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The Ends of Tn*10* Are Not IS*3*

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By heteroduplex and hybridization analysis we showed that the inverted repetition (here called IS*10*) at the ends of the translocatable tetracycline resistance element Tn*10* is not IS*3*, as had previously been reported by Ptashne and Cohen (J. Bacteriol. **122**:776-781, 1975). Further analysis confirmed the homology between IS*3* and the $\alpha\beta$ sequence of F and demonstrated that IS*10* was not present in the genomes of *Salmonella typhimurium* LT2 or *Escherichia coli* K-12.

The translocatable tetracycline element Tn*10* is bounded by a 1,400-base-pair inverted repetition. When Tn*10* was originally described (16), it was also shown that a mutant which had lost the drug resistance phenotype had suffered an insertion of 1,400 base pairs within the element homologous to the inverted repetition at the ends of the element. A great deal of additional evidence has subsequently accumulated supporting the idea that the 1,400-base-pair inverted repetitions are themselves independently active in transposition and in other illegitimate recombination events (9, 14a, 20). This idea fit nicely with the published observation that the 1,400-base-pair inverted repetition was homologous with the known insertion sequence IS*3* (14). IS*3* had been identified as an element which caused insertion mutations in the *lac* operon of *Escherichia coli* (6, 11), and was shown to be homologous to the $\alpha\beta$ sequence frequently encountered on F and R plasmids (8).

The first indication that there might have been some error in this picture was the finding by Ohtsubo and Ohtsubo (12) that there was a difference between $\alpha\beta$ sequences and the ends of Tn*10* in that the former are cleaved twice by the specific restriction endonuclease *Hin*dIII, whereas the latter are not cleaved at all. In this paper we show that IS*3* and the inverted repetition in Tn*10* share no homology whatsoever, although we confirmed the homology between IS*3* and $\alpha\beta$. We also show that there are no sequences normally present in the chromosomes of either *E. coli* or *Salmonella typhimurium* homologous to the ends of Tn*10*, whereas several sequences homologous to IS*3* can be detected. Since the inverted repetition of Tn*10* does, nevertheless, appear to have the properties of an IS sequence, we propose the name IS*10* to denote the family of sequences homologous to the 1,400-base-pair inverted repetition in Tn*10*.

Homology among sequences alleged to be related to IS*3* was tested by two different methods. In one, bacteriophage λ derivatives which contain two elements were constructed so as to be compared in both orientations with respect to each other. Then single strands of the phages were examined in an electron microscope under conditions which allowed the intramolecular reannealing of any homologous sequences, forming visible stem and loop structures. In the other method, plasmids containing either one or the other of the allegedly homologous elements were used as hybridization probes. Hybridization of radioactive plasmid DNA with restriction digests of lambda phages containing the other elements was assessed by the blot hybridization method of Southern (18).

Origin of the various 1,400-base-pair elements tested. (i) IS*3*. The IS*3* element is defined by an insertion mutation in the *lac* operon of *E. coli* (11). We obtained a lambda phage derivative (λ*plac*5 MS505) as a gift from M. Malamy, and all of the IS*3*-containing derivatives were made by genetic crosses with this phage. The lengths of the insertions were confirmed with an electron microscope and were found to correspond to about 1,400 base pairs each.

(ii) $\alpha\beta$. The $\alpha\beta$ sequence is defined as a 1,400-base-pair sequence which recurs twice in the F factor (8). Skurray et al. (17) made in vitro recombinant plasmids between the small plasmid pSC101 and partial *Eco*RI digests of F DNA. Two of these, pRS26 and pRS31 (kindly provided by A. J. Clark), contain the $\alpha\beta$ sequence near coordinate 0 of F and were used here.

IS*10* (the inverted repetition of Tn*10*). Tn*10* is defined as the tetracycline resistance element acquired from an R plasmid by phage

P22 (20). A coliphage derivative carrying Tn*10* was made by a series of genetic crosses and transposition events (3, 9). Derivatives carrying only part of the element (i.e., one or more copies of the inverted repetition IS*10*) were found as spontaneous drug-sensitive derivatives of a λ::Tn*10* phage (14a). The two derivatives used (del268 and del270) carried only IS*10* material and do not, insofar as we can tell, contain any residual material from the nonrepeated portion of Tn*10* (14a). These derivatives were used as isolated and were also introduced into derivatives containing IS*3* by genetic crosses.

A plasmid derivative carrying IS*10* was made by in vitro recombination methods. A fragment of a λ::Tn*10* insertion containing an entire copy of IS*10* was inserted into the colicin E1-derived plasmid vector pBR322. The inserted fragment is the left *Hin*dIII junction fragment of λcI171::Tn*10* (9) and includes, in addition to IS*10*, about 500 base pairs of λcI gene material and about 400 base pairs of nonrepeated Tn*10* sequence. Its structure was verified by restriction analysis.

Failure of IS*3* and IS*10* to show intramolecular annealing. The two ends of Tn*10* were shown to be inverted repetitions of each other because they anneal intramolecularly to form a stem and loop structure easily observed when the DNA is spread for electron microscopy under conditions which allow visualization of both single-stranded and duplex DNA (16, 19). To test the homology between IS*3* and the ends of Tn*10* (IS*10*), two lambda phages were constructed with contained IS*3* in one orientation and IS*10* in one of each of the two possible orientations. If the two elements were homologous, then one or the other of these phage should have shown a stem and loop. However, no stem and loop structure was observed with either combination of orientation (Fig. 1). Even when mixtures of DNA containing these IS*3* and IS*10* phage DNAs and λ::Tn*10* DNA were analyzed, only stems with loops of Tn*10* length were observed; the IS*3*:IS*10* stem would have flanked a loop with a length about twice that of the Tn*10* loop. We therefore concluded that IS*3* and IS*10* are not homologous.

Blot hybridization: *αβ* hybridizes to IS*3* but IS*10* fails to hybridize either *αβ* or IS*3*. An independent method of assessing homologies is by hybridization on nitrocellulose paper. We carried out hybridization experiments between derivatives of lambda carrying either IS*10* or IS*3* and small plasmids carrying either IS*10* or *αβ*. The form of the experiments was to digest the lambda phage DNA with endo R·*Eco*RI, electrophorese the fragments through an agarose gel, and transfer the DNA after denaturation to nitrocellulose paper by the method of Southern (18). The filters, which had an image of the separated DNA fragments, were then exposed to ^{32}P-labeled denatured plasmid DNA under conditions favoring hybridization, and the washed filters were autoradiographed.

The results of two such experiments are shown in Fig. 2. The labeled DNA of an *αβ*-containing plasmid hybridized specifically to IS*3*: lanes 1 and 2 of Fig. 2A contain the DNA of λ phages which differ only by the presence of IS*3* in the lane 1 DNA. Lanes 3 through 5 show

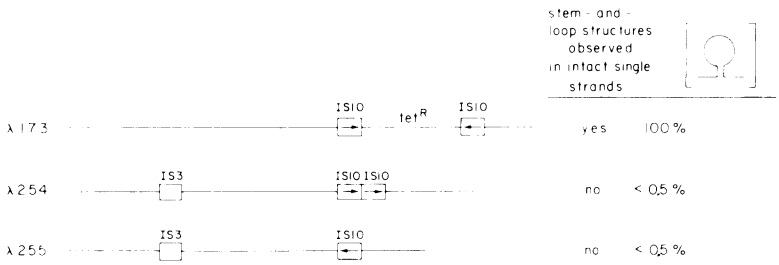

FIG. 1. *Failure of IS3 and IS10 to show intramolecular annealing. DNA from each phage was prepared for electron microscopy by using the formamide spread method of Davis et al. (4), with the modification that annealing was done at a low DNA concentration (less than 0.5 μg/ml). At least 200 intact single-stranded DNA molecules were scored in each assay for the presence of a stem and loop structure characteristic of intramolecular annealing. Mixtures of λ254 plus λ173 and λ255 plus λ173 were spread as controls: only stems with Tn10-size loops were seen. λ254 is λplac5-MS505::IS3 b1453 rex173::Tn10(del268) cI857; λ255 is λplac5-MS505::IS3 b1453 rex173::Tn10(del270) cI857; λ173 is b221 rex173::Tn10 cI857. The characterization of the del268 and del270 derivatives of λrex173::Tn10, including the fact that they are oppositely oriented, is fully described by Ross et al. (14a). λ254 and λ255 were obtained by standard genetic crosses starting with the original λrex173::Tn10del derivatives, λb1453 (7) and λplac5-MS505 cI857 (a gift of M. Malamy [11]). The size, position, and orientation of the remnants of Tn10 in λ254 and λ255 were checked by heteroduplex analysis with the parents. The size and position of the MS505::IS3 insertion in λ254 and λ255 were checked by heteroduplex analysis with λplac5 and by genetic crosses with known lac deletion mutations as described by Sodergren and Fox (17a).*

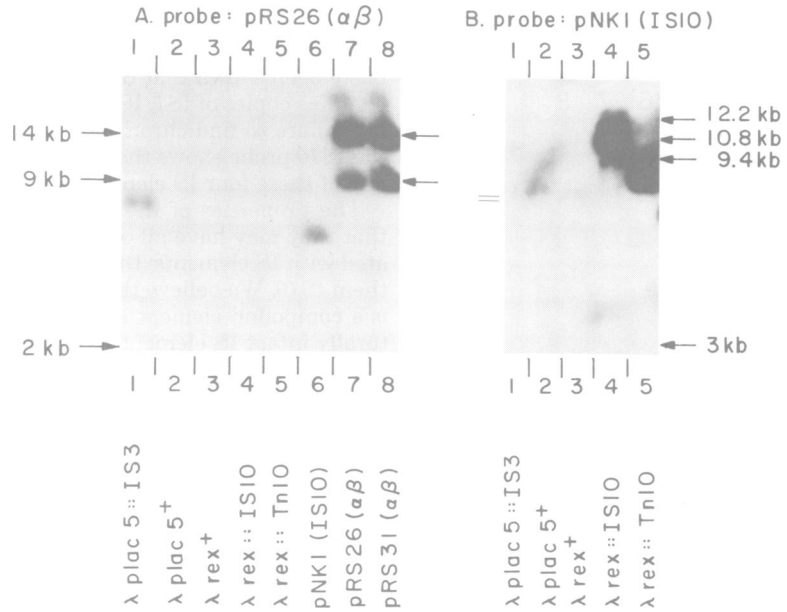

FIG. 2. *Blot hybridization of IS3-containing λ DNAs with ^{32}P-labeled plasmid DNA probes. λ phage DNAs were prepared, digested with EcoRI, and electrophoresed through 0.5% agarose gels as described previously (9). The gels were blotted onto nitrocellulose paper and hybridized to nick-translated plasmid DNA (17) essentially as described by Botchan et al. (2). Each lane contained about 1 μg of phage DNA; about 0.5 μg of denatured ^{32}P-labeled plasmid DNA (containing about 10^7 cpm) was used for each filter. Origins and genotypes of phages and plasmids are given in the text. The plasmid vector portions of pNK1 and pRS26 are partially homologous. The pair of narrow horizontal lines at the left of Fig. 2B indicate the position of weak hybridization observed in all lanes of this blot after extensive overexposure (see text).*

that the same radioactive probe failed to hybridize to IS*10* or even to the entire Tn*10* element. Lanes 7 and 8 show a positive control of *Eco*RI fragments of the plasmids totally homologous to the radioactive probe.

Figure 2B shows the results of identical experiments using as the radioactive probe a plasmid containing one end of Tn*10* (IS*10*). Lanes 1 and 2 show, again, two phages differing only by the presence of IS*3*. Both show weak hybridization at the same position (Fig. 2B). This weak hybridization is attributable to the fact that the probe was made from a plasmid containing about 500 base pairs of DNA from the λcI gene. Lanes 3 through 5 show phages which contained no Tn*10* DNA, one end of Tn*10*, and all of Tn*10*, respectively. Lanes 4 and 5 show extensive hybridization in addition to the weak band seen in lanes 1 and 2. Since these are the lanes containing DNA derived from Tn*10*, they show that the probe is specific for Tn*10* and that the failure to see hybridization in lane 1 can only mean that IS*10* and IS*3* are not homologous.

These results directly contradict the claim of Ptashne and Cohen (14) of homology between IS*3* and the ends of Tn*10*, while confirming the existence of homology between IS*3* and αβ as first reported by Hu et al. (8).

Absence of IS*10* from the chromosomes of *S. typhimurium* and *E. coli*. Since the ends of Tn*10* (IS*10*) are not IS*3*, but nevertheless appear to have some (if not all) of the properties of authentic IS elements, it was of interest to determine whether IS*10* was present in the chromosomes of *S. typhimurium* LT2 and *E. coli* K-12. This was done by performing blot hybridization experiments on total bacterial DNA, using radioactive IS*10*-containing plasmid DNA as a probe. An otherwise isogenic Tn*10* insertion mutant of each bacterial species was included as a control. Two restriction enzymes were used, each of which splits Tn*10* into two IS*10*-containing fragments (9). The results (Fig. 3) show clearly that the probe hybridized only with bacterial DNA containing Tn*10*; hybridization occurred at two positions as expected. Therefore IS*10* is not normally present in the chromosome of either *S. typhimurium* or *E. coli*. A similar result has been reported for transposon Tn*5*; no sequences homologous to Tn*5* are present in the *E. coli* genome (1).

The absence of IS*10* from the bacterial chromosomes confirmed the nonidentity of IS*10* and IS*3*; parallel experiments (not shown) showed

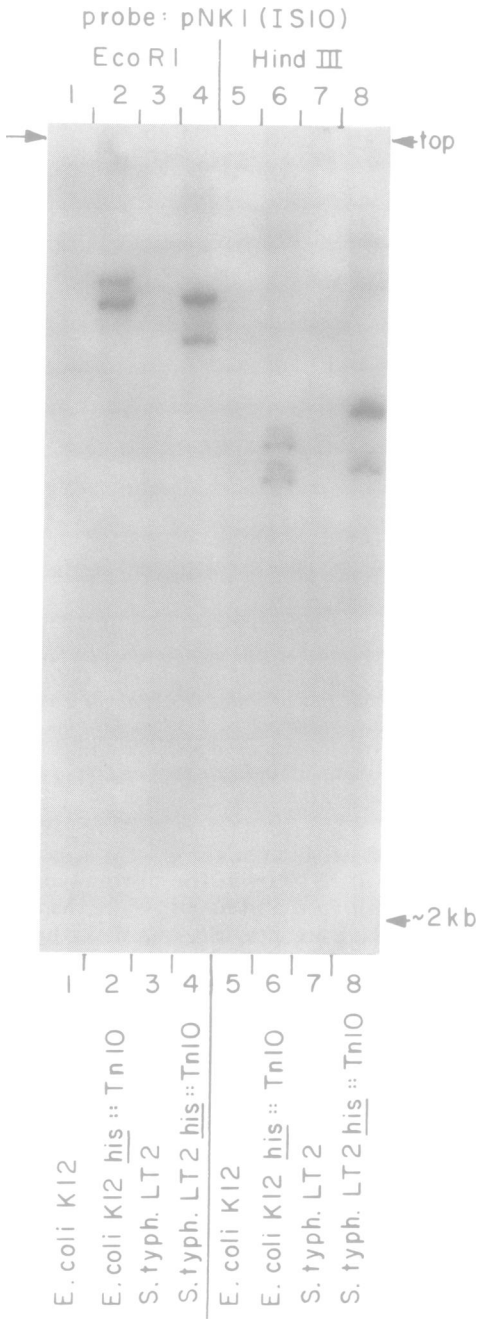

FIG. 3. *Blot hybridization of total bacterial genome DNA with ^{32}P-labeled IS10 plasmid probe. Total bacterial DNA was extracted essentially as described by Ebel-Tsipis et al. (5); about 1 μg of each DNA was digested with EcoRI or HindIII, electrophoresed, blotted, and hybridized to ^{32}P-labeled plasmid probe as described in the legend to Fig. 2.*

that both species have several copies of IS*3* in their genomes. Furthermore, since it has previously been shown that the *E. coli* K-12 genome contains copies of IS*1*, IS*2* (11, 15), and IS*4* (13), the failure to find chromosomal homology with the IS*10* probe shows that IS*10* is not related to any of these four IS elements.

The properties of the ends of Tn*10* indicate that they may have all of the properties associated with IS elements; therefore, we have called them IS*10*. We believe that Tn*10*, like Tn*9* (10), is a compound element consisting of two structurally intact IS elements flanking a unique sequence encoding a drug resistance determinant. However, the IS element in Tn*10* is not IS*3*, and the report of Ptashne and Cohen (14) must be in error. The reason for the error is not clear.

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