Two Naturally Occurring Transposons Indistinguishable from Tn7

By P. T. BARTH AND NAOMI DATTA

Department of Bacteriology, Royal Postgraduate Medical School, Du Cane Road,
London W12 0HS

(Received 16 February 1977; revised 26 April 1977)

Two plasmids from different sources, determining trimethoprim and streptomycin resistances, harbour transposons which we designate Tn71 and Tn72. These transposons are indistinguishable from Tn7 in the resistances determined, in their molecular masses and in the number and relative positions of their sites susceptible to the restriction enzymes *EcoRI*, *HindIII* and *BamHI*. We conclude that Tn7 has been naturally spread among plasmids.

INTRODUCTION

A transposon is a specific DNA sequence carrying a recognizable genetic determinant or determinants, such as drug resistances, that is freely transposable from one replicon to another. Transposition is a non-reciprocal event that occurs equally in the presence or absence of the requirements of normal recombination, i.e. a functional *recA* gene and regions of extensive homology between the participating DNA sequences (Heffron, Rubens & Falkow, 1975*a*; Kopecko & Cohen, 1975; Gottesman & Rosner, 1975; Barth *et al.*, 1976).

Since the original recognition of a transposon carrying an ampicillin resistance determinant (TnA or Tn1) from plasmid RP4 (Hedges & Jacob, 1974), many others have been recognized (for review, see Cohen, 1976). Because of their ubiquity and ability to move freely from plasmid to plasmid, transposons are thought to have played a large part in the rapid evolutionary spread of bacterial drug resistances during the last two decades. TnA certainly appears to be widely spread: its DNA sequence homology (Heffron *et al.*, 1975*b*) is present in, and its TEM β -lactamase (Matthew & Hedges, 1976) is produced by a variety of unrelated plasmids that confer ampicillin resistance.

We have recently been studying transposon C [TnC, now redesignated Tn7 by a self-appointed committee (Cohen, 1976)]. Tn7, derived from R483, carries determinants for resistances to trimethoprim (Tp^r) and streptomycin/spectinomycin (Sm^r) (Barth *et al.*, 1976). We were interested to find out whether Tn7 was also spread amongst other naturally occurring plasmids. We therefore looked for other plasmids that conferred Tp^r and Sm^r and tested them for the transposability of these determinants. Two such plasmids were found. We measured the molecular weights and mapped the restriction enzyme-susceptible sites on the transposons they harboured. On these criteria, the two new transposons were indistinguishable from Tn7.

METHODS

Materials and media. Materials and growth media were as described previously (Coetzee, Datta & Hedges, 1972; Barth & Grinter, 1974). Restriction enzymes were obtained from Miles Laboratories.

Bacterial strains. Strains of *Escherichia coli* K12 used were: w3110T⁻ (*thy dra*); J53 (F⁻ *pro met*) (Bachmann, 1972); J53-I, a nalidixate-resistant derivative of J53 (Coetzee *et al.*, 1972); HH26 (Barth *et al.*, 1976) and HH27. Strains HH26 and HH27 are *recA thy*⁺ derivatives of C600T⁻ and w3110T⁻ respectively. The *recA* marker was introduced from the Hfr *recA* strain MA1079, selecting for Thy⁺ transconjugants. The *Proteus mirabilis* strain was PM13 (Coetzee, 1972).

Table 1. *Bacterial plasmids*

Plasmid	Characters conferred*	Origin		References
		Bacterial	Geo-graphical	
R483	Tp ^r Sm/Sp ^r Colla I pili Incl α	<i>Escherichia coli</i>	Kent	Datta & Barth (1976)
R721	Tp ^r Sm/Sp ^r I pili Incl δ †	<i>E. coli</i>	London	Jobanputra & Datta (1974)
pBW1	Tp ^r Sm/Sp ^r I pili Incl δ ‡	<i>E. coli</i>	Herts	B. West (unpublished)
RP4	Ap ^r Tc ^r Km ^r IncP	<i>Pseudomonas aeruginosa</i>	Glasgow	Datta <i>et al.</i> (1971)
R702	Sm/Sp ^r Tc ^r Km ^r Su ^r IncP	<i>Proteus mirabilis</i>	U.S.A.	Hedges & Jacob (1974)

* Plasmid character abbreviations as in Novick *et al.* (1976).

† The plasmid used here was separated by transformation from a smaller, accompanying plasmid (Falkow *et al.*, 1974).

‡ Personal communication, Hilary Richards.

Bacterial plasmids used are given in Table 1.

Isolation of transposition plasmids. *Escherichia coli* K12 J53 strains were constructed carrying two plasmids, the prospective donor plasmid, R721 or pBW1, and the prospective recipient, RP4. These strains were mated with *P. mirabilis*, and the mixtures were plated on MacConkey agar incorporating trimethoprim (10 µg ml⁻¹), 4 % (v/v) lysed horse blood (to allow the trimethoprim to be actively antibacterial in the MacConkey medium) and polymyxin B (25 µg ml⁻¹) (to prevent the growth of *E. coli* while permitting that of *P. mirabilis*). Trimethoprim-resistant *P. mirabilis* transconjugants, recognized as Lac⁻ colonies on the selection plates, were purified on MacConkey plates, again incorporating Tp and lysed blood. Purified clones were identified by biochemical tests and their resistance patterns were defined by disc tests. This method selects transposition plasmids when the transposon donor is unable to replicate in *P. mirabilis* (see Results).

Plasmids were transferred from *P. mirabilis* to *E. coli* K12 J53-1 by a similar technique, using nalidixic acid in place of polymyxin.

Retransposition from plasmid to chromosome. RP4 plasmids containing a transposon were transferred by conjugation to the *recA* strain, HH26. R702, another IncP plasmid, was then introduced by selection of sulphonamide-resistant transconjugants. Isolated clones were tested for retention of Tp^r; such clones were tested for the markers of RP4. The genetic location of Tp^r (chromosomal or plasmid) was established by tests for transfer frequency and linkage.

Isolation of plasmid DNA. Strains of W3110T⁻ carrying plasmids were labelled with [³H]- or [¹⁴C]thymine, lysed with sarkosyl, and their supercoiled DNA was isolated by ethidium bromide-CsCl equilibrium centrifugation as previously described (Barth & Grinter, 1974). The isolated DNA was dialysed overnight against a continuous flow of 5 l TNE buffer (50 mM-Tris/50 mM-NaCl/1 mM-EDTA, pH 7.5).

Cleavage of plasmid DNA with restriction enzymes. Plasmid DNA was supplemented with 0.125 vol. of 0.1 M-MgCl₂/1 M-Tris buffer, pH 7.5, plus 0.01 vol. of the appropriate restriction enzyme, and incubated at 37 °C for 1 h.

Sucrose gradient sedimentation analysis. Molecular weights of plasmid DNAs were measured by sedimentation, together with a suitable marker DNA, through neutral sucrose gradients. These were isokinetic (approx. 5 to 20 %, w/v) and generated by a freeze-thaw method (Barth & Grinter, 1977). Molecular weights were calculated as described by Barth & Grinter (1974).

RESULTS

Identification of plasmids possibly harbouring Tn7

We hold a collection of over 1000 R plasmids, collected from many bacterial and geographical sources. Among these, only 30 determine Tp resistance. Of these, only three determine linked Tp and Sm resistance, namely R483, R721 and pBW1 (Table 1).

Table 2. *Molecular masses of intact and restriction enzyme-cloven transposition plasmids*

Molecular masses (Mdal) were measured by sucrose gradient analyses using ³H-labelled RP4-Tn71 or RP4-Tn72 and ¹⁴C-labelled RP4 as a marker. The insertion sites refer to the map of RP4 given in Fig. 1.

Plasmid		RP4-Tn71	RP4-Tn72
Molecular mass		44.5	44.0
<i>Eco</i> RI fragments	1	29.9	30.4
	2	14.6	14.1
<i>Hind</i> III fragments	1	39.0	23.6
	2	3.6	18.9
	3	1.9	2.0
<i>Bam</i> HI fragments	1	21.9	37.9
	2	21.9	5.8
	3	0.75	0.75
Insertion site into RP4		24.1	8.3

Test for the presence of transposons

Plasmid R483 was previously shown to contain Tn7 (Barth *et al.*, 1976). R721 and pBW1 were found to be non-transferable to *P. mirabilis* strain PM13, like other I-pilus-determining plasmids (Datta & Hedges, 1972). This enabled us to demonstrate the transposition of resistance genes to RP4, a plasmid capable of replication in *P. mirabilis*. This method was used previously to detect transposition of Tn7 from R483 (Barth *et al.*, 1976). Donor bacteria carrying both RP4 and either R721 or pBW1 were mated with strain PM13; selection was made for the transfer of Tp^r (see Methods). All Tp^r transipients carried, not only the RP4 markers, but also the unselected Sm^r marker. Resistant *P. mirabilis* transconjugants were used as donors to J53-1, selection being for Tp^r and Km^r separately. All *E. coli* transconjugants tested carried ApKmTcSmTp^r, indicating that the TpSm resistance genes were transposed to RP4 from both R721 and pBW1, as previously shown with R483.

Retransposition in a recA host

We tested the ability of the RP4 derivatives, carrying TpSm^r from R721 or pBW1, to transpose these latter markers to another replicon in a *recA* host. R702, incompatible with RP4, was therefore transferred to HH26, carrying these derivatives. In approximately 50 % of transconjugants, the resident RP4 plasmid, including its transposed Tp^r marker, was eliminated. In the remainder, Tp^r was retained, although RP4, recognizable by its Ap^r, had been eliminated. [Hedges & Datta (1973) demonstrated a similar frequency of retention of the Tp^r of R483, i.e. Tn7, when the incompatible plasmid JR66a was transferred to an R483⁺ strain.]

To determine the location of the retained Tp^r, clones carrying it were used as donors to another *recA* strain, HH27, selecting for Km^r (an R702 marker) or Tp^r. Transconjugants selected for Km^r were 1000-fold more numerous than those selected for Tp^r, and of 10 isolated Km-selected clones, none was Tp^r. All clones tested, selected on Tp plates, carried the resistance markers of R702. In these transconjugants, Tp^r was linked to R702, as evidenced by co-transfer in conjugation. It was not possible, in these tests, to demonstrate Sm/Sp^r, linked to Tp^r in transposition, since it was masked by the Sm/Sp^r of R702.

Plasmid molecular weights

Parental plasmids. The molecular masses of plasmid DNAs isolated from strains carrying R721 and pBW1, determined by sucrose gradient analysis, were 48 and 45 Mdaltons (Mdal) respectively. Thus they are different from R483 (62 Mdal; Barth *et al.*, 1976) and from each other.

Fig. 1. A map of plasmid RP4 showing the observed sites of insertion of Tn71 and Tn72. The positions of resistance genes, transfer genes and sites susceptible to restriction enzymes on RP4 were determined by Barth & Grinter (1977). The positions and orientations of Tn71 and Tn72 were calculated from the data shown in Table 2, using the assumed position of the *EcoRI* site on each transposon described in the text. The internal scale is shown in 3 Mdal units.

Transposition plasmids. RP4 has a molecular mass of 36 Mdal. We have previously shown that transposition of TpSm^r from R483 into RP4 leads to a molecular mass gain of about 8.5 Mdal which we have used as an estimate of the size of Tn7 (Barth *et al.*, 1976). We measured the molecular mass of two plasmids derived from RP4 that had acquired the resistances of R721 or pBW1 as described above. They were 44.5 and 44.0 Mdal respectively (Table 2) i.e. gains of 8.5 and 8.0 Mdal above the molecular mass of RP4. These gains are, within experimental error, the same as that of Tn7. We designate these transposons from R721 and pBW1 as Tn71 and Tn72 respectively.

Restriction enzyme sites

***EcoRI*.** We have previously shown that Tn7 and RP4 each have a single site susceptible to *EcoRI*, thus RP4-Tn7 transposition plasmids have two such sites. The molecular masses of the two DNA fragments obtained from *EcoRI* cleavage of such plasmids depend upon the site of insertion of Tn7 (Barth *et al.*, 1976; Barth & Grinter, 1977). RP4-Tn71 and RP4-Tn72 plasmid DNA samples were treated with *EcoRI* and analysed by sucrose gradient sedimentation. Each sample gave two DNA fragments (Table 2). Thus Tn71 and Tn72 each have a single *EcoRI* susceptible site.

***HindIII*.** RP4 has a single *HindIII* site whereas Tn7 has two, separated by 2.0 Mdal (Barth & Grinter, 1977). When RP4-Tn71 and RP4-Tn72 were treated with *HindIII* each gave three fragments including a fragment of about 2 Mdal (Table 2). Thus Tn71 and Tn72 each have two *HindIII* sites 2 Mdal apart. (The data for Tn71, but not Tn72, could also be interpreted as indicating a distance of 3.6 Mdal between its *HindIII* sites. In view of the other close similarities of Tn71 with Tn7 we think this possibility is less likely.)

***BamHI*.** RP4 has a single *BamHI* site; Tn7 has two, separated by 0.7 Mdal (Barth & Grinter, 1977). The analyses of *BamHI* generated fragments of RP4-Tn71 and RP4-Tn72 show that Tn71 and Tn72 also have two cut sites separated by 0.75 Mdal (Table 2).

Sites of insertion of Tn71 and Tn72 into RP4

The combination of *EcoRI* and *HindIII* data for each plasmid allow one to calculate the site of insertion of its transposon on a circular map of RP4 (whereas the data from either

enzyme alone do not discriminate between insertion sites suitably placed on either side of the RP4 cut site). The (*Eco*RI, *Hind*III and *Bam*HI) restriction sites on RP4 have been located relative to one another, but precise mapping of transposon insertion depends upon knowing the position of one of the restriction sites on each transposon. The position of the *Eco*RI site on Tn7 was deduced to be about 2.7 Mdal from one of its ends (Barth & Grinter, 1977). We have assumed this same position for Tn71 and Tn72 in order to calculate their insertion sites (Table 2, Fig. 1). This argument and method of calculation are fully expounded in Barth & Grinter (1977).

Relative positions of restriction sites on Tn71 and Tn72

The data on restriction fragments (Table 2) also enable one to calculate the relative positions of the restriction sites on Tn71 and Tn72. Thus we find that each transposon has the same sequence of restriction sites as Tn7. From the 'left' end of each transposon these are: *Eco*RI, *Hind*III, *Bam*HI, *Hind*III and *Bam*HI, their distances from that end being 2.7, 4.0, 5.3, 5.9 and 6.0 Mdal, respectively, for Tn71, and 2.7, 4.1, 5.5, 6.1 and 6.3 Mdal, respectively, for Tn72. Within experimental error (bearing in mind the indirect nature of the measurements) these values are not significantly different from the distances of 2.7, 3.8, 5.2, 5.8 and 5.9 Mdal estimated for Tn7 (Barth & Grinter, 1977).

Orientation of Tn71 and Tn72 insertion

Because of the asymmetry of the restriction sites on these transposons, the above calculations enable one to determine their orientation of insertion. We have previously found that Tn7 is inserted in the same orientation in all the (now more than 40) transposition RP4 plasmids that we have examined (Barth & Grinter, 1977, and unpublished data). Tn71 and Tn72 were also found to be in this orientation (Fig. 1).

DISCUSSION

We have identified two plasmids from different sources, determining Tp^r and Sm^r, from which we demonstrated transposition of these markers, initially to RP4. Sequential transposition of Tp^r, from the RP4 derivatives was demonstrated in a *recA* host, to the bacterial chromosome and thence to R702. Thus we conclude that these sequences are transposed by a specific mechanism, independent of the *recA* gene product. We have designated the transposons from R721 and pBW1 as Tn71 and Tn72 respectively. They are indistinguishable from Tn7 on the following criteria: (i) they determine the same resistances; (ii) they have the same molecular mass; (iii) each has a single *Eco*RI site, two *Hind*III sites and two *Bam*HI sites; (iv) these restriction sites are in the same order and at the same relative distances from one another.

Tn7 has multiple sites of insertion into plasmid RP4. These are not randomly placed around its circular map, but tend to concentrate into 'hot spots' (Barth & Grinter, 1977). In the two transposition plasmids we have examined, Tn71 and Tn72 were inserted into RP4 at two of these 'hot spots' (Fig. 1). This is consistent with our conclusion that Tn71 and Tn72 are very closely related or identical to Tn7.

The plasmids originally carrying Tn7, Tn71 and Tn72 are all clearly distinguishable by molecular weight and other properties (Table 1). They belong to two incompatibility groups, although all determine I pili. R721 and pBW1 are very similar and may have a common origin. The *E. coli* strain carrying R721 was isolated from a human urinary tract infection whereas pBW1 came from *E. coli* from a trimethoprim-treated calf, so there is no obvious epidemiological relationship between them. However, R483 differs from R721 and pBW1 in its incompatibility properties as well as molecular weight, and it is improbable that all three plasmids are descended from a common ancestor that had already acquired Tn7. Thus we

suggest that Tn7 has been transposed in nature between compatible plasmids. We doubt whether there is any significant correlation between the presence of Tn7 (or Tn71 or Tn72) and the production of I pili, since Tn7 is readily transposable to the *E. coli* chromosome and to plasmids of other incompatibility groups (Barth *et al.*, 1976). Further search may reveal this transposon in other naturally occurring plasmids.

Evidence for the world-wide distribution of TnA is well documented (Heffron *et al.*, 1975*b*; Matthew & Hedges, 1976). Other transposons encoding resistance to antibiotics have been described, for example Tn10 carries tetracycline resistance (Kleckner *et al.*, 1975). Tetracycline resistance genes are very common in plasmids, but information as to whether this has resulted from the spread of a particular transposon is lacking. The observations reported here are further evidence that transposons may be responsible for the rapid spread of drug resistances.

We are grateful to Nigel Grinter for technical assistance and to Mrs Beryl West, Wellcome Research Laboratories, Berkhamstead, Hertfordshire, for pBW1. Transposon numbers were allocated by the Plasmid Reference Centre, Stanford University, U.S.A. This work was supported by a grant from the Medical Research Council.

REFERENCES

- BACHMANN, B. J. (1972). Pedigrees of some mutant strains of *Escherichia coli* K12. *Bacteriological Reviews* **36**, 525-557.
- BARTH, P. T. & GRINTER, N. J. (1974). Comparison of the deoxyribonucleic acid molecular weights and homologies of plasmids conferring linked resistance to streptomycin and sulphonamides. *Journal of Bacteriology* **120**, 618-630.
- BARTH, P. T. & GRINTER, N. J. (1977). A map of plasmid RP4 derived by insertion of transposon C. *Journal of Molecular Biology* **113**, 455-474.
- BARTH, P. T., DATTA, N., HEDGES, R. W. & GRINTER, N. J. (1976). Transposition of a DNA sequence encoding trimethoprim and streptomycin resistances from R483 to other replicons. *Journal of Bacteriology* **125**, 800-810.
- COETZEE, J. N. (1972). Genetics of the *Proteus* group. *Annual Review of Microbiology* **26**, 23-54.
- COETZEE, J. N., DATTA, N. & HEDGES, R. W. (1972). R factors from *Proteus rettgeri*. *Journal of General Microbiology* **72**, 543-552.
- COHEN, S. N. (1976). Transposable genetic elements and plasmid evolution. *Nature, London* **263**, 731-738.
- DATTA, N. & BARTH, P. T. (1976). Hfr formation by I-pilus-determining plasmids in *Escherichia coli* K12. *Journal of Bacteriology* **125**, 811-817.
- DATTA, N. & HEDGES, R. W. (1972). Host ranges of R factors. *Journal of General Microbiology* **70**, 453-460.
- DATTA, N., HEDGES, R. W., SHAW, E. J., SYKES, R. B. & RICHMOND, M. H. (1971). Properties of an R factor from *Pseudomonas aeruginosa*. *Journal of Bacteriology* **108**, 1244-1249.
- FALKOW, S., GUERRY, P., HEDGES, R. W. & DATTA, N. (1974). Polynucleotide sequence relationships among plasmids of the I compatibility complex. *Journal of General Microbiology* **85**, 65-76.
- GOTIESMAN, M. M. & ROSNER, J. L. (1975). Acquisition of a determinant for chloramphenicol resistance by coliphage lambda. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 5041-5045.
- HEDGES, R. W. & DATTA, N. (1973). Plasmids determining I pili constitute a compatibility complex. *Journal of General Microbiology* **77**, 19-25.
- HEDGES, R. W. & JACOB, A. E. (1974). Transposition of ampicillin resistance from RP4 to other replicons. *Molecular and General Genetics* **132**, 31-40.
- HEFFRON, F., RUBENS, C. & FALKOW, S. (1975*a*). Translocation of a plasmid DNA sequence which mediates ampicillin resistance: molecular nature and specificity of insertion. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 3623-3627.
- HEFFRON, F., SUBLETT, R., HEDGES, R. W., JACOB, A. E. & FALKOW, S. (1975*b*). Origin of the TEM betalactamase gene found on plasmids. *Journal of Bacteriology* **122**, 250-256.
- JOBANPUTRA, R. S. & DATTA, N. (1974). Trimethoprim resistance factors in enterobacteria from clinical specimens. *Journal of Medical Microbiology* **7**, 169-177.
- KLECKNER, N., CHAN, R. K., TYE, B.-K. & BOTSTEIN, D. (1975). Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. *Journal of Molecular Biology* **97**, 561-575.
- KOPECKO, D. J. & COHEN, S. N. (1975). Site-specific *recA*-independent recombination between bacterial plasmids: involvement of palindromes at the recombinational loci. *Proceedings of the National Academy of Science of the United States of America* **72**, 1373-1377.
- MATTHEW, M. & HEDGES, R. W. (1976). Analytical isoelectric focusing of R factor-determined β -lactamases: correlation with plasmid compatibility. *Journal of Bacteriology* **125**, 713-718.
- NOVICK, R. P., CLOWES, R. C., COHEN, S. N., CURTISS, R., III, DATTA, N. & FALKOW, S. (1976). Uniform nomenclature for bacterial plasmids: a proposal. *Bacteriological Reviews* **40**, 168-189.