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traY and *traI* Are Required for *oriT*-Dependent Enhanced Recombination between *lac*-Containing Plasmids and λp*lac5*

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Recombination between F42*lac* and λp*lac5* is typically 20- to 50-fold more efficient than recombination between chromosomal *lac* and λp*lac5*. This enhancement of recombination requires *trans*-acting factors located in the promoter-distal and promoter-proximal regions of the main *traY*-to-*traI* (*traZ*) operon. By testing the ability of deletion mutants of *tra* to support enhanced recombination, we have identified *traY* as the only essential gene from the promoter-proximal region of *tra*. The possibility of a direct role for the *traJ* gene product has been ruled out. We also report that *traI* is the only gene from the promoter-distal end of the *traY* to *traI* operon that is required for recombination enhancement. Of the two proposed domains of *traI*, we conclude that the *oriT*-nicking activity is essential, whereas the helicase activity is largely dispensable. The possibility of a third *traI* activity is also discussed.

Recombination levels seen in F42*lac* × λp*lac5* transductional crosses are normally 20- to 50-fold greater than levels seen in chromosomal *lac* × λp*lac5* crosses (19, 21). The occurrence of this enhanced transductional recombination requires the presence of a functional RecBCD enzyme (20, 21) and the constitutive expression of the F-factor *tra* regulon (17, 24). The sole *cis*-acting site required for this enhancement is *oriT*, the origin of F-factor conjugal transfer, which must be present on the same plasmid as the resident *lac* gene copy that is to undergo recombination with λp*lac5* (25). This enhancement of recombination also requires the participation of *trans*-acting gene products from the promoter-proximal and promoter-distal regions of the main *traY*-to-*traI* (*traZ*) operon of the F factor (25). This same RecBCD enzyme and *tra*-dependent enhancement is also seen when recombination occurs between F42*lac* and the chromosomal *lac* region in a heterozygous *lac* merodiploid (33).

Enhanced recombination between two different *lacZ* alleles has been monitored by two assays. The first assay involves scoring for the production of viable Lac$^+$ transductants as a measure of the recombination events that proceeded to completion. The second assay involves determining the units of β-galactosidase produced per CFU as an indication of recombination events that proceeded to the point where a DNA strand with the wild-type sequence of the *lacZ* gene can be transcribed and translated, i.e., a transcribable intermediate (3). The increase in transcribable intermediate produced as the result of *tra*-dependent recombination enhancement is formed only via the RecBCD pathway of recombination, since strains using the RecE or RecF pathways show no *tra*-dependent increase in transcribable intermediate levels (18). This intermediate may or may not undergo the further processing needed to produce a viable Lac$^+$ transductant. Typically, transcribable intermediate levels for enhanced recombination are 20- to 50-fold higher than nonenhanced levels, whereas the increase in viable Lac$^+$ transductants is less dramatic.

We have explored further the requirement for *trans*-acting *tra* gene products in recombination enhancement. In this report, we present data showing that the *traY* gene product and the nicking domain of the *traI* gene product are essential for this enhancement of recombination. These findings are discussed in the context of a model for enhanced transductional recombination. We also discuss the possibility of a new conjugal *tra* activity located past the helicase domain of *traI*.

MATERIALS AND METHODS

Bacterial strains, phages, and plasmids. The *Escherichia coli* K-12 strains used in these experiments are listed in Table 1. A *lacZ118* version of λp*lac5* was prepared by heat induction of RDP102. Phage titers were determined with KL528 as previously described (19). All plasmids used in this study are listed in Table 2, and the *tra* chimeric plasmids are diagrammed in Fig. 1. The design of several of the *tra*-containing plasmids was greatly aided by availability of *tra* sequence data (5, 9, 10, 28).

In vitro DNA manipulations and electrophoresis. Restriction enzymes, T4 DNA ligase, and S1 nuclease were purchased from Bethesda Research Laboratories, New England BioLabs, or International Biotechnologies Inc. and used essentially according to the instructions provided by the manufacturers. DNA fragment concentrations and ratios in ligation reactions were adjusted by the method of Dugaiczyk et al. (7), and the reactions were incubated at 16°C for 10 to 16 h. Either low-melting-point agarose from Bethesda Research Laboratories or Gene Clean from Bio 101 was used for the isolation of DNA restriction fragments, when required, during plasmid constructions. Low-melting-point agarose was used at 0.6 or 0.8%, and DNA fragments were processed essentially as described by Maniatis et al. (14). Gene Clean was used according to the instructions of the manufacturer. DNA samples were subjected to electrophoresis in horizontal submarine gel boxes (Bio-Rad) at 0.5 to 7 V/cm in 40 mM Tris–20 mM acetic acid–10 mM EDTA or TBE (89 mM Tris, 2.75 mM EDTA, 89 mM boric acid). Agarose concentrations between 0.5% and 0.8% were used. Gels were stained in 0.8 μg of ethidium bromide per ml in

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TABLE 1. Strains of *E. coli*

Strain	Relevant genotype and plasmids	Source or reference
KL528	F⁻ Δ(*lac-pro*) *supF trp pyrF his rpsL thi*	Porter et al. (19)
KL791	F⁻ Δ(*lac-pro*) *met his trp rpsL thi* (λ *ind*)	Porter (17)
KL765	F⁻ *lacZ813 lacI3 pro met his trp rpsL thi* (λ *ind*)	Porter et al. (19)
KL771	F42*lacZ813 lacI3*/Δ(*lac-pro*) *met his trp rpsL thi* (λ *ind*)	Porter et al. (19)
RDP102	F⁻ Δ(*lac-pro*) *leu thi acrA*(?) *supE44*(λ *cI857 Sam7plac5I⁻Z118Y⁻*)	Porter et al. (19)
RDP116	F42*lac*/KL791	Porter (19)
RDP195	KL791(pRPZ118)	Seifert and Porter (25)
RDP196	RDP195(pRPZ117)	Seifert and Porter (25)
RDP270	RDP195(pRPZ128)	This work
RDP271	RDP274(pRPZ128)	This work
RDP272	RDP195(pRPZ130)	This work
RDP273	RDP274(pRPZ130)	This work
RDP274	RDP195(pRS31)	This work
RDP275	RDP195(pPM3, pRPZ128)	This work
RDP276	RDP195(pRPZ131, pRPZ128)	This work
RDP277	RDP195(pRPZ132, pRPZ128)	This work
RDP278	RDP195(pPM55, pRPZ128)	Thi work
RDP279	RDP195(pPM362, pRPZ128)	This work
RDP280	JCFL41Km^r/KL791	This work
RDP291	RDP195(pPM3)	This work
RDP292	RDP291(pRPZ127)	This work
RDP293	RDP291(pRPZ133)	This work
RDP295	RDP294(pRPZ135)	This work

TBE and photographed with 302-nm light from a transilluminator.

Cell growth and transformation. Cells were routinely grown in LB medium (16). Transformations were performed by the method of Kushner (12) or by a laboratory protocol for preparing and transforming frozen competent cells. Transformed cells were plated onto LB agar supplemented with the appropriate antibiotic(s). The antibiotic concentrations used for counterselection or plasmid selection and maintenance were as follows: rifampin, streptomycin, and spectinomycin at 100 μg/ml; ampicillin and neomycin at 50 μg/ml; and tetracycline and chloramphenicol at 20 μg/ml.

Plasmid DNA extraction and purification. Large-scale plasmid extractions were performed by the alkaline sodium dodecyl sulfate method of Birnboim and Doly (4). The plasmid DNA extracts were generally subjected to one or two rounds of CsCl-ethidium bromide centrifugation. Ethidium bromide was extracted with NaCl-saturated isopropanol, and the DNA-containing fractions were dialyzed extensively against 10 mM Tris (pH 8)–1 mM EDTA at 4°C.

TABLE 2. Plasmids

Plasmid	Parent plasmid	Relevant *tra* genes[a]	Useful markers	Source or reference
pRPZ114	R1	None	Km^r	Seifert and Porter (25)
pRPZ117	pRPZ114	*traJYALEKBPVSTDI(Z)*	Km^r	Seifert and Porter (25)
pRPZ109	pRPZ114	*traJYALEKBPV*	Km^r	Seifert and Porter (25)
pPRZ118	pMF3	*oriT*	Ap^r *lacZ118 lacI3*	Seifert and Porter (25)
pRS31	pSC101	*traSTDI(Z)*	Tc^r	Skurray et al. (26)
pPM3	pRS31	*traI(Z)*	Tc^r	Manning et al. (15)
pPM362	pRS31	pRS31 with *traI*::Tn*5*	Tc^r Km^r	Manning et al. (15)
pPM55	pRS31	5′ deletion of *traI*	Tc^r	Manning et al. (15)
pRPZ124	pBR325	*traJYAL*	Ap^r Cm^r	Seifert and Porter (25a)
pRPZ125	pRPZ114	*traJYAL*	Ap^r Cm^r	Seifert and Porter (25a)
pRPZ127	pBR322	*traJY*	Ap^r Cm^r	This work
pRPZ128	pRPZ114	*traJY*	Km^r Cm^r	This work
pRPZ129	pBR322	*traJ*	Ap^r Cm^r	This work
pRPZ130	pRPZ114	*traJ*	Km^r Cm^r	This work
pRPZ131	pPM3	*traI* truncated at *Sac*I site	Tc^r Spc^r/Str^r	This work
pRPZ132	pPM3	*traI* truncated at *Eco*RV site	Tc^r Spc^r/Str^r	This work
pRPZ133	pRPZ127	*traY*	Cm^r Spc^r/Str^r	This work
pRPZ134	pRPZ126	None	Ap^r Tc^r	Chen and Porter (6) and this work
pRPZ135	pRPZ134	*traY*	Ap^r Tc^r Km^r	This work
JCFL41	JCFL0	*tra*$^+$ except *traI41*	*lacZ118 lacI3*	Achtman et al. (2)
JCFL41Km^r	JCFL41	*tra*$^+$ except *traI41*	Km^r *lacZ118 lacI3*	This work

[a] See Fig. 1 for the *tra* gene content of plasmids.

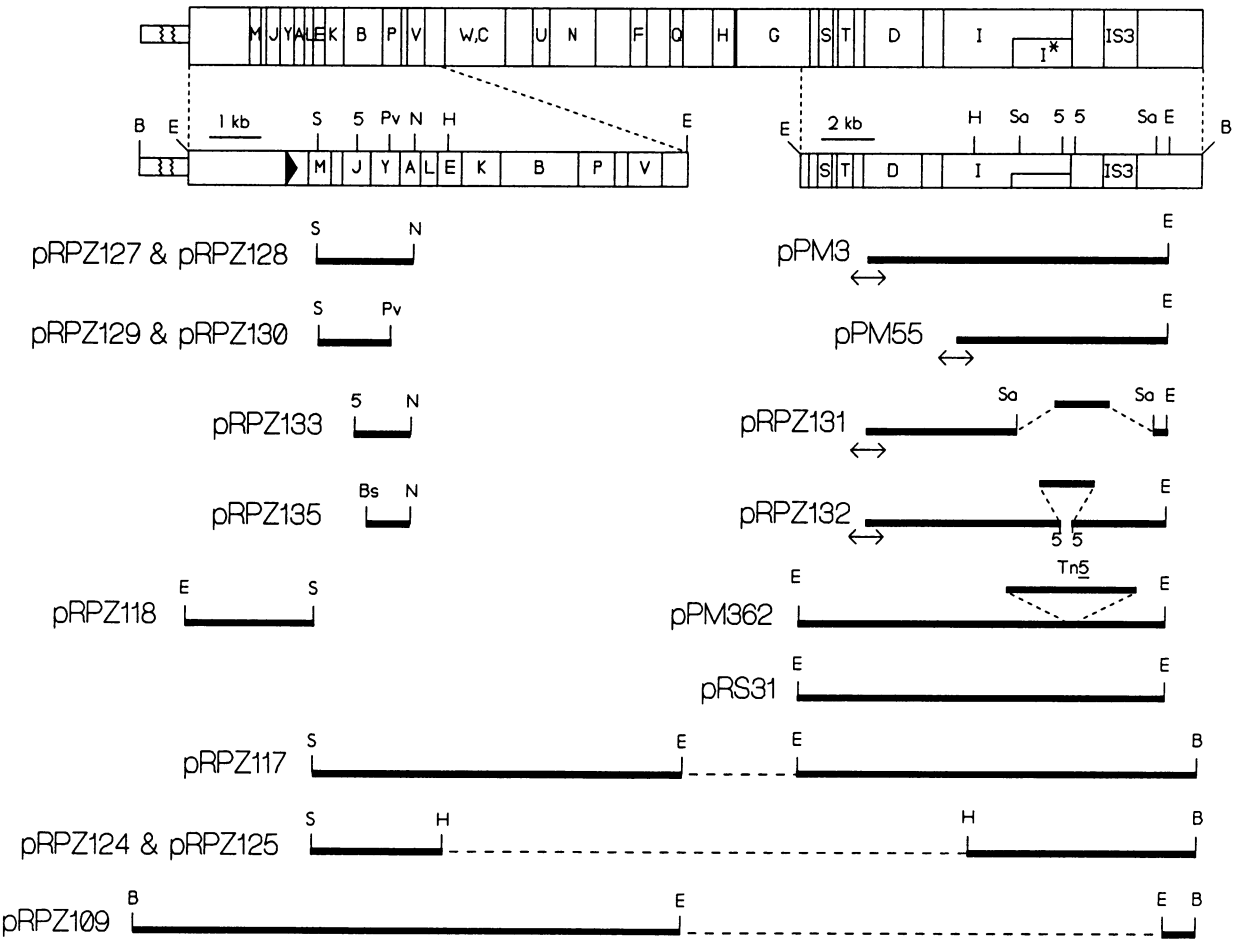

FIG. 1. Physical and genetic map of the *tra* regulon. The top line depicts the entire *tra* regulon, and the second line shows the regions relevant to this work; the scale is expanded for the left portion of the second line. The rest of the lines show the regions of *tra* included in our *tra*-containing plasmids, thick lines indicate segments of *tra* that are carried on the plasmid(s) listed to the left of each line. Double-headed arrows indicate that the exact endpoint of *tra* DNA in these plasmids is not known. The disruptions shown in pRPZ131 and pRPZ132 are the omega fragment (see the text), and the disruption in pPM362 is the result of a Tn*5* insertion. Restriction sites are labeled as follows: B, *Bam*HI; Bs, *Bst*EII; E, *Eco*RI; 5, *Eco*RV; H, *Hpa*I; N, *Nae*I; Pv, *Pvu*I; S, *Sal*I; Sa, *Sac*I.

Small-scale plasmid extractions were performed by the rapid boiling method of Holmes and Quigley (11) or a scaled-down version of the alkaline sodium dodecyl sulfate lysis method. DNA samples were occasionally extracted with phenol and chloroform before precipitation with isopropanol.

Recombination–β-galactosidase assays. Recombination assays were performed as previously described (17). Briefly, cells were grown exponentially at 37°C in supplemented 56/2 minimal salts medium to about 2×10^8 cells per ml and infected with λ*plac5* (*lacZ118*) at a multiplicity of infection of 5. Samples were taken at 1 h and plated onto minimal lactose plates to score for Lac^+ recombinants and onto LB plates to determine total CFU. Samples were also taken at 3 h to assay for β-galactosidase enzyme units (EU) and CFU. One EU is the amount of enzyme activity needed to hydrolyze 1 nmol of *o*-nitrophenyl-β-D-galactopyranoside in 1 min at 28°C (3).

Plasmid constructions. The mini-R1-*traJY* plasmid, pRPZ128, was constructed as follows. pRPZ124 is a pBR325 derivative containing a *Bam*HI-*Sal*I fragment from a deleted version of the *tra* regulon (Fig. 1). A 1.9-kb fragment containing the chloramphenicol acetyltransferase gene from Tn*9* was cloned at the *Sal*I site boundary between *tra* sequence and pBR325 sequence after the original chloramphenicol acetyltransferase gene of pBR325 had been inactivated by partial deletion. *Bam*HI linkers were ligated to a 3.8-kb *Nae*I fragment from this pRPZ124 derivative that contains 118 bp of DNA from the Tc^r gene of pBR325, the 1.9-kb chloramphenicol acetyltransferase gene-containing fragment of Tn*9*, the 3′ 312 bp of *traM*, all of *traJ* and *traY*, and the first 155 bp of the coding region of *traA*. After restricting with *Bam*HI, this fragment was ligated into the *Bam*HI site of pBR322 to make pRPZ127. Finally, the 3.8-kb *Bam*HI fragment was removed from pRPZ127 and cloned into the *Bam*HI site of the mini-R1 vector pRPZ114 (25) to make pRPZ128.

The mini-R1-*traJ* plasmid pRPZ130 was then derived from pRPZ127. pRPZ127 was restricted with *Pvu*I and treated with S1 nuclease. This removes about 1 kb of pBR322, the remainder of *traA*, and the 3′ 282 bp of *traY*. *Bam*HI linkers were added to a ligation reaction mix containing the *Pvu*I- and S1-treated preparation of pRPZ127, and an isolate

TABLE 3. *traJ* and *traY* satisfy the promoter-proximal *tra* requirement for recombination enhancement[a]

Strain	*tra* plasmids	*tra* genes	Relative Lac^+ transductants	Relative EU
RDP195	None	None	1.0	1.0
RDP196	pRPZ117	*traJYALEKBPVTDI(Z)*	11.5	35.9
RDP272	pRPZ130	*traJ*	0.3	0.08
RDP270	pRPZ128	*traJY*	0.86	0.92
RDP274	pRS31	*traI(Z)*	0.13	0.41
RDP273	pRS31	*traI(Z)*	0.29	0.45
	pRPZ130	*traJ*		
RDP271	pRS31	*traI(Z)*	4.9	23.6
	pRPZ128	*traJY*		

[a] Strain RDP195 contains a *lacZ813 lacI3* version of the mini-F-*lac-oriT* plasmid pRPZ118 (25). The other strains are derivatives of RDP195 that contain one or two additional plasmids. Recombination assays involve infecting each strain with λ *c*I857 *Sam7placZ118* at a multiplicity of 5 and then plating for Lac^+ recombinants and assaying for β-galactoidase activity (EU) as described in Materials and Methods. Values for the other strains have been normalized to those for RDP195; the actual values for RDP195 are 3.43 ± 1.07 Lac^+ transductants per 10^3 CFU and 0.12 ± 0.04 EU per 10^9 CFU. The ratio of the standard deviation to the average for RDP195 is typical of each of the other strains represented. Each value is based on a minimum of three experiments.

TABLE 4. *traJ* has no direct role in recombination enhancement[a]

Strain	*tra* plasmids	*tra* genes	Relative Lac^+ transductants	Relative EU
RDP291	pPM3	*traI(Z)*	1.0	1.0
RDP275	pPM3	*traI(Z)*	7.7	30.5
	pRPZ128	*traJY*		
RDP293	pPM3	*traI(Z)*	18.5	71.4
	pRPZ133	*traY*		
RDP295	pRPZ132	*traI* (*Eco*RV truncation)	6.9	15.1
	pRPZ135	*traY*		
RDP292	pPM3	*traI(Z)*	29.5	32.3
	pRPZ127	*traJY*		

[a] Each of the strains herein contains a *lacZ813 lacI3* version of the mini-F-*lac-oriT* plasmid pRPZ118 (25) and additional plasmids; the segments of *tra* regulon carried by these additional plasmids are shown in Fig. 1. Recombination assays involve infecting each strain with λ *c*I857 *Sam7placZ118* at a multiplicity of 5 and then plating for Lac^+ recombinants and assaying for β-galactosidase activity (EU) as described in Materials and Methods. Values for the other strains have been normalized to those for RDP291; the actual values for RDP291 are 0.39 ± 0.16 Lac^+ transductants per 10^3 CFU and 0.34 ± 0.03 EU per 10^9 CFU. The ratio of the standard deviation to the average for RDP291 is typical of each of the other strains represented. Each value is based on a minimum of three experiments.

containing *Bam*HI sites flanking the chloramphenicol acetyltransferase gene and *traJ* was obtained and named pRPZ129. The *Bam*HI fragment of pRPZ129 was cloned into the *Bam*HI site of pRPZ114 to make pRPZ130. The *tra* sequence contained in each of these plasmids is shown in Fig. 1.

RESULTS

***traJ* and *traY* satisfy the promoter-proximal *tra* requirement for enhancement.** Previous work with pRPZ125 (Fig. 1) showed that this plasmid provides all of the promoter-proximal *trans*-acting *tra* functions for enhanced recombination (18a). This observation limits the potentially required gene(s) from this region to *traJ*, *traY*, *traA*, and *traL*. Everett and Willetts (8) have shown by an in vivo λ *oriT* nicking assay that *traY* is required to nick at *oriT*. Since enhanced recombination is *oriT* dependent, we decided to investigate the need for *traY* in enhanced recombination. To this end, pRPZ128 (miniR-*traJY*) and pRPZ130 (mini-R1-*traJ*) were tested for the ability to provide sufficient *tra* promoter-proximal activity for enhancement.

RDP274 is an *E. coli* strain that contains pRS31 and pRPZ118. pRS31 is a pSC101-*tra* chimera (26) containing all of the promoter-distal functions needed for recombination enhancement, and pRPZ118 is a mini-F-*lac-oriT* plasmid (25). pRPZ128 and pRPZ130 were introduced into RDP274 to yield RDP271 and RDP273, respectively. Recombination between pRPZ118 and λ*plac5* was monitored by the transcribable intermediate assay (EU/CFU) and by scoring for Lac^+ transductants as described in Materials and Methods. The data obtained from these recombination assays are presented in Table 3.

RDP195 contains only pRPZ118 and was used as the background strain for these recombination assays. RDP270, RDP272, and RDP274 are negative control strains in which only a plasmid containing *tra* promoter-proximal or promoter-distal activity is present in a given strain. RDP196 is a positive control strain that contains both pRPZ118 and pRPZ117, a mini-R1 derivative which carries all of the *trans*-acting *tra* functions needed for enhancement (25). By either measure of recombination, RDP271 is comparable to RDP196 and markedly above RDP273, which shows nonenhanced levels of recombination. It is clear from these experiments that loss of *traY* from the cell abolishes enhancement of recombination and that *traJ* alone is not sufficient to promote enhancement.

To confirm that *traJ* is expressed from pRPZ130, we tested the ability of this plasmid to complement a *traJ90* derivative of F42*lac* in a conjugation assay. Neither pRPZ130 nor pRPZ114 has any effect on wild-type F42*lac* conjugation efficiency, whereas *traJ* expression from pRPZ130 was confirmed by the ability of that plasmid to efficiently complement F42*lac traJ90* for conjugational transfer (data not shown).

Does *traJ* have a direct role in enhancement? Although the data obtained with RDP271 and RDP273 demonstrate that *traY* is essential to recombination enhancement, there remained the possibility of a direct role for *traJ*. To address this question, we constructed two plasmids that we hoped would express *traY*, in the absence of *traJ*, at a level sufficient to support enhanced recombination. The first plasmid, pRPZ133, was made by replacing the *Sca*I-*Eco*RV fragment of pRPZ127 with the omega fragment of pHP45Ω (22). The omega fragment contains transcription termination signals as well as translation stop codons in all three reading frames. This construction removes the 5′ 422 bp of *traJ* and leaves *traY* as the only complete *tra* gene on the plasmid. pRPZ133 was transformed into RDP291 to create RDP293. Also contained in these strains is pPM3, a pRS31 derivative which provides promoter-distal activity sufficient for enhanced recombination (see below).

Data from recombination assays performed on RDP293 are presented in Table 4. Also presented are data from assays on strains RDP292 and RDP275, in which pRPZ127 (pBR322-*traJY*) or pRPZ128 (mini-R1-*traJY*) is used to provide promoter-proximal activity. A comparison of the results with RDP292 and those with RDP275 demonstrates that TraY protein participation in recombination enhancement is not strongly gene dosage dependent. The data obtained with RDP293 clearly indicate that *traY* provides all of the activity

required of the promoter-proximal region of *tra* and also suggest a significant degree of independence of *traY* expression from TraJ activation.

To further explore this question we tested pRPZ135. pRPZ135 is a plasmid that contains a promoterless *traY* gene downstream of the λpL promoter of the pBR322-based plasmid pRPZ134, an *Hpa*I deletion product of pRPZ126 (6). The salient feature of pRPZ135 is that *traY* is under control of the λ *c*I repressor and is independent of transcriptional activation by TraJ. Transformation of pRPZ135 into RDP294 created RDP295. Because pRPZ135 is Tc^r, pRPZ132 was used instead of pPM3 to provide promoter-distal activity because pRPZ132 is Spc^r and can be selectively maintained in the same cell as pRPZ135 by using spectinomycin. Since both pRPZ135 and pRPZ118 are Ap^r, we relied on the stability of the mini-F replicon after this strain was constructed. The continued presence of pRPZ118 in this strain was confirmed by monitoring recombination levels between this plasmid and UV-irradiated λp*lacZ118* (data not shown), a *tra*-independent process (17, 21). pRPZ135 was stably maintained by growth in the presence of neomycin.

Recombination assays were carried out with RDP295 (Table 4). The fact that pRPZ135 is able to provide promoter-proximal activity confirms that *traJ* does not have a direct role in enhanced recombination.

Promoter-distal *tra* requirement(s) for enhanced recombination. The plasmid pRPZ117 supports enhanced recombination, whereas pRPZ109 does not (25) (Fig. 1). This means that one or more of the genes *traT*, *traD*, and *traI* from the promoter-distal region of the *traY*-to-*traI* operon is required. Since the product of *traT* is involved in surface exclusion (32), it seemed unlikely that this protein would be involved. Conversely, the products of *traD* and *traI* are active in donor conjugal DNA synthesis (32) and are thus more likely candidates for a role in *tra*-mediated recombination enhancement. Of particular interest is *E. coli* DNA helicase I, the product of *traI* (1). There is evidence that this gene product contains both the DNA helicase activity and the TraZ component of the site-specific nicking complex that acts at *oriT* (29).

Recombination assays were performed by using pPM3 to provide promoter-distal activity. pPM3 is a deletion derivative of pRS31 and contains a complete coding sequence only for *traI* (15). pPM3 provides for full enhancement of recombination between pRPZ118 (mini-F-*lac-oriT*) and λp*lac5* when pRPZ128 (mini-R1-*traJY*) is also present (Table 5). Also, the products of *traT* and *traD* are shown to not be required for recombination enhancement.

Traxler and Minkley (29) carried out a study of the distal region of the main *tra* operon that focused on the *traI* gene and included a mapping revision for that area. On the basis of their results, they removed the *traZ* gene from the *tra* map and postulated that the amino-terminal domain of the *traI* gene product provides the TraZ component of the TraYZ *oriT* nicking activity. Further, Abdel-Monem et al. (1) have described a carboxy-terminal portion of the *traI* gene product from plasmid pPM55 which retains helicase activity. To determine whether one or both of these postulated domains of the *traI* gene product provide activity essential to enhancement of recombination, we performed recombination assays involving both pPM55 and a *traI41* derivative of F42*lac*.

The ability of the carboxy-terminal domain of DNA helicase I to provide the required *traI* activity was tested by carrying out an assay for recombination enhancement with strain RDP278. RDP278 contains pPM55 (15), a deletion derivative of pRS31 that expresses a 140-kDa polypeptide that represents the carboxy-terminal portion of the normal *traI* gene product. This 140-kDa polypeptide retains DNA helicase activity (1) but lacks *traI*-complementing ability (15). Traxler and Minkley (30) described a plasmid that produces a 150-kDa carboxy-terminal polypeptide from *traI* that retains no nicking activity; it is most likely that the pPM55-encoded protein lacks this activity as well. Recombination data for RDP278 are found in Table 5. By both assays it is clear that pPM55 does not provide the activity required of the promoter-distal end of *tra* for enhanced recombination.

TABLE 5. The nicking activity of the *traI* gene product is required for recombination enhancement[a]

Strain	*tra* plasmids	*tra* genes	Relative Lac$^+$ transductants	Relative EU
RDP195	None	None	1.0	1.0
RDP196	pRPZ117	*traJYALEKBPVSTDI(Z)*	6.8	31.1
RDP275	pPM3	*traI(Z)*	9.7	39.3
	pRPZ128	*traJY*		
RDP276	pRPZ131	*traI* (*Sac*I truncation)	2.2	10.2
	pRPZ128	*traJY*		
RDP278	pPM55	*traI* (5′ deletion)	1.3	0.83
	pRPZ128	*traJY*		
RDP277	pRPZ132	*traI* (*Eco*RV truncation)	7.8	43.5
	pRPZ128	*traJY*		
RDP279	pPM362	*traI*::Tn*5*	3.2	12.7
	pRPZ128	*traJY*		

[a] Strain RDP195 contains a *lacZ813 lacI3* version of the mini-F-*lac-oriT* plasmid pRPZ118 (25). The other strains are derivatives of RDP195 that contain one or two additional plasmids; the segments of *tra* regulon carried by these additional plasmids are shown in Fig. 1. Recombination assays involve infecting each strain with λ *c*I857 *Sam7*p*lacZ118* at a multiplicity of 5 and tthen plating for Lac$^+$ recombinants and assaying for β-galactosidase activity (EU) as described in Materials and Methods. Values for the other strains have been normalized to those for DP195; the actual value for RDP195 are 1.51 ± 0.60 Lac$^+$ transductants per 10^3 CFU and 0.35 ± 0.02 EU per 10^9 CFU. The ratio of the standard deviation to the average for RDP195 is typical of each of the other strains represented. Each value is based on a minimum of three experiments.

To test the ability of the postulated *oriT* nicking domain of the *traI* gene product to support enhanced recombination, we assayed recombination in a strain that contains a derivative of JCFL41, the *traI41* mutant of F42*lac* (2). JCFL41 transfers at a very low efficiency and has no genetic markers that readily permit its detection once it has been homogenotized to *lacZ813*. For this reason, we carried out a series of manipulations that allowed us to place a neomycin phosphotransferase gene approximately 4 kb upstream of the *lac* operon of JCFL41. RDP280 contains this new derivative of JCFL41, which is called JCFL41Km^r.

traI41 appears to be a missense mutation in *traI* that has lost helicase activity (2, 31) while retaining nicking activity in a λ *oriT* nicking assay (8). RDP280 demonstrates recombination values essentially identical to those of KL771, which contains the *tra*$^+$ version of F42*lac* (Table 6). These data show that the nicking activity of the TraI protein is essential for recombination enhancement.

To explore this hypothesis, we tested the ability of pRPZ131 to support enhanced recombination in RDP276, a strain that also contains pRPZ118 (mini-F-*lac-oriT*) and pRPZ128 (mini-R1-*traJY*). pRPZ131 was made by substituting the *Sac*I fragment of pPM3 with the omega fragment of pHP45Ω (22). This construction deletes the 3′ 2,053 bp of

TABLE 6. Effect of *traI41* mutation on recombination between F42*lac* and λp*lac5*[a]

Strain	*lac* location	Relative Lac$^+$ transductants	Relative EU
KL765	Chromosomal *lac*	1.0	1.0
KL771	F42 *lac tra*$^+$	29.4	51.2
RDP280	F42 *lac traI41* (JCFL41Km^r)	16.4	42.4

[a] Each of the strains herein contains a *lacZ813 lacI3* version of the *lac* operon located at the indicated location; KL771 and RDP280 are Δ(*lac-pro*) strains. Recombination assays involve infecting each strain with λ *c*I857 *Sam7placZ118* at a multiplicity of 5 and then plating for Lac$^+$ recombinants and assaying for β-galactosidase activity (EU) as described in Materials and Methods. Values for the other strains have been normalized to those for the chromosomal *lac* strain KL765; the actual values for KL765 are 2.63 ± 1.19 Lac$^+$ transductants per 10^3 CFU and 0.02 ± 0.0005 EU per 10^9 CFU. The ratio of the standard deviation to the average for KL765 is typical of each of the other strains represented. Each value is based on a minimum of three experiments.

traI and is predicted to produce a 105-kDa amino-terminal *traI* polypeptide. Traxler and Minkley (30) have shown that the amino-terminal 96 kDa of TraI retains *oriT* nicking activity but is devoid of helicase activity. We expect the truncated *traI* polypeptide from pRPZ131 to have similar properties. The data for recombination assays with the pRPZ131-containing strain RDP276 are shown in Table 5.

A comparison of the recombination data for RDP275 and RDP276 shows that pRPZ131 is about fourfold less active than pPM3 in providing promoter-distal *tra* function. Nevertheless, it still increases recombination levels 1 order of magnitude over nonenhanced levels in the transcribable intermediate assay. RDP276 is also about fourfold lower than RDP275 in the viable Lac$^+$ recombinant assay. Because of the smaller difference between enhanced and nonenhanced recombination levels seen when assaying Lac$^+$ colonies, RDP276 shows only a twofold increase over RDP195 by this criterion. Thus, the data obtained with JCFL41Km^r and with both pRPZ131 and pPM55 provide genetic evidence that the nicking activity of the TraI protein is essential for *tra*-mediated enhanced recombination, whereas the helicase activity is largely dispensable.

Since enhanced recombination and conjugal transfer are related events, the importance of testing mutant *traI*-containing plasmids for *traI* complementation in a mating experiment is evident. Therefore, several of these *traI* sequence-containing plasmids were tested for their ability to complement JCFL41Km^r for conjugal transfer. From these experiments, pPM3, which carries the wild-type *traI* gene, was found to complement *traI41* efficiently (data not shown). Conversely, pRPZ131 and pPM55 were completely unable to complement *traI41*. This is consistent with the inability of these plasmids to support enhanced recombination.

Genetic evidence for an additional *tra* activity. In an effort to generate a deletion mutant of *traI* that lacked helicase activity but contained more of the coding region of this gene than pRPZ131, we constructed pRPZ132. This plasmid was made by replacing the 511-bp *Eco*RV fragment of pPM3, which codes for the terminal 129 amino acid residues of *traI*, with the omega fragment of pHP45Ω. A second plasmid, pPM362 (15), contains a Tn*5* at the extreme carboxy end of *traI* and produces a slightly truncated TraI polypeptide (29). pRPZ132 and pPM362 were introduced into RDP270 to produce RDP277 and RDP279. Data from recombination assays performed with these strains are presented in Table 5; both plasmids provide the *traI* activity required for enhancement. However, data from conjugal transfer experiments showed that neither of these plasmids is able to complement *traI41* for transfer (data not shown).

As stated previously, the helicase domain of TraI is located in a 140-kDa carboxy-terminal polypeptide that includes the regions that were altered in pPM362 and pRPZ132. One might expect these constructions to lack DNA helicase activity, and the inability of pPM362 and pRPZ132 to complement *traI41* for transfer is consistent with this expectation. However, Traxler and Minkley (30) have reported that deletion of the 511-bp *Eco*RV fragment, which was deleted to make pRPZ132 and which was the target for Tn*5* insertion in pPM362, results in production of a 165-kDa protein that retains wild-type levels of ATPase activity (used as a measure of helicase activity) but does not complement *traI171* for transfer. The nature of the ATPase-helicase activity of pPM362 has not been studied. One plausible explanation of these data is that there exists an additional activity of *traI* in the carboxy-terminal region of the protein. Deletion of or insertion into this region of *traI* abolishes this putative activity and renders these *traI* mutants incapable of conjugal transfer but has little effect on their ability to support recombination enhancement.

DISCUSSION

This study was undertaken in an attempt to identify the *trans*-acting *tra* components required for enhanced recombination. Genes in the promoter-proximal region of the main *tra* operon of the F factor were initially analyzed through a determination of the ability of two deletion plasmids to complement pRS31 in a recombination assay involving mini-F-*lac-oriT* (pRPZ118) and λp*lac5*. pRPZ128 (mini-R1-*traJY*) is fully proficient in complementing pRS31, which means that the proximal requirements are sufficiently met by *traJ* and *traY*. The inability of pRPZ129 (mini-R1-*traJ*) to complement pRS31 demonstrates that *traJ* alone does not provide the promoter-proximal activity; thus, *traY* is essential to recombination enhancement.

After these results were obtained, we decided to ask whether the role of *traJ* is direct or simply regulatory in nature. To answer this question, we used the pBR322-based *traY* plasmid pRPZ133 instead of a mini-R1 based plasmid in hopes that a higher copy number would compensate for loss of the positive regulatory role attributed to TraJ (32) and provide for adequate expression of *traY*. Not only was this plasmid able to provide all of the activity required of the promoter-proximal end of *tra*, but the strain used to test this plasmid, RDP293, gave transcribable intermediate levels higher than those of any other strain previously tested.

traJ was also shown to be dispensable in this system by placing a promoterless version of *traY* under the control of λpL, thus removing *traY* from *traJ* regulation. Such a situation was obtained with pRPZ135. The ability of this type of construction to produce *traY* protein has been demonstrated (13). The fact that pRPZ135 provides for enhanced recombination confirms that *traJ* does not play a direct role in enhanced recombination. It should be noted that this plasmid is able to express enough *traY* to support enhancement in spite of the presence of wild-type *c*I repressor produced by the λ *ind* lysogens present in the strains used in this study.

traJ has been shown to be essential for enhancement of F42*lac* × λp*lac* recombination (17). Given the data in this paper, it is evident that *traJ* has only an indirect role of promoting expression of *tra*. Further, the ability of pRPZ133

to express *traY* in quantities sufficient for enhancement suggest that initiation of transcription from P_{traY} is at least partially independent of TraJ activation.

Lahue and Matson (13) have described a system in which purified TraY protein is able to bind to an *oriT*-containing DNA fragment in vitro. Using this system, they were unable to obtain evidence for purified TraI protein binding to the TraY protein-*oriT* complex or for in vitro nicking at *oriT*. These authors suggest that their result may indicate the need for an additional *tra* protein to obtain the nicking reaction at *oriT*. In our enhanced recombination system we have shown that TraY and TraI are the only F-encoded proteins that are required to activate *oriT*. It should be noted that the purified TraI protein used by Lahue and Matson did not contain TraI*, a *traI* translational restart product previously thought to be *traZ* (9, 15, 29). Furthermore, the need for one or more host proteins in *oriT* nicking has not been discounted.

To determine the extent of the *tra* genetic information that is needed from the promoter-distal end of the main *tra* operon, we used several deletion and insertion derivatives of the pSC101-*tra* chimera pRS31. pPM3 is a deletion derivative of pRS31 that provides all of the genetic information needed from this region of *tra*. Since the only known *tra* gene on pPM3 since the demise of *traZ* is *traI*, we have concluded that *traI* is the only gene needed from the promoter-distal end of the main *tra* operon.

Our model for enhanced recombination requires the action of the TraYI nicking complex at *oriT*. The likelihood that the TraI protein contains domains for both *oriT* nicking (in conjunction with TraY protein) and for DNA helicase activity prompted us to investigate the requirement for each domain. These experiments involved both a *traI41* derivative of F42*lac* (JCFL41Km^r) and the use of various derivatives of pRS31 or pPM3 together with pPRZ118 (mini-F-*lac-oriT*) and pRPZ128 (mini-R1-*traJY*). JCFL41Km^r and pPM55 were of particular interest. pPM55 includes a 5′-truncated version of the *traI* gene that produces a protein with DNA helicase but no nicking activity; it also fails to support recombination enhancement. The mutant TraI protein produced by JCFL41Km^r lacks DNA helicase activity but retains the ability to support *oriT* nicking in the λ *oriT* assay; JCFL41Km^r shows recombination enhancement levels comparable to those obtained with the *traI*$^+$ version of F42*lac*. Combined, these results provide a strong indication that enhancement of recombination has an absolute requirement for the nicking activity of TraI and is largely independent of the ability of the protein to unwind DNA.

This conclusion is largely supported by the results obtained with the other pRS31 and pPM3 derivatives in the mini-F-*lac-oriT* × λp*lac5* assay. Although the reduction in the extent of recombination enhancement observed when either pRPZ131 or pPM362 was used could be interpreted as reflecting some dependence on the DNA helicase activity, it is also possible that perturbations of overall protein structure are responsible for the reduction. These perturbations could have an indirect effect on the nicking activity or could influence the stability of the protein. Conversely, the data derived from the full-length protein of *traI41*, a protein already shown to have wild-type levels of nicking activity, provide a much more straightforward picture.

Our working model for recombination enhancement has been that recombination is initiated by the production of a nick at *oriT* by the TraYI (or the TraYZ) endonuclease, and the results obtained from analysis of several of the *traI* mutants support this notion. This nicking reaction is proposed to be the first step in allowing the RecBCD enzyme to enter the duplex circular DNA molecule. In vitro work with RecBCD enzyme has shown that nicks and small gaps in duplex DNA do not allow the enzyme to enter the duplex. Thus, either the in vitro data do not provide a completely accurate representation of the situation in vivo, or the nick at *oriT* is processed to a type of structure that allows entry by the RecBCD enzyme. Preliminary data indicate that the occurrence of a double-strand break at *oriT* is not likely.

That the helicase activity of TraI is dispensable allows us to eliminate from our model the otherwise viable possibility that the action of helicase I produces recombinogenic single strands. Therefore, we propose that RecBCD assumes its DNA helicase function upon entering the duplex. This is in accord with other models for recombination in which the helicase activity of RecBCD is postulated to act early in the recombination event. The separated single strands produced by the RecBCD enzyme may be substrates for RecA protein binding, and such bound single-stranded DNA may serve as the basis for facilitated recombination initiation. Alternatively, RecBCD may need to encounter one or more Chi sites before producing displaced single-stranded tails that are recombinogenic (27). The possibility of Chi site involvement in recombination enhancement is supported by preliminary data obtained with strains containing altered RecBCD enzyme (23). Although this model is in accord with other recombination models in which the RecBCD helicase activity is postulated to act early in the recombination event, it does not preclude an additional activity for this enzyme, such as resolution of Holliday-type structures, which could occur later in the reaction.

Approximately 700 bp between *traI* and the beginning of *finO* have not been characterized to date. In addition, 387 bp after the *Eco*RV site in *traI* are not necessary for either of the two known activities of *traI*. We tested two plasmids that had been altered in one or both of these regions. pRPZ132 is deleted for the 3′ 387 bp of *traI* and 124 bp of the *traI-finO* intercistronic region. pPM362 has suffered a Tn*5* insertion in the extreme end of *traI*, an event which is likely to have a polar effect on any putative genes downstream of *traI*.

We find it quite interesting that although these two plasmids are efficient in supporting enhanced recombination, neither is able to complement *traI41* in conjugal transfer to even a slight extent. Traxler and Minkley (30) have shown that there is no *tra* activity downstream of the *Eco*RV site in *traI* that is required for *oriT* nicking, which is consistent with our results and the part of our model that invokes *tra*-dependent nicking at *oriT*. They also reported that plasmids lacking this intercistronic region are unable to complement the deletion *traI171*. Thus, the inability of pRPZ132 and probably pPM362 to complement *traI41* is not due to negative complementation.

One possible explanation of these results is that there exists a third *tra* activity located downstream of the helicase domain of *traI*. Since *traI41* would still express any putative cistron downstream of *traI*, it is not likely that inactivation of the region downstream of *traI* in pPM362 or pRPZ131 has caused the transfer-deficient phenotype seen when these plasmids are used to complement *traI41*. On the other hand, one would expect that a modified *traI* protein that still contained all of the wild-type *traI* activities would complement *traI41* at least to a modest extent. This is not the case for pPM362 or pRPZ132. For this reason, we are led to believe that there exists a third activity for *traI* that is at least partly contained in the last 387 bp of the gene. This activity is required for conjugal transfer but not for enhancement of transductional recombination.

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