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# General Recombination in *Escherichia coli* K-12: In Vivo Role of RecBC Enzyme

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**Heterozygous *lacZ*$^-$ merodiploids of *Escherichia coli* K-12 have been used to study the role of the RecBC enzyme in general recombination. The transcribable intermediate assay detects the product of early steps in recombination without requiring the formation of viable recombinant colonies. Recombination is initiated by infection with λ *precA*$^+$. We have found that transcribable intermediate formation in crosses between F42 *lac* and chromosomal *lac* is dependent on F fertility functions and an active RecBC enzyme. Thus, the products of the *recB* and *recC* genes are required in early steps of recombination between these two substrates. Introduction of the F42 *lac* donor DNA by conjugation immediately after infection with λ *precA*$^+$ abolishes the requirement for an active RecBC enzyme.**

The function of the RecBC enzyme, also known as exonuclease V, in general recombination in *Escherichia coli* K-12 has been difficult to determine. This enzyme is composed of the products of two genes, *recB* and *recC* (9, 12). It has been shown in vitro to act as an ATP-dependent exonuclease, a DNA-dependent ATPase, an ATP-stimulated endonuclease, and a DNA helicase (11, 22, 28). However, its biochemical role in the recombination process remains unclear.

Strains of *E. coli* that contain a *recB* or *recC* mutation have been found to be reduced in genetic recombination when viable recombinant colony formation is tested after Hfr conjugation (1) or P1-mediated generalized transduction (31). On the other hand, viable recombinant colony formation resulting from recombination between λ p*lac5* and a chromosomal *lac* gene was shown to have no significant dependence on the *recB* gene product (21).

Birge and Low (1) developed an essay for detecting the product of early steps in recombination. Two different ochre alleles of the *lacZ* gene are allowed to recombine into a structure (the transcribable intermediate), where a recombinant *lacZ*$^+$ gene is present in a form that can be transcribed by RNA polymerase. This DNA structure, however, may not necessarily undergo the additional processing required to give rise to a viable recombinant colony. This transcribable intermediate assay was used to show that recombination to this stage after an Hfr × F$^-$ cross was not *recB* or *recC* dependent (1). It was concluded that the products of the *recB* and *recC* genes were required for a late step in the recombination process associated with Hfr conjugation.

Recombination to a transcribable intermediate between λ p*lac5* and chromosomal *lac* was not affected by a *recB21* mutation (20), just as this mutation did not affect the production of viable recombinant colonies in this type of cross (21). However, recombination between F42 *lac* and λ p*lac5* was shown to be 20- to 50-fold higher than that in chromosomal *lac* × λ p*lac5* crosses for both transcribable intermediate and viable recombinant colonies (20). This relative enhancement of recombination was found to be dependent on the presence of a functional RecBC enzyme, implying a role for RecBC enzyme in early steps of recombination in this case (20). Conjugation of the F42 *lac* DNA into the cell where recombination was being tested immediately before infection with λ p*lac5* abolished the requirement for the RecBC enzyme (20). This suggested that some feature of the recently transferred F42*lac* DNA was eliminating the requirement for RecBC enzyme in the early steps of recombination. It was suggested that the relevant feature might be the single strandedness of the recently transferred F42 *lac* DNA.

The constitutive expression of the *tra* regulon of F42 *lac* has also been found to be required for enhanced recombination with λ p*lac5* (18, 23). The *tra* dependence of enhanced recombination involves a *cis*-acting component, thought to be the *oriT* site on F42 *lac*, and *trans*-acting functions encoded for in the promoter-proximal and promoter-distal portions of the *tra* regulon (H. S. Seifert and R. D. Porter, Proc. Natl. Acad. Sci. U.S.A., in press).

The work described here was done to determine whether recombination between F42 *lac* and the chromosomal *lac* gene in a stable heterozygous merodiploid also shows *tra*-dependent enhancement and whether it requires a functional RecBC enzyme. The *tra* and RecBC enzyme dependence of recombination obtained by introducing the donor F42 *lac* DNA molecule into the chromosomal *lac* strain via conjugation immediately before initiating recombination was also studied.

## MATERIALS AND METHODS

**Bacterial and phage strains.** The *E. coli* strains used in this study are listed in Table 1. New strains were produced via P1 *vir* transduction, conjugation, or plasmid transformation. Phage λ derivatives used in β-galactosidase assays were made by heat induction from the lysogenic strains listed in Table 1. Titers of lysates were determined on strain KL528 as previously described (19).

The *recB* deletion strains were made by transducing λ *ind thyA*$^-$ derivative of KL765, RDP229, with P1 *vir* grown on strain AC114 (6), kindly provided by A. Chaudhury. Transductants were selected for Thy$^+$ on minimal 56/2 agar containing the appropriate amino acids, thiamine, and glucose to produce RDP230 (*recB*$^+$) and RDP231 [Δ(*argA-recB*)]. These two strains were then transduced with P1 *vir* grown on JC10289, selecting for Tc$^r$ on LB agar containing 20 μg of tetracycline per ml. The transductants were screened

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TABLE 1. Strains of *E. coli*

| Strain | Relevant characteristics | Reference, source, or comment |
|---|---|---|
| AC114 | W3110 Δ(*argA-recB*) | A. Chaudhury |
| J53F⁻(R1) | F⁻ *metF63 pro-22* (R1) | Plasmid Reference Center, Stanford University |
| JC10289 | AB1157 Δ(*srl-recA*)*306* | (7, 32) |
| KL528 | F⁻ Δ(*lac-pro*) *supF trp pyrF rpsL thi* λ⁻ | (18) |
| KL550 | RDP100 (λ cI857 *S*am7 *lacI5p*⁻ Z⁺Y⁻ | (18) |
| KL765 | F⁻ *lacZ813 lacI3 pro met his trp rpsL thi* (λ *ind*) | (18) |
| KL791 | F⁻ Δ(*lac-pro*) *met his trp rpsL thi* (λ *ind*) | (17) |
| RDP100 | F⁻ Δ(*lac-pro*) *leu thr acrA* (?) *supE44* | (18) |
| RDP162 | F⁻ *gal* (λ cI857 *S*am7 p*recA*⁺) | Phage from C. Radding |
| RDP207 | F⁻ *lacZ813 lacI3 pro met his trp thyA rpsL nalA* | Trimethoprim-resistant derivative of KL765 |
| RDP213 | KL791 (pRPZ100 *lacZ118lacI3*) | This work |
| RDP214 | F42 *lacZ118 lacI3*/Δ(*lac-pro*) *recA1 mal rpsE* λ^r | This work |
| RDP215 | F42 *lacZ*⁺*lacI3*/Δ(*lac-pro*) *recA1 mal rpsE* λ^r | Lac⁺ revertant of RDP214 |
| RDP229 | RDP207 λ *ind* | This work |
| RDP230 | F⁻ *lacZ813 lacI3 pro met his trp rpsL nalA* (λ *ind*) | This work |
| RDP231 | F⁻ *lacZ813 lacI3 pro met his trp* Δ(*argA-recB*) *rpsL nalA* (λ *ind*) | This work |
| RDP232 | F⁻ *lacZ813 lacI3 pro met his trp* Δ(*srl-recA*)*306 rpsL nalA* (λ *ind*) | This work |
| RDP233 | F⁻ *lacZ813 lacI3 pro met his trp* Δ(*argA-recB*) Δ(*srl-recA*) *rpsL nalA* (λ *ind*) | This work |
| RDP234 | RDP232(pRPZ100 *lacZ118 lacI3*) | This work |
| RDP235 | RDP233(pRPZ100 *lacZ118 lacI3*) | This work |
| RDP236 | F42 *lacZ118 lacI3*/RDP232 | This work |
| RDP237 | F42 *lacZ118 lacI3*/RDP233 | This work |
| RDP238 | RDP236(R1) | This work |
| RDP239 | RDP237(R1) | This work |
| RDP240 | RDP232(pRPZ113) | This work |
| RDP241 | RDP233(pRPZ113) | This work |
| RDP242 | RDP232(pRPZ114) | This work |
| RDP243 | RDP233(pRPZ114) | This work |

for the presence of the (*srl-recA*)*306* deletion by replica plating on MacConkey-Sorbitol agar and on LB agar containing 0.2 μg of mitomycin C per ml. The resulting *recA* deletion strains were RDP232 (*recB*⁺) and RDP233 (*recB* deletion). RDP232 and RDP233 were mated overnight in LB at 37°C with a strain containing F42 *lacZ118 lacI3* to produce the heterozygous Lac⁻ merodiploids RDP236 and RDP237. Plasmid R1 was introduced into these merodiploids by conjugation after growing J53F⁻(R1), RDP236, and RDP237 separately to $1.5 \times 10^8$ cells per ml. Matings were begun by mixing equal volumes of J53F⁻(R1) and each of the recipient strains and then incubating them in a slowly shaking water bath at 37°C for 2 h. Mating mixes were then streaked out on LB agar containing 50 μg of ampicillin and 100 μg of streptomycin per ml. Colonies were screened for recipient markers a well as for Cm^r. These strains are RDP238 and RDP239.

**Plasmid isolation and transformation.** The plasmids used in this study are listed in Table 2. Heterozygous Lac⁻ merodiploids were also made containing a mini-F *lac* plasmid, pRPZ100 *lacZ118 lacI3*. This plasmid lacks the *tra* regulon of F, but contains the origin of F replication and *bla* gene from pMF3 (16). It also contains the entire *lac* operon from pFB140 (5). This plasmid was derived from pRPZ100 *lacZ*⁺*lacI3* (23) by homogenotization. In RDP232, transformation was by the process of Kushner (14). Cells were spread on LB agar containing 50 μg of ampicillin per ml. The transformants were purified and screened for markers, and the presence of the plasmid was detected by running vertical agarose screening gels (10). RDP233 [the Δ(*argA-recB*) strain] was refractory to transformation. This was probably due to the reduced viability of the *recB* mutant (3, 4), the large size of the plasmid (38.2 kilobases), and the low concentration of plasmid DNA. The overnight $CaCl_2$ method of Dagert and Ehrlich (8) was used with modifications to transform this strain. These modifications include a higher concentration of DNA, more competent cells, and a 3-h period at 37°C for the expression of Ap^r before plating. Cells were spread on LB agar with 25 μg of ampicillin per ml rather than 50 μg/ml; 25 μl of 4% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside was also added to each plating tube. This allowed visualization of those cells where the polypeptides from the two ochre *lacZ* alleles were undergoing protein complementation and producing a faint blue color. Transformants were purified and screened for markers as well as the presence of the plasmid.

The *finO*⁺ mini-R1 derivative pRPZ113 (Seifert and Porter) was used to transform RDP232 and RDP233 to provide repression of F42 *lac* fertility functions in conjugation experiments. pRPZ114 [Δ*finO*] was used as a negative control to be sure that any effect was not due to the presence of the mini-R1 replicon. Transformation was by the procedure of Kushner (14), with selection on LB agar containing 40 μg of kanamycin sulfate per ml.

**DNA extraction.** Plasmid pRPZ100 *lacZ118 lacI3* was extracted from 6 liters of stationary cells (RDP213) grown in LB. DNA isolation was by the method of Birnboim and Doly (2). However, the volumes of the solutions were increased 2.5 times to account for the increased cell density and the single-copy nature of the plasmid. DNA was purified through two CsCl-ethidium bromide gradients and dialyzed against TE buffer. Purified plasmid DNA was analyzed on horizontal 0.5% agarose slab gels.

**β-Galactosidase recombination assays.** Recombination in *recA*⁻ merodiploids was initiated by infection with λ p*recA*⁺ at various multiplicities of infection (MOIs) as previously described (33). Control infections were also done with λ p*lacZ*⁺ to determine differences in constitutive β-galactosidase production. Assays involving conjugation were done in a similar manner. Recipient cells were first infected with λ p*recA*⁺ at an MOI of 5. After phage adsorption at 37°C for 15 min, 5 ml of RDP215 (containing F42 *lacZ*⁺) or

TABLE 2. Plasmid list

| Plasmid | Parent replicon | Relevant characteristics | Reference, source, or comment |
|---|---|---|---|
| pMF3 | F | Ap^r | (16) |
| pFB140 | pBR322 | Lac⁺ Ap^r | (5) |
| pRPZ100 | pMF3 | Lac⁻ Ap^r | (23) |
| pRPZ113 | R1 | *finO*⁺ Km^r | Mini-R1 (23a) |
| pRPZ114 | pRPZ113 | Δ*finO* Km^r | (23a) |

RDP214 (containing F42 *lacZ118*) were added to 10 ml of recipient cells. Incubation was continued at 37°C in a slowly shaking water bath for 3 h after mating was initiated. Samples of the mating mixes with F42 *lacZ118* were added to cold chloramphenicol, and CFU were plated as described previously (20). Viable colonies were spread on LB agar containing 100 μg of streptomycin per ml so that only recipient cells were counted. The matings using F42 *lacZ*$^+$ were spread, at 1 h after mating, on LB-str agar and on minimal 56/2 agar supplemented with lactose, thiamine, 100 μg of streptomycin, and the appropriate amino acids. After incubation at 37°C, colonies were counted, and the percentage of CFU that were Lac$^+$ was used to normalize the enzyme unit (EU)/CFU values obtained in the matings with F42 *lacZ118* to each strain's ability as a recipient in conjugation.

β-Galactosidase assays were performed on the cell samples as previously described (1, 20) with a 3-min incubation step at 57°C to inactivate any β-galactosidase activity resulting from the complementation of the two ochre *lacZ* polypeptides (20). One EU equals the amount of β-galactosidase that hydrolyzes 1 nmol of *o*-nitrophenyl-β-D-galactopyranoside in 1 min at 28°C (1).

**Media.** LB medium (17) was used as a liquid culture medium or hardened with 1.5% agar in plate whenever a rich medium was required. Modified half-strength minimal medium 56 (56/z) supplemented as described previously (15) and containing 0.4% sterile glycerol as a carbon source and 0.4 ml of sterile 1 M MgSO4 per 100 ml of medium, was used for liquid growth medium in the β-galactosidase assays.

**Chemicals and media.** Tryptone, yeast extract, MacConkey agar base, and agar were obtained from Difco Laboratories. *o*-Nitrophenyl-β-D-galactopyranoside, Brij 58, ampicillin, kanamycin, tetracycline, sugars, and other biochemicals were from Sigma Chemical Co. Streptomycin sulfate was obtained from Eli Lilly & Co. All other chemicals were reagent grade.

## RESULTS

**Comparison of F42 *lac* and mini-F *lac* recombination with chromosomal *lac*.** The first studies were done to determine whether (i) enhancement of transcribable intermediate formation occurred when F42 *lac* recombined with the chromosome in a stable merodiploid and (ii) whether this recombination was *recB* dependent. Strains containing either F42 *lac*, RDP236, and RDP237 or mini-F *lac*, RDP234, and RDP235 were infected with λ *precA*$^+$ at various MOIs to initiate recombination. β-Galactosidase assays were carried out as described above (Fig. 1). The EU/CFU values in the *recB*$^-$ mutant were normalized to the production of β-galactosidase in these strains after infection with λ p*lacZ*$^+$ at an MOI of 5. λ p*recA*$^+$ MOIs are shown from 0.2 to 20 PFU/ml. MOI greater than 20 were not used due to "lysis from without" at higher MOIs.

The data in Fig. 1 show that recombination between F42 *lac* and the chromosomal *lac* gene, RDP236, is six- to eightfold higher than for the mini-F *lac* × chromosomal *lac* cross, RDP234, in the linear portions of the curves at low MOIs. This difference in recombination potential for the two plasmids with a chromosomal *lac* gene is very similar to the difference in their ability to recombine with λ p*lac5* (23).

Recombination between F42 *lac* and chromosomal *lac* shows some *recB* dependence at all MOIs of λ p*recA*$^+$. The EU/ml value for the Δ(*argA-recB*) mutant, RDP237, is always six- to eightfold lower than for the isogenic *recB*$^+$ strain, RDP236. On the other hand, recombination between

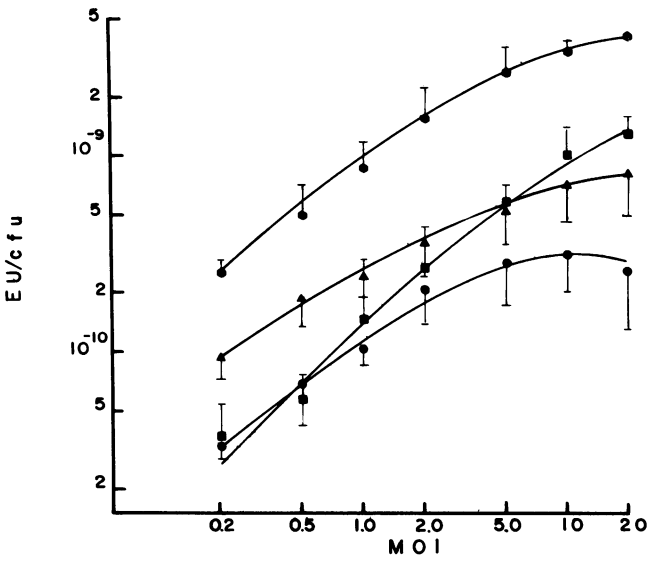


FIG. 1. Effect of Δ(*argA-recB*) on recombination between chromosomal *lac* and F42 *lac* or mini-F *lac*. λ p*recA*$^+$ was used to infect strains at the MOIs shown, and β-galactosidase assays were performed as described in the text. λ p*lacZ*$^+$ control infections were at an MOI of 5. Each point represents the average of six experiments, with standard deviations shown by the error bars. Symbols: ●, RDP236 F42 *lac*/*recB*$^+$; ▲, RDP237 F42 *lac*/Δ(*argA-recB*); ■, RDP234 *recB*$^+$(pRPZ100*lac*); ●, RDP235 Δ(*argA-recB*) (pRPZ100*lac*).

mini-F *lac* and the chromosome is not *recB* dependent at low MOIs. Only at MOIs of 5 or greater is an appreciable difference seen between the *recB*$^+$ RDP234 and Δ(*argA-recB*) RDP235 strains. Interestingly, the reduction in recombination between F42 *lac* and chromosomal *lac* observed in the *recB*$^-$ mutant, RDP237, does not decrease to the level seen with mini-F *lac* in the *recB*$^+$ strain, RDP235. There is an approximately threefold difference in the recombination level observed for RDP237 and the level observed for the *recB*$^-$ mini-F *lac* strain at all λ p*recA*$^+$ MOIs. The possible significance of these observations is discussed below.

R1 repression of F fertility reduces the enhancement of recombination between F42 *lac* and λ p*lac5* (18). The plasmid R1 carries a functional *finO*$^+$ gene. The product of this gene, along with the *finP* product, negatively controls the transcription of the *tra* regulon of F (30). F is naturally *finO*$^-$ and therefore constitutively expresses its fertility functions. If R1 is present in the same cell, these *tra* functions are repressed. We used this plasmid as another means of determining whether recombination between F42 *lac* and chromosomal *lac* was also dependent on F fertility as well as *recB*. Strains were constructed that contained both F42 *lac* and R1 and were *recB*$^+$ (RDP238) or contained the *recB* deletion (RDP239). The strains were infected at various MOIs with λ p*recA*$^+$ and β-galactosidase assays performed as described above (Fig. 2). The data for the strains containing only F42 *lac*, RDP236 and RDP237, are repeated from Fig. 1 to allow direct comparison. The presence of R1 does reduce recombination between F42 *lac* and the chromosome twofold in the *recB*$^+$ strain, RDP238. The added presence of the *recB* deletion (RDP239) further reduces recombination, but only to the level seen in the F42 *lac* Δ(*argA-recB*) strain, RDP237. Repression of *tra* function by R1 does not reduce recombination completely, i.e., to the level seen when *tra* is deleted in mini-F-*lac* (Fig. 1, RDP234 and RDP235). This

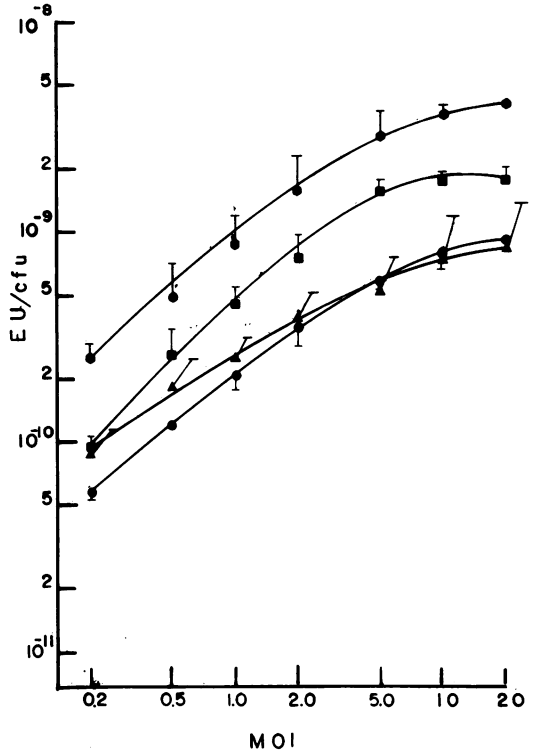


FIG. 2. Effect of R1 repression of F fertility functions on recombination. λ *precA*$^+$ was used to infect cells at the MOIs shown, and β-galactosidase assays were performed as described in the text. λ *placZ*$^+$ control infections were at an MOI of 5. Each point represents the average of six experiments with standard deviations shown by the error bars. Symbols: ●, RDP236 F42 *lac*/*recB*$^+$; ■, RDP238 F42 *lac*/*recB*$^+$(R1); ▲, RDP237 F42 *lac* Δ(*argA-recB*); ●, RDP239 F42 *lac*/Δ(*argA-recB*)(R1).

confirms that the high level of recombination observed between F42 *lac* and the chromosomal *lac* gene is also dependent on expression of F fertility functions as well as on the presence of an active RecBC enzyme.

**Conjugation alleviates *recB* reduction of F42 *lac* recombination.** As mentioned above, Porter et al. (20) did an experiment in which the F42 *lac* DNA is introduced into a cell by conjugation at the same time as it is infected by λ *plac5*, and they showed that recombination in a *recB21* mutant was 10-fold higher in this situation than it was in a *recB21* strain with a stabilized F42 *lac*. A similar type of experiment is described here. In this case, the F42 *lac* DNA is introduced via conjugation immediately after infection with λ *precA*$^+$ (Table 3). It should be noted that corrections were made of the data for all matings to account for differences in mating efficiency of each recipient strain. Stable merodiploids were also tested in parallel to allow for comparison within each experiment.

First, recombination in the stable merodiploid strains was approximately eightfold higher in the *recB*$^+$ strain, RDP236, than in the *recB*$^-$ strain, RDP237. This is comparable to the difference between these two strains shown in Fig. 1. However, when the F42 *lac* molecule was introduced via conjugation, recombination was increased sevenfold in the *recB*$^+$ recipient, RDP232, and 21-fold in the *recB*$^-$ recipient, RDP233. These results indicate that the reduction in recombination to the transcribable intermediate stage in a *recB*$^-$ strain for a stable F42 *lac* × chromosomal *lac* cross is largely alleviated when the F42 *lac* has been recently introduced into the cell by conjugation. This result is similar in the findings of Birge and Low (1) for the *recB* dependence of transcribable intermediate production in Hfr conjugation. These results also indicate, however, that an F42 *lac* recently introduced into a cell by conjugation has a higher potential for the initiation of recombination than a stabilized F42 *lac*, even when active RecBC enzyme is present.

Second, the effect of F fertility repression was also examined in this system. Rather than using R1, a mini-R1 derivative lacking the R1 conjugation factors, but retaining *finO*$^+$, pRPZ13 (23a), was used in the recipient strains. RDP240(*recB*$^+$) and RDP241 [Δ(*argA-recB*)] are the pRPZ113-containing derivatives that were used in matings with F42 *lac*. The presence of the mini-R1 derivative had little effect on recombination between a chromosomal *lac* gene and an incoming F42 *lac* in either the *recB*$^+$ or the *recB*$^-$ strain (Table 3). Thus, it would appear that F fertility repression plays no role in the initiation of recombination when the F42 *lac* DNA is introduced via conjugation. A mini-R1 derivative containing a deletion of the *finO* gene, pRPZ114 (23a), was also used in the same recipients to be sure that the effect of mini-R1 *finO*$^+$ was not due solely to the presence of the R1 replicon. With this plasmid present, recombination levels were the same as those with mini-R1 *finO*$^+$ (data not shown).

## DISCUSSION

It has been very difficult to determine the exact nature of the role played by the *E. coli* RecBC enzyme in general

TABLE 3. Effect of mating in F42 *lacZ* on RecBC enzyme requirement in recombination[a]

| Strain or cross | Genotype | | EU/CFU × $10^9$ |
|---|---|---|---|
| | *recB*$^+$ | Δ(*recB*) | |
| RDP236 | F42 *lacZ*/*lacZ* | | 5.25 (0.12) |
| RDP214 × RDP232 | F42 *lacZ* × F$^-$ *lacZ* | | 36.4 (4.7)[b] |
| RDP214 × RDP240 | F42 *lacZ* × F$^-$ *lacZ* (pRPZ113) | | 20.0 (6.0)[b] |
| RDP237 | | F42 *lacZ*/*lacZ* | 0.65 (0.02)[c] |
| RDP214 × RDP233 | | F42 *lacZ* × F$^-$ *lacZ* | 13.9 (3.9)[d] |
| RDP214 × RDP241 | | F42 *lacZ* × F$^-$ *lacZ* (pRPZ113) | 23.4 (7.1)[d] |

[a] The results shown are the averages for three or four experiments with standard deviations within parentheses.
[b] EU/CFU values have been corrected for the mating efficiency of these strains. This was done by dividing raw EU/CFU by the percentage of the Lac$^+$ transconjugants per CFU obtained in control matings with RDP215.
[c] EU/CFU values have been corrected for the reduced viability of the Δ*recB* strains. This was done by dividing EU/CFU by the ratio of EU/CFU obtained in λ *placZ*$^+$ infections in the Δ*recB* versus *recB*$^+$ strains.
[d] Both corrections were done on these two strains.

recombination. In vitro data on the activities of the RecBC enzyme have supported a role in unwinding duplex DNA preparatory to recombination (28, 29). Arguments have also been presented for a role in the resolution of recombination intermediates (1, 25, 26). The studies presented here involve only transcribable intermediate assays and therefore do not address the possibility that the RecBC enzyme plays a role in the later processing steps of recombination. The data presented, however, tend to support the hypothesis that the RecBC enzyme acts at early stages in the recombination process in at least some specific situations.

The data presented herein show that fully enhanced recombination between a stable F42 *lac* and a chromosomal *lac* gene requires both constitutive expression of the *tra* regulon of F42 *lac* and an active RecBC enzyme. Both of these factors have previously been shown to be required for fully enhanced recombination between F42 *lac* and λ p*lac*5 (1, 18, 21, 23).

The requirement for both *tra* expression and an active RecBC enzyme in enhanced recombination between F42 *lac* and λ p*lac*5 has led to a model for their coordinate involvement in recombination enhancement (18, 23, 23a). As the RecBC enzyme normally has no means of entry into a double-stranded circular DNA molecule (13), it has been proposed that the nicking reaction at *oriT* may allow the RecBC enzyme to enter the F42 *lac* molecule either directly at *oriT* or at some other site (18, 23, 23a). The helix unwinding activity of the RecBC enzyme could then provide single-stranded DNA substrates that would facilitate the initiation of recombination (28). Alternatively, the RecBC enzyme might encounter one or more *cis*-acting sites within the molecule that are active in recombination initiation once it has gained entry into the molecule (24, 27).

The data presented here allow us to make some refinements of our understanding of the coordinate role of *tra* and the RecBC enzyme in recombination enhancement. The requirement for RecBC enzyme participation in *tra*-dependent recombination enhancement is not absolute (Fig. 1). This comes from comparing the results for F42 *lac* and mini-F *lac* in the Δ(*recB*) strain. The EU/CFU-versus-MOI curves are roughly parallel for these two strains, but the F42 *lac* is uniformly two- to threefold higher in its potential to recombine with the chromosomal *lac* gene. This reflects a difference in recombination potential that appears to be *tra* dependent, but that does not depend on the RecBC enzyme. This difference between F42 *lac* and mini-F *lac* in the absence of a functional RecBC enzyme could be due to the participation of another enzyme activity in *tra*-dependent enhancement. It could also be due to an effect of some non-*tra* DNA sequences that are present on F42 *lac*, but not on mini-F *lac*. We feel that this latter possibility is unlikely, since only a 2.5-kilobase DNA fragment containing the *oriT* site must be added to mini-F *lac* to allow it to manifest a high level of transductional recombination enhancement (23a).

There is a role for the RecBC enzyme in recombination between mini-F *lac* and chromosomal *lac* that is independent of *tra*-dependent enhanced recombination (Fig. 1). The ability of mini-F *lac* to recombine with chromosomal *lac* is the same in either the *recB*$^+$ or the Δ*recB* strain at low MOI values. However, at high MOIs of λ p*recA*$^+$, recombination between mini-F *lac* and chromosomal *lac* in a *recB*$^+$ background is two- to fourfold higher than that in the Δ(*argA-recB*) strain (Fig. 1; RDP234 and RDP235). It is possible that another recombination pathway is actually responsible for the recombination observed between mini-F *lac* and λ chromosomal *lac*. The alternate recombination pathway may be capable of carrying out low levels of recombination in the absence of an active RecBC enzyme, but may not be capable of dealing with a high level of recombination activity demanded at high MOIs of λ p*recA*$^+$ without the participation of the RecBC enzyme in some sort of secondary role.

We therefore conclude that the RecBC enzyme plays a role in the initiation of recombination in a stable merodiploid under two different sets of conditions. The first set of conditions in which the RecBC enzyme plays a role in initiation is when *tra*-dependent enhancement is occurring. Although the requirement for the RecBC enzyme is not absolute in this case, the *tra*-dependent enhancement is uniformly more pronounced in the presence of an active RecBC enzyme over a wide range of MOIs for λ p*recA*$^+$. The second set of conditions in which the recBC enzyme plays a role in the initiation of recombination is when high levels of *recA* protein are provided in the absence of *tra*-dependent enhancement. When the *tra*-dependent component of enhancement is not functioning, low levels of recombination do not appear to require the RecBC enzyme for initiation. The initiation of recombination does, however, proceed more efficiently in the presence of an active RecBC enzyme when the pressure to recombine increases at higher λ p*recA*$^+$ MOIs.

Two conclusions can be drawn from the comparison of recombination initiation in stable merodiploids versus F42 *lac* exconjugants. The first conclusion is that the RecBC enzyme plays little role in the recombination between a chromosomal *lac* gene and an F42 *lac* that has recently been introduced by conjugation (Table 3). This conclusion is in agreement with similiar observations involving Hfr conjugation (2). The second conclusion is that higher levels of recombination initiation are achieved in an F42 *lac* exconjugant than in a stable merodiploid exhibiting *tra*-dependent recombination enhancement (Table 3). This may mean that some physical aspect of the recently conjugated F42 *lac* DNA mimics the DNA substrate created by the action of RecBC enzyme on the F42 *lac* DNA in the *tra*-dependent enhancement in the stable merodiploid. If this is the case, it then can be concluded that conjugation results in the generation of more of that DNA substrate than can be produced by the RecBC enzyme. The simplest explanation for the recombinogenic nature of the recently conjugated F42 *lac* DNA is the single-stranded nature of this DNA substrate. The high level of recombination in the exconjugants may also mean that the recently transferred F42 *lac* DNA serves as a substrate for recombination initiation by a different mechanism than is utilized in the stable merodiploid. In these experiments, the presence of a functional *finO*$^+$ gene has little, if any, effect. This may indicate either that fertility repression is unimportant in the recombination that occurs in a recipient cell after conjugation or that recombination initiation occurs before the expression of the *finP* gene on the incoming F42 *lac*.

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