Recombination in *recA* Cells Between Direct Repeats of Insertion Element IS1

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The IS1 sequences that flank the Tn9 chloramphenicol acetyltransferase gene as direct repeats recombine after transformation into an *Escherichia coli recA* strain. The recombination requires the λp_L promoter on the plasmid. A plasmid that contains mutant IS1 elements does not recombine. These results indicate that this recombination requires an IS1-specific gene product. The recombinational activity of IS1 may resolve transient cointegrates formed during the transposition of IS1. I discuss a possible role for the λp_L promoter.

Insertion sequences are mobile genetic elements that translocate to new positions in the same or different genomes. In addition, insertion sequences catalyze DNA rearrangements in their vicinity including deletions (8, 34) and inversions (8). The insertion sequence IS1 is a 768-base-pair transposable sequence (18, 27) that transposes by a *recA*-independent mechanism to a large number of sites.

The transposition of Tn3 from one replicon to another occurs in two steps (2, 15, 17, 35). The first step is the generation of a cointegrate intermediate that contains direct repeats of Tn3. The second step is a site-specific recombination between the Tn3 elements that resolves the cointegrate intermediate. This step generates the target replicon containing an insertion of Tn3 and regenerates the original donor replicon. The resolution reaction is catalyzed by the *tnpR* polypeptide encoded by Tn3 (17, 19, 29-31).

Although the results of experiments with Tn3 have provided models for transposition which require a cointegrate intermediate, it is not clear whether other transposons and insertion sequences also require cointegrate intermediates for their transposition. For example, Galas and Chandler (14) concluded that IS1 either transposes directly or forms cointegrates that are not transposition intermediates. They base their conclusion on the observation that IS1-generated cointegrate molecules are formed at a frequency of 10% of the transposition frequency, and that cointegrate molecules, once formed, do not readily resolve. They reasoned that, due to their stability, if cointegrates were transposition intermediates, then their frequency would have to be much higher to account for the transposition frequency.

In contrast, the results of Reif and Arber (33) suggest that IS1 normally transposes via a cointegrate intermediate. They cloned a kanamycin resistance gene into the single IS1 *Pst*I site. The mutant (IS1 *kan*) exhibited a wild-type transposition frequency in *rec*⁺ cells, but not in *recA*⁻ cells. In the *rec*⁻ cells, IS1 transposition was decreased 100-fold, whereas the frequency of cointegrate formation was elevated to levels comparable to the transposition frequency in *rec*⁺ cells. Cointegrates were rare in *rec*⁺ cells. This result suggests that the *recA* gene product has the ability to resolve IS1 *kan* cointegrates and can therefore substitute for a resolvase activity that is supplied by IS1, but not the mutant IS1 *kan*. The results presented in this paper support the results of Reif and Arber (33).

Tn9 consists of a sequence that encodes resistance to

chloramphenicol (*cat*) flanked by direct repeats of IS1 (1, 21, 32). There are three long open reading frames that I have designated A, B, and C (Fig. 1). Machida and co-workers (22) suggest that the A and B reading frames (which they designate *ins a* and *ins b*) encode polypeptides that are required for IS1 transposition.

The IS1 elements of Tn9 recombine in a *recA*⁻ strain under some conditions. Since the IS1s of Tn9 are directly repeated, they resemble an IS1-generated cointegrate, and the recombination between the direct repeats of IS1 of Tn9 resembles a resolution reaction similar to that observed for Tn3 (2, 15, 17, 35). The results of experiments presented here suggest that an IS1 gene product is required for the recombination between direct repeats of IS1 that are formed during the transposition of IS1.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages are listed in Table 1. A *recA::Tn10* allele that has an insertion of Tn10 into the *recA* gene (Gellert, unpublished data) was introduced into N721 and N1106 by P1 transduction with P1 *cm clr100* (26) grown on N1680. Transductants were found by selection for resistance to 25 μ g of tetracycline per ml. Tetracycline-resistant cells were tested for sensitivity to UV light. Lambda lysogens were constructed by incubating cells at 37°C for 20 min with λ at a multiplicity of infection of 1 before plating on LB plates (26) seeded with λ b2c to select for λ immunity. The resulting strains are N1694 and N1713.

Manipulation of DNA. Plasmid extraction was by the method of Birnboim and Doly (5). Restriction endonucleases, ligase, and polymerase were purchased from either New England Biolabs or Bethesda Research Laboratories. Restriction enzyme digests were done as recommended by the suppliers. The conditions for DNA polymerase I large fragment were as described previously (23). Ligations were done in a 100- μ l volume containing 10 mM Tris (pH 7.4), 10 mM dithiothreitol, 10 mM MgCl₂, 10 mM ATP, and one Weiss unit (37) of T4 DNA ligase. Ligations were done at 14°C for at least 1 h or at room temperature overnight.

Electrophoresis of DNA was on 0.7% agarose gels in Tris-acetate buffer (23) or on 0.5% acrylamide gels in TBE (23). DNA was extracted from agarose gels by the "freeze-squeeze" method of Thuring and co-workers (36), except that 10 μ g of tRNA per ml was added to the gel extract to insure quantitative precipitation of small amounts of DNA.

Hybridization of a ³²P-labeled probe to restriction enzyme digests of chromosomal DNA was performed by standard

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FIG. 1. Relative location in base pairs of major *IS1* open reading frames determined from the sequence (18, 26). Also included are a few restriction sites and their locations in base pairs.

procedures (23). The probe for *IS1* was nick translated pSM1, which is a 5.4-kilobase plasmid derived from R100 that contains one *IS1* sequence (27). Previous experiments demonstrate that the only significant homology between pSM1 and the *Escherichia coli* chromosome is the *IS1* (28).

Transformations. Batch transformations were done as follows. Exponentially growing cells were pelleted, washed in 10 mM NaCl, and suspended in 0.5 volume of cold 30 mM CaCl₂. After 20 min on ice, the cells were concentrated 10× in cold 30 mM CaCl₂, and 0.5 µg of DNA was added to 0.5 ml of cells. The mixture was incubated on ice for 1 h before a heat pulse for 2 min at 42°C or 4 min at 30°C. The cells were added to 2.5 ml of LB broth (25) and shaken at 37 or 30°C for 1 h. A 100-µl sample was plated on LB plates containing 50 µg of kanamycin per ml. A 15-ml sample of LB broth containing 50 µg of kanamycin per ml was added to the remaining 2.4-ml culture. The DNA population of the batch culture was analyzed after growth for 16 to 18 h.

DNA used for the transformation of *E. coli* W was purified by electrophoresis on agarose gels from a *recA*⁺ strain of *E. coli* W containing the plasmid pRK248 (3, 4) and the appropriate pMCR plasmid. DNA used for the transformations of *E. coli* K-12 was purified in a similar manner from an *E. coli* K-12 strain.

Plasmid construction. The strategies for plasmid construction and plasmid structures are given in Fig. 2. The two *IS1* elements of the pMCR plasmids are identical, except for the deletion of the terminal 75 base pairs of the left element. I have designated them *IS1/a* and *IS1/b* for ease of reference.

RESULTS

Transformation of pMCR16. The plasmid pMCR16 contains a derivative of Tn9 downstream from *p_L* and the *N* gene (Fig. 2B). The left end of *IS1/a* has a deletion that ends in the *IS1 PvuII* site, which removes the left inverted repeat and thus interrupts the A reading frame without altering the B or C reading frames (Fig. 1). This plasmid was used to transform a λ lysogenic, *recA* derivative of *E. coli* W (N1694) that does not contain any chromosomal *IS1* elements (Fig. 3). This strain was chosen to assay for potential *IS1* recombination since *E. coli* K-12 strains contain multiple *IS1* elements (Fig. 3), and thus these strains may also contain an *IS1* repressor or inhibitor.

The plasmid population from a batch culture of kanamycin-resistant transformants obtained after the transformation of pMCR16 into N1694 contained many smaller plasmids in addition to the parental plasmid (Fig. 4, lane 3).

A clone containing one of the plasmids smaller than pMCR14 which was generated after transformation was

subjected to restriction enzyme analysis (Fig. 5). The plasmid has lost the restriction sites that lie to the left of *IS1*. One *IS1*-specific restriction site is retained. I conclude that this plasmid most likely has a deletion that begins at *IS1/b* and extends beyond *p_L* (Fig. 2). Restriction digests of other deletion plasmids indicate that they also carry deletions from the end of *IS1/b* (data not shown).

The population also contained a plasmid that migrates at a position expected for pMCR14 (Fig. 4, lanes 2 and 3). Isolation of this plasmid from an agarose gel and restriction analysis with *HaeII* (Fig. 6) demonstrate that it has a structure identical to pMCR14, the result expected from a recombination between the direct repeats on pMCR16.

Requirements for recombination. The right inverted repeats of both *IS1/a* and *IS1/b* on pMCR16 were deleted to create pMCR26 to test the role of *IS1*-encoded gene products on the *IS1*-catalyzed reactions. (Fig. 2). The mutation (ΔT) destroys the C reading frame and alters the B reading frame. The A reading frame is unchanged. The DNA population that results from a transformation of pMCR26 into N1694 does not contain a plasmid with the structure expected from a recombination of the direct repeats of *IS1* on pMCR26, although it does contain deletion derivatives of pMCR26 (Fig. 4, lane 4). The DNA population after the transformation of pMCR24, a plasmid that has the ΔT mutation in *IS1/b* only, contains a product with the structure expected for a plasmid that has recombined the *IS1* elements on pMCR24 as well as deletion derivatives of pMCR24 (Fig. 4, lane 5). Thus, it appeared that one copy of the wild-type *IS1* B or C genes was essential for *IS1*-catalyzed recombination between direct repeats of *IS1*.

The conversion of pMCR16 to smaller plasmids after transformation requires the presence of *p_L* on the plasmid. Neither the *IS1*-catalyzed deletions nor the recombination

TABLE 1. Bacteria, plasmids, and phages

Strain	Genotype	Source or reference
Bacteria		
N721	<i>E. coli</i> K-12 strain MO <i>rpsL thi</i> , isogenic with HfrH	Maquat and Reznik (24)
N1106	<i>E. coli</i> strain W, prototroph containing no <i>IS1</i> s	Indiana University
N1680	$\Delta lac ara(Am)$ <i>galK(Am) galE</i> <i>trp(Am) recA::Tn10</i>	M. Gellert
N1694	λ^+ , <i>recA::Tn10</i> derivative of N1106	This work
N1713	λ^+ , <i>recA::Tn10</i> derivative of N721	This work
Plasmids^a		
pHUB4	<i>Kan^r</i> , contains λ <i>p_L</i> and <i>N</i> gene	N. Franklin (3, 4)
pRK248cIts	<i>c1857</i> allele in pRK248, <i>ter^r</i>	N. Franklin (3, 4)
pSM1	One <i>IS1</i> , no selectable phenotype	H. Ohtsubso (27)
Phages		
λ	Wild type	Laboratory collection
λ b2 c	Δatt	Laboratory collection
λ cm1	$\lambda::Tn9$ in <i>b2</i> region	J. L. Rosner (16, 31)
P1 cm <i>clr100</i>	P1::Tn9	M. M. Howe (26)

^a For the structure of plasmids constructed in this work, see Fig. 2.

FIG. 2. Structure of plasmids. The open boxes represent IS1 DNA. The heavy line represents the Tn9 drug resistance region, and the thin line represents pHUB4 and λ sequences. The direction of transcription from p_L and the *cat* gene are indicated by an arrow, as is the direction of replication. The arrow within the IS1 indicates orientation; the left end is as defined by Ohtsubo and Ohtsubo (27). Restriction sites are abbreviated as follows: B, *Bam*HI; Bg, *Bgl*II; P, *Pvu*II; R, *Eco*RI; S, *Sal*I; T, *Tth*111I.

could be detected after transformation with pMCR18, which lacks p_L (Fig. 7, lanes 3 and 4).

The recombination and deletion events probably require transcription from p_L . I assume that a burst of transcription occurred in cells after transformation; the incoming plasmids containing p_L apparently titrate cytoplasmic *cI* repressor.

To test the role of p_L , I transformed pMCR16 into N1106 (Table 1) containing the compatible, *cI*-bearing plasmid pRK248. The heat pulse for transformation was either 30°C (Fig. 8, lane 7) or 42°C (lane 8). A heat pulse at 42°C would be expected to inactivate the thermolabile *cI* repressor.

FIG. 3. Southern blot hybridization of IS1 sequences. Chromosomal DNA was digested with *Eco*RI and *Hind*III for *E. coli* strains W (lane 1), W *recA*::Tn10 (lane 2), W (λ cm1) (lane 3), and K-12 (lane 4). Neither enzyme cuts within IS1; however, digestion with *Eco*RI separates the two IS1s on Tn9. The probe was 32 P-labeled pSM1, which contains one IS1 sequence (26). W (λ cm1) serves as a control of the W hybridizations since λ cm1 contains a complete Tn9 and thus two copies of IS1. Comparison of lanes 1 and 2 with lane 4 demonstrates multiple IS1 sequences in *E. coli* K-12, whereas *E. coli* W is lacking any significant homology to IS1.

Although both transformations gave rise to deletion plasmids and pMCR14, the cells heat pulsed at 42°C contain less parental plasmid than those heat pulsed at 30°C, suggesting that pMCR16 is more reactive when *cI* is thermally inactivated during the transformation process.

pMCR16 recombines after transformation into N1694, but not into N1713. This result could reflect a differential expression of p_L in the two strains, or some other metabolic difference. Another explanation is that the reaction does not occur readily in cells in which IS1 has become established. Therefore, I isolated strains of N1721 and N1106 (Table 1) containing both pMCR16 and pRK248. The *cI* repressor produced by pRK248 in these strains was inactivated by

FIG. 4. Transformations with pMCR plasmids. Lanes 1 and 2 are batch transformations of N1713 with pMCR16 and pMCR14, respectively, which serve as standards. Lanes 3 through 5 are batch transformations of N1694 with pMCR16 (lane 3), pMCR24 (lane 4), and pMCR26 (lane 5).

FIG. 5. Restriction enzyme digest of a deletion derivative of pMCR16. Lanes 1 and 2 are untreated pMCR16 and pMCR14, respectively. Lane 3 is untreated deletion plasmid. Lanes 4 through 7 are deletion plasmids treated with *Eco*RI (lane 4), *Bam*HI (lane 5), *Bgl*II (lane 6), and *Pvu*II (lane 7).

placing the cells at 42°C for 20 min followed by 1 h at 37°C. The cultures were then allowed to grow at 30°C for 16 to 18 h until they reached saturation. No detectable *IS*/*I*-catalyzed events occurred in either strain even though the strains used are *rec*⁺ (Fig. 8). Other time courses of induction were tried with the same result (data not shown).

DNA was purified on agarose gels before transformation since the smaller plasmids observed after the transformation of N1694 with pMCR16 may exist as contaminants in the transforming DNA. To test whether small amounts of contaminating plasmids exist in the DNA samples before purification on agarose gels, DNA was extracted from the area of the gel that would contain these plasmids, as well as the area that contains pMCR16. Only the DNA extracted from the

FIG. 6. Restriction digest of pMCR16 and the product recovered from a gel. Lane 1 is an *Hinf*I digest of pBR322 for size markers. Lane 2 is an *Hae*II digest of pMCR16. Lanes 3 and 4 are *Hae*II digests of pMCR14 and the product recovered from a gel which has the same apparent mobility as pMCR14. The plasmids in lanes 3 and 4 appear to be identical and differ from the plasmid in lane 2 by the absence of an *IS*/*I* *Hae*II fragment (as judged by the intensity of the resulting bands) and the internal *Hae*II fragment from the drug resistance region.

FIG. 7. Requirement for the λ *p*_L promoter. Lanes 1 and 3 are the transformation of N1713 with pMCR16 and pMCR18, respectively. Lanes 2 and 4 are these same plasmids transformed into N1694. pMCR18, which lacks the λ *p*_L promoter, does not appear to react after introduction into N1694.

area of the gel that contained pMCR16 resulted in the growth of a batch culture after transformation of N1694. The possibility remains that very small amounts of DNA cannot be extracted from agarose gels. However, a gel slice that contains 10 ng of either pMCR14 or pMCR18 transformed N1694.

If the smaller plasmids that appear in the DNA population after the transformation of N1694 with pMCR16 are contaminants of the transforming DNA, then these plasmids must have a selective advantage over pMCR16. However, when N1694 is transformed with mixtures of pMCR16 and either pMCR14 or pMCR18, the relative amount of pMCR14 or pMCR18 to pMCR16 is directly proportional to the amount of these plasmids in the original mixture (data not shown). Since pMCR14 has the structure expected for a plasmid that has recombined the direct repeats of *IS*/*I* on pMCR16, pMCR18 has the structure of a plasmid that has undergone an *IS*/*I* deletion, and these plasmids do not have a selective advantage over pMCR16, it can be assumed that the smaller plasmids seen in this study are not merely selected out of the transforming plasmid DNA.

FIG. 8. Induction of the *cI* repressor in pRK248 strains bearing pMCR16. Lanes 1 and 2 are pMCR16 and pMCR14, respectively. Lanes 3 and 5 represent isolates of N721 and N1106 harboring pMCR16 and pRK248. Lanes 4 and 6 represent the induction of the *cI* repressor in these strains as described in the text. Lane 7 is a transformation of pMCR16 into N721 containing pRK248 where the pulse was at 30°C for 4 min. Lane 8 is an identical transformation, except that the heat pulse was at 42°C for 2 min.

DISCUSSION

The experiments described here demonstrate that the direct repeats of IS/ contained on pMCR16 will recombine. The recombination is no longer detectable when pMCR26, which contains the ΔT mutation in IS/a and IS/b, is the substrate. The related plasmid pMCR24, which contains the ΔT mutation only in IS/b, continues to recombine. Thus, it is not likely that the mutation inactivates a site required for recombination. Rather, it seems likely that the ΔT mutation affects the activity of an IS/-encoded gene product. All of the above plasmids promote IS/-generated deletions.

The ΔT mutation fuses IS/ and drug resistance region sequences with the result that 36 codons from the 5' portion of the IS/ B reading frame are eliminated and replaced with 57 codons from the Tn9 drug resistance region. The substitution does not affect the predicted conformation of the IS/ B protein as determined by the rules of Chou and Fasman (7). The fusion protein, therefore, may retain some activity. The IS/ C protein is completely eliminated since the deletion removes the IS/ C start codon.

The mechanism that leads to IS/-promoted deletion formation is more likely to resemble the pathway that leads to the formation of cointegrates than the pathway that resolves cointegrates. Sequence analysis of IS/-generated deletions in the *lac* operon demonstrate that they begin at the end of IS/ and terminate at locations that are hot spots for IS/ and Tn9 insertion (6, 13). Thus, transposase activity probably generates these deletions since deletion endpoints reflect transposase specificity. I thus propose that the ability of pMCR26 to form deletions represents the transposase activity of the IS/ A and IS/ B proteins, in accordance with the findings of Machida et al. (22). The IS/ C protein or the C-terminal domain of the IS/ B protein may be responsible for IS/-catalyzed recombination.

The activity that results in the recombination between direct repeats of IS/ can be mutated without affecting the activity that promotes deletion formation. I thus further propose that the IS/ recombinational activity represents an IS/ resolvase that is responsible for resolving IS/ cointegrates formed during the transposition of IS/.

Requirement for transcription. The requirement for transcription in the generation of deletion plasmids is understandable if the activity that leads to deletion formation is catalyzed by transposase. Several workers report that IS/ preferentially transposes into regions of high A+T content, that is, into regions that are easily denaturable (9, 11, 20, 25). Transcription of the plasmids used in this study may provide transient single-strandedness, which facilitates transposase-catalyzed events. Alternatively, transcription may stimulate the overproduction of IS/-encoded gene products that are normally repressed.

The IS/ resolution step may normally occur simultaneously with the replication step of transposition. Occasional molecules that escape resolution are no longer the proper substrate for IS/ resolvase and thus are stable cointegrates. Transcription from p_L during transformation of pMCR16 into N1694 may provide a more normal substrate for recombination.

recA-independent recombination of plasmids that do not contain insertion sequences has also been reported (10, 12). Thus, the small plasmids seen in this study could be generated by a *recA*-independent pathway and selected for during the transformation process. The presence of the strong p_L promoter and the *N* gene on the plasmid may inhibit plasmid establishment by interfering with replication. However, the

deletion plasmids observed after transformation of N1694 with pMCR16 do not represent plasmids selected out of a subpopulation of plasmids contained in the transforming DNA, since the area of an agarose gel that would contain these plasmids fails to transform N1694. Moreover, experiments designed to test whether these smaller plasmids have the required selective advantage over pMCR16 demonstrate that they do not. The formation of deletion derivatives of pMCR16 may be a transposase-catalyzed event similar to those observed by Calos and Miller (6).

Similarly, pMCR14 does not have a selective advantage over pMCR16. Further, the recombination between the direct repeats of IS/ on pMCR16 can be prevented by introducing the ΔT mutation. The IS/-catalyzed recombination resembles the homologous recombination observed between direct repeats of IS50 (38) and probably represents an IS/ resolvase activity.

IS/ repressor? The plasmid pMCR16 is subject to IS/-catalyzed rearrangements after transformation into N1694, but not into N1713. The only apparent difference between these strains is the presence of IS/s in N1713 that are lacking in N1694. Neither recombination nor deletion formation is detectable in W or K-12 strains that contain pMCR16 and pRK248, even when p_L is induced by thermal inactivation of *cl*. These results indicate that IS/ may encode a repressor or inhibitor of IS/-catalyzed events since IS/-bearing plasmids are unreactive in strain W, once established. Another explanation is that the induction does not mimic closely enough the conditions of transformation.

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ADDENDUM

After submitting this paper, we found that mutations in the IS/ A and IS/ B reading frames prevent IS/-generated deletion formation without affecting the ability of the direct repeats of IS/ to recombine.

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