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# The transposons Tn*501*(Hg) and Tn*1721*(Tc) are related

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## SUMMARY

Internal sequences of Tn*501*(Hg) and Tn*721*(Tc) have been compared by hybridization. In spite of the difference in the resistance they code for, there is extensive homology between the two elements. This homology resides in the transposon-coded genes that are necessary for transposition and indicates that the elements are closely related.

## 1. INTRODUCTION

It is now apparent that transposable elements must play a fundamental role in evolution (see, for example, Calos & Miller, 1980). But the origins and inter-relationships of the elements themselves are not well understood. This report shows that two transposons that code for diverse resistance characters have considerable internal homology, and indicates how one particular group of transposable elements may have evolved.

The transposon Tn*501* carries genes that code for resistance to mercuric ions (Bennett *et al.* 1978) and Tn*1721* carries genes that code for resistance to tetracycline (Schmitt, Bernhard & Mattes, 1979). The 38 base pair inverted repeats at the termini of these two elements are almost identical, and the identity extends to the sequences adjoining the repeats (see Fig. 1). This high degree of homology between the terminal sequences strongly suggests that Tn*501* and Tn*1721* are closely related in spite of the difference in phenotypes that they confer. The relationship has been investigated further by comparing the internal sequences of the elements by hybridization, using both the method of Southern (1975), and heteroduplex analysis.

## 2. METHODS

The plasmids were pUB781 (a ColE1:Tn*501* recombinant – Bennett *et al.* 1978), pRSD1 (a naturally-occurring Tn*1721*-containing plasmid – Schmitt *et al.* 1979) and pJOE120 (a pUB781::Tn*1721* recombinant). Plasmid DNA was isolated from strains carrying the appropriate plasmids by CsCl/ethidium bromide equilibrium centrifugation of cleared lysates, essentially as described by Cornelis, Bennett & Grinsted (1978). Hybridization was carried out either by the visualization of heteroduplexes as described by Burkhardt *et al.* (1978) (which should give duplexes with DNA of about 85% homology and over), or by hybridization of $^{32}$P-labelled DNA to DNA fragments that had been separated on agarose gels and transferred to nitrocellulose filters, as described by Southern (1975). DNA was labelled by nick-translation in the presence of ($\alpha$-$^{32}$P) dATP (Maniatis, Jeffrey & Kleid, 1975) and the conditions of the 'Southern hybridizations' were 40% formamide in 2XSSC, with incubation at 45 °C, for 16 h. This should show up duplexes that are at least 85% homologous.



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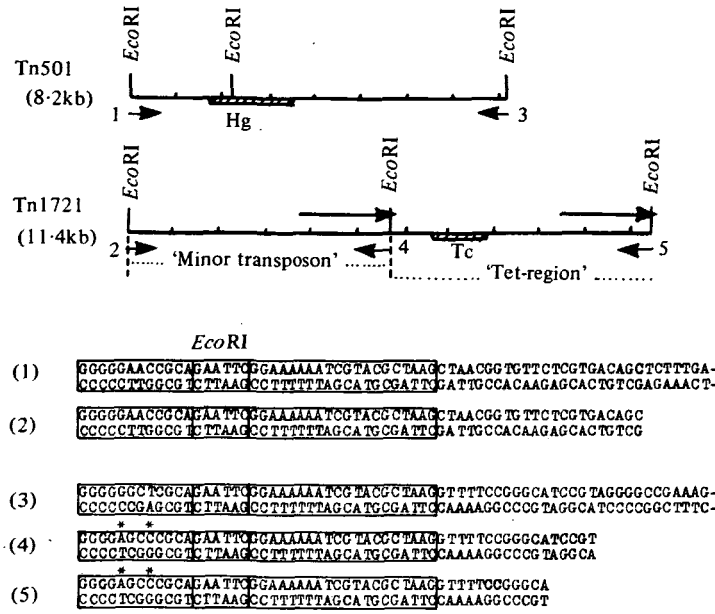


Fig. 1. Maps and terminal sequences of Tn*501* and Tn*1721*. The data are taken from Bennett *et al.* (1978) and Brown *et al.* (1980 and unpublished) for Tn*501*, and Schmitt *et al.* (1979) and Schöffl *et al.* (1980) for Tn*1721*. The transposon Tn*1721* contains a second copy of one of the inverted repeats. This divides the element into two segments: these are the 'minor transposon', which can transpose independently, and the *tet*-region (Schmitt *et al.* 1981). The sequence at the right-hand end of the *tet*-region is a direct repetition of the right-hand end of the minor transposon (this homology is indicated on the map of Tn*1721* by the heavy arrows); the remainder of the *tet*-region is homologous with the region of RP4 that codes for resistance to tetracycline (Scmitt *et al.* 1981). In the maps, the boxes indicate those sequences where insertional inactivation of the resistance markers can occur, according to Grinsted *et al.* (1978), and the small numbered arrows show the origin and direction of the sequences shown. In the sequences, asterisks show base pairs in Tn*1721* that are different from those in the corresponding sequence in Tn*501*, and the boxed base pairs show the inverted repeats.

## 3. RESULTS

There is an *Eco*RI site within the inverted repeats of both Tn*501* and Tn*1721* (see Fig. 1), so the sequences that comprise the elements can be almost exactly excised from host replicons by the action of this enzyme. The *Eco*RI fragments that make up the two elements were isolated; the fragments from Tn1721 were then digssted with *Bgl*I and the resulting fragments hybridized with radioactive *Eco*RI fragments of Tn*501* (Fig. 2). The smaller *Eco*RI fragment of Tn*501* (the 'left-hand' end of the element as drawn in Fig. 1) hybridized only with the fragment from the left-hand end of Tn*1721*. This homology is that seen in the sequences shown in Fig. 1, an interpretation confirmed by the heteroduplex analysis shown below. (The extent of this homology is, in fact, 82 bp – F. Schöffl *et al.* 1980.) The larger of the *Eco*RI fragments of Tn*501* hybridized with all of the fragments of the 'minor transposon' segment and with the two fragments from the right-hand end of Tn*1721* (Fig. 2). This homology at the right-hand end of the element

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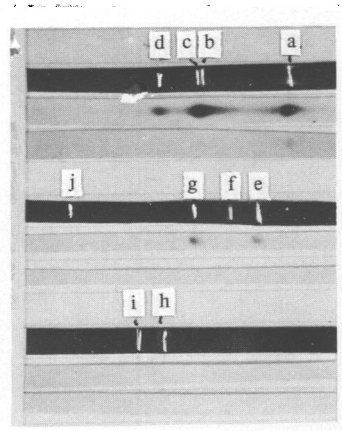


Fragment-1 filter
Big fragment probe
Small-fragment probe

Fragment-2 filter
Big fragment probe
Small fragment probe

Fragment-3 filter
Big-fragment probe
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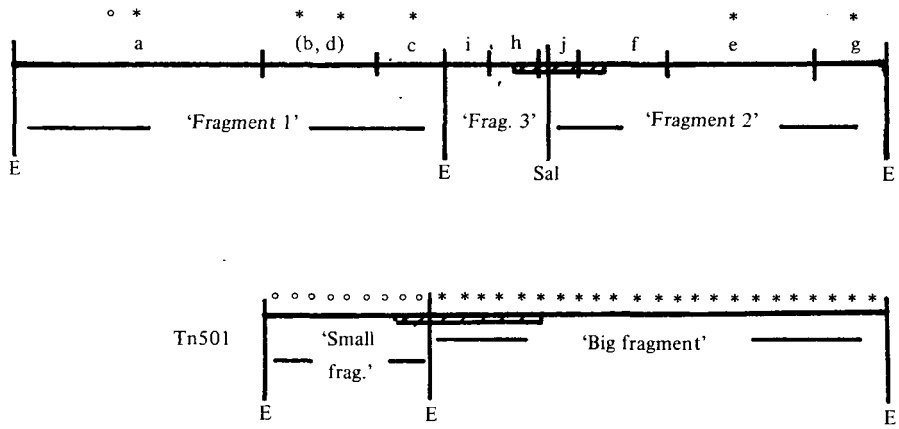





Fig. 2. Hybridization of $^{32}$P-labelled fragments from Tn*501* with fragments from Tn*1721*. Fragments of Tn*501* and Tn*1721* were excised from pUB*781* and pRSD1 respectively using *Eco*RI (plus *Sal*GI in the case of Tn*1721*, to give distinguishable fragments). The fragments of Tn*1721* were further digested with *Bgl*I, separated on agarose gels and transferred to nitrocellulose filters, and then hybridized with $^{32}$P-labelled Tn*501* fragments. The maps show the origin of the fragments and also give a summary of the data: * indicates hydridization of the large *Eco*RI fragment of Tn*501* with a particular band, and ° hybridization of the small *Eco*RI fragment.



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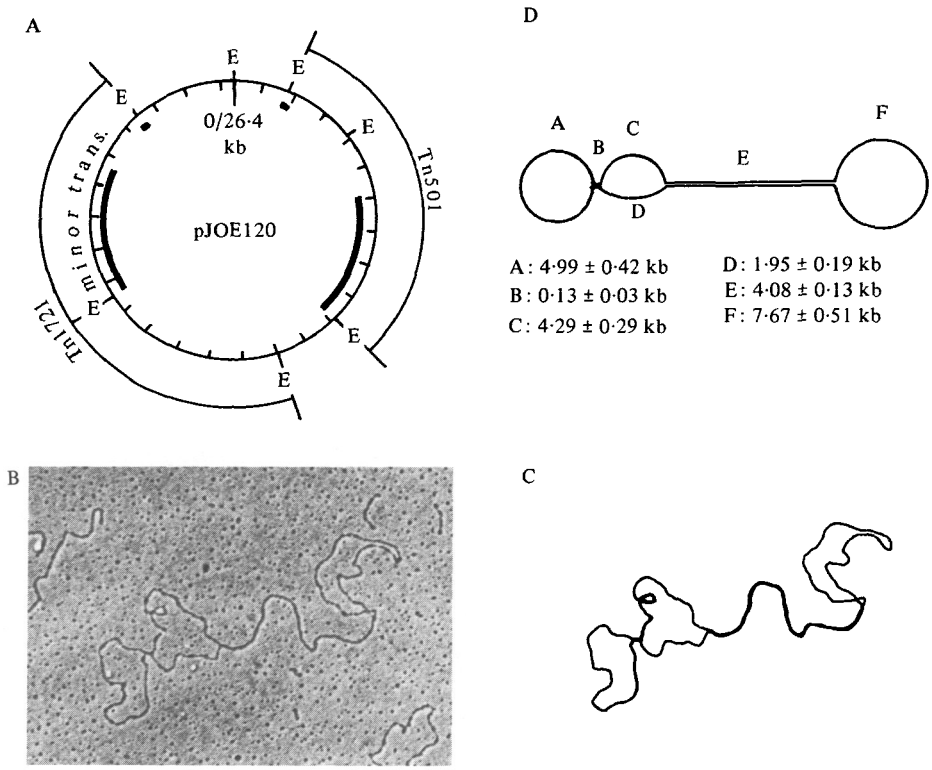


Fig. 3. Heteroduplex analysis of Tn*501* and Tn*1721*. The analysis was done by denaturing and re-annealing pJOE120, a double-recombinant plasmid which contains both transposons. A. Map of pJOE120, showing positions of the transposons. The heavy bars indicate the regions of homology shown in B. The *Eco*RI sites in the plasmid are represented by the letter E. B. Electronmicrograph of re-annealed single-stranded circular molecule of pJOE120 showing two regions of homology and three single-stranded loops. C. Tracing of B: thin line, single-stranded; thick line, double-stranded. D. Diagram of C (not to scale) showing single- and double-stranded regions and their lengths. These contour lengths are average values obtained from 20 different molecules ± standard deviation.



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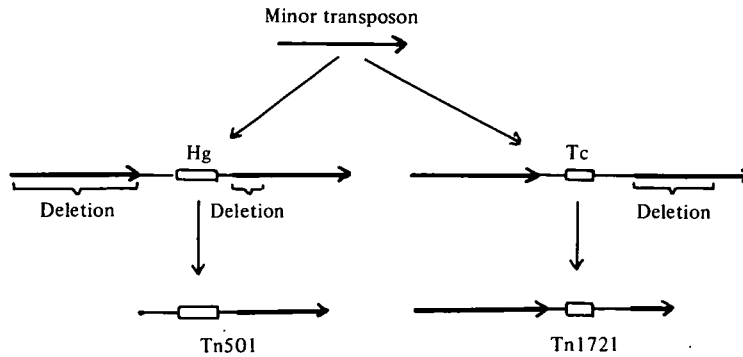


Fig. 4. Possible genealogy of Tn*501* and Tn*1721*. It is postulated that a transposable element very like the minor transposon of Tn*1721* flanks the appropriate genes, thus generating a transposon that contains those genes. Deletion could then result in the formation of the known transposons.

Table 1. *Inverted repeat sequences of transposons that generate a 5 bp direct repeat of host DNA*

| Element | Sequence | Reference |
|---|---|---|
| TN*3* | GGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAG | Takeya *et al.* 1979 |
| *γδ* | GGGGTTTGAGGGCCAATGGAACGAAAACGTACGTT | Reed *et al.* 1979 |
| Tn*551* | GGGGTCCGAGCGCACGAGAAATTTGTATCGATAAG | Khan & Novick 1980 |
| Tn*501* | GGGGGGCTCGCAGAATTCGGAAAAAATCGTACGCTAAG | Brown *et al.* 1980 |
| Tn*1721* | GGGGAGCCCGCAGAATTCGGAAAAAATCGTACGCTAAG | Schöffl *et al.* 1980 |

Underlined letters show the homology with the Tn*3* when the sequences are aligned as shown.

is due to the identity of this sequence with that at the right-hand end of the 'minor transposon' (see Fig. 1).

The overall implication of the data shown in Fig. 2 is that the minor transposon segment of Tn*1721* and the larger *Eco*RI fragment of Tn*501* are extensively homologous. The extent of this homology was investigated by the formation of heteroduplexes between the two transposons. This was done by re-annealing the single-stranded DNA of a plasmid that contains both elements in opposite orientation. The result and its interpretation are shown in Fig. 3: the small amount of homology at the extreme left-hand-side of the elements (see Fig. 1) shows up (B in Fig. 3D) and, apart from a substitution loop (C and D in Fig. 3D; from Tn*501* and Tn*1721* respectively) adjoining this terminal homology, the minor transposon and Tn*501* form a heteroduplex under the conditions used here and are at least 85% homologous. Since the right-hand-ends of the minor transposon and of the tet region of Tn*1721* are identical (see Fig. 1), it follows that the 2kb at the righthand ends of Tn*501* and Tn*1721* are also homologous. (Reannealed molecules that show this homology rather than Tn*501* with the minor transposon have also been seen – data not shown.)

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## 4. DISCUSSION

The data above show that there is a continuous sequence of about 4kb in common between Tn*501* and Tn*1721*. This sequence comprises the major part of the large *Eco*RI fragment of Tn*501* and the 'minor transposon' segment of Tn*1721*. The minor transposon can transpose independently of the rest of Tn*1721* (Schmitt *et al.* 1981), and there can be no doubt that all the transposon-coded genes that are necessary for transposition of this element are contained within this segment. The small *Eco*RI fragment of Tn*501* is predominantly concerned with mercury-resistance (Grinsted *et al.* 1978; P. M. Bennett, personal communication), so transposon-coded genes necessary for the transposition of Tn*501* are contained in the large *Eco*RI fragment of this element. Thus, it is the genes responsible for transposition that are common to both Tn*1721* and Tn*501*. This suggests that a common ancestor of the two elements may have been an element that was similar to the minor transposon, as shown in Fig. 4.

The transposons Tn*501* and Tn*1721* are both flanked by a 5 bp direct repeat of host DNA (Brown *et al.* 1980; Schöffl *et al.* 1980). Other large transposable elements containing extensive sequences that are necessary for transposition are also flanked by a 5 bp direct repeat (for references see Table 1). The inverted repeat sequences of these elements are related to those of Tn*501* and Tn*1721* (Table 1).

Such similarity is *prima facie* evidence for a common origin for this group of elements. The data in this report show that an element like the minor transposon, once elaborated, could generate the members of this class of transposable element. Hence, we predict that, in addition to the homology seen at the ends of these elements (Table 1), there will be homology in the genes that code for transposition functions. This has already been seen in Tn*3* and gammadelta (Reed & Steitz 1981).

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