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Transposon-specified site-specific recombination

(Tn*1*/Tn*3*/γδ/Tn*501*/transposition mechanism)

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ABSTRACT **Cointegrate DNA molecules containing two copies of a transposable element appear to be intermediates in the transposition process. These structures are resolved by site-specific recombination to yield the normal end products of transposition. The transposable element γδ (Tn*1000*) synthesizes a product interchangeable with the Tn*1*/*3* *tnp*R protein in promoting Tn*1*/*3* site-specific recombination. These data support the hypothesis that cointegrates containing directly repeated copies of Tn*1*/*3* are obligatory intermediates in interreplicon transposition of Tn*1*/*3*. In addition, we show here that the reaction is independent of the element-encoded *tnp*A gene product. Tn*501*, which specifies mercury resistance, also produces cointegrates as intermediates in interreplicon transposition. The appearance of Tn*501*-specified recombination activity that can act on these cointegrates requires growth of cells in the presence of Hg^{2+}.**

Models of transposition suggested by Shapiro (1) and by Arthur and Sherratt (2) have implicated cointegrate DNA molecules containing two directly repeated copies of the transposable element as obligatory intermediates during transposition from one circular replicon to another. Deletion mutants of the closely related transposons Tn*1* and Tn*3* that transpose to give such cointegrate structures have already been described (2, 3). One such transpositional cointegrate, containing a Tn*1* derivative (Tn*103*) deleted in the *tnp*R gene, can be stably propagated in a *rec*A$^-$ strain; however, the cointegrate structure undergoes site-specific recombination to yield the normal end products of transposition in the presence *in trans* of a complementing *tnp*R$^+$ gene of Tn*1* or Tn*3* (2, 4).

Because this cointegrate plasmid (pAA131; ref. 2; Fig. 1) contains a wild-type transposase, (it is *tnp*A$^+$ *tnp*R$^-$), we were unable to conclusively determine whether the product of the *tnp*A gene in addition to the *tnp*R product is necessary for site-specific recombination, though indirect evidence indicated that this was unlikely (2). Analysis of Tn*3* mutants by Heffron and coworkers (5), Arthur and Sherratt (2), and Sherratt *et al.* (4) has identified a region of Tn*1*/*3* that contains DNA sequences necessary *in cis* for Tn*3*-specified recombination [the internal resolution site (5)]. Although our experiments indicated that the *tnp*R gene product acts at such a site to mediate the recombination event (2, 4), the results of Heffron and coworkers did not obviously indicate such a conclusion. Much of the published work on the role of the *tnp*R gene product has focused on its ability to repress both its own synthesis and that of the *tnp*A gene product (5–8). Here we show that tnpR protein is the only element-encoded product necessary to act at the internal resolution site to mediate the site-specific recombination. Its role as a repressor likely results from binding the resolution site and thus regulating transcription of the *tnp*R and *tnp*A genes from promoters located in the internal resolution site region. This appears to be the case for γδ (Tn*1000*) (9). We also demonstrate that γδ (9), Tn*1*/*3*, and another related transposable element, Tn*501* [specifying Hg resistance (Hg^R)], likewise encode analogous site-specific recombination systems whose major function is to resolve intermediates in interreplicon transposition.

MATERIALS AND METHODS

Escherichia coli K-12 strains JC5446 (*trp his rps*E *rec*A γδ$^-$), MG1240 (*rps*L *rec*A γδ$^+$), K1470 (*rps*L *rec*A γδ$^+$), C600 (*thr leu thi*), W1485 (CGSC#5024), RR1012 (*rec*A γδ$^+$), and RR1031 (*rec*A γδ$^-$) and methods for their cultivation have been described (2, 9). Strain JC5466 has been shown to be γδ$^-$ both by DNA hybridization and by its inability to complement transposition of the *tnp*A$^-$ *tnp*R$^-$ γδ deletion on pRR1. The plasmids have been described, as have the methods for their isolation and manipulation (2, 4, 5, 9, 10). In some experiments, most of the chromosomal DNA was removed from single-colony Na-DodSO_4 lysates (2) by spinning the unheated lysate in a microcentrifuge for 20 min at 12,000 × *g*. Such supernatants (NaDodSO_4-cleared lysates) were run directly on agarose gels. Electron microscopy was by the method of Davis *et al.* (11). Selection for Hg^R Tn*501*-containing clones was on minimal agar containing $HgCl_2$ at 10 μg/ml.

Plasmid pRR1 was generated from pOX14 (pBR322 onto which γδ was transposed from the F plasmid; ref. 12) by deleting the 3.2 kilobases of DNA between the two *Xho* I sites within γδ. The *Eco*RI linker insertion mutations of pBR322::γδ were generated by cleaving supercoiled pOX14 DNA (20 μg) with *Hae* III until ≈20% of the molecules were converted to the linear form. These were then purified and treated with *Eco*RI methylase, and *Eco*RI linkers were attached and subsequently cleaved with *Eco*RI (13). The resulting DNA fragments were then ligated and used to transform W1485. The plasmids containing *Eco*RI linker inserts were designated pRR12 and pRR17 [with inserts at ≈2500 and 3600, respectively, on the γδ map of Guyer (12)]. The transposition phenotypes of the three γδ mutants, pRR1, pRR12, and pRR17 were identified by using an F derivative deleted in the region containing γδ (pOX38) (14) in strains with γδ present (RR1012) or absent (RR1031) on the chromosome (9).

RESULTS

The transposable elements that generate 5-base-pair (bp) direct repeats (Tn*1*, Tn*3*, γδ, Tn*501*) share significant DNA sequence homology at their termini. In addition, limited sequence analysis data (9, 10) suggest that a similar genetic organization exists for γδ and Tn*3* and that the end of γδ designated δ (12) cor-

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Abbreviations: bp, base pair(s); Hg^R, Sm^R, Tc^R, Cm^R, and Tp^R, mercury-, streptomycin-, tetracycline-, chloramphenicol-, and trimethoprim-resistant, respectively.

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responds to the region encoding transposase in Tn3. To investigate functions encoded by the γδ element, we isolated mutations in γδ by insertion of an *Eco*RI linker octamer into random sites in a γδ-containing plasmid. Our experiments examined the ability of these γδ mutants to undergo normal and complemented transposition. The results, in conjunction with sequence analysis data on γδ (9), are consistent with the γδ deletion derivative, pRR1, being a deletion of the NH_2 terminus of *tnp*A, all of *tnp*R, and the internal resolution site (i.e., it does not transpose unless complemented and then cannot resolve even in the presence of *tnp*R). Likewise, pRR12, an *Eco*RI linker insertion into γδ, appears to introduce a mutation in the *tnp*R gene making the plasmid *tnp*R$^-$, thus explaining the high frequency of transposition and the failure to resolve in γδ$^-$ recipients. Finally, another *Eco*RI linker insertion into a γδ-containing plasmid, pRR17, appears to be *tnp*A$^-$ in that transposition is observed only in donors carrying a complementing γδ on the chromosome.

Complementation of Tn*1/3* Site-Specific Recombination by γδ and its Derivatives. We have analyzed the ability of γδ and its mutant derivatives to resolve Tn*1*-generated cointegrate structures. Plasmid pAA131 (Fig. 1*B*) is a transpositional cointegrate resulting from transposition of the deleted Tn*1* derivative Tn*103* from the streptomycin-resistant (Sm^R) plasmid RSF1010 into the tetracycline-resistant (Tc^R) gene of pACYC184 [chloramphenical-resistant (Cm^R) Tc^R]. pAA131 is *tnp*A$^+$ *tnp*R$^-$ *bla*$^-$ internal resolution site-positive and can be efficiently resolved into pACYC184::Tn*103* and RSF1010::Tn*103* by *tnp*R$^+$ derivatives of Tn*1* and *3* (2, 4). Derivatives of plasmid F on which γδ normally resides had been used in those experiments of Heffron *et al.* (5, 6) and of Chou *et al.* (7) that did not report a role of Tn*1/3* *tnp*R product in cointegrate resolution, suggesting a role for the related γδ protein in those experiments. The results are shown in Fig. 2: the presence of wild-type γδ either on pBR322 or on F′ *lac* in a *rec*A$^-$ cell (e.g., pOX14, lanes F and G; F′*lac*, lanes N and O) results in efficient resolution of pAA131 into its recombinational products. Mutants of γδ lacking the functional equivalent of the Tn*1/3* *tnp*R gene (pRR1 and pRR12; lanes H, I, L, and M) do not complement pAA131 resolution whereas a *tnpA*$^-$ *tnpR*$^+$ derivative of γδ (pRR17, lanes J and K) complements resolution just as a *tnpA*$^-$ *tnpR*$^+$ derivative of Tn*1/3*. The identity of cointegrates and resolution products was confirmed genetically after transformation of plasmid DNA isolates from cells whose lysates are shown.

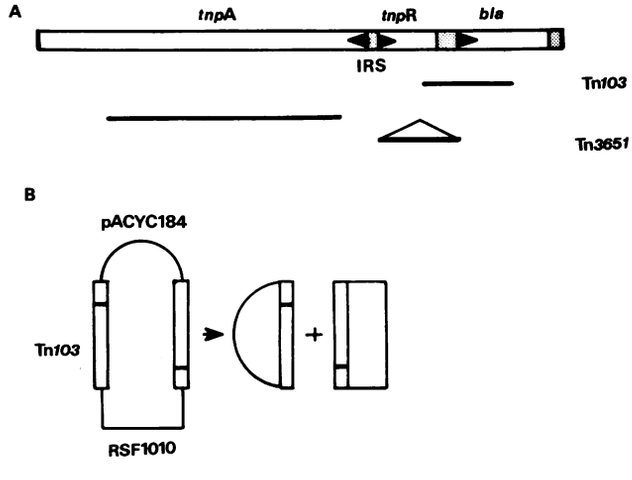

FIG. 1. (*A*) Map of the 4957-bp transposon Tn*3*, showing the mutants used in this study. Tn*3651* carries a deletion in the *tnp*A gene and a 1150-bp insertion of plasmid F DNA at the *Bam*HI site within *tnp*R. The internal resolution site (IRS) is located between the right-hand end point of the *tnp*A deletion in Tn*3651* and the beginning of the *tnp*R gene. Tn*103* is derived from Tn*1*. ▨, Intercistronic regions; ▶, direction of transcription. (*B*) Schematic map of the cointegrate plasmid pAA131 produced by transposition of Tn*103* from RSF1010::Tn*103* into pACYC184. pAA131 is Cm^R Sm^R. Tn1/3 site-specific recombination generates pACYC184::Tn*103*(Cm^R) and RSF1010::Tn*103*(Sm^R).

We therefore conclude that γδ produces a gene product that is interchangeable with the Tn*1/3* *tnp*R product for site-specific recombination. In contrast, γδ does not appear to efficiently complement transposition of *tnp*A$^-$ derivatives of Tn*3* (unpublished observations).

A Functional Tn*3* *tnp*A Gene Is Not Required for *tnp*R-Mediated Site-Specific Recombination. We next investigated whether the *tnp*A product plays a role in resolution of cointegrate structures. A *tnp*A$^-$ *tnp*R$^-$ internal resolution site-positive derivative of Tn*3* was first constructed. The starting material was plasmid RSF1365 (pMB8::Tn*3*; *tnpA*$^-$ internal resolution site-positive *tnpR*$^+$; ref. 4). A *tnp*R$^-$ derivative of this plasmid was constructed by inserting an 1150-bp *Bgl* II fragment derived from *Eco*RI fragment 6 of plasmid F (14) into the *Bam*HI site of the Tn*1/3* *tnp*R gene. Dimeric molecules of one such plasmid, pCHG7, were transformed into a *rec*A$^-$ strain, where they were stably propagated as dimers (Fig. 3, lanes A, N, and Y). The presence of complementing *tnp*R$^+$ *tnp*A$^+$ and *tnp*R$^+$ *tnp*A$^-$ derivatives of Tn*1/3* or γδ in the same cell all resulted in efficient conversion to monomers (lanes H, I, M, O, S–W), whereas a *tnp*R$^-$ *tnp*A$^+$ derivative of Tn*1* (tracks G and X) did not complement conversion of pCHG7 dimers to monomers. We therefore conclude that Tn*1/3* site-specific recombination does not require the presence of transposase. *tnp*A and *tnp*R gene products appear to be separately and independently involved in the two separable steps (i.e., cointegrate formation and cointegrate resolution) of interreplicon transposition.

Formation and Resolution of Cointegrate Plasmids during Tn*501* Interreplicon Transposition. Because transposon Tn*501* (8.2 kb; Hg^R) has inverted repeat termini that are similar to those of Tn*3* (15), we examined whether this transposon forms cointegrate intermediates that are resolved by a Tn*501*-specified site-specific recombination system. The assay depended on the fact that the trimethoprim-resistant (Tp^R) *Inc*W conjugative plasmid R388 mobilizes neither pACYC184 nor various ColE1 derivatives during conjugal transfer. If such a nonmobilizable plasmid carries Tn*501*, then transposition into R388 may generate a cointegrate intermediate that can be transferred during conjugation.

The results of such experiments are shown in Fig. 4. Either pACYC184::Tn*501* or a nonmobilizable ColE1::Tn*501* derivative were transformed into a *rec*A$^-$ R388$^+$ strain. Interreplicon Tn*501* transposition into R388 was assayed by mating the donor population with a *rec*A$^-$ recipient. The transposition frequency is given by the fraction of Tp^R transconjugants that are Hg^R. Such transconjugants were then scored for the nontransposon marker carried by the nonconjugative plasmid [colicin immunity (Iea$^+$) for ColE1 and Cm^R for pACYC184]. In experiments in which donor cells had not been previously treated with Hg^{2+}, the majority of Tp^R Hg^R derivatives clearly inherit the marker of the nonconjugative plasmid, consistent with the idea that the end product of Tn*501* transposition into R388 in these donor cells is a cointegrate molecule. Surprisingly, on analysis (Fig. 4, lanes B and E), the recipient cells contained not cointegrate molecules but plasmids of the size expected for their resolution products—i.e., R388::Tn*501* and either ColE1::Tn*501* or pACYC184::Tn*501*. It therefore seemed that the transposition events assayed in these experiments had generated stable cointegrate plasmid molecules in the donor cells but that these were efficiently resolved to the final transposition products after con-

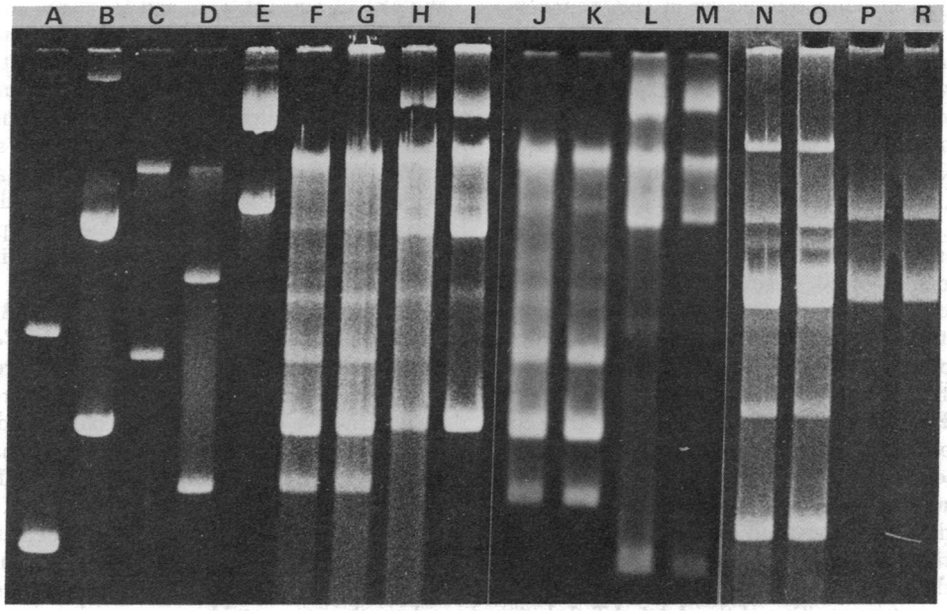

FIG. 2. Analysis by 0.8% agarose gel electrophoresis of Tn*1* cointegrate resolution by γδ derivatives. Lanes A–E contain purified plasmid and lanes F–R are $NaDodSO_4$- cleared lysates of *recA*$^-$ cells containing the various plasmids. Lanes: A, pRR1 DNA; B, pBR322::γδ DNA (pOX14); C, RSF1010::Tn*103* DNA; D, pACYC184::Tn*103* DNA; E, pAA131 DNA; F and G, pOX14/pAA131; H and I, pRR12/pAA131; J and K, pRR17/pAA131; L and M, pRR1/pAA131; N and O, F′*lac*/pAA131; P and R, pAA131.

jugal transfer to the recipient. Control experiments showed that most R388::Tn*501* plasmids were transfer proficient and did not efficiently mobilize ColE1::Tn*501* or pACYC184::Tn*501*. Three mechanisms for this cointegrate resolution during or after (or both) conjugal transfer seem feasible: (*i*) Cointegrate resolution results from derepression on transfer of a Tn*501* recombination system (a sort of zygotic induction). (*ii*) It is the result of transfer-dependent site-specific recombination. (*iii*) Tn*501*-specified site-specific recombination activity is inducible by Hg^{2+}, as is mercury reductase (16); i.e., selection for Hg^R transconjugants on Hg^{2+}-containing plates results in the appearance of recombination activity that can efficiently resolve the cointegrate molecules.

Experiments to distinguish these possibilities are also shown in Fig. 4. When donor cells that had been previously grown on Hg^{2+}-containing plates were used, the transposition frequency was similar to that in the experiments described above, although most Tp^R Hg^R transconjugants were now chloramphenicol sensitive and showed only R388::Tn*501* plasmid DNA (lanes M, N, and X). Moreover, if selection was made for Tp^R Cm^R transconjugants directly, they occurred at the same frequency as Tp^R Hg^R transconjugants, yet analysis of their plasmid DNA showed

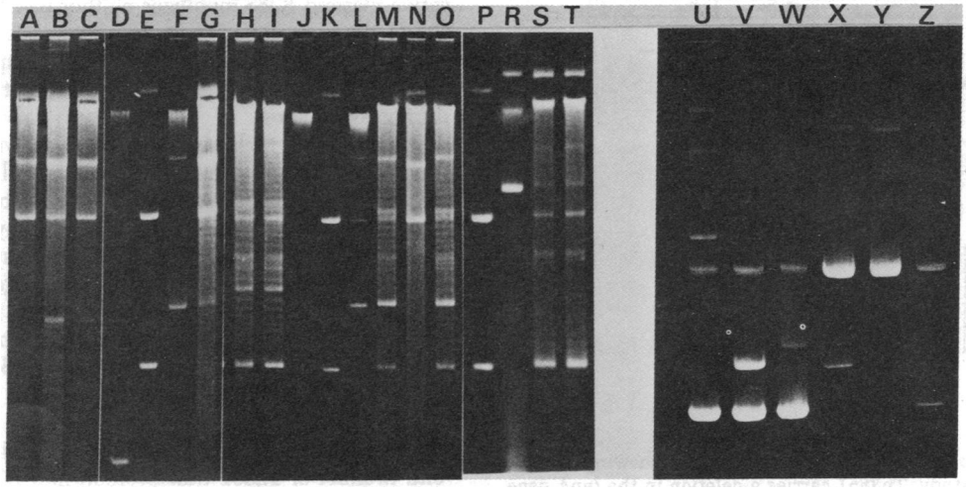

FIG. 3. Analysis by 0.8% agarose gel electrophoresis of Tn*1/3* site-specific recombination. The stability of pCHG7 dimers (*tnpA*$^-$ *tnpR*$^-$ internal resolution site-positive) in JC5466 (*recA*$^-$) derivatives carrying a variety of Tn*1/3* and γδ derivatives is shown. Lanes A–C, G–I, M–O, S, and T are $NaDodSO_4$-cleared lysates of single colonies; lanes D–F, J–L, P, and R are markers; and lanes U–Z contain plasmid DNA isolated from the appropriate clones. Identities of pCHG7 monomers and dimers were confirmed by electron microscopy. pDS4153 is a *tnpA*$^-$ *tnpR*$^+$ internal resolution site-positive derivative of ColK::Tn*1*. Lanes: A, pCHG7 dimers in JC5466 (i.e., PK133); B and C, PK133/pACYC184; D, pACYC184 DNA; E, pCHG7 monomer DNA; F, pACYC184::Tn*103* DNA; G, PK133/pACYC184::Tn*103*; H and I, PK133/pACYC184::Tn3; J, chromosomal DNA; K, pCHG7 monomer DNA; L, pDS4153 DNA; M, PK133/pDS4153; N, PK133; O, PK133/pDS4153; P, pCHG7 monomer DNA; R, pSC101::γδ DNA; S and T, PK133/pSC101::γδ; U, PK133/pSC101::γδ; V, PK133/pDS4153; W, PK133/pACYC184::Tn*3*; X, PK133/pACYC184::Tn*103*; Y, pCHG7 dimer DNA; Z, pCHG7 monomer DNA.

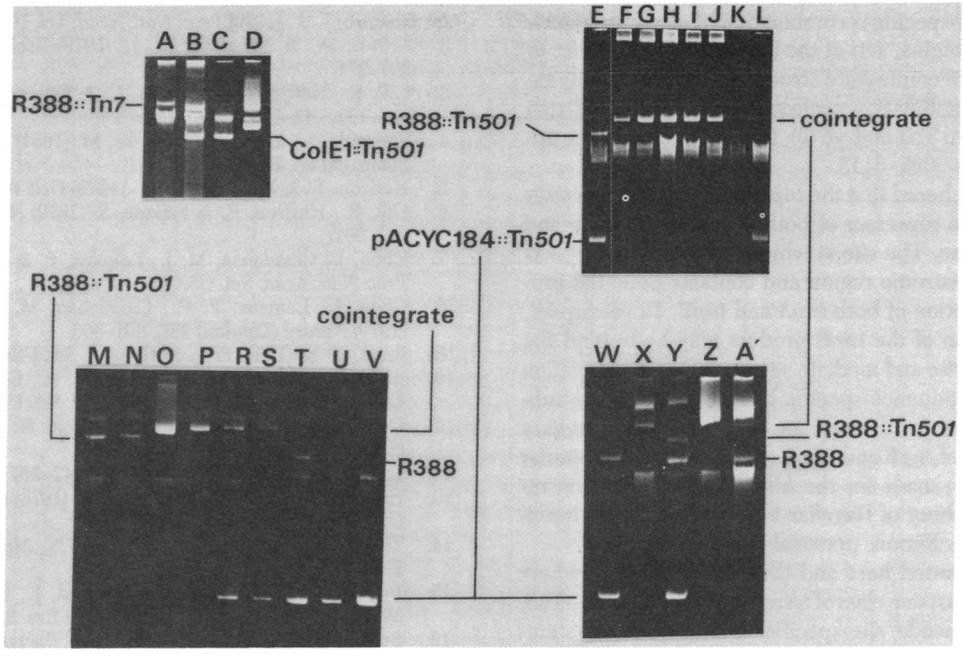

FIG. 4. Analysis of Tn*501* transposition by 0.8% agarose gel electrophoresis. Lanes: A, R388::Tn*7* marker DNA; B, ColE1::Tn*501*/R388::Tn*501*DNA isolated from the Tp^R Hg^R (Iea^+) transconjugant; C, ColE1::Tn*501* marker; D, ColE1::Tn*7* marker; E, pACYC184::Tn*501*/R388::Tn*501* DNA in $NaDodSO_4$-cleared lysate of Tp^R Hg^R (Cm^r) transconjugant; F–J, R388::Tn*501* cointegrate DNA in $NaDodSO_4$-cleared lysate of Tp^R Cm^R transconjugants (no exposure to Hg^{2+}); K and L, pACYC184::Tn*501* marker DNA (from $NaDodSO_4$-cleared lysate); M, N, and X, R388::Tn*501* DNA isolated from Tp^R Hg^R (Cm^S) transconjugant; O, P, and Z, R388::Tn*501*::pACYC184::Tn*501* cointegrate DNA isolated from Tp^R Cm^R transconjugant (no exposure to Hg^{2+}); R and S, cointegrate DNA/R388::Tn*501*/pACYC184::Tn*501* after partial resolution by growth of Tp^R Cm^R transconjugant (see lanes O, P, and Z) on suboptimal Hg^{2+} (1 μg/ml); T, U and Y, R388/PACYC184::Tn*501* DNA isolated from donor cells; V and W; pACYC184::Tn*501* DNA; A', R388::Tn*501*/pACYC184::Tn*501* DNA in Hg^{2+} (10 μg/ml)-grown cells derived from Tp^R Cm^R transconjugants (lanes O, P, and Z). In all cases, the identities of individual plasmids were confirmed genetically and physically. Cells that contained multiple plasmids were separated by transformation or conjugation, and single plasmid species were then characterized by assaying markers carried and their size and restriction endonuclease patterns. For example, 50/50 Tp^R transformants derived from DNA in lanes O, P, and Z were Cm^R Hg^R. In contrast, 0/50 Tp^R transformants from DNA shown in lane A' were Cm^R and 0/50 Cm^R transformants were Tp^R; all transformants were Hg^R in addition to being either Cm^R or Tp^R. In lane A,' the broad diffuse band behind the R388::Tn*501* band contains predominantly R388::Tn*501* open circles.

no evidence of cointegrate breakdown (lanes F–J, O, P, and Z). Analysis of plasmid DNA from such Tp^R Cm^R transconjugants after subsequent subculture on Hg^{2+}-containing media showed essentially complete cointegrate resolution (lane A') whereas the plasmid isolated from cells subcultured in the absence of Hg^{2+} was primarily cointegrate molecules. These observations strongly support our view that cointegrate molecules are an obligatory intermediate in the interreplicon transposition of at least some transposable elements. The finding that efficient resolution of Tn*501* promoted cointegrates requires growth on Hg^{2+} is intriguing, particularly in view of the derepression of mercury reductase synthesis by mercury salts (16) and the occurrence of transposition to yield cointegrates in the absence of growth on mercury. Other experiments (unpublished) have shown that growth on Hg^{2+}-containing medium can increase the frequency of interreplicon transposition, showing an apparent triple adaptive response of Tn*501* to Hg^{2+}, derepression of mercury reductase activity, increase in Tn*501* gene copies by transposition, and spread of the transposon to different replicons.

DISCUSSION

Our data extend the functional similarity of Tn*1/3* and $\gamma\delta$ and further support the idea that interreplicon transposition of some translocatable elements proceeds through an obligatory cointegrate intermediate as suggested previously (1, 2). Although transposable element-promoted cointegrate molecules have also been shown for Tn*5*, Tn*903*, Mu, and IS*1* (1, 17–19), it appears that cointegrates are not necessary transposition intermediates for these latter elements. In the absence of element-specified site-specific recombination, *rec*A-mediated homologous recombination can act, albeit inefficiently compared with the Tn*1/3*–$\gamma\delta$ system, to separate cointegrate replicons. We suggest that transposons that reside predominantly on plasmids and have evolved to transpose between plasmids efficiently, have acquired (or will acquire) site-specific recombination systems if their transposition proceeds through obligatory cointegrate intermediates. Insertion sequence elements, which are often present as part of the bacterial chromosome, may have less opportunity for interreplicon transposition: their intrareplicon transposition has little use for site-specific recombination if it proceeds by the models decribed in refs. 1 and 2.

The observation that $\gamma\delta$ has an efficient *rec*A-independent site-specific recombination system suggests that Hfr strains formed by transpositions of $\gamma\delta$ from plasmid F onto the chromosome (or vice versa) or by recombination (homologous or site specific) between F and chromosomal $\gamma\delta$ sequences will be unstable: $\gamma\delta$ site-specific recombination will quickly excise the F plasmid and the cell will revert to F^+. Thus, the population will contain a mixture of F^+ and Hfr forms. This mechanism appears, at least for some *E. coli* strains, to be responsible for a significant portion of chromosomal transfer (20). Hfr strains formed by transposition of IS*2* or IS*3* from plasmid F onto the chromosome [*rec*A-independent and rare (21)] or by *rec*A-dependent homologous recombination (most Hfr strains; ref. 21) between F and chromosomal IS*2* or IS*3* may be much more stable, although the F plasmid will still be subject to *rec*A-mediated excision.

The *tnp*R protein is the only Tn*1/3*-encoded product in-

volved in Tn1/3 site-specific recombination: it alone, or in conjuction with host proteins, acts at the internal resolution site to mediate resolution of cointegrate structures. Recent results indicate that the recombination occurs within a 19-bp A-T-rich sequence common to Tn*3* and γδ (9); in Tn*3*, this sequence appears at coordinates 3095–3113.

It must be remembered that the *tnp*R gene product was originally identified as a repressor of both its own expression and that of the *tnp*A gene. The site at which the *tnp*R product acts is within the intercistronic region and contains potential promoters for transcription of both *tnp*A and *tnp*R. In retrospect, the primary function of the *tnp*R product may be to bind the internal resolution site and mediate site-specific recombination (9). This efficient sequence-specific binding to both the substrate and the product of the reaction may have the subsidiary effect of repression of *tnp*R and *tnp*A gene expression. A similar suggestion has been made for the *hin* promoter inversion resulting in the switching of flagellar type in *Salmonella typhimurium* (ref. 22; M. Simon, personal communication).

The results presented here and those presented elsewhere (9) suggest that at least one class of transposable elements—Tn*3*, γδ, and Tn*501*—encode site-specific recombination systems that play a role in the amplification and transmission of the element throughout the population.

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