TRANSPOSITION OF PLASMID-BORNE Tn*10* ELEMENTS DOES NOT EXHIBIT SIMPLE LENGTH-DEPENDENCE

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ABSTRACT

The transposition frequencies of Tn*10* elements from the bacterial chromosome to an F epitome decrease 40% for every kilobase increase in transposon length. The basis for this relationship is not known. We have now examined complemented transposition of defective Tn*10* elements off small multicopy plasmids. We find that length dependence in this situation is either reduced or absent, depending on the specific class of transposition events involved. These observations can be interpreted as evidence against the model that chromosomal length dependence occurs because of decay of a transposition-associated replicative complex. This interpretation is consistent with unrelated experiments suggesting that Tn*10* transposition is normally nonreplicative. Alternative explanations of length dependence phenomena are discussed.

TRANSPOSITION rates for chromosomal insertions of Tn*10*- and Tn*9*-like elements decrease exponentially as the length of the element increases (MORISATO *et al.* 1983; CHANDLER, CLERGET and GALAS 1982). Tn*10* transposition decreases by approximately 40% per kilobase increase in length; for Tn*9* the rate of decrease is 50% per kilobase. CHANDLER, CLERGET and GALAS (1982) proposed two explanations for the exponential relationship between transposition rate and transposon length. First, transposon DNA might behave as a three-dimensional random coil whose two ends, with or without transposase protein attached, find one another by collision. Second, some feature of transposition might be processive, beginning at one end of the transposon and proceeding to the other with a constant probability of decay per unit length of DNA. CHANDLER, CLERGET and GALAS (1982) originally suggested that this feature might be transposition-associated DNA replication, with the fork aborting at a constant probability per unit length. In fact, this feature might be any process, replicative or nonreplicative, in which transposase, bound to one transposon end, moves along the DNA to the second transposon terminus with a constant probability per unit distance that the complex will

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fall off the intervening DNA or become irreversibly bound to it (CHANDLER, CLERGET and GALAS 1982; THOMPSON, CAMIEN and WARNER 1976).

We have now examined the length dependence of transposition by complemented function-defective Tn*10* elements off of small multicopy plasmids, and we find results that differ from those obtained with self-driven transposition of chromosomal Tn*10* elements. We suggest that the differences can be most easily explained if length dependence arises by mechanisms other than decay of transposition-linked replication.

MATERIALS AND METHODS

Mating-out transposition assay: The mating-out assay is described in more detail by FOSTER *et al.* (1981). Briefly, single colonies of F′ donor strains containing transposon plasmids and function donors (see figure legends) were grown into saturated cultures; were subcultured; were regrown briefly with minimal agitation into exponentially growing, conjugation-proficient cultures; were mated for 90 min with a late-log culture of the recipient strain and the mating mixture was plated on selective plates to determine the titer of bacteria carrying both recipient and transposon markers. Several transformants of each plasmid-containing donor strain were tested in each experiment, and the data from the several cultures were averaged. In these experiments, mating efficiencies are highly reproducible from one donor culture to another. Thus, relative average titers of the desired exconjugant types are assumed to reflect directly the relative transposition rates of the corresponding transposons in the donor.

Plasmid constructions: Standard recombinant DNA techniques were used, essentially as described in MANIATIS, FRITSCH and SAMBROOK (1982). All enzymes were purchased from New England Biolabs. BAL31 digestions were performed as described by WAY and KLECKNER (1984).

RESULTS

Effects of deletions and insertions on transposition of plasmid elements: Tn*10* transposition from a plasmid into the pOX38 conjugative episome was examined using derivatives of a multicopy donor plasmid containing four Tn*10* ends (pNK615, Figure 1). The four ends are oriented as two pairs of inverted repeats, with a KanR marker present between one pair and a LacZ marker present between the other. The segments that separate the two pairs on either side are also marked, with AmpR and HisD respectively. Each of the four marked segments is roughly 3–4 kb in length. When transposition functions are provided in *trans* from a λ prophage containing an IS*10* transposase overproducer construction, the absolute and relative rates of transposition of different segments can be determined by selection and screening for appropriate markers (see below). In several cases the structures of genetically identified transposition products have been confirmed by restriction analysis of the pOX38::Tn derivatives (not shown).

Each of the different segments on pNK615 transposes at a different frequency (Figure 2, legend); however, no conclusions about length dependence can be drawn from these differences, because the intrinsic activity of each terminus on the plasmid is likely to be different. Tn*10* terminus activity varies as much as 30-fold, depending on the nature of the immediately adjacent nucleotides (M. A. DAVIS and N. KLECKNER unpublished results) and the presence or absence of transcription crossing into the terminus from either direc-

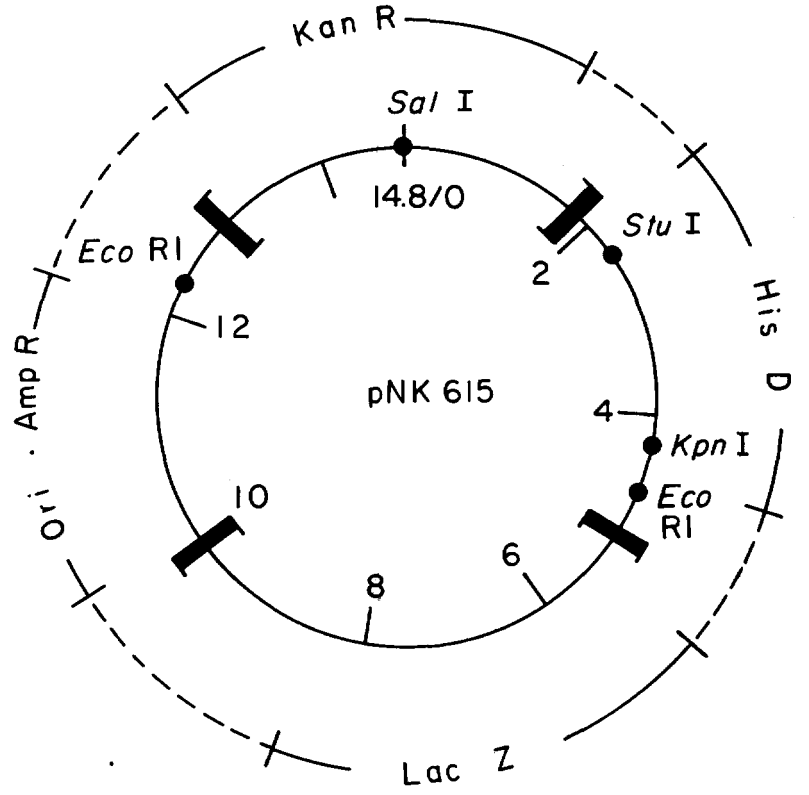

FIGURE 1.—Structure of the 14.8 kb plasmid pNK615. Each of the four Tn*10* termini consists of the outermost 70 bp of IS*10* (right). The two pairs of termini bracket 4.0 kb KanR and 5.0 kb LacZ segments, respectively. The HisD and AmpR-Ori segments are each 2.9 kb. pNK615 was constructed by insertion of the *Eco*RI fragment from pNK289 (FOSTER *et al.* 1981) into the unique *Eco*RI site of pNK419 (WAY and KLECKNER 1984).

tion (WAY and KLECKNER 1984; M. A. DAVIS, R. W. SIMONS and N. KLECKNER, unpublished results). Length dependence, therefore, was examined by construction and analysis of derivatives of pNK615 in which the length of one of the four marked segments was altered by deletion or by insertion of random *E. coli* fragments. Transposition of segments in the altered derivatives was compared with transposition of the corresponding segments in the parental plasmid.

The structures and transposition properties of altered plasmids are shown in Figure 2. (Numbers in parentheses are probably artificially low; the corresponding plasmid insert makes its host grow poorly.) The general finding from these experiments is that insertion or deletion of DNA at the *Sal*I, *Eco*RI or *Kpn*I sites does not reduce or increase transposition of segments containing the inserted fragment in the way predicted from length-dependence experiments with chromosomal insertions. In particular:

1. Insertions into the *Sal*I site of 1.5, 7.5, 9 and 12 kb do decrease transposition of the smallest KanR segment in an exponential fashion, but the rate

Site of Alteration	Plasmid (pNK)	Extent of Alteration (kb)	Relative Transposition Frequencies of: 3.8 kb	12 kb	12 kb [size]
none	615	none	≡1	≡1	≡1
Sal I	1086	+1.5	0.8	1.2	1.0
	1090	+7.5	0.25	0.7	0.8
	1087	+9	0.2	1.4	0.7
	1092	+12.5	0.1	0.5	0.6
Eco RI	1091	+4.5	0.8	1.0	0.4
	1094	+12	(0.2)	(0.25)	(0.05)
Kpn I	1082	−0.2	0.9	1.3	0.8
	1084	−1.5	0.9	0.8	1.2
	1089	+1.0	1.0	0.9	1.0

FIGURE 2.—Structures and properties of pNK615 derivatives. pNK615 was altered by deletion of DNA from the unique *Kpn*I site using BAL31 (pNK1082), by deletion of DNA between the *Kpn*I and *Stu*I sites (pNK1084) and by insertion of random *Sal*I-, *Kpn*I- and *Eco*RI-generated restriction fragments of *E. coli* K12 into pNK615's unique *Sal*I site (pNKs 1086, 1087, 1090 and 1092), unique *Kpn*I site (pNK1089), and *Eco*RI site near the AmpR gene (pNK1082) respectively. Transposition of different DNA segments from pNK615 and derivatives into the pOX38 conjugative episome (GUYER *et al.* 1981) was measured and a mating-out assay was carried out as described in MATERIALS AND METHODS and by WAY and KLECKNER (1984) and FOSTER *et al.* (1981). Plasmids were introduced into donor strain NK6660 (*recA*⁻ pOX38) that carries a λ prophage and a transposase overproducer construction, λ1046 (MORISATO, *et al.* 1983; WAY and KLECKNER 1984). The recipient was NK6641 (*ΔlacproXIII recA*⁻ *strA* λ^R *mal*⁻; MORISATO *et al.* 1983; FOSTER *et al.* 1981). The recipient also carried a multicopy CamR plasmid, pNK259, that is incompatible with pNK615 and others. Presence of this plasmid prevented overreplication of pOX38::Tn exconjugants containing the pNK615 origin on the transposed segment. Kanamycin-resistant exconjugants were selected in the presence of chloramphenicol and were scored for LacZ and AmpR phenotypes. Relative frequencies of exconjugants carrying transposon markers are given. In the particular experiment shown, 0.01 ml of the parental pNK615 mating mix gave about 200 transpositions of the small KanR segment, and 20 transpositions each of the large KanR-AmpR-LacZ (KHL) and KanR-HisD-LacZ (KDL) segments (average of five transformants, see MATERIALS AND METHODS).

of decrease is only 15% per kilobase, rather than the 40% per kilobase observed for chromosomal transposition (Figure 3). Furthermore, these insertions have no effect on transposition of the two larger segments that include the KanR segment. If all transposition events decrease exponentially with increasing transposon length, transposition of the larger elements should decrease at the same rate as transposition of the smaller elements.

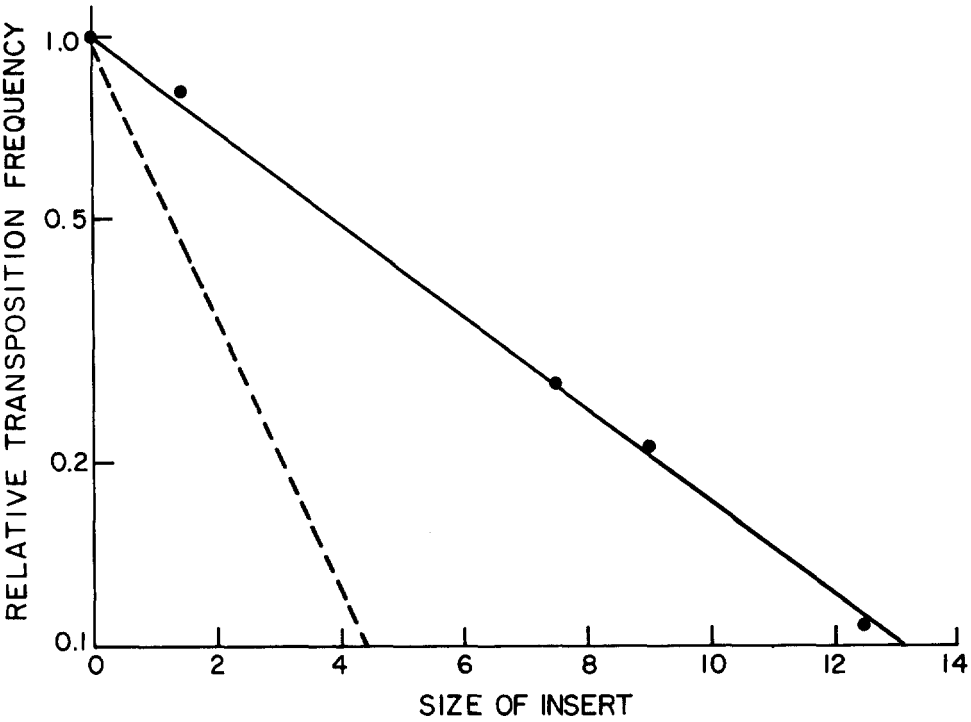

FIGURE 3.—Transposition of KanR segment as a function of length. Transposition frequencies of KanR segments from plasmid carrying DNA inserts at the *Sal*I site (pNK1086, 1090, 10087 and 1092) relative to that of pNK615 are plotted on a logarithmic scale against the size of the insert on a linear scale. Data are from Figure 2. Solid line corresponds to a decrease of 16% per kilobase increase in transposon length; dashed line corresponds to a decrease of 40% per kilobase increase as found for chromosomal Tn*10* insertions (MORISATO *et al.* 1983).

2. Insertions of 4.5 and 12 kb into the *Eco*RI site have little or no effect on transposition of segments carrying the insertions. Even more paradoxically, both insertions decrease transposition of the large segment that does *not* contain the insert. Transposition of the KHL segment from pNK1091 is decreased by twofold, which is a reproducible and significant effect. Transposition of the same segment from pNK1094 is decreased at least four-fold relative to that of other segments, assuming that transposition of all segments is nonspecifically depressed four or fivefold due to a deleterious effect of the inserted fragment.

3. Small insertions and deletions into the *Kpn*I site have no detectable effect on transposition frequencies, where differences of up to 70% would have been expected among the derivatives tested from the chromosomal length dependence relationship.

Transposition off a transposon plasmid dimer: We also examined transposition of DNA segments from a dimeric derivative of pNK1084 (= pNK615 with a small, irrelevant deletion at the *Kpn*I site). Because each of the four Tn*10* termini of pNK1084 is present on this plasmid twice (Figure 4), one can compare transposition of the shortest segment between a given pair of ends

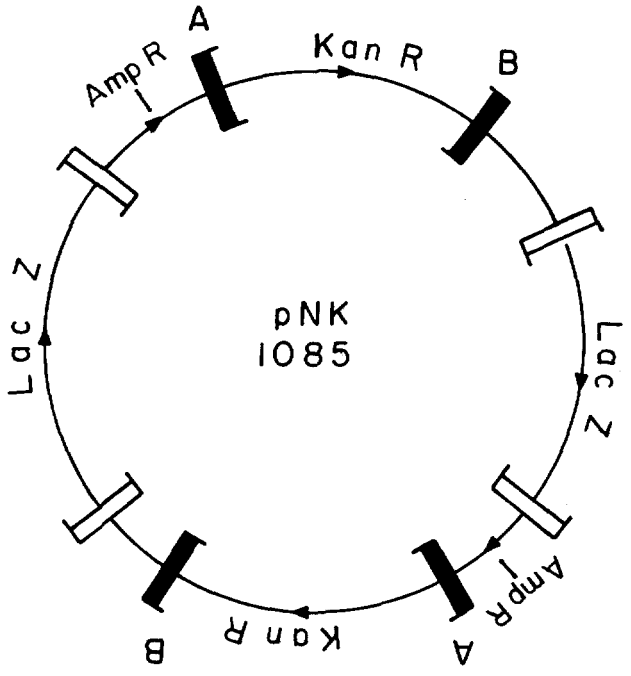

FIGURE 4.—pNK1085 is a dimeric form of pNK1084 (Figure 2) obtained and purified in a *recA*⁻ host.

and transposition of what amounts to the same segment containing an insert of pNK1084. These two segments should not differ in the intrinsic properties of their termini but only in the length and nature of the material between them. Consider the termini marked *A* and *B* flanking the KanR segment in Figure 4. An *A* and a *B* terminus can promote transposition of the KanR segment alone (segment X) or of a larger unit containing two KanR segments and a LacZ segment (segment Y). Among transpositions involving termini *A* and *B*, about 80% (110 of 138) were transpositions of the small KanR segment alone (X); while 20% (28 of 138) were transpositions of two KanR segments plus the intervening material (Y). Once again, the results are contrary to those expected from chromosomal length dependence assays. Since element Y is about 13.5 kb longer than element X, length dependence of the type observed for chromosomal Tn*10* elements would have predicted a several hundred-fold drop in transposition frequency.

DISCUSSION

The above experiments demonstrate that the simple length dependence observed for self-driven Tn*10* transposition from the bacterial chromosome is not seen during complemented transposition of Tn*10* from a small multicopy plasmid. Transposition of one short element exhibits greatly reduced length dependence, while transposition of the larger elements is usually not affected at all by changes in length. This discrepancy with the previous results is not

likely to reflect a difference between complemented and self-driven transposition, because transposition of chromosomal elements is length-dependent even when driven by transposition functions provided in *trans* from a transposase overproducer plasmid (MORISATO *et al.* 1983).

One interpretation of these results is that Tn*10* transposition of a multicopy plasmid is mechanistically fundamentally different from transposition off of the chromosome—for example, that chromosomal transposition is replicative while most or all plasmid transposition is not—or that the replication process differs significantly in the two cases. This does not seem very likely, primarily because there has been no other indication that chromosomal and plasmid transposition differ significantly. The frequency of Tn*10* transposition per donor genome is the same for chromosomal and plasmid elements, as judged by comparing transposition of a particular *hisG*::Tn*10* insertion from a chromosomally integrated λ *hisG*::Tn*10* prophage with transposition of the identical insertion off of a multicopy *hisG*::Tn*10* plasmid (N. KLECKNER, unpublished results). More importantly, the relative frequencies of different Tn*10*- and IS*10*-promoted DNA rearrangements, which differ from one another by several orders of magnitude, are virtually identical for both chromosomal and plasmid-borne Tn*10* elements (WEINERT *et al.* 1984; M. SHEN, E. A. RALEIGH and N. KLECKNER, unpublished results.

We would like to propose, instead, that neither chromosomal length dependence nor the less dramatic length dependence observed for one plasmid segment is due to decay of a passing replication fork, and that both effects reflect the process by which a complex is formed between transposase protein and two transposon ends. According to this interpretation, the difference between chromosomal and plasmid transposition results from differences in the nature of the two donor molecules.

Chromosomal length dependence could reflect the probability of collision between the two terminus-bearing segments on a randomly coiled bacterial chromosome (MORISATO *et al.* 1983). If so, reduction or disruption of length dependence on a small plasmid could result from more compact coiling of the small circular molecule. Alternatively, length dependence could reflect decay of a transposase/terminus complex searching in one dimension for a second terminus. In this case, searching on a small circular plasmid might be different from searching on the long, linear bacterial chromosome. For example, searching might continue around and around the plasmid until an effective site is found. Alternatively, smooth one-dimensional movement might alternate with intramolecular jumps between one segment of the plasmid and another. Evidence is already available that IS*10* transposase can search a target and select stronger termini over weaker ones; and intramolecular "jumps" have been proposed to account for the behavior of Int and Resolvase proteins (MORISATO and KLECKNER 1984; KRASNOW, MATZUK and COZZARELLI 1983; BERG, WINTER and VON HIPPEL 1982). Disruption of smooth searching and/or the possibility of transposase movement "the wrong way around" a plasmid molecule could account for the total absence of length dependence in longer transposon segments. It could also account for the inhibitory effect in two cases of insert-

ing DNA outside the transposing segment, although this effect might also be due to nonspecific inhibition of adjacent terminus activity by promoters on the inserted sequences.

Consistent with the notion that length dependence is not related to a replicative process is independent genetic evidence that Tn*10* transposition does not involve DNA replication. (KLECKNER *et al.* 1984; J. BENDER and N. KLECKNER, unpublished results) have shown that artificially constructed heteroduplex Tn*10* elements present on appropriate λ phages give rise to mixed colonies when they transpose into hosts lacking mismatch correction enzymes. The simplest interpretation of these results is that Tn*10* DNA is excised from the donor molecule and is inserted more-or-less intact into the target chromosome.

In the case of bacteriophage Mu, there is direct evidence that transposition length dependence does not result from abortive DNA replication but from a prior step. FAELEN *et al.* (1985) have recently demonstrated that transposition by Mu, which is known to involve replication of the Mu genome, exhibits an exponential decay with increasing genome length similar to, although slightly less severe than, that observed with Tn*9* and Tn*10* elements. However, measurements of phage replication following prophage induction revealed that increasing Mu genome length results in a decrease in the absolute amount of Mu-specific DNA synthesis to levels of less than one or two copies per genome, even after 60 min, suggesting that the length-sensitive step is formation of a complex between the two termini or some other prereplicative event.

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