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Genetics

Enhanced recombination between λp*lac5* and F42*lac*: Identification of *cis*- and *trans*-acting factors

(*Escherichia coli* recombination/*tra* regulon/*oriT*/RecBC enzyme)

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ABSTRACT **Enhanced transductional recombination between specialized transducing phage λp*lac5* and plasmid F42*lac* depends on *cis*- and *trans*-acting factors. By constructing a series of recombinant molecules, the *cis*-acting site required for enhanced recombination has been identified as *oriT*, the origin of conjugational transfer of the F sex factor of *Escherichia coli*. The *trans*-acting factors are located in the promoter-proximal and the promoter-distal regions of the *traY*-to-*traZ* operon.**

Recombination levels scored in crosses between λp*lac5* specialized transducing phage and plasmid F42*lac* are 20- to 50-fold higher than recombination levels scored in a similar cross between λp*lac5* and a chromosomally located *lac* gene (1). The enhanced recombination shown by F42*lac*, a derivative of the *Escherichia coli* F sex factor containing the *E. coli* *lac* operon, can be assayed by plating Lac^+ transductants on selective media or by using the "transcribable intermediate" assay of β-galactosidase production (2). The transcribable intermediate is defined as a stage in the recombination process where the transcription and translation of a recombinant *lacZ* gene can occur, but where the additional processing required to give rise to a Lac^+ colony may or may not occur (2). The use of transcribable intermediate and Lac^+ colony assays divides the recombination process into two steps and sometimes provides valuable additional information regarding mechanism.

Previous reports have determined that the enhanced recombination involves a *cis*-acting component (1), is *recB* dependent (1, 3), and requires the presence (4) and expression (5) of the *tra* regulon of F42*lac*. In addition, UV irradiation of the transducing phage roughly equalizes the recombination potential of F42*lac* and a chromosomal *lac* gene (1). This UV stimulation is essentially *recB* independent (3) but requires a functional single-strand DNA binding protein in the case of a chromosomal *lac* gene (6).

A mini-F-*lac-tra* plasmid, pRPZ105, shows recombination levels similar to F42*lac* (4). The enhanced recombination shown by pRPZ105, along with the observation that *tra* expression is required for the enhancement (5), suggests that some function(s) carried and expressed by the *tra* regulon are involved in the enhancement. The requirement for *tra* and *lac* to be on the same molecule also suggests that some part of the enhancement is *cis* dependent. The only known *cis*-acting element in *tra* is *oriT*, the origin point of conjugal DNA transfer of the F sex factor of *E. coli* (7). This site and the *traM*, *traY*, *traD*, *traI*, and *traZ* genes are known to be required for donor conjugal DNA synthesis (DCDS) (8). It has previously been proposed that *oriT* and at least some of the related *tra* genes are involved in recombination enhancement (4, 5). A series of plasmids was constructed to determine whether *oriT* is the *cis*-acting site required for the enhancement and to begin a determination of which *tra* functions may be required in *trans* for the enhancement.

MATERIALS AND METHODS

Bacterial Strains, Bacteriophages, and Media. All *Escherichia coli* K-12 strains and phage λp*lac5* derivatives used in this work have been described (refs. 1, 4, and 9; see the legend to Table 1). Bacteria were grown in modified minimal medium 56 supplemented with glycerol and growth factors (1, 10) for recombination assays. LB medium (11) was used for all other purposes.

Nick Translation and Colony Hybridization. Labeling of plasmid DNA with [α-P^{32}]dATP by nick-translation was performed by the method of Maniatis *et al.* (12). Colony hybridizations of replica-plated colonies were done by the procedure described by Hanahan and Meselson (13).

β-Galactosidase/Recombination Assays. Recombination assays were conducted as described (4). λp*lac5* was used at a multiplicity of infection of 5. Enzyme units of β-galactosidase were determined 3 hr after infection by the method utilizing the chromogenic substrate *O*-nitrophenyl-β-D-galactopyranoside (1, 2).

Plasmid Constructions. Procedures for plasmid DNA isolation, restriction endonuclease digestions, agarose gel electrophoresis, ligation, and bacterial transformation were as described (4).

Partial *Eco*RI digests of pRPZ105 (mini-F-*lac-tra*; Fig. 1) were used to produce pRPZ108 and pRPZ109. These plasmids differ from pRPZ105 only in that various *Eco*RI fragments from the F factor *tra* region have been removed as shown in Fig. 2.

pRPZ108 was digested with *Sal* I and *Bam*HI to generate an 8-kilobase (kb) *oriT*-containing fragment and a 19-kb fragment that contains a deleted version of the *traJ* to *traZ* region. These two fragments were separately inserted into *Bam*HI/*Sal* I-digested pBR325 (14) to make pRPZ115 and pRPZ116, respectively (see Fig. 2).

A 2.5-kb *Eco*RI–*Sal* I fragment containing *oriT* was isolated from pRPZ115 and ligated with a mini-F-*lac* plasmid (pRPZ100; Fig. 3) which had been partially digested with *Eco*RI and *Sal* I. Lac^+ recombinant plasmids that had the *oriT* sequence inserted were identified by the hybridization of transformants with α-P^{32}-labeled pRPZ115. pRPZ118 and pRPZ119 are the mini-F-*lac-oriT* plasmids that were isolated (see Fig. 3).

A series of mini-R1 cloning vectors was constructed to allow the cloning of putative *trans*-acting *tra* sequences in a low-copy replicon (15). Plasmid R1 DNA was partially digested with *Eco*RI and ligated under dilute conditions. A 24-kb plasmid was isolated (pRPZ113; Fig. 4) that carries kana-

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Abbreviations: kb, kilobase; DCDS, donor conjugal DNA synthesis; IS, insertion sequence.

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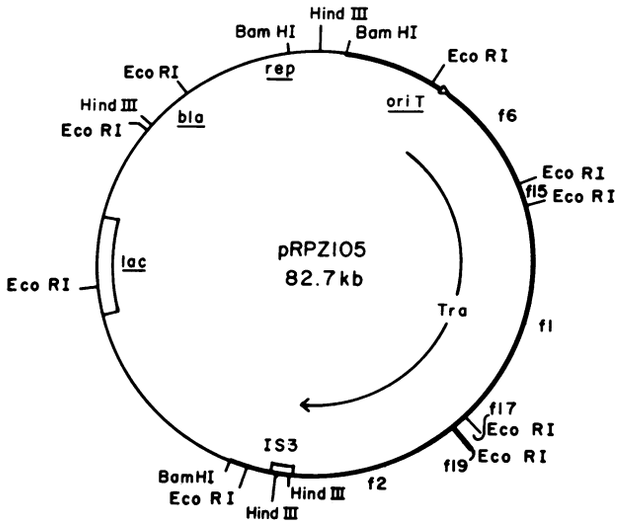

FIG. 1. Physical and genetic map of pRPZ105. The thin line represents pRPZ100 sequences. The thick line represents sequences from the 44.5-kb Tra$^+$ fragment from pED851 (see ref. 4 for construction). *rep* is the origin of replication of the F factor. *bla* represents the β-lactamase gene. Tra indicates the transfer regulon *tra*. *oriT* is the origin of conjugal transfer. f6, f15, etc. are F factor *Eco*RI fragment designations.

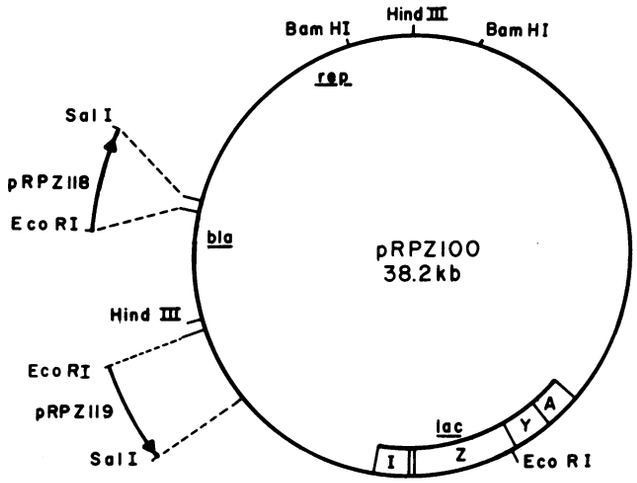

FIG. 3. Physical and genetic map of mini-F-*lac-oriT* plasmids. ---, Sites of insertions; ⟶, *oriT*. All other symbols are as indicated in the previous figure legends.

mycin resistance (Km^r) as the only drug-resistance marker. pRPZ113 carries a functional *finO* gene which repressed the expression of F factor *tra* DNA cloned into it (data not shown). The *finO* gene product from another F-like plasmid acts in conjunction with the F factor *finP* gene product to repress the expression of the F factor *traJ* gene (16). *traJ* expression is required for the expression of most of the rest of the *tra* genes (17), and enhanced recombination does not occur in the absence of *traJ* expression (5). Therefore, *Pst* I deletion derivatives of pRPZ113 were constructed that no longer contain a functional *finO* gene. All of these deletion derivatives were missing a 4.15-kb *Pst* I fragment. The plasmid containing the largest deletion that retained a full replication region (*cop* control) was named pRPZ114. Fig. 4 shows the physical and genetic maps of pRPZ113 and pRPZ114.

The 19-kb *Bam*HI–*Sal* I *tra* fragment from pRPZ116 was inserted into pRPZ114 to make pRPZ117 (see Figs. 2 and 4). Thus, pRPZ117 is a mini-R1 derivative containing the *traJ*-to-*traZ* region of the F factor, with several of the central F factor *Eco*RI fragments from the *tra* region deleted.

RESULTS

Deletion Derivatives of pRPZ105. To begin the analysis of which *tra* function(s) are involved in recombination enhancement, partial *Eco*RI deletions of the *tra* regulon of pRPZ105 were made. Two deletion derivatives of pRPZ105 were isolated, pRPZ108 and pRPZ109 (see Fig. 1 for a map of pRPZ105 and Fig. 2 for the deletions). pRPZ108 is deleted of F factor *Eco*RI fragments f15, f1, f17, and f19 (18). pRPZ109 is deleted of F factor *Eco*RI fragment f2 as well as

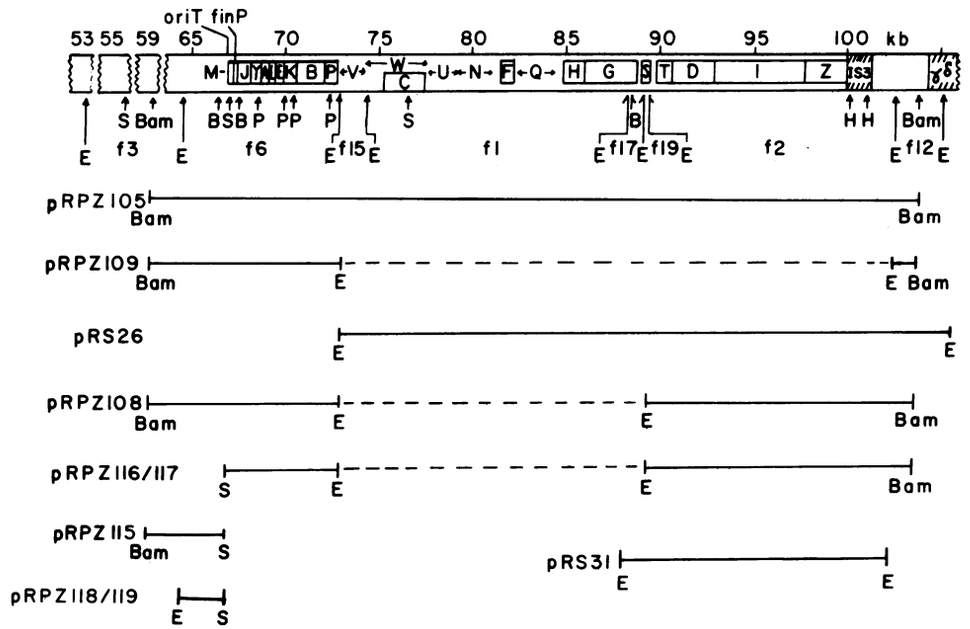

FIG. 2. A physical and genetic map of the *tra* regulon. The top line of the figure shows the location of restriction sites and mapped genes from the F plasmid transfer regulon and surrounding DNA (adapted from ref. 8). E, *Eco*RI; S, *Sal* I; Bam, *Bam*HI; B, *Bgl* II; H, *Hin*dIII; and P, *Pst* I (only the *Pst* I sites in f6 are shown). The *traM* gene lies between *oriT* and *traJ* and is interrupted by a *Sal* I site. The solid lines below the map indicate the sequences derived from this region that are contained in the plasmids listed to the left of the lines. ---, Deleted *tra* sequences.

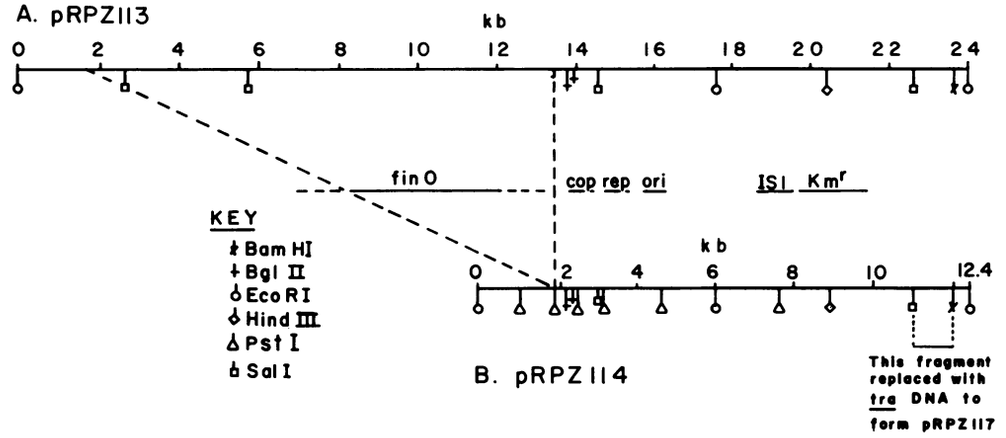

FIG. 4. Physical and genetic map of mini-R1 plasmids. Symbols representing restriction enzyme sites are listed in the key. *Pst* I sites are only shown for pRPZ114. *cop* is a copy number polypeptide, *rep* is a positive regulator of replication, *ori* is the origin of replication, IS*1* is an insertion sequence, and *Km*^{r} is the kanamycin-resistance gene (15). *finO* is the gene for part of the *finO–finP* repressor system and is only present in pRPZ113. – – –, Sequences deleted from pRPZ113 to make pRPZ114.

all of the fragments deleted from pRPZ108 (Fig. 2). These particular deletion derivatives were chosen because they still retain F factor *Eco*RI fragment f6, which contains *oriT* and the transcriptional control region of the *tra* regulon (19).

pRPZ108 and pRPZ109 were made *lacZ813* by homogenotization of *lac* alleles in KL765 (4). The resulting plasmids were transformed into KL791 to make strain RDP189 (containing pRPZ108) and strain RDP191 (containing pRPZ109). pRS26, a pSC101 derivative containing F factor *Eco*RI fragments f15, f1, f17, f19, f2, and f12 (18), was transformed into RDP191 to make RDP192. pRS31 is a pSC101 derivative that contains F factor *Eco*RI fragments f17, f19, and f2 (18). pRS31 was transformed into RDP191 to make RDP212.

Table 1, group II, shows the recombination data from λp*lac5* crosses with these plasmid-containing strains. Table 1, group I, reports recombination data for chromosomal *lac*, F42*lac*, mini-F-*lac* (pRPZ100), and mini-F-*lac-tra* (pRPZ105) for comparison. It should be noted that plasmid copy number does appear to account for part of the enhancement differential seen when comparing the recombination of chromosomal *lac* versus F42*lac* with λp*lac5* (4, 5, 20). Therefore, the most valid basis for comparison with the mini-F-*lac* derivatives described herein is mini-F-*lac* itself (pRPZ100). An examination of the values in Table 1, group I, shows that mini-F-*lac* gives recombination levels about 4-fold higher than chromosomal *lac*, whereas F42*lac* is 5- to 7-fold higher than mini-F-*lac*. This latter factor is the actual *tra*-dependent enhancement with which we are concerned here.

pRPZ108 showed fully enhanced recombination levels in the transcribable intermediate assay, but showed only 2-fold higher levels than pRPZ100 showed in the Lac$^+$ transductant assay. pRPZ108 contains all of the genes required for DCDS, as listed above, but is missing several genes involved in pilus production (8). Therefore, pRPZ108 contains all of the infor-

Table 1. Recombination assays

E. coli strain	*lac* gene and relevant plasmids	Lac$^+$/10^3 cfu	EU/10^9 cfu
	Group I		
KL765	chromosomal *lac*	0.15 (0.02)	0.031 (0.002)
KL771	F42*lac*	2.7 (0.78)	0.93 (0.071)
RDP157	pRPZ100 (mini-F-*lac*)	0.57 (0.11)	0.14 (0.033)
RDP159	pRPZ105 (mini-F-*lac-tra*)	9.1 (1.7)*	0.92 (0.076)
	Group II		
RDP189	pRPZ108	1.1 (0.15)	1.7 (0.30)
RDP191	pRPZ109	0.53 (0.06)	0.28 (0.008)
RDP192	pRPZ109 & pRS26	4.2 (0.32)	4.2 (1.4)
RDP212	pRPZ109 & pRS31	2.5 (0.55)	0.97 (0.045)
	Group III		
RDP195	pRPZ118	0.60 (0.18)	0.23 (0.075)
RDP196	pRPZ118 & pRPZ117	6.5 (1.1)	18.5 (6.1)
RDP205	pRPZ118 & pRS26	0.25 (0.075)	0.11 (0.03)

Strain KL765 (9) is F$^-$ *lacZ813 lacI3 pro met his trp rpsL thi* (λ*ind*). All other strains are plasmid-containing derivatives of KL791 (5), which is F$^-$ Δ(*lac-pro*) *met his trp rpsL thi* (λ*ind*). Each *lac*-containing plasmid in these strains is *lacZ813 lacI3*. The recombination assays involved infecting each strain with λ*cI857Sam7placZ118* at a multiplicity of infection of 5. EU refers to β-galactosidase enzyme units. All numbers for both Lac$^+$ transductants and EU have been normalized to colony forming units (cfu) to facilitate comparisons. Each value shown is an average of at least three experiments and the standard deviations are indicated in parentheses.

*The Lac$^+$ transductant value reported for RDP159 is lower than has been reported for this strain previously (4). The value shown here is correct for pRPZ105. The previously reported values were actually measured for a plasmid that had picked up an insertion from an unknown source.

mation necessary to initiate enhanced recombination but appears to be partially blocked in the maturation of recombinant structures into viable Lac$^+$ DNA molecules (see *Discussion*).

pRPZ109 failed to show enhanced recombination levels in either assay (compare RDP191 with RDP157). As pRPZ109 differs from pRPZ108 only in the absence of F factor *Eco*RI fragment f2, one or more functions coded for by that fragment are required for the initiation of enhanced recombination. RDP192 contains both pRPZ109 and pRS26. These two plasmids provide the cell with all of the F factor *Eco*RI fragments from the *tra* region. This combination of plasmids showed enhanced recombination by both assays and demonstrated that the f2-encoded function(s) required for the initiation of enhanced recombination can be provided to the recombining DNA molecule in *trans*. RDP212 contains both pRPZ109 and pRS31 and showed enhanced recombination in both assays. This result demonstrates that fully enhanced recombination can be obtained in the absence of F factor *Eco*RI fragments f15 and f1. The higher levels of recombination seen in both assays with RDP192 versus RDP212 may result from some manifestation of the ability of pRPZ109 and pRS26 to recombine with each other and form a cointegrant. It also might result from a differential expression of *tra* genes from pRS26 and pRS31.

Separation of *cis*- and *trans*-acting Factors. pRPZ108 contains a single *Sal* I site in the *tra* portion of the plasmid. This *Sal* I site interrupts the coding sequence of *traM* (21). *oriT* and the 5′ end of *traM* are to the left of the *Sal* I site, whereas all other known *tra* functions have been mapped to the right of the *Sal* I site (see Fig. 2). Our working hypothesis has been that enhancement requires the action of *trans*-acting *tra* gene products at *oriT*. Therefore, we used this *Sal* I site in *traM* as a dividing point for the subcloning of the *tra* region.

pRPZ118 and pRPZ119 are mini-F-*lac-oriT* plasmids that contain a 2.5-kb *Eco*RI–*Sal* I fragment with *oriT* and no other known *tra* functions (see Figs. 2 and 3). Previous reports have described an orientation-dependent instability of TraYZ endonuclease-activated *oriT* sequences in pBR322 (22). A similar orientation effect of *Tra* in mini-F-*lac* plasmids also has been reported (4). pRPZ119 was expected to be an unstable replicon in the presence of *tra* functions; however, no instability of pRPZ119 was detected in the presence of a mini-R1 derivative that contained the complete *tra* region from the F factor (data not shown). Both pRPZ118 and pRPZ119 could be mobilized to a recipient cell via conjugation at high efficiency by this mini-R1-*tra* derivative (data not shown) so the *oriT* site appears to be functional in both plasmids. pRPZ118 was chosen for recombination studies since the orientation of *oriT* in relation to *lac* was the same as in F42*lac* and pRPZ105. pRPZ118 was made *lacZ813* by homogenotization in KL765 and transformed into KL791. This strain is RDP195.

Two different plasmids were used to provide some *tra* gene products to pRPZ118 in *trans*. pRPZ117 is a mini-R1 derivative that contains the *traJ*-to-*traZ* portion of the *tra* regulon minus the central *Eco*RI fragments from the *tra* region (see *Materials and Methods* and Fig. 2). pRPZ117 was transformed into RDP195 to yield strain RDP196. pRS26 is a pSC101 derivative that contains F factor *Eco*RI fragments f15, f1, f17, f19, f2, and f12 (ref. 18; see Fig. 2). pRS26 was transformed into RDP195 to yield strain RDP205.

Table 1, group III, shows data from λp*lac5* crosses with RDP195, RDP196, and RDP205. pRPZ118 (mini-F-*lac-oriT*) by itself showed nonenhanced recombination levels (compare to pRZP100). The strain containing both pRPZ117 and pRPZ118 showed high recombination levels by both assays. The enhanced recombination seen in the strain containing both pRPZ117 and pRPZ118 (RDP196) did not result from some function provided by the mini-R1 vector, as pRPZ114 itself had no effect on the recombination between pRPZ118 and λp*lac5* (data not shown). The result with pRPZ117 plus pRPZ118 is also in contrast to that found with pRPZ108, which showed enhanced recombination only in the transcribable intermediate assay. pRPZ108 contains the same *tra* information as pRPZ117 plus pRPZ118 (the 5.5-kb *Bam*HI–*Eco*RI fragment found in pRPZ108, but not in pRPZ117 plus pRPZ118, contains no known *tra* genes) except that pRPZ108 contains a functional *traM* gene. The strain containing both pRPZ118 and pRS26 (RDP205) showed nonenhanced recombination levels with both assays. This result indicates that a *trans*-acting function carried by pRPZ117 but not by pRS26 is required for enhanced recombination (see *Discussion*).

DISCUSSION

The recombination assays reported here show that enhanced transductional recombination is dependent on the presence of an *oriT* sequence in *cis* to the recombining *lac* gene and more than one *trans*-acting *tra* gene. In addition, the enhancement of recombination has been shown to be independent of DNA transfer *per se* (see RDP196 in Table 1) as it occurs when segments of the *tra* regulon required for conjugation are missing.

The recombination assays that used pRPZ118 (mini-F-*lac-oriT*) as the recipient DNA molecule indicate that the *oriT* site is the only part of the *tra* regulon that must be in *cis* to the recombining *lac* gene for enhanced recombination to occur. This *oriT*-promoted enhancement occurs, however, only if *oriT* is activated by *trans*-acting *tra* genes contained in the promoter proximal and promoter distal regions of the *traY* to *traZ* operon. The localization of a *trans*-acting *tra* function in the promoter proximal portion of the *traY*-to-*traZ* operon comes from comparing the recombination levels scored when pRPZ118 is in the same cell as pRPZ117 or pRS26. pRPZ117 complementation of pRPZ118 produces high levels of recombination while pRS26 does not. The only *tra* sequences carried by pRPZ117 but not pRS26 are contained on the 5.8-kb *Sal* I–*Eco*RI fragment from the promoter proximal region of the *traY*-to-*traZ* operon (see Fig. 2). This 5.8-kb fragment contains the coding sequences for *finP* and for *tra* genes *J*, *Y*, *A*, *L*, *E*, *K*, *B*, and *P* (8, 19). As *traY* is the only one of these genes whose product is known to act at *oriT* (23, 24), it is likely that the *traY* gene product is the *trans*-acting factor coded for by the promoter proximal region of the *traY*-to-*traZ* operon.

pRPZ109 differs from pRPZ108 only in that it lacks F factor *Eco*RI fragment f2 (see Fig. 2). The observation that pRPZ108 shows enhanced levels of transcribable intermediate while pRPZ109 does not leads to the conclusion that f2 also encodes a *trans*-acting factor required for recombination enhancement. F factor *Eco*RI fragment f2 carries the coding sequences for *tra* genes *T*, *D*, *I*, and *Z*. *traD*, *traI*, and *traZ* have been postulated to be involved in DCDS (8, 24). *traZ* is thought to code for part of the TraYZ endonuclease that acts at *oriT* (23); therefore, it is a likely candidate for a role in recombination enhancement. *traI* is known to be the gene for helicase I of *E. coli* (25), while the *traD* gene product is thought to have a role in the facilitation of DNA transfer during conjugation (24). The possible role of these genes in recombination enhancement is currently under investigation.

The discrepancy between the transcribable intermediate assay and the Lac$^+$ transductant assay for pRPZ108 was initially somewhat puzzling. This plasmid seems to be able to initiate enhanced recombination, but is partially blocked in some step leading to the maturation of recombinant molecules into stable Lac$^+$ genes. However, pRPZ118 complemented by pRPZ117 shows enhanced recombination in both

assays. The only difference in these two situations is the presence or absence of a functional *traM* gene. F42*lac*, pRPZ105, and pRPZ109 plus pRS26 all have a functional *traM* gene and yet show enhanced recmbination in both assays. These latter three cases, however, all have F factor *Eco*RI fragments f15, f1, f17, and f19, which are missing in pRPZ108 and pRPZ117. Some function encoded by this segment of *tra* DNA may interact with the *traM* gene product to modify or inhibit its activity. The combination of pRPZ109 and pRS31 shows enhanced recombination by both assays and differs from pRPZ108 only in that F factor *Eco*RI fragments f17 and f19 are present. It has been suggested that the *traM* gene product acts as a trigger for DCDS when a donor cell interacts with a potential recipient (26). Unrestrained *traM* activity might lead to abortive DCDS that could destroy any unresolved recombinant structures that are encountered. It also has been suggested that the *traS* gene product acts as an inhibitor of the triggering of DCDS (26). Although this inhibition of the triggering of DCDS by the *traS* gene product is normally thought of in terms of abortive matings between two F factor-containing cells, it may also play a role here in that pRPZ108 is deleted for the *traS* gene. The possiblity of an interaction between the *traM* and *traS* gene products is strengthened by the data obtained with pRPZ109 plus pRS31. pRS31 contains F factor *Eco*RI fragments f17, f19, and f2 and has been shown to express the *traS* gene (27). pRPZ109 plus pRS31 shows enhanced recombination in both assays and differs from pRPZ108 only in that F factor *Eco*RI fragments f17 and f19 are present.

Some role for insertion sequence *3* (IS*3*) in recombination enhancement cannot be rigorously ruled out as the IS*3* element immediately adjacent to the F factor *tra* regulon (see Fig. 2) is present in every plasmid or combination of plasmids that demonstrates enhanced recombination with λp*lac5*. We do feel, however, that a significant role for IS*3* is unlikely, as it would have to be interacting in *trans* with the *oriT*-containing DNA fragment in several of the situations that we have described. It is certainly not sufficient in and of itself as pRPZ118 plus pRS26 would otherwise demonstrate recombination enhancement.

The data reported here and elsewhere (4, 5) allow the formulation of a tentative model for the molecular basis of recombination enhancement. The first step is TraYZ endonuclease nicking at *oriT*. This nicking allows the RecBC enzyme (the protein product of the *E. coli recB* and *recC* genes; formerly called exonuclease V) to enter the DNA molecule either at *oriT* or at some other site in the plasmid. Once in the duplex, the RecBC enzyme can travel along the duplex molecule by unwinding it (28). The unwinding activity of the RecBC enzyme can produce single-stranded loops (29), which are potential substrates for recA protein. Alternatively, the RecBC enzyme may encounter *cis*-acting sites in the duplex that stimulate its recombination functions. Either possibility involves the participation of the RecBC enzyme at an early stage in the recombination process.

This model relies on a nick at *oriT* being sufficient for the RecBC enzyme to gain more frequent access to the DNA duplex. Although gapped circular molecules are a good substrate for the nuclease activities of the RecBC enzyme (30), they do not appear to be a good substrate for its unwinding activity *in vitro* (A. Taylor, personal communication). Little is known about the structure of the nick at *oriT*, so it is not possible to determine if the RecBC enzyme would be able to enter the duplex molecule at an *oriT* nick. Stahl *et al.* have proposed that a double-strand break may be required for the RecBC enzyme to enter a duplex DNA molecule (31). The data presented here does not rule out the possibility of a double-strand break at *oriT* after nicking.

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