272. Transfer experiments using several of these transconjugants, in turn, as donors confirmed the linkage. Since no recombination between the chloramphenicol resistance determinant of pACYC184 and pUB307 was demonstrated in similar experiments, we concluded that the recombination observed was as a consequence of the residual *TnA* sequences carried on pUB3032 and pUB3033.

Recombination between R388 and pUB3032, pUB3033 or pBR322

Plasmids pUB3032 and pUB3033 carry the left-hand end of *TnA*, incorporating the left-hand IR sequence and an intact copy of the *tnpA* gene. Their ability to mediate recombinations *trans* was also assessed. Plasmid pBR322 is a non-conjugative plasmid that carries the right-hand IR sequence and the *bla* gene of *TnA* (Fig. 1). R388 is a conjugative IncW plasmid that confers resistance to sulphonamides and trimethoprim, carries no *TnA* sequences, and is unable to mobilize pBR322 or pACYC184 (data not shown). Plasmids pUB3032 and pUB3033 were introduced separately into *E. coli* UB5201(pBR322) by transformation. Plasmid R388 was then introduced, by conjugation, into the transformants. The progeny were held on Dorset egg slopes for 4 weeks, after which time they were mated, on nutrient agar, with *E. coli* JC6310. The transfer frequencies measured are presented in Table 2. In these experiments R388 transferred at a frequency of approximately 10^{-1} . Transconjugants were tested to determine which non-selected resistance determinants had been co-inherited; all of the 24 carbenicillin resistant transconjugants tested were also resistant to tetracycline and trimethoprim, but not to chloramphenicol, indicating that pBR322 alone had been mobilized by R388 in the presence of transposase. All of the 18 transconjugants selected for the transfer of chloramphenicol resistance displayed resistance to trimethoprim, indicating recombination between either pUB3032 or pUB3033 and R388. One chloramphenicol resistant transconjugant was also resistant to carbenicillin and tetracycline in addition to trimethoprim. In subsequent transfer experiments with this transconjugant as donor, all antibiotic resistance determinants transferred in the initial mating remained linked, irrespective of which was chosen to select for transfer of the putative fusion product. This recombinant plasmid presumably arose as the product of two separate recombination events involving all three plasmids.

To test genetic linkage of the resistance markers in the presumed fusions, four representatives of each type of transconjugant were mated, on solid medium, with *E. coli* UB5201. Mating products were selected on supplemented minimal medium with nalidixic acid and trimethoprim. In addition to trimethoprim resistance, all transferred either chloramphenicol resistance or carbenicillin and tetracycline resistance, as appropriate, indicating recombination of R388 with pUB3032, pUB3033 or pBR322. Two independent isolates of the 12 clones tested failed to exhibit sulphonamide resistance as normally conferred by R388, indicating insertion in this gene.

Table 3. Transfer of pUB2280, pUB2284, pUB3032, pUB3033 and pUB3078 markers mobilized by pUB307

Mobilization frequencies were calculated as the ratio of the frequency of inheritance of the appropriate non-conjugative marker to the total frequency of inheritance of resistance to kanamycin, the selection imposed to measure the transfer of pUB307. The plasmid host strain was *E. coli* UB5201.

Plasmids in donor strain	Relative transfer frequencies	
	Cm/Km	Tp/Km
pUB2280, pUB307	—	$< 2 \times 10^{-8}$
pUB2284, pUB307	—	$< 4 \times 10^{-8}$
pUB3032, pUB2280, pUB307	2×10^{-6}	4×10^{-7}
pUB3033, pUB2280, pUB307	4×10^{-6}	1×10^{-6}
pUB3032, pUB2284, pUB307	2×10^{-5}	1×10^{-5}
pUB3033, pUB2284, pUB307	7×10^{-6}	1×10^{-7}
pUB3032, pUB3078, pUB307	1×10^{-6}	$< 3 \times 10^{-7}$
pUB3033, pUB3078, pUB307	8×10^{-7}	$< 1 \times 10^{-7}$

Recombination between pUB307 and either pUB2280 or pUB2284 requires TnA IR sequences

In a parallel set of experiments, the ability of the IncP plasmid pUB307 to mobilize pUB2280, pUB2284 and pUB3078 (Fig. 1) was tested. In this experimental set, pUB2280, pUB2284 or pUB3078 was together with either pUB3032 or pUB3033 and pUB307 in the donor strain. Plasmids pUB2280, pUB2284 and pUB3078 are non-self-transmissible R388 derivatives which confer resistance to trimethoprim. Plasmid pUB2280 carries the left-hand 1000 bp of TnA, while pUB2284 carries the right-hand 450 bp of TnA (see Fig. 1). Plasmid pUB3078 carries no sequence derived from TnA. None of these plasmids is normally mobilized by pUB307 (Table 3). The six donor strains were individually mated, on nutrient agar, with UB2272. Transconjugants were selected separately with kanamycin, chloramphenicol and trimethoprim (Table 3). All crosses displayed transfer of resistance to kanamycin and to chloramphenicol. While transfer of trimethoprim resistance was observed from strains carrying pUB2280 and pUB2284, no transfer of trimethoprim resistance from strains carrying pUB3078 was detected.

On testing for the acquisition of non-selected markers, it was demonstrated that if the initial selection imposed was for the transfer of trimethoprim resistance, this was always accompanied by the co-transfer of kanamycin or of tetracycline, usually both, implying recombination of pUB307 with either pUB2280 or pUB2284. Similarly, if chloramphenicol resistance was employed as the primary selection, kanamycin or tetracycline resistance, usually both, were acquired, indicating recombination between pUB307 and either pUB3032 or pUB3033. In this set of experiments, no products carrying markers derived from all three plasmid types were seen. Finally, when these transconjugants were, in turn, outcrossed to another strain of *E. coli* (UB5201), all the markers acquired in the first mating were transferred as linked sets, irrespective of which marker was used for the primary selection. All recombinant plasmids transferred with frequencies between 10^{-2} and 10^{-3} per donor.

Restriction endonuclease analysis of plasmid recombinants

Recombination products were subjected to restriction endonuclease analysis. Three recombinants of pBR322 and R388 were analysed in detail, as were one each of the recombinants of R388 and either pUB3032 or pUB3033. The enzymes employed in this analysis included *Bam*HI, *Bgl*II, *Eco*RI, *Hind*III and *Pst*I, and these were used both singly and in combination. Fig. 2(a) illustrates the results for an R388::pUB3033 recombinant, pUB3073, while Fig. 2(b) indicates the molecular configuration of the recombinant that was deduced from the data. In all cases where a detailed analysis of the fusion products was undertaken, the recombinant plasmids carried the appropriate number of restriction sites consistent with simple plasmid fusions. Furthermore, in all cases examined to date the junction between R388 and the mobilized plasmid carrying the TnA IR sequence involves the same region of the mobilized

Fig. 2. (a) Agarose gel (0.8%) of fragments of pUB3073 generated by restriction endonuclease digestion. Tracks 1 and 12 are λ BglII fragments used as size standards. The enzymes employed were: *Pst*I (track 2); *Eco*RI (track 3); *Hind*III (track 4); *Bam*HI (track 5); *Pst*I + *Eco*RI (track 6); *Pst*I + *Hind*III (track 7); *Pst*I + *Bam*HI (track 8); *Eco*RI + *Hind*III (track 9); *Eco*RI + *Bam*HI (track 10); and *Hind*III + *Bam*HI (track 11). (b) Map deduced from the restriction endonuclease data presented in (a). The double lines denote pUB3033 sequences and the thin line denotes R388 sequences. The TnA sequence is shown as a bold line. Arrows denote the points of recombination. L, left-hand IR sequence; B, *Bam*HI; E, *Eco*RI; H, *Hind*III; P, *Pst*I.

plasmid, namely that accommodating the IR sequence of Tn802, while the R388 junction varies from one recombinant to another (Fig. 2). To ensure that the remaining fusion products conformed to the same general pattern, each recombinant isolated was examined with a *Hind*III + *Eco*RI double digestion. The results were totally consistent with the above findings.

Restriction endonuclease analysis of the recombinants of pUB307 and either pUB2280, pUB3032 or pUB3033 showed them also to be simple replicon fusions. Again the point of fusion in the target replicon, in this case pUB307, varied from one recombinant to another, while the point of fusion on the mobilized plasmid appeared to be site-specific, consistent with the

Fig. 3. Agarose gel (0.8%) of restriction endonuclease fragments of R388/pBR322 recombinants. Fragments were generated by *Hind*III + *Eco*RI double digestion. Track 1, pBR322; tracks 2–6, R388/pBR322 recombinants; track 7, R388.

involvement of the TnA IR sequence. Major duplications of, or deletions of sequences from, any of the parental plasmids have not been observed, although the resolution of this analysis is not sufficient to determine whether small duplications or deletions have occurred.

The molecular analysis of the fusion products revealed that insertions occur at diverse sites in R388 (Fig. 3) and pUB307 (data not shown). Interestingly, fusion products isolated independently from the same original donor strain display a marked tendency to give an identical pattern of restriction fragments, implying a high degree of sibling isolation, in which case the frequencies calculated for fusion formation are upper limits.

In comparison to the other 'single-ended' TnA fusions described, fusions between pUB2284 and pUB307 were more difficult to demonstrate, and although some fusion products apparently incorporate the entire pUB2284 sequence, others do not. In these cases, although all the pUB307 sequences could be accounted for, not all of the pUB2284 sequence was present [indicated by the observation that the *Bgl*II site of pUB2284 (Fig. 1) was not present on the fusion product (data not shown)], implying either premature termination of the replication of this plasmid during the fusion process, or secondary deletion of part of the pUB2284 sequence after fusion of the entire plasmid with pUB307.

DISCUSSION

TnA belongs to a family of transposons which share similar structures and transposition functions (Kleckner, 1981; Schmitt *et al.*, 1981; Grinsted *et al.*, 1982; Diver *et al.*, 1983; Heffron, 1983). Recently it has been reported that derivatives of two members of this family, Tn21 (Avila *et al.*, 1984) and Tn1721 (Motsch & Schmitt, 1984), possessing only one IR sequence, can, when provided with appropriate transposase, promote plasmid fusions, albeit at a lower frequency than transposition of the intact element. In the case of Tn21, the frequency of replicon fusion is about 10-fold lower than normal transposition; with Tn1721, transposition of the intact transposon is about 100 times more frequent than replicon fusions mediated by one end of the element.

The results reported in this paper indicate that the transposition system of TnA can also mediate plasmid fusions via a single end of the element. [Since the work described here was completed Arthur *et al.* (1984) have also reported that TnA can mediate one-ended plasmid fusions.] Either end of TnA may be utilized, as witnessed by fusions involving pUB3032, pUB3033 and pUB2280, which all carry the left-hand end of TnA, and those involving pBR322 and pUB2284, which both carry the right-hand end of TnA. That one end of TnA is necessary for recombination is demonstrated by the inability of pUB3078 to form fusions with pUB307 under conditions in which pUB2280 and pUB2284 did so. Furthermore, the fact that pBR322, pUB2280 and pUB2284 underwent fusion with pUB307 only in the presence of either pUB3032 or pUB3033 indicates that the recombination requires the *tnpA* gene product.

The frequency of TnA-mediated one-ended recombinations is at least 1000-fold lower than the frequency of transposition of Tn596 in a comparable system, i.e. when complemented with either pUB3032 or pUB3033. Heffron *et al.* (1977) reported a 1000-fold reduction in the mobilization of ampicillin resistance on plasmids carrying TnA elements from which the left-hand IR sequence has been lost. However, the transposase gene was provided on a plasmid carrying TnA with a deletion in the *bla* and *tnpR* genes but with both IR sequences intact. No analysis of the products was reported; our results suggest that the most likely method of mobilizing the *bla* gene in these systems would have been by a double transposition event, such as we observed when assessing the ability of pUB3032 and pUB3033 to complement transposition of Tn596, and not by a 'single-ended' plasmid fusion.

In the systems we have used to study recombinations mediated by a single end of TnA, transposase production was derepressed, since the deletions giving rise to pUB3032 and pUB3033 caused the loss of the *tnpR* gene. Additionally, the transposase gene was present on a multi-copy plasmid (a derivative of pACYC184). Yet, recombinations could not be demonstrated in every cell-line tested, even though these were apparently identical, indicating that in comparison with the normal transposition of TnA, recombinations mediated by one end of the element are rare events. The demonstration that from a single donor strain there is an apparently high probability of sibling isolation leads us to the conclusion that the frequency of plasmid fusion must be lower than the apparent frequency observed.

The phenomenon of plasmid fusions mediated by a single end of TnA is not confined to a single plasmid type, since both the IncP plasmid pUB307 and the IncW plasmid R388 are capable of mobilizing various non-conjugative plasmids carrying either the left- or right-hand IR sequence of TnA when transposase is provided in *cis* or in *trans*, as appropriate. Thus the data presented here are consistent, in general, with the observation that plasmids incorporating a single IR sequence derived from this family of transposons are capable of fusing with other replicons, providing that the appropriate transposase function is supplied. This type of recombination is independent of the host recombination system since, in these experiments, a *recA* cell was used as the donor strain. It is also independent of the transposon-encoded site-specific recombination system since the complementing system is *tnpR*.

In cells carrying RSF1596, which carries Tn596, a TnA derivative with a small internal deletion but with both IR sequences intact (Heffron *et al.*, 1977), and either plasmid pUB3032 or pUB3033, chloramphenicol resistance encoded by either pUB3032 or pUB3033 was only ever seen to be mobilized by pUB307 via transposition cointegrates generated by Tn596; simple fusions between either pUB3032 or pUB3033 and pUB307 were never detected in these particular experiments, even though we would have expected to detect these events with the number of chloramphenicol resistant transconjugants examined. Thus 'normal' transposition would appear to have suppressed one-ended recombinations.

It is worth noting that whereas pUB3032 and pUB3033 mediated, with reasonable ease, plasmid fusions of both themselves and either pBR322 or pUB2280 with either R388 or pUB307, it was much more difficult to detect fusions between pUB3032 and pUB2284, mediated by either pUB3032 or pUB3033, in that plasmid recombination occurred in less than half of the cell lines constructed. The reason for this is not known, but may reflect the somewhat larger size of pUB2284, a factor that may also be relevant to the isolation of recombinants that incorporated less than the whole of pUB2284.

The finding that the *tnpA* gene product of TnA can mediate inter-replicon recombinations via a single IR sequence appears to be more consistent with an asymmetric or processive model of transposition (Galas & Chandler, 1981; Grindley & Sherratt, 1979; Harshey & Bukhari, 1981) than with a symmetric model (Arthur & Sherratt, 1979; Shapiro, 1979), and, indeed, it has been argued that the generation of recombinants mediated by a single IR sequence of Tn21 provides evidence in support of asymmetric models of transposition, the corollary being that symmetric models must be incorrect (Avila *et al.*, 1984). However, Motsch & Schmitt (1984) point out that the low frequency of replicon fusion and the apparent inability of the system to form cointegrate-type intermediates leaves open the possibility that different processes may be responsible for the two types of recombination. In general, we would concur with the latter point of view, since our observations point to differences between recombinations mediated by a single end of TnA and the transposition of a complete TnA element. The one-ended recombinations studied here are different from normal transposition systems both in form and in end-product. The former recombination events, mediated by TnA components, are rare, and do not transpose a discrete, defined element, while an element bounded by TnA IR sequences, present as inverted repeats, does transpose as a discrete sequence, and at a frequency which is several orders of magnitude greater. It is, therefore, difficult to escape the conclusion that the presence of the second IR sequence as an inverted repeat not only helps to define the element, but also plays a significant role in determining its transposition frequency. The mechanism of recombination mediated by a single IR sequence of TnA, and of true TnA transposition, although involving common components, are quite clearly and necessarily different in several aspects, so there is no compelling reason to believe that the initiation mechanisms must be identical. Hence, although the recombinations reported here are likely to be mediated by a mechanism akin to the asymmetric models of transposition, this does not preclude a symmetric mechanism for true transposition.

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REFERENCES

- ARTHUR, A. & SHERRATT, D. J. (1979). Dissection of the transposition process: a transposon-encoded site-specific recombination system. *Molecular and General Genetics* **175**, 267–274.
- ARTHUR, A., NIMMO, E., HETTLE, S. & SHERRATT, D. (1984). Transposition and transposition immunity of transposon Tn3 derivatives having different ends. *EMBO Journal* **3**, 1723–1730.
- AVILA, P., DE LA CRUZ, F., WARD, E. & GRINSTED, J. (1984). Plasmids containing one inverted repeat of Tn21 can fuse with other plasmids in the presence of Tn21 transposase. *Molecular and General Genetics* **195**, 288–293.
- BENNETT, P. M. & RICHMOND, M. H. (1976). Translocation of a discrete piece of deoxyribonucleic acid carrying an *amp* gene between replicons in *Escherichia coli*. *Journal of Bacteriology* **126**, 1–6.
- BENNETT, P. M., GRINSTED, J. & RICHMOND, M. H. (1977). Transposition of TnA does not generate deletions. *Molecular and General Genetics* **154**, 205–211.
- BIRNBOIM, H. C. & DOLY, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* **7**, 1513–1523.
- BOLIVAR, F., RODRIGUEZ, R. L., GREENE, P. J., BETLACH, M. C., HEYNECKER, H. C., BOYER, H. W., CROSA, J. H. & FALKOW, S. (1977). Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* **2**, 95–113.
- CHOU, J., LEMAUX, P. G., CASADABAN, M. J. & COHEN, S. N. (1979). Transposition protein of Tn3: identification and characterisation of an essential repressor-controlled gene product. *Nature, London* **282**, 801–806.
- COHEN, S. N., CHANG, A. C. Y. & HSU, L. (1972). Non chromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. *Proceedings of the National Academy of Sciences of the United States of America* **69**, 2110–2114.
- DATTA, N. & HEDGES, R. W. (1972). Trimethoprim resistance conferred by W plasmids in enterobacteriaceae. *Journal of General Microbiology* **72**, 349–355.
- DIVER, W. P., GRINSTED, J., FRITZINGER, D. G., BROWN, N. L., ALTENBUCHNER, J., ROGOWSKY, P. & SCHMITT, R. (1983). DNA sequences of and complementation by the *tnpR* genes of Tn21, Tn501 and

- Tn1721. *Molecular and General Genetics* **191**, 189–193.
- DODD, H. M. & BENNETT, P. M. (1983). R46 encodes a site-specific recombination system interchangeable with the resolution function of TnA. *Plasmid* **9**, 247–261.
- GALAS, D. J. & CHANDLER, M. (1981). On the molecular mechanism of transposition. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 4858–4862.
- GILL, R., HEFFRON, F., DOUGAN, G. & FALKOW, S. (1978). Analysis of sequences transposed by complementation of two classes of transposition-deficient mutants of Tn3. *Journal of Bacteriology* **136**, 742–756.
- GRINDLEY, N. & SHERRATT, D. J. (1979). Sequence analysis of IS1 insertion sites. *Cold Spring Harbor Symposia on Quantitative Biology* **43**, 1257–1261.
- GRINSTED, J., BENNETT, P. M., HIGGINSON, S. & RICHMOND, M. H. (1978). Regional preference of insertion of Tn501 and Tn802 into RPI and its derivatives. *Molecular and General Genetics* **166**, 313–320.
- GRINSTED, J., DE LA CRUZ, F., ALTENBUCHNER, J. & SCHMITT, R. (1982). Complementation of transposition of *tnpA* mutants of Tn3, Tn21, Tn501 and Tn1721. *Plasmid* **8**, 276–286.
- HARSHEY, R. M. & BUKHARI, A. (1981). A mechanism of DNA transposition. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 1090–1094.
- HEFFRON, F. (1983). Tn3 and its relatives. In *Mobile Genetic Elements*, pp. 223–260. Edited by J. A. Shapiro. New York: Academic Press.
- HEFFRON, F., BEDDINGER, P., CHAMPOUX, J. J. & FALKOW, S. (1977). Deletions affecting the transposition of an antibiotic resistance gene. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 702–706.
- HERITAGE, J. & BENNETT, P. M. (1984). The role of TnA transposase in transposition immunity. *Plasmid* **12**, 218–221.
- HUMPHRIES, G. O., WILLSHAW, G. A., SMITH, H. R. & ANDERSON, E. S. (1976). Mutagenesis of plasmid DNA with hydroxylamine: isolation of mutants of multicopy plasmids. *Molecular and General Genetics* **145**, 101–108.
- JENKINS, S. T. & BENNETT, P. M. (1978). Effect of mutations in deoxyribonucleic acid repair pathways on the sensitivity of *Escherichia coli* K-12 strains to nitrofurantoin. *Journal of Bacteriology* **125**, 1214–1216.
- KLECKNER, N. (1981). Transposable elements in prokaryotes. *Annual Review of Genetics* **15**, 341–404.
- MOTSCH, S. & SCHMITT, R. (1984). Replicon fusion mediated by a single-ended derivative of transposon Tn1721. *Molecular and General Genetics* **195**, 281–287.
- NOVICK, R. P., CLOWES, R. C., COHEN, S. N., CURTISS, R., DATTA, N. & FALKOW, S. (1976). Uniform nomenclature for bacterial plasmids: a proposal. *Bacteriological Reviews* **40**, 168–189.
- ROBINSON, M. K., BENNETT, P. M. & RICHMOND, M. H. (1977). Inhibition of TnA translocation by TnA. *Journal of Bacteriology* **129**, 407–414.
- SCHMITT, R., ALTENBUCHNER, J. & GRINSTED, J. (1981). Complementation of transposition functions encoded by transposons Tn501 (Hg^r) and Tn1721 (Tc^r). In *Molecular Biology, Pathogenicity, and Ecology of Bacterial Plasmids*, pp. 359–370. Edited by B. Levy, R. C. Clowes & E. C. Koenig. New York: Plenum.
- SHAPIRO, J. (1979). Molecular model for the transposition and replication of bacteriophage mu and other transposable elements. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 1933–1937.
- WALLACE, L. J., WARD, J. M. & RICHMOND, M. H. (1981). The location of sequences of TnA required for the establishment of transposition immunity. *Molecular and General Genetics* **184**, 80–86.