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ENTITLED Insertion Orientation and Distribution Preferences of Tn3-Mediated Transposition in a Transposition Immune System

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science

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Insertion Orientation and Distribution Preferences of Tn3-Mediated Transposition in a Transposition Immune System

by

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INTRODUCTION

Transposons are DNA sequences that are able to insert an identical copy of themselves into a new DNA site. Typically, transposons encode enzymes that bring about transposition. In general, transposons range anywhere from 750-40,000bp in length and do not require DNA sequence homology with the recipient DNA in order to transpose(18). Although transposons generally appear to insert randomly within recipient DNA, certain DNA regions tend to be transposed into more frequently and are termed transposition "hot spots". Transposons are responsibe for much of the genetic diversity in bacteria and can confer characteristics such as antibiotic resistance or toxin production to certain strains(3). The frequency of transposition is characteristic for each transposon. Low frequency transposons transpose at a frequency of about 10^{-6} per cell division. High frequency transposons transpose at a frequency of about 10^{-1} per cell division(17).

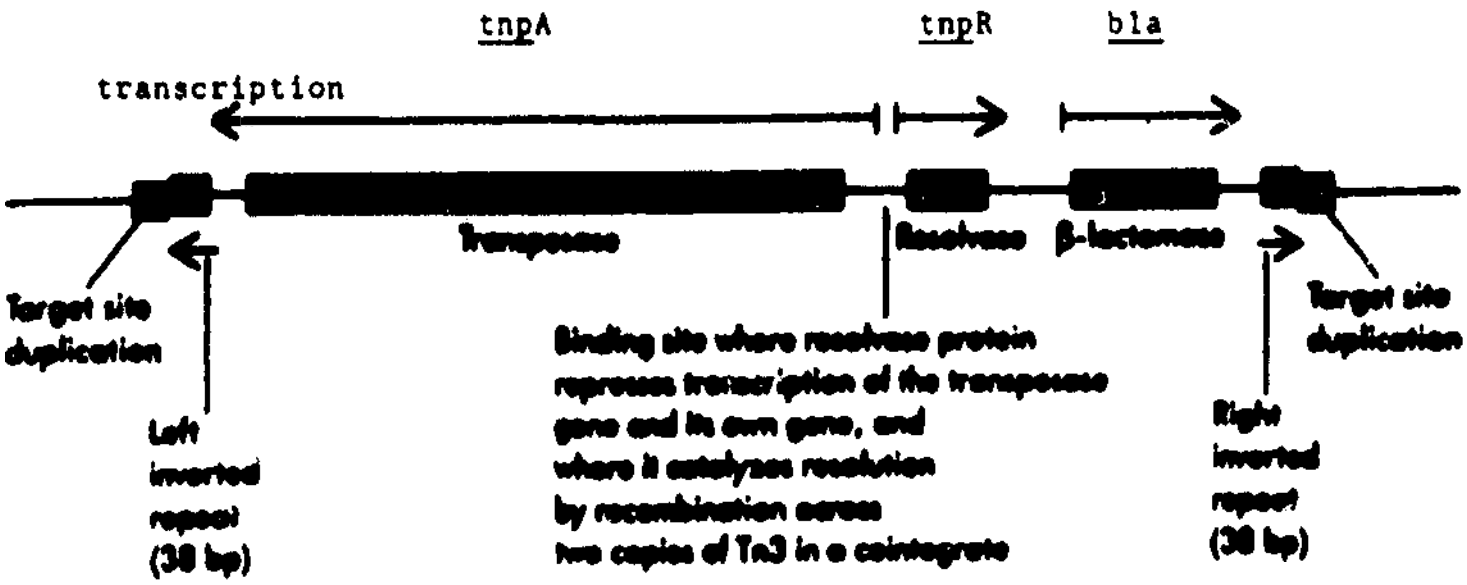
There are three main categories of transposable elements in bacteria.

1. insertion sequences(IS) 2. transposons 3. transposable bacteriophage such as bacteriophage Mu. IS elements are transposable elements which carry no known genetic information other than that necessary to transpose. Thus, key components are sequences of 20-40 nucleotides of inverted repeats at the ends of the element and the presence of one or more genes that encode the transposase function responsible for catalyzing insertion of the element into a new DNA site. Currently, the known function of IS elements is to induce genetic changes via activation of dormant adjacent genes, deletions, inversions or chromosomal fusions(18). The second

category of transposable elements are known as transposons. Transposons encode genetic information, such as antibiotic resistance, in addition to that necessary for transposition. Frequently these transposons are flanked by two complete IS elements which suggests that they are created when two IS elements flank one or more cellular genes and mobilize the entire complex. Transposons flanked by IS elements are called composite transposons(18). Two examples of this type are Tn9 and Tn10. Tn9 encodes chloramphenicol resistance and is bound by direct repeats of IS1. Tn10 encodes tetracycline resistance and is flanked by IS10 inverted repeats. Transposition in these cases is mediated by transposase produced by the intact IS elements(2).

However, not all transposons are flanked by IS elements. The related transposons Tn1 and Tn3 are two such examples. These transposons lack IS elements and are defined by terminal inverted repeat sequences of 38bp in length. Tn1/Tn3 are very closely related, differing in only a few restriction enzyme sites(11). Tn3 has been sequenced. It is 4957bp in length and contains three genes plus an internal res site (fig.1)(18). The first gene, tnpA encodes the transposase enzyme, the second gene, tnpR encodes the resolvase enzyme, and the third gene, bla, encodes the enzyme B-lactamase, which destroys the B-lactam antibiotics like penicillin, ampicillin and carbenicillin.(2,5,6).

fig.1(18) STRUCTURE OF Tn3

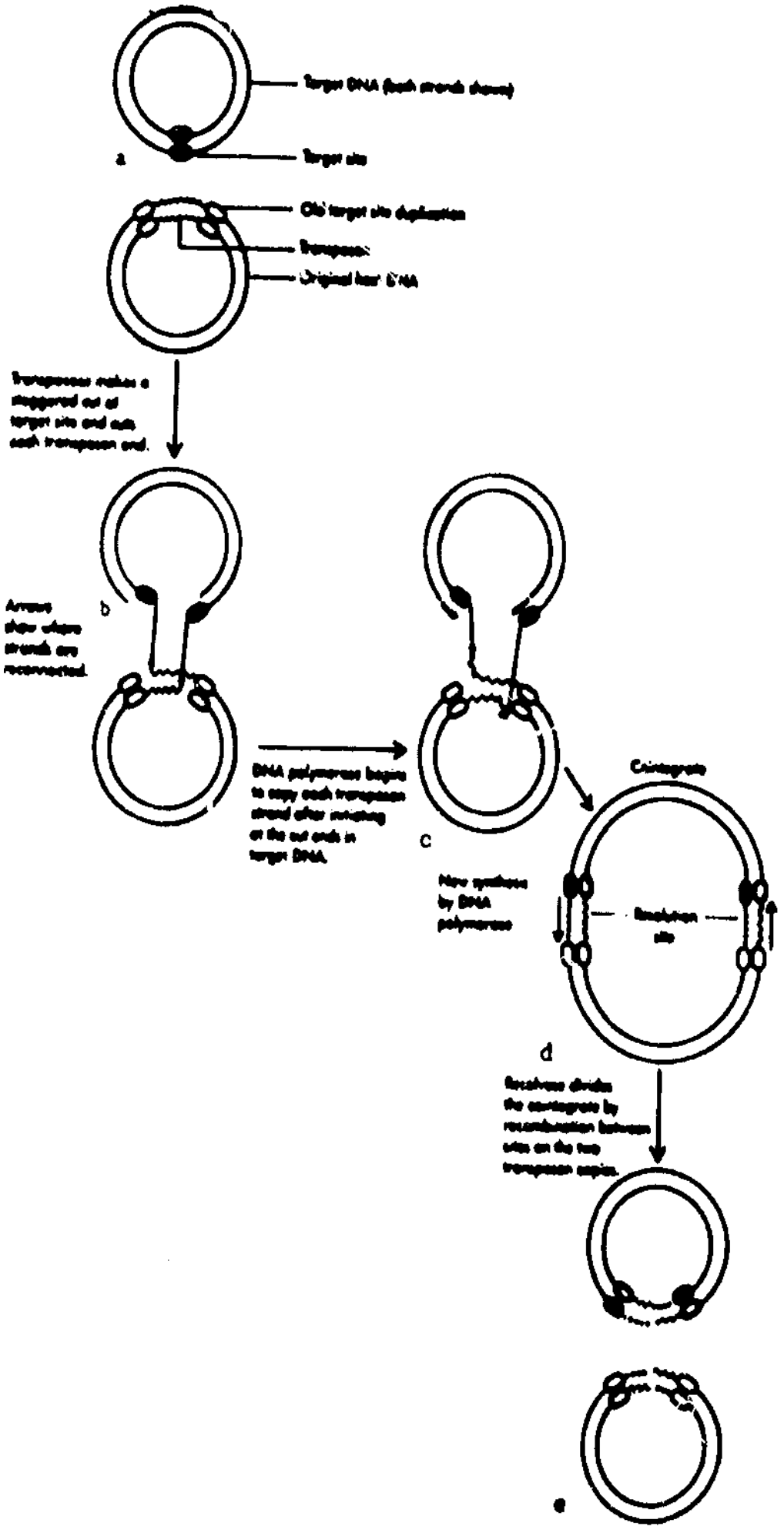


The enzyme transposase is the product of the first gene of Tn3. It is a thermal labile enzyme, which is active at 32^{0}C, but inactive at 37^{0}C. Since transposons move precisely, carrying none of the adjacent DNA, it is believed that transposase recognizes both transposon ends, thus explaining the need for inverted repeated ends(3,9). The current model for the role of transposase is that it binds to both ends of the transposon and also the target DNA sequence. It then makes 5bp staggered cuts in the target DNA (like a restriction enzyme) and also makes one cut at each transposon end, but on different strands. It is possible that cellular enzymes may also be involved in this step (fig.2 step a). The freed transposon ends are then ligated to the target site ends (which are also 5bp long), connecting the two DNA molecules, which are bridged at each end by one strand of the original transposon (fig.2 step b)(12). Now the single stranded DNA at the target site and also the transposon DNA must replicate semi-conservatively via DNA polymerase forming a structure called a cointegrate (fig. 2 step c). This model of transposition explains the 5bp target site direct repeats characteristic of Tn3 insertions(3). Cointegrates can best be described as a fusion of the donor and recipient replicon via two direct repeats of the transposon (fig.2 step d)(12). The second Tn3 encoded gene product, resolvase, catalyzes site-specific recombinaton between the two transposon copies at the res sites, producing separate DNA molecules, each with one copy of the transposon (fig.2 step e). Thus, in cases where Tn3 is tnpR$^-$, a cointegrate structure will remain as the final product as long as the cell is deficient in homologous recombination. Transposon sequences are long enough to allow resolution of cointegrates via rec-mediated

homologous recombination.(9,18).

A second function of the tnpR gene product is a regulatory one. The resolvase protein can repress transcription of the transposase gene and its own gene in trans by binding to specific DNA sequences in the A-T rich(70-80% A and T) res region. This region is located between the tnpA and tnpR genes and contains the promotors for both tnpR and tnpA(5,6). Thus, levels of transposase and resolvase enzymes are regulated by resolvase. The level of transposition is increased in tnpR$^-$ versions of Tn1/Tn3, and it has recently been observed that the frequency of transposition can be significantly increased(up to 1000 fold) when more transposase is provided(18). An interesting discovery made recently was that transposons tend to be much more active just after replication. It is believed that this burst of activity is due to the fact that the transposase promoter, which contains the DNA sequence GATC, is methylated at the adenine nucleotide. The complementary strand, also GATC, is methylated as well. In the methylated state, the promoter is relatively inactive. However, after DNA replicates, the adenine in the newly synthesized GATC is not methylated for a minute or so and the promoter is very active. Thus, a small burst of transposase is made just after replication attributing to the increased frequency of transposition(18).

fig. 2 Model of Transposition



Another means of regulating transposition besides suppressed levels of transposase is transposition immunity. Transposition immunity is defined as the greatly reduced ability of a replicon carrying a copy of Tn1/Tn3 to acquire a second copy of Tn1/Tn3 by transposition(8). Unlike the <u>trans</u> regulating action of resolvase, transposition immunity is <u>cis</u> dominant, suggesting that a sequence within the transposon is responsible. Thus, a short sequence almost anywhere within a plasmid can greatly reduce transposition elsewhere within that plasmid(13). Transposition frequency is reduced 10^3 to 10^8 fold in a transposition immune molecule. Recent findings have suggested that the inverted repeat ends are essential in immunity(10,13). Studies have shown that the presence of either 38bp end of Tn3 can confer immunity and that presence of both ends does not significantly increase immunity. Kans et al. conducted experiments in which deletions were made within the 38bp terminal repeats. They found that some deletions conferred only partial immunity while others conferred no immunity(10). A current theory for the mechanism of transposition immunity has been proposed by Lee et al. It suggests that all, or at least a large part, of the recipient molecule is scanned by the transposition complex before transposition is initiated. When the transposition complex encounters a "hot spot" for insertion, transposition is initiated in this vicinity. If, however, the transposition complex encounters a copy of its terminal repeat in the recipient DNA during its scanning of the recipient it may dissociate itself(13). Kans et al. have obtained results consistent with this theory. When they constructed plasmids containing more than one partial immunity sequence they found that the presence of multiple ends

resulted in increased levels of transposition immunity(10).

One can ask whether the only effect of a resident Tn1/Tn3 in a recipient molecule is to reduce the frequency of transposition or whether there is also an effect on the kinds of transpositions made at the lower frequency imposed by transposition immunity. Such information may help to determine the mechanism of transposition immunity. In order to address this question, the Muster lab constructed recipient molecules carrying a deleted version of Tn1. Two such constructs are PCMX948 and PCMS1075(fig.3). These constructs were formed as follows. First, a Pst I deletion derivative of Tn1 carrying the left 0.99Kbp of Tn1 and the right 0.45Kbp of Tn1 was made(Tn1Δpp)(fig.4). This derivative contains no promotors, no functional genes, and no res site, but it does contain the 38bp inverted terminal repeats. Second, in the presence of TnpA(transposase) provided in trans, a streptomycin resistant plasmid carrying Tn1Δpp was inserted into the cosmid PACM forming a cointegrate capable of co-transducing streptomycin resistance(plasmid marker) and chloramphenical resistance(cosmid marker)(15). PACM carries Tn9(Chloramphenical resistance determinant) and is deleted from the b2 region into the N gene, resulting in a molecule which is approximately 79% of the weight of λ and exists as a plasmid, but is encapsidated in phage virions in the presence of N gene product(16). Third, after mapping the insertion site and transposon orientation, transduction of rec^+ E. coli to Chloramphenical resistance allowed the cointegrates to be exposed to homologous recombination functions. Lysates were made from rec^+ transductants and used to transduce rec^- cells to Chloramphenical resistant

and Streptomycin sensitive. These structures have lost plasmid DNA via recombination between the direct repeats of Tn1*app* in the molecule. Physical analysis of cosmid DNA proved these structures were PλCM::Tn1*app*(4,15).

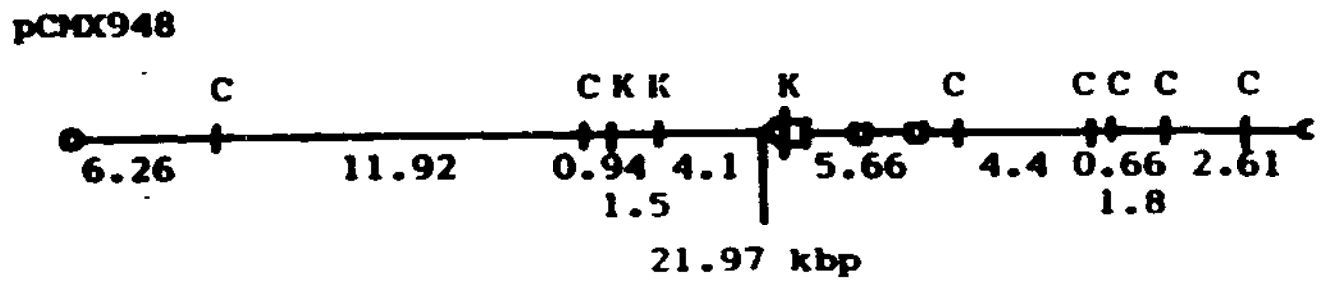
These constructs were shown to behave as transposition immune molecules. When RSF1596 plasmid(pMB8::Tn3*Δ*596)(fig. 5) was introduced into cells containing PλCM, PCMX948 or PCMX1075 plus a source of *TnpA*, and the frequency of cointegrates was measured in a *rec*$^-$*res*$^-$*tnpR*$^-$ system, the frequency of transposition into PCMX948 and PCMX1075 was $\geq$ 500 times lower than into PλCM(4,16)

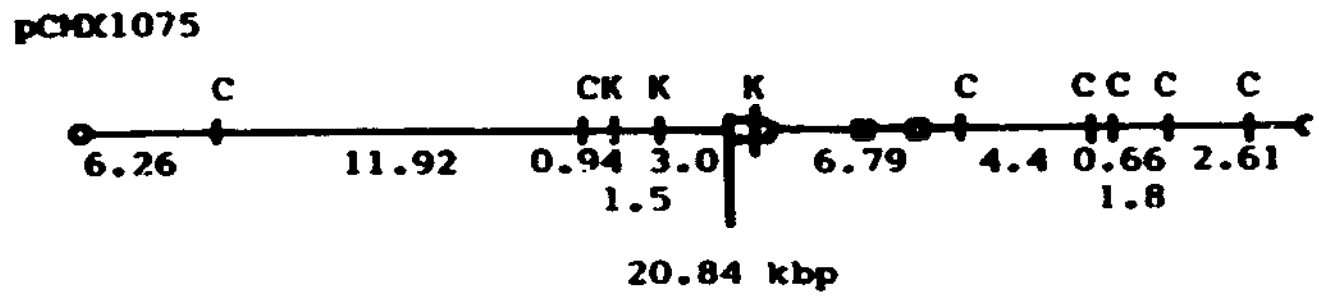
I have analyzed PCMX948::Tn3*Δ*596 and PCMX1075::Tn3*Δ*596 cointegrates to answer the following questions:

1. Is the distribution of insertion sites in PCMX948 and PCMX1975 similar to or different from the distribution of insertion sites in PλCM?

2. Is the preferred orientation of insertions into PCMX948 and PCMX1075 similar to or different from the preferred orientation of insertions into PλCM?

fig. 3 (3) Linearized Maps of PCMX948 and PCMX1075

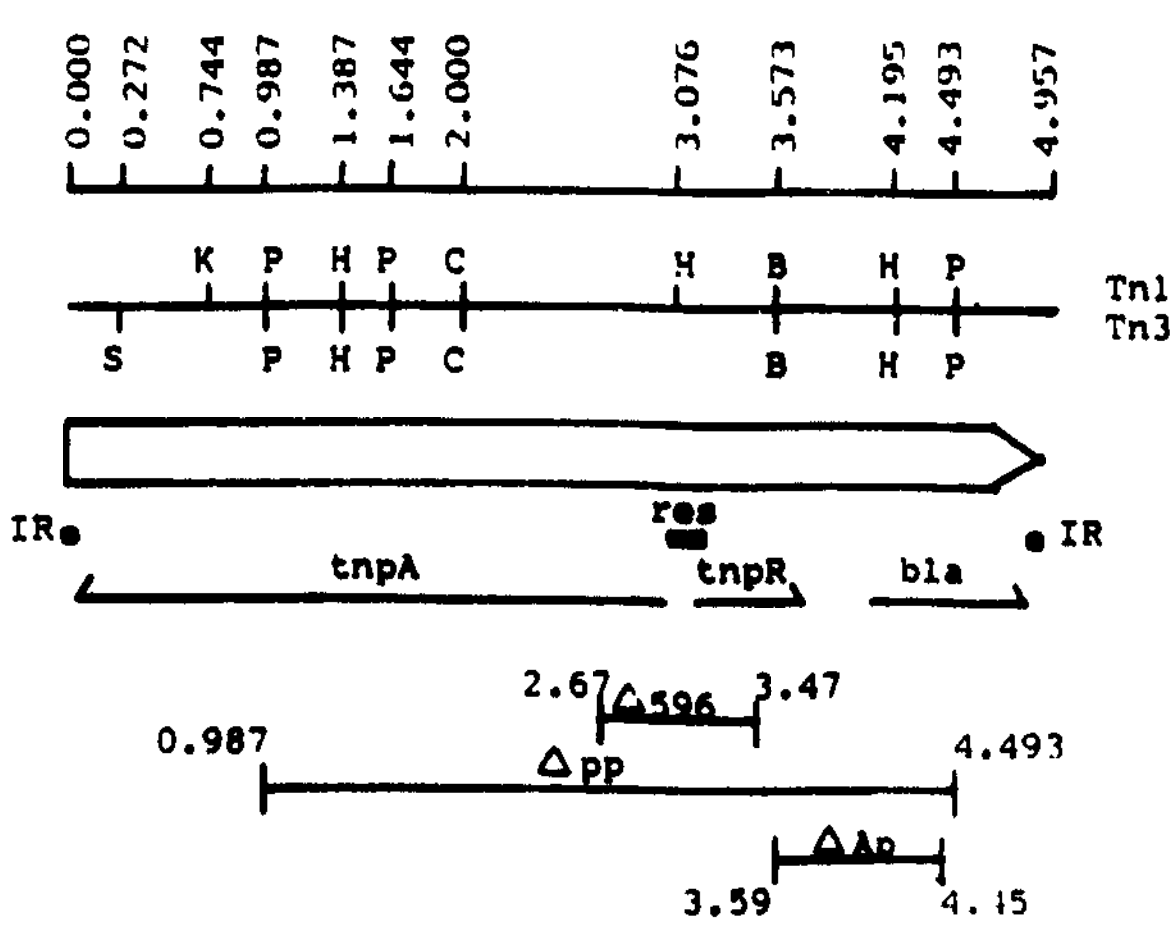




KEY

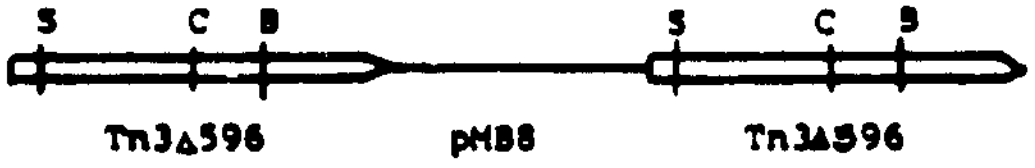
C = Cla I cleavage site
K = Kpn I cleavage site

Numbers indicate fragment sizes (Kbp)
Resident Tn1App insertions are shown



Note

Restriction endonuclease cleavage sites are shown for KpnI(K); Cla I(C); Pst I(P); Hinc II(H); and Bam HI(B). IR is the 38bp inverted repeat. Regions encoding for the 3 gene products are illustrated. Numbers represent kilobase pairs and areas of deletions are shown accordingly.



Note

Structure represents RSF1596 in the cointegrate state.

S = Sst I cleavage site

C = Cla I cleavage site

B = Bam HI cleavage site

MATERIALS AND METHODS

Bacterial Strains/Plasmids/Cosmids/Bacteriophage

E. coli MV12(Gill,R) *thi*⁻*leu*⁻*trp*⁻*recA* was the background used to get cointegrates. XJS214 is MV12 carrying the plasmids RSF1596 and RSF103(4). Plasmid RSF1596 is PMB8::Tn3Δ596. The Tn3 is deleted in *tnpA*, *res* and *tnpR*.(figs .1,4) and was used as a source of donor transposons. Plasmid RSF103 is RSF1010::Tn1ΔAp(fig. 4). The Tn1 is deleted in *tnpR* and *bla*(figs. 1,4). It is *TnpA*⁺*Res*⁺ and was used as a source of *tnpA* to get a cointegrate. The cosmids PCMX948 and PCMX1075 are described in the introduction and illustrated in fig. 4. PλCM is deleted in sequences from 22.64Kbp to 35.25Kbp on the standand λ map (7). Tn9 (2.64Kbp) is present at 22.64 Kbp of λ and carries the chloramphenicol resistance(Cm^r) gene. Bacteriophage λYS19 (*b2 red3 c*I857) was used as a helper virus to get cosmid lysates, and lysates carrying cosmid::RSF1596 cointegrates.

Media

Strains containing cointegrates were grown routinely on tryptone-yeast extract(TYE) agar supplemented with 50ug/ml chloramphenicol. The presence of carbenicillin resistance(Cb^r) was determined using TYE supplemented with 500ug/ml carbenicillin.

Formation of Cointegrates

PλCM::RSF1596, PCMX948::RSF1596 and PCMX1075::RSF1596 cointegrates were isolated as follows. XJS214 was transduced to Cm^r with lysates of PλCM, PCMX1075 or PCMX948 at 32^0C. After 48 hours, mass lysates were

made from the transduction plates (approximately 1000colonies/plate) using λYS19 helper phage to provide N gene function. Mass lysates were used to transduce E. coli MV12 to Cm^r. Cm^r transductants were tested for Cb^r(bla$^+$) by replica plating. Transpositon frequency was determined by calculation of Cb^rCm^r transductants/Cm^rtransductants. Cb^rCm^r transductants carried putative cosmid::Tn3&596 cointegrates.

Isolation of Cointegrate Structures

Cointegrate DNA was isolated by an alkaline extraction method modified from Birmboim-Doly and Maniatis(1,14).

Restriction Endonuclease Analysis of Cointegrates

Restriction endonuclease digests were performed as recommended by the supplier (Bethesda Research Laboratory). Usually, 4-5 units of enzyme were used to digest 2-3ug of the appropriate DNA at 37^0 C for 1 hour. Enzyme-digested DNA was then analyzed by electrophoresis through a 0.7% agarose gel submerged in TEB buffer (0.09M boric acid, 0.09M Tris base, 2.55mM Na_2-EDTA). An electric current of 50mamps (approximately 90 volts) was applied for approximately 2.5 hours. DNA fragments were visualized by staining with ethidium bromide for 15 minutes and observing under UV light. All gels were photographed with a polaroid camera using type 55 film. A standard curve was constructed by plotting known DNA fragment sizes (logKbp) vs. distance migrated (mm). Unknown DNA fragment sizes were determined by measuring the distance they migrated (mm) in the gels, and applying this value to the standard curve to determine

the new molecular weight. A list of specific restriction enzymes used in this analysis and their cleavage sites is found in table 1.

table 1. Restriction Enzyme DNA Cleavage Sites

Restriction Enzyme	DNA Cleavage Site
Bam H1	5'-G···GATCC-3' 3'-CCTAG···G-5'
Bgl II	5'-A···GATCT-3' 3'-TCTAG···A-5'
Cla I	5'-AT···CGAT-3' 3'-TAGC···TA-5'
Eco RI	5'-G···AATTC-3' 3'-CTTAA···G-5'
Kpn I	5'-GGTAC···C-3' 3'-C···CATGG-5'
Pvu I	5'-CGAT···CG-3' 3'-GC···TAGC-5'
Sst I	5'-GAGCT···C-3' 3'-C···TCGAG-5'

RESULTS AND DISCUSSION

A total of 116 independent insertions of RSF1596 into PλCM were analyzed with respect to insertion site in PλCM sequences, and orientation of Tn3Δ596 with respect to PλCM sequences. Some of these insertions were mapped prior to the commencement of this project. The distribution of RSF1596 insertions on the PλCM map is shown in figs.6.7, and the orientation of these insertions(leftward or rightward) with respect to PλCM sequences listed in figs. 6, 7 and table 2.

A total of 72 independent insertions of RSF1596 into PCMX948 and a total of 63 independent insertions of RSF1596 into PCMX1075 were analyzed with respect to insertion in cosmid sequences and orientation of Tn3Δ596 in cosmid sequences. The distribution of RSF1596 insertions on the map of PCMX 948 and PCMX1075 are shown in figs. 6,8,9. The orientation of their insertions with respect to cosmid sequences can be noted in figs. 6,8,9 and table 2.

Distribution of RSF1596 Insertions in PλCM Sequences

Distribution of RSF1596 insertions in PλCM sequences is not random. Figs. 6,7 indicate several "hot spots" for RSF1596 insertions. The most obvious hot spots include the regions from 3-4Kbp, 6-9Kbp, 10-12Kbp, 19-22.6Kbp, 40-42Kbp and 44-46Kbp on the standard λ map(7). When distribution of insertions is examined considering insertions oriented leftward and rightward separately, the same general pattern is observed. Considering leftward insertions separately, hot spots are observed in the

regions from 3-4Kbp, 6-9Kbp, 10-12Kbp and 40-42Kbp on the standard map. Differences were noted in the 19-22.6Kbp region where leftward insertions only revealed a hot spot at 19-20Kbp of λ, and in the 44-46Kbp region where leftward insertions only revealed a hot spot at 43-44Kbp and 45-46Kbp of λ. Considering only rightward insertions, hot spots are observed in the regions from 3-4Kbp, 19-22.6Kbp, 40-42Kbp and 44-46Kbp. Differences were noted in the 6-9Kbp region where no hot spot appears. It is difficult to determine whether there is a hot spot at 10-12Kbp on the standard λ map. Since relatively few rightward insertions were found, it is more difficult to judge the presence of hot spots.

Regions of low insertion frequency by RSF1596 into PΛCM were also noted. Two such regions can be accounted for by the way in which cointegrates were selected. Few inserts in Tn9 were observed since a functional Cm^r determinant was required to isolate and identify cointegrates. Few inserts in the 35.5-38Kbp region were observed because this region contains sequences necessary for λ-mediated DNA replication. An additional cold spot at 13-16Kbp was observed. Since virion coat proteins are encoded in this region, and these functions were provided by YS19 helper bacteriophage, no selection against inserts in this region was expected.

Orientation Preference of RSF1596 Insertions in PΛCM Sequences

On a random basis, one would expect 50% of the RSF1596 insertions to be in each orientation with respect to PΛCM sequences. This analysis showed, however, that 59.9% of all insertions were in a leftward orientation when RSF1596 inserted into PΛCM via TnpA-mediated

recombination. This preference was noted in each of the five independent experiments done at different times in order to aquire cointegrates. For each set of experiments, a preference for the leftward orientation of 58.3%, 57.9%, 56.2%, 58.8% and 66.7% was observed. Thus, this preference probably reflects the way the donor transposon-transposase complex interacts with the DNA sequences in PλCM, rather than the result of a chance preference in a single experiment.

Distribution of RSF1596 Insertions in PCMX948 and PCMX1075

If transposition events in the presence of transposon immunity are similar to non-immune transpositions, only occuring at a lower frequency, one would expect a distribution of insertions in PCMX948 nad PCMX1075 similar to that described for PλCM. The distribution of RSF1596 insertions in PCMX948 is shown in figs.6,8. Several differences in distribution are observed when compared with RSF1596 insertions into PλCM(fig.6.7). Hot spots in PCMX948 are noted at 1-2Kbp, 7-19Kbp, 14-15Kbp, 20-22Kbp, and 44-47Kbp on a standard λ map(7). The first and third hot spots for inserts in PCMX948 are not seen for inserts in PλCM. In fact, in PλCM, 14-15Kbp is a cold spot for insertions. The hot spots in PλCM at 3-4Kbp and 40-42Kbp may also be absent in PCMX948. Another difference that should be noted is that more RSF1596 insertions into Tn9 were found in PCMX948 sequences compared to PλCM sequences.

The distribution of insertions of RSF1596 into PCMX948 when leftwar and rightward inserts are considered separately is also shown in fig.6,8. For leftward inserts, hot spots are observed at 1-2Kbp,7-10Kbp, 20-22Kbp

and 44-47Kbp, reflecting hot spots seen for total inserts into PCMX948. However, a hot spot at 14-15Kbp is not represented in leftward inserts. For rightward insertions of RSF1596 into PCMX948 hot spots are noted at 1-2Kbp, 7-10Kbp, 14-15Kbp and 44-45Kbp, reflecting hot spots seen for total inserts into PCMX948. The hot spot seen tor total insertions into PCMX948 at 20-22Kbp is not present for rightward insertions.

The distribution of RSF1596 insertions into PCMX1075 is shown in figs. 6,8,9. Insertion hot spots are noted at 1-7Kbp, 8-12Kbp, 22-22.6Kbp, 40-42Kbp and 44-47Kbp. This pattern differs from that seen for RSF1596 insertions into PACM or PCMX948. The first hot spot represents a broad region of the recipient. The second hot spot may be similar to those seen in PACM and PCMX948. The third hot spot at 22-22.6Kbp is much narrower than the similar hot spots in PACM and PCMX948. The last two hot spots are similar to those seen in PACM.

When leftward and rightward insertions of RSF1596 into PCMX1075 are considered separately, the patterns are generally similar to eachother and to that of total insertions into PCMX1075. However, the hot spot at 22-22.6Kbp was exclusively due to leftward insertions and that at 40-42Kbp was mainly due to rightward insertions.

Insertion orientation preference was examined for RSF1596 insertions into PCMX948 and PCMX1075. The results are shown in figs. 6,8,9 and table 2. For insertions into PCMX948, the preference was for a leftward orientation (55.6%). This level is not as high as that seen with PACM (59.5%). Chi Square analysis of our data comparing PCMX948 to PACM shows that differences in insertion orientation are not significantly different and can thus be attributed to chance differences. Thus, it

appears in this case that the leftward orientation of the resident Tn1ΔppIs not having a significant effect on the orientation of the Tn3Δ596 insertion. For insertions into PCMX1075 there was a slight preference for the leftward orientation (52.4%). This is a decrease from that seen in PΛCM (59.9%). However, Chi Square analysis of our data comparing PCMX1075 to PΛCM shows that differences in insertion orientation are not significantly different and can thus be attributed to chance differences. Thus, it appears in this case that the rightward orientation of the resident Tn1ΔppIs not having a significant effect on the orientation of the Tn3Δ596 insertion.

Effect of Resident Tn1ΔppSequences on Distributionn and Orientation of RSF1596 Insertions Mediated by TnpA

Presence of Tn1ΔppIn PΛCM in either orientation(PCMX948 or PCMX1075) appears to have an effect on distribution of RSF1596 insertions. The 3-4Kbp insertion hot spot seen in PΛCM, appears to be present in PCMX1075 but not PCMX948. The 6-9Kbp hot spot in PΛCM appears to be displaced to 7-10Kbp in PCMX948 and to 8-10Kbp in PCMX1075. The 10-12Kbp hot spot of PΛCM is absent in PCMX948 and may be present in PCMX1075. The 13-19Kbp cold region of PΛCM is reflected in PCMX1075 but not PCMX948 which has a hot spot at 14-15Kbp. The 19-22.6Kbp hot spot of PΛCM is less prominant in PCMX948 (20-22Kbp) and replaced by a 22-22.6Kbp hot spot in PCMX1075. The 40-42Kbp hot spot in PΛCM may be present in PCMX1075 but is not present in PCMX948. The 44-46Kbp hot spot in PΛCM is also present in both PCMX948 and PCMX1075.

In some cases, there appears to be an insertion orientation preference

attached to a particular hot spot. In PλCM, most of the inserts in the 6-9Kbp, 19-20Kbp, and 40-42Kbp regions are leftward, which is the preferred orientation (59.9%). The 20-22.5Kbp hot spot contained mostly rightward insertions. In PCMX948, leftward insertions are seen preferentially in the 20-21Kbp and 45-47Kbp hot spots while rightward insertion account for the 14-15Kbp hot spot. In PCMX1075 the 22-22.6Kbp hot spot results from mostly leftward insertions, and the 40-42Kbp hot spot from mostly rightward insertions.

Clear differences in insertion patterns are seen when comparing RSF1596 insertions into PλCM with or without Tn1ΔApp. The patterns for PCMX948 and PCMX1075 also show differences. It should be noted that when a PλCM::Tn1ΔAp derivative, PXJS972, acted as a recipient for RSF1596 insertions, a distribution pattern very similar to that of PλCM was observed. PλCM::Tn1ΔAp shows relatively little transposition immunity when challenged with RSF1596 (a 5 fold decrease from PλCM::RSF1596 cointegrate frequency)(16). These results suggest that altering the insertion pattern may be important in expression of transposition immunity. It is also possible that resident Tn1ΔApp transposon sequences in PλCM can affect the orientation of TnpA-mediated RSF1596 insertions. However, our data is not as strong as that obtained for RSF1596 insertions into PXJS972.

Although the expression of transposition immunity appears to alter the distribution pattern of RSF1596 insertions into PXCXM948 and PCMX1075, it is not clear why this should be so, or whether the position of the resident Tn1ΔApp exerts any influence. The effect of the PλCM sequences surrounding the resident Tn1ΔApp has yet to be examined. PλCM::Tn1ΔApp

constructs with the resident Tn1*app* inserted at different sites in PλCM have been made, and can be used to address the question. Mapping a larger number of RSF1596-PCMX948 and RSF1596-PCMX1075 cointegrates might clarify differences in distribution patterns. Mapping insertions into PλCM::Tn1*app* constructs with resident transposons at the exact same site but opposite orientation might help determine whether the orientation of the resident transposon affects the orientation of RSF1596 insertions into PCM::Tn1*app*. Preliminary data involving Tn1*app* inserted at 14.5Kbp of into PλCM in both orientations suggests that RSF1596 inserts do show a preference to insert in the direction of the resident transposon(16).

Table 2. Orientation of Tn3(Δ596) Insertions

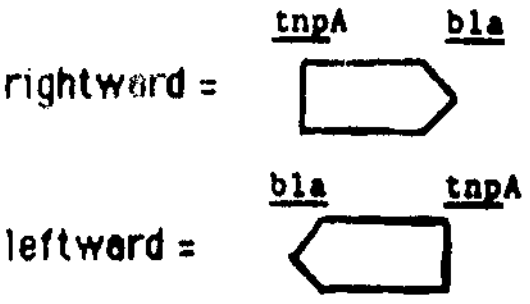
Cointegrate	Total insertions	Insertions Oriented Left	Insertions Oriented Right	%Left	%Right
PΔCM-RSF1596[a]	116	69	47	59.5	40.5
PCMX948-RSF1596[b]	72	40	32	55.6	44.4
PCMX1075-RSF1596[c]	63	33	30	52.4	47.6

a - PΔCM is a non-immune recipient for transposition (contains no Tn*4pp* resident)

b - PCMX948 is a transposition immune recipient which contains a resident Tn*4pp* insert oriented leftward.

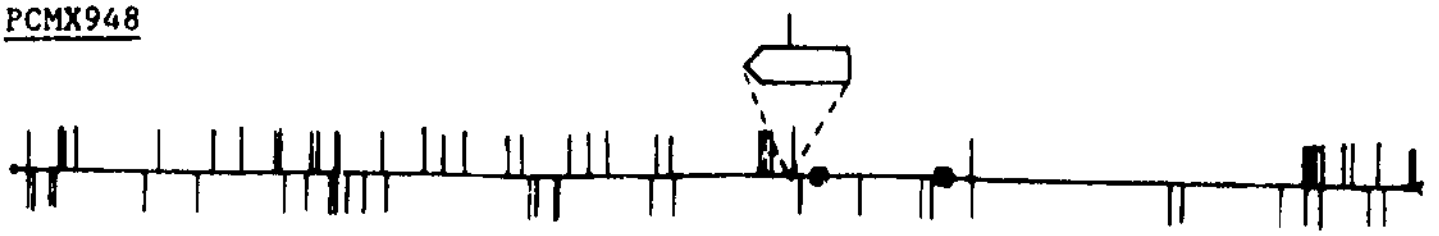
c - PCMX1075 is a transposition immune recipient which contains a resident Tn*4pp* insert oriented rightward.

note: standard direction of Tn1/Tn3 is from the *tnpA* end to the *bla* end (fig. 1)

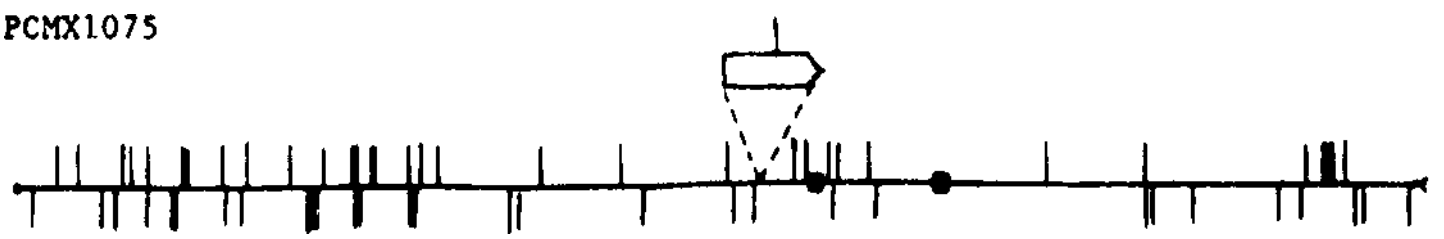


PΔCM

PCMX948



PCMX1075

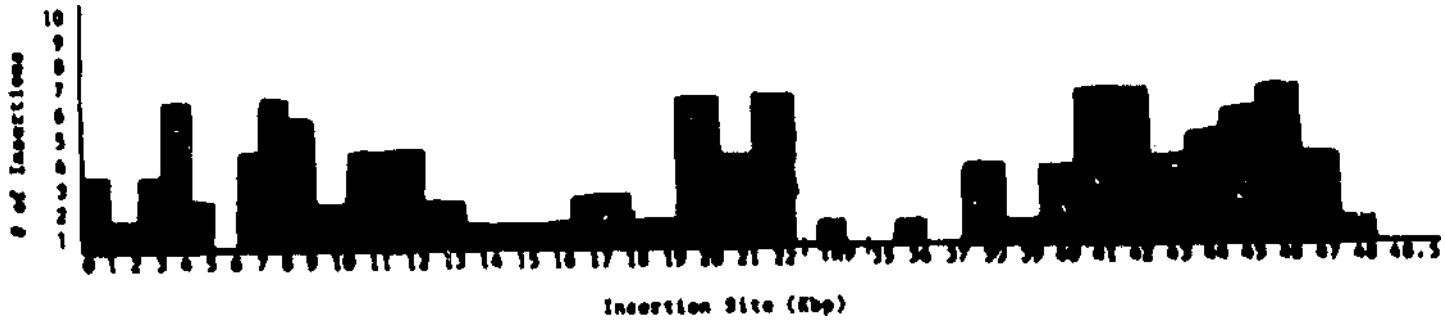


KEY

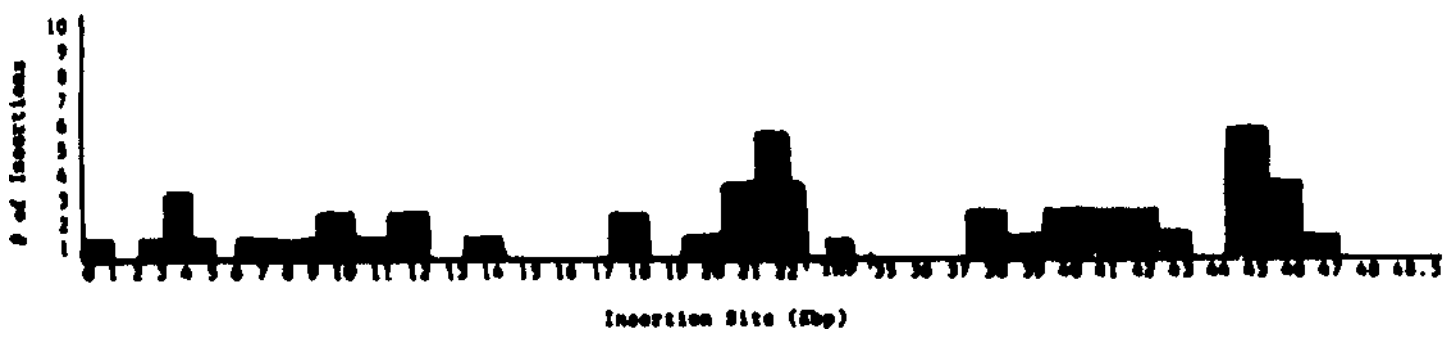
- 1Kbp of λ DNA
- Tn9 (2.64Kbp)
- a = non-immune case (contains no resident transposon)
- b = immune case (contains resident Tn*3pp insert at 21.97Kbp as illustrated)
- c = immune case (contains resident Tn*3pp insert at 20.84Kbp as illustrated)

note : lines above the map represent leftward orientation of the Tn3(Δ596) insert: lines below the map represent rightward orientation of the Tn3(Δ596) insert.

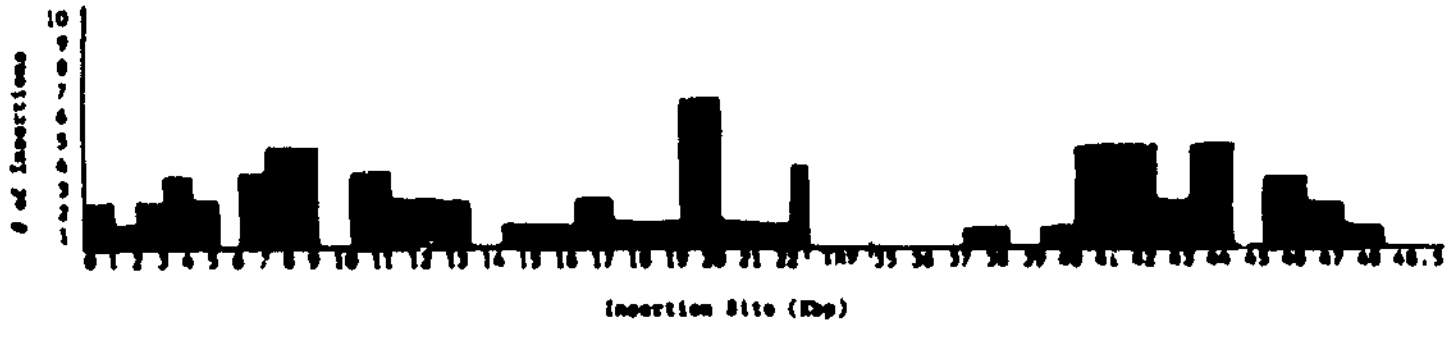
a) Total Tn3Δ596 Insertions



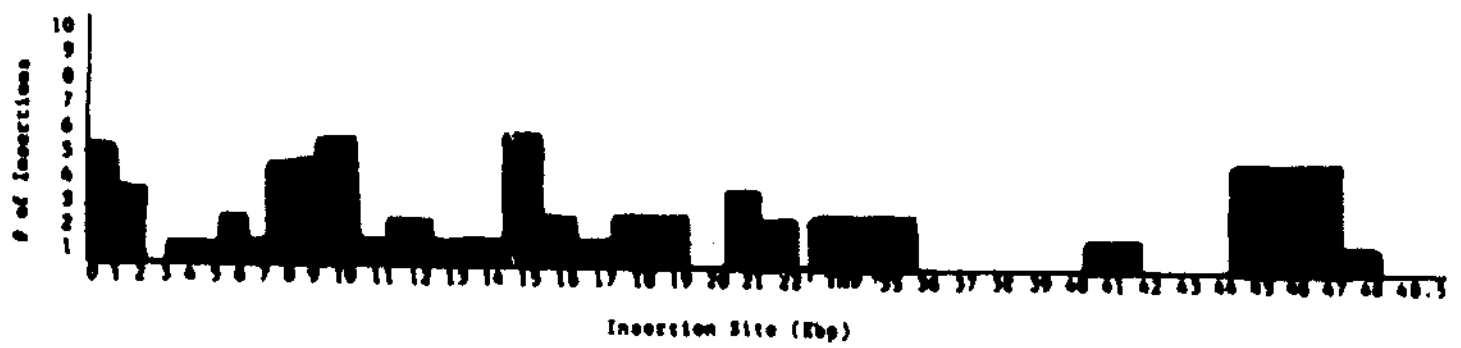
b) Tn3Δ596 Insertions Oriented Rightward



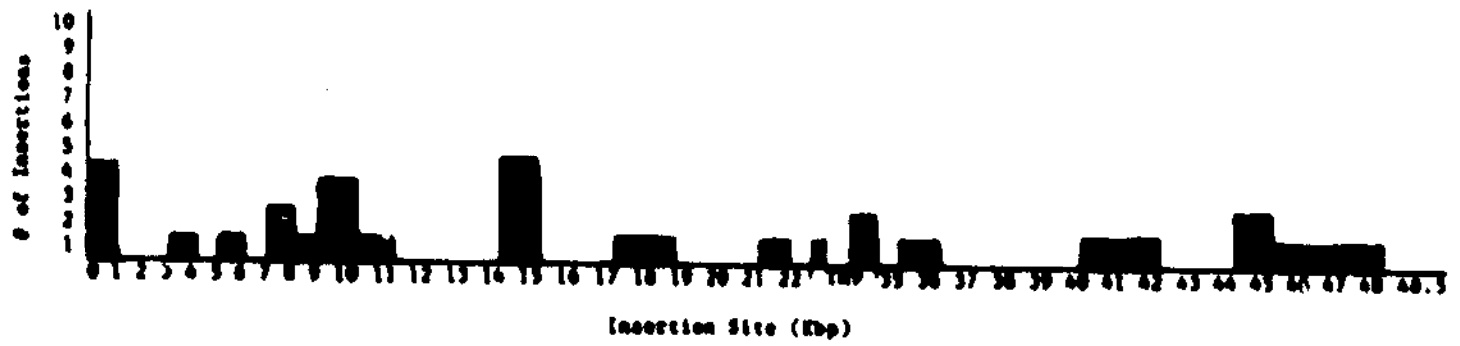
c) Tn3Δ596 Insertions Oriented Leftward



a) Total Tn3Δ596 Insertions



b) Tn3Δ596 Insertions Oriented Rightward



c) Tn3Δ596 Insertions Oriented Leftward

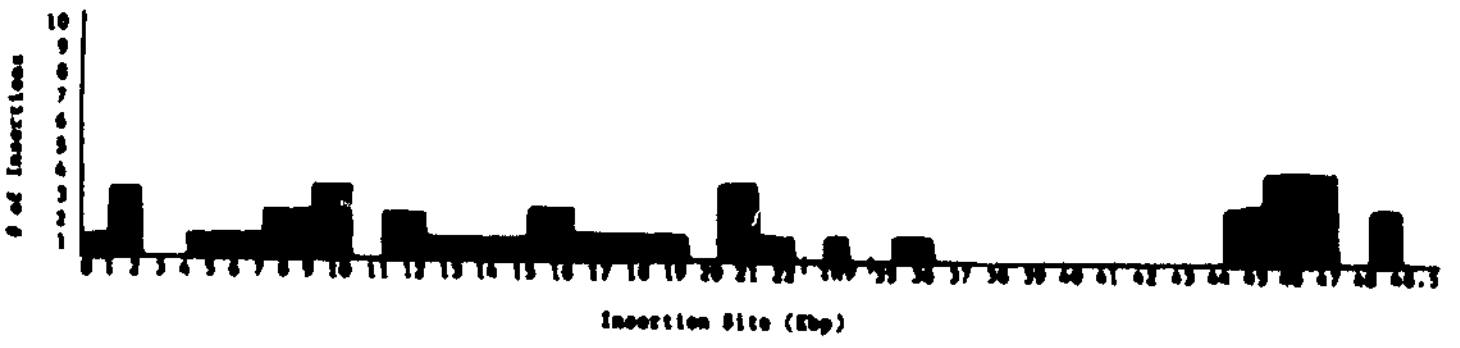
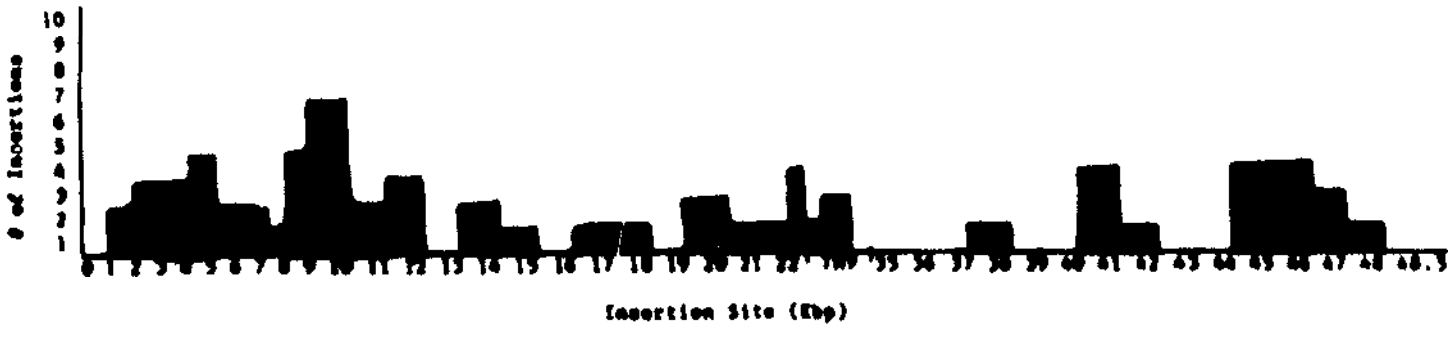
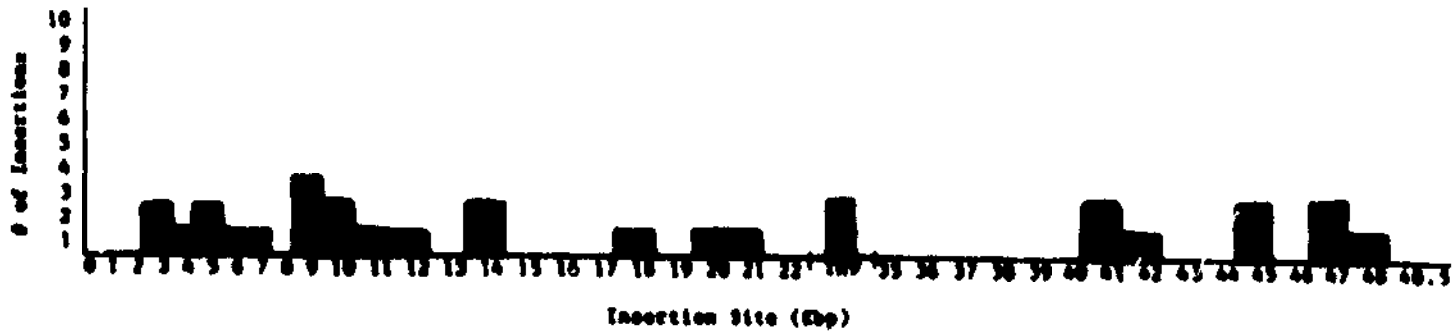


fig. 9 Tn3Δ596 Insertions into PCMX1075

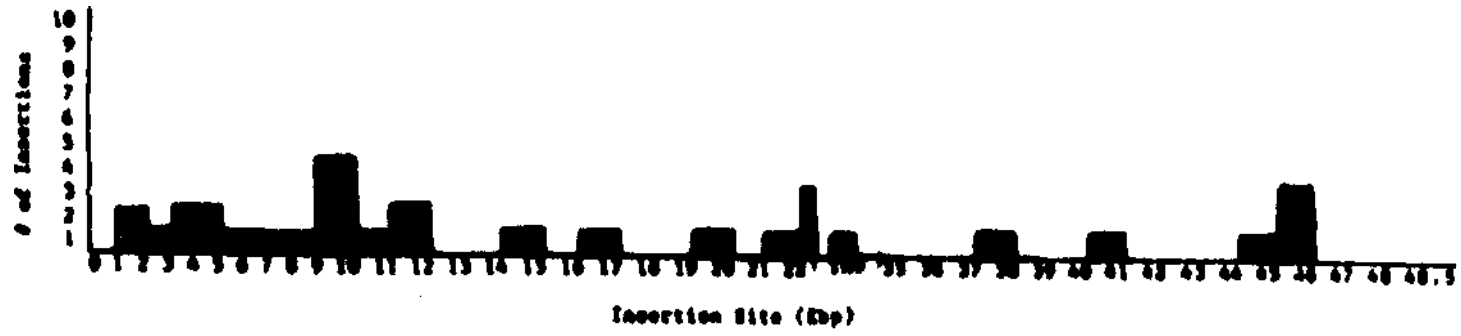
a) Total Tn3Δ596 Insertions



b) Tn3Δ596 Insertions Oriented Rightward



c) Tn3Δ596 Insertions Oriented Leftward



REFERENCES

1. Bornboim, H.C., and Doly,C. (1979) A Rapid Alkaline Extraction Procedure for Screening Recombinant Plasmid DNA. Nuleic Acids Res. 1, pp. 1513-1523.

2. Calos, Michele P., and Miller, Jeffrey H. (1980) Transposable Elements. Cell. Vol. 20, pp. 579-595.

3. Cohen, Stanley H., and Shapiro, James A. (1980) Transposable Genetic Elements. Scientific American. Vol.242, No. 2, pp.40-49.

4. Getty, Pat. Senior Thesis, 1987

5. Gill, Ronald E., Heffron, Fred, and Falkow, Stanley (1979) Identification of the Protein Encoded by the Trnsposable Element Tn3 Which is Required for its Transposition. Nature. Vol. 282, pp.797-801.

6. Heffron, Fred, and McCarthy, Brian J. (1979) DNA Sequence Analysis of the Transposon Tn3: Three Genes and Three Sites Involved in Transposition of Tn3. Cell. Vol. 18, pp.1153-1163.

7. Hendrix, Roger W., Roberts, Jeffrey,W., Stahl, Franklin W., Weisberg, Robert A. (1983) Lambda II. Cold Spring Harbor Laboratory.

8. Heritage, Hahn, and Bennett, Peter (1984) The Role of TnA Transposase in Transposition Immunity. Plasmid. Vol. 12, pp. 218-221.

9. Ichikawa, Hitoshi, Ikeda, Kaoru, Wishart, William, and Whtsubo, Eichi (1987) Specific Binding of Transposase to Terminal Inverted Repeats of Transposable Element Tn3. Proc. Natl. Acad. Sci. U.S.A. Vol. 84, pp.8220-8224.

10. Kans, Jonathan A., and Casadaban, Malcolm J. (1989) Nucleotide Sequences Required for Tn3 Transposition Immunity. J. of Bacteriology. April. pp. 1904-1914.

11. Kitts, Paul, Symington, Lorraine, Burke, Mary, Reed, Randall, and Sherratt, David (1982) Transposon-specified Site-specific Recombination. Proc. Natl. Acad. Sci. U.S.A. Vol. 79, pp 46-50

12. Kostriken, Richard, Morita, Carol, and Heffron, Fred (1981) Transposon Tn3 Encodes a Site-specific Recombination System Identification of Essential Sequences, Genes, and Actual Site of Recombination. Proc. Natl. Acad. Sci. U.S.A. Vol. 78, No. 7, pp 4041-4045

13. Lee, Chao-Hung, Bhagwat, Ashok, and Heffron, Fred (1983) Identification of a Transposon Tn3 Sequence Required for Transposition Immunity. Proc. Natl. Acad. Sci. U.S.A. Vol. 80, pp 6765-6769.

14. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1983). Molecular Cloning, pp.368-369. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.

15. Muster, Carol J., MacHaltie, Lorne A., Shapiro, James A. (1983) P CM System: Observation on the Roles of Transposable Elements in Formation and Breakdown of Plasmids Derived from Bacteriophage Lambda Replicons. J. of Bacteriology.

16. Muster, personal communication.

17. Strickberger, Monroe W. (1985) Genetics. Macmillan Publishing Co. pp. 468-474, 442-443.

18. Watson, James D. (1987) Molecular Biology of the Gene Benjamin/Cummings Publishing Co. pp 201-201, 332-337.