SPECIALIZED TRANSDUCTION WITH λ*plac5*: INVOLVEMENT OF THE RecE AND RecF RECOMBINATION PATHWAYS

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ABSTRACT

Several aspects of the recombination resulting from λ*plac5* transduction were investigated in strains of *Escherichia coli* K-12 that use the RecE or RecF recombination pathways. In a RecBC pathway strain, F42*lac* recombination with λ*plac5* is 20- to 50-fold higher than chromosomal *lac* times λ*plac5* recombination, and this recombination enhancement is largely dependent on constitutive expression of F42*lac* fertility functions. Here, it was observed that F42*lac* fertility functions do not effect the ability of F42*lac* to recombine with λ*plac5* in a RecE or RecF pathway strain. Therefore, the enhancement observed in a Rec$^+$ (or RecBC pathway) strain is directly dependent on the *recBC* gene product. The end product of recombination between λ*plac5* and either F42*lac* or chromosomal *lac* in RecE and RecF pathway strains was monitored by scoring for addition and substitution transductants. It was observed that the percentage of addition transductants was lower in all cases for RecE and RecF pathway strains as compared with RecBC pathway or a *recB* strain. It is concluded that the introduction of *sbcA* or *sbcB* into a *recB* strain produces a change in recombination mechanism that is reflected in the nature of the end product of recombination.

A number of recent studies have examined the involvement of various recombination genes of *Escherichia coli* K-12 in specialized transduction with λ*plac5* (PORTER, MCLAUGHLIN and LOW 1978; PORTER, LARK and LOW 1981; PORTER, WELLIVER and WITKOWSKI 1982; PORTER 1982). In some cases the nature of the recombination end product has been examined by determining the relative proportions of addition and substitution transductants. These studies have sometimes also included transcribable intermediate assays as well as determinations of viable Lac$^+$ transductants. The transcribable intermediate assay involves measuring the level of wild-type β-galactosidase produced during the early stages of recombination between two different *lacZ* alleles. The wild-type β-galactosidase produced is thought to result from the transcription and translation of a transcribable intermediate stage in the recombination process which may or may not undergo the necessary further processing required to produce a viable recombinant cell (BIRGE and LOW 1974). The assay provides a means of examining the levels of recombination initiation obtained when

different combinations of recombining DNA substrates are used in strains with varying configurations of recombination genes.

The presence of a function *recA* gene product is essential for *lac* times *lac* recombination (including transcribable intermediate) in all cases thus far tested (BIRGE and LOW 1974; PORTER, MCLAUGHLIN and LOW 1978; PORTER, LARK and LOW 1981; PORTER 1982). The involvement of the *recB recC* gene product, however, appears to be much more complex. When a chromosomal *lac* gene is the recipient DNA substrate in λ*plac5* transduction, the yield of viable Lac^+ transductants per colony-forming unit (cfu) is only reduced two- to threefold in a *recB* strain as compared with a Rec^+ (or RecBC pathway) strain (PORTER, WELLIVER and WITKOWSKI 1982). This is in sharp contrast to the case for Hfr conjugation, where the formation of viable Lac^+ recombinants is reduced more than 100-fold in a *recB* strain (BIRGE and LOW 1974). The nature of the end product of chromosomal *lac* times λ*plac5* recombination also appears to show essentially no *recB recC* dependence as the ratio of addition to substitution transductants is not affected by the presence or absence of a functional *recB recC* gene product (PORTER, WELLIVER and WITKOWSKI 1982). From these observations it was concluded that the *recB recC* gene product has little if any role in chromosomal *lac* times λ*plac5* recombination.

It has also been observed that F42*lac* recombines with λ*plac5* 20- to 50-fold more frequently than chromosomal *lac* recombines with λ*plac5* in a Rec^+ strain (PORTER, MCLAUGHLIN and LOW 1978; PORTER, LARK and LOW 1981). This enhanced recombination is accompanied by a change in the nature of the recombination end product as the percentage of addition transductants is considerably higher than it is for chromosomal *lac* times λ*plac5* recombination (PORTER, WELLIVER and WITKOWSKI 1982). This enhanced recombination between F42*lac* and λ*plac5* disappears in a *recB* strain (PORTER, MCLAUGHLIN and LOW 1978; PORTER, WELLIVER and WITKOWSKI 1982), and the percentage of addition transductants is the same for both F42*lac* and chromosomal *lac* in a *recB* strain (PORTER, WELLIVER and WITKOWSKI 1982). The enhanced recombination has also been shown to be almost totally dependent on the constitutive expression of the *tra* regulon of F42*lac* (PORTER 1981).

The RecE and RecF recombination pathways of *E. coli* function to restore the level of recombination resulting from Hfr conjugation to essentially wild-type levels in the absence of a functional *recB recC* gene product (HORII and CLARK 1973; GILLEN, WILLIS and CLARK 1981) which is essential for the operation of the RecBC recombination pathway. These pathways appear to play a significant role in recombination resulting from Hfr conjugation only when either the *sbcA* gene (for the RecE pathway) or the *sbcB* gene (for the RecF pathway) is mutated in a strain lacking a functional *recB recC* gene product. Previous work with λ*plac5* transduction in RecE pathway (*recB21 sbcA8*) and RecF pathway (*recB21 sbcB15*) strains has shown that recombination with chromosomal *lac* is somewhat greater than in a RecBC pathway strain, whereas recombination with F42*lac* is somewhat reduced in comparison with a Rec^+ (or RecBC pathway) strain (PORTER, MCLAUGHLIN and LOW 1978; PORTER 1982). In this present study we have examined two additional questions regarding λ*plac5* transduction in RecE and RecF pathway strains. The first ques-

tion is whether or not recombination between F42*lac* and λ*plac5* in RecE and RecF strains involves any component of the F42*lac* fertility-dependent enhancement seen in RecBC strains. This work has involved looking at recombination between λ*plac5* and *traJ*$^+$ and *traJ*$^-$ versions of F42*lac* in the appropriate strains. The *traJ* gene of the F factor encodes a positive regulatory protein whose presence is required for the expression of essentially all of the other *tra* genes (WILLETTS 1977). The *traJ90* amber mutation used here reduces the conjugal transfer ability of F42*lac* about 10^5-fold and largely eliminates the enhancement of recombination between F42*lac* and λ*plac5* in a Rec$^+$ strain (PORTER 1981). These assays have involved measuring both transcribable intermediate and Lac$^+$ colony formation. We have also examined the ratios of addition and substitution transductants in RecE and RecF pathway strains in order to ascertain their effects on the nature of the recombination end product in λ*plac5* transduction.

MATERIALS AND METHODS

Bacterial and phage strains: The *E. coli* K-12 strains used in this study are listed in Table 1. The newly constructed strains identified with this work were simple derivatives of previously described strains and were made using standard conjugation and P1*vir* transduction procedures (MILLER 1972). The complete pedigree of all strains is available upon request. The λ*plac5* derivatives used in this study were prepared from the lysogenic strains shown in Table 1 by heat induction and titered on KL528 or RDP101 as previously described (PORTER, LARK and LOW 1981).

Media: LB medium (MILLER 1972) was used for either liquid culture medium or agar plates whenever a rich medium was desired. Assays for Lac$^+$ transductants involved platings on modified minimal medium 56 supplemented with lactose and other required growth factors (LOW 1973).

Transcribable intermediate assays: Bacterial cultures were grown to approximately 2×10^8 cells/ml at 37° in half-strength modified minimal medium 56 (LOW 1973) supplemented as previously described (PORTER, MCLAUGHLIN and LOW 1978) with the addition of 0.4 ml of sterile M $MgSO_4$ per 100 ml of medium after autoclaving (PORTER 1981). It should be noted that this medium contains 0.4% glycerol as a carbon source. Portions of each culture were then infected with the appropriate λ*plac5* derivative at a multiplicity of infection (moi) of five, and incubation was continued in a shaking waterbath at 37° to provide moderate aeration. All of the strains used in these assays contained a λ*ind* prophage to repress the lytic functions of λ*plac5*. At 1 hr after infection, platings were done on minimal lactose plates and LB plates to determine Lac$^+$ transductants and cfu, respectively. At 3 hr after infection, culture samples were taken for β-galactosidase assays and cfu platings were repeated. β-galactosidase assays were performed as previously described with a 3-min treatment at 57° to inactivate complement enzyme (BIRGE and LOW 1974; PORTER, MCLAUGHLIN and LOW 1978). One enzyme unit (EU) equals that amount of enzyme that hydrolyzes 1 nmol of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) in 1 min at 28° (BIRGE and LOW 1974).

Addition/substitution transductant assays: Transductions of Δ(*gal-bio*) strains were done with genetically disabled (N$^-$N$^-$P$^-$) λ*plac5* derivatives (PORTER, LARK and LOW 1981) at moi of 0.1 to rule out site-specific recombination at *att*λ. Addition and substitution transductants were distinguished by printing grids of transductants on LB plates spread with 10^9 λ*c*71 phage as previously described (PORTER, WELLIVER and WITKOWSKI 1982).

Chemicals and media: Tryptone, yeast extract, MacConkey agar base and agar were obtained from Difco. ONPG, Brij-58, drugs, sugars and other biochemicals were obtained from Sigma Chemical Company. All other chemicals were reagent grade.

RESULTS

Effect of recB vs. traJ90 *on enhanced recombination*

It has previously been reported that the enhanced levels of recombination

TABLE 1

Strains of Escherichia coli

Strain	Relevant properties	Source/reference/comments
KL528	F⁻ Δ(*lac-pro*) *supF trp pyrF his rpsL thi* λ⁻	PORTER, LARK and LOW (1981)
KL550	RDP100 (λ*c*I857 *Sam7 plac5* I⁻Z⁺Y⁻)	PORTER, LARK and LOW (1981)
KL551	RDP100 (λ*c*I857 *Sam7 plac5* I⁻Z118Y⁻)	PORTER, LARK and LOW (1981)
KL759	RDP100 (λ*c*I857 *Nam7 Nam53 Pam80 plac5* I⁻Z⁺Y⁻)	PORTER, LARK and LOW (1981)
KL760	RDP100 (λ*c*I857 *Nam7 Nam53 Pam80 plac5* I⁻Z118Y⁻)	PORTER, LARK and LOW (1981)
KL765	F⁻ *lacZ813 lacI3 pro met his trp rpsL thi* (λ*ind*)	PORTER, LARK and LOW (1981)
KL771	F42*lacZ813 lacI3*/KL791	PORTER, LARK and LOW (1981)
KL791	F⁻ Δ(*lac-pro*) *met his trp rpsL thi* (λ*ind*)	PORTER (1981)
KL798	F⁻ *lacZ813 lacI3 pro met his recB21 sbcA8 rpsL thi* (λ*ind*)	Used in PORTER, MCLAUGHLIN and LOW (1978)
KL799	F⁻ Δ(*lac-pro*) *met his recB21 sbcA8 rpsL thi* (λ*ind*)	Used in PORTER, MCLAUGHLIN and LOW (1978)
RDP100	F⁻ Δ(*lac-pro*) *leu thi acrA*(?) *supE44*	PORTER, LARK and LOW (1981)
RDP101	F⁻ Δ(*lac-pro*) *leu thi supE44*	PORTER, LARK and LOW (1981)
RDP112	F⁻ *lacZ813 lacI3* Δ(*gal-att*λ*-bio*) *met rpsL thi* λ⁻	PORTER, LARK and LOW (1981)
RDP114	F42 *lacZ813 lacI3*/Δ(*lac-pro*) Δ(*gal-att*λ*-bio*) *met rpsL thi* λ⁻	PORTER, LARK and LOW (1981)
RDP128	F42 *lacZ813 lacI3 traJ90*/KL791	PORTER (1981)
RDP172	F⁻ *lacZ813 lacI3 met his trp recB21 rpsL thi* (λ*ind*)	This work
RDP173	F⁻ Δ(*lac-pro*) *met his trp recB21 rpsL thi* (λ*ind*)	This work
RDP174	F42 *lacZ813 lacI3*/RDP173	This work
RDP175	F42 *lacZ813 lacI3 traJ90*/RDP173	This work
RDP176	F42 *lacZ813 lacI3*/KL799	This work
RDP177	F42 *lacZ813 lacI3 traJ90*/KL799	This work
RDP178	F⁻ *lacZ813 lacI3 met trp recB21 sbcB15 rpsL thi* (λ*ind*)	This work
RDP179	F⁻ Δ(*lac-pro*) *met trp recB21 sbsB15 rpsL thi* (λ*ind*)	This work
RDP180	F42 *lacZ813 lacI3*/RDP179	This work
RDP181	F42 *lacZ813 lacI3 traJ90*/RDP179	This work
RDP182	F⁻ *lacZ813 lacI3* Δ(*gal-att*λ*-bio*) *met recB21 sbcA8 rpsL thi* λ⁻	This work
RDP183	F42 *lacZ183 lacI3*/Δ(*lac-pro*)Δ(*gal-att*λ*-bio*) *met recB21 sbcA8 rpsL thi* λ⁻	This work
RDP184	F⁻ *lacZ813 lacI3* Δ(*gal-att*λ*-bio*) *met recB21 sbcB15 rpsL thi* λ⁻	This work
RDP185	F42 *lacZ813 lacI3*/Δ(*lac-pro*)Δ(*gal-att*λ*-bio*) *met recB21 sbcB15 rpsL thi* λ⁻	This work

seen with F42*lac* times λ*plac5* in a Rec$^+$ strain are not observed in a *recB* strain (PORTER, MCLAUGHLIN and LOW 1978; PORTER, WELLIVER and WITKOWSKI 1982). It has also been demonstrated that constitutive expression of the *tra* regulon on F42*lac* is required for essentially all of the recombination enhancement between F42*lac* and λ*plac5* in a Rec$^+$ strain (PORTER 1981). The first question to be examined here is whether or not *tra* expression has any effect on F42*lac* times λ*plac5* recombination in a *recB* strain.

The *traJ90* mutation of F42*lac* (ACHTMAN, WILLETTS and CLARK 1971) is an amber mutation that reduces the conjugal transfer ability of F42*lac* about 10^5-fold and largely eliminates the enhancement of recombination between F42*lac* and λ*plac5* in a nonsuppressing strain (PORTER 1981). Derivatives of F42*lac* with the proper *lac* alleles and either *tra*$^+$ or *traJ90* were placed in a *recB* strain containing a deletion of the normal *lac* region of the genome. Assays for transcribable intermediate production and viable Lac$^+$ transductants were run on these strains as described in MATERIALS AND METHODS with comparable Rec$^+$ strains and a chromosomal *lac recB* strain run in parallel for comparison. The results of these experiments are shown in Table 2 in terms of viable Lac$^+$ colonies per 10^3 cfu and EU of β-galactosidase per 10^9 cfu.

The comparison of KL765 and KL771 reveals a typical 20- to 40-fold enhancement for λ*plac5* recombination with F42*lac* as compared with chromosomal *lac* in a Rec$^+$ strain for both transcribable intermediate and viable Lac$^+$ colony formation. The results with RDP128 show that F42*lac traJ90* in a Rec$^+$ strain gives about fourfold more recombination with λ*plac5* than does chromosomal *lac*. Previous work has indicated that the copy number of F42*lac* may provide approximately half of that difference (PORTER 1981, 1982). Examination of the data for RDP172 shows that chromosomal *lac* times λ*plac5* recombination in a *recB* strain is only reduced about threefold as compared with a Rec$^+$ strain (KL765) in terms of viable Lac$^+$ transductants per cfu, which is in agreement with previous work (PORTER, WELLIVER and WITKOWSKI 1982). The viable Lac$^+$ transductants per cfu for the *tra*$^+$ and *traJ90* versions of F42*lac* (RDP174 and RDP175, respectively) indicate that *tra* has no effect on F42*lac* times λ*plac5* recombination in a *recB* strain. The values for Lac$^+$ transductants per cfu for either version of F42*lac* in a *recB* strain are approximately half the value for chromosomal *lac* in a Rec$^+$ strain and 50% higher than chromosomal *lac* in a *recB* strain. All of these results are consistent with a model in which the recombination enhancement is totally dependent on a functional *recB recC* gene product and in which *recB* has little other effect on recombination levels between λ*plac5* and *lac* genes in the infected cell.

The EU/cfu values obtained in *recB* strains appear anomalously high at first scrutiny. The viability problems of *recB* strains are such, however, that β-galactosidase is most likely being made in cells that can not divide enough times to give rise to a colony (CAPALDO, RAMSEY and BARBOUR 1974). In each of these experiments, a portion of each culture is infected with λ*placZ*$^+$ as a control for the β-galactosidase expression ability of each strain (data not shown). The EU/cfu levels for λ*placZ*$^+$ infections are significantly higher for *recB* strains than for Rec$^+$ strains. When that differential is used for a correc-

TABLE 2

Recombination in recB *strains*[a]

Strain	Relevant properties	Lac$^+$/10^5 cfu: Non-UV-irradiated λ*plac*	Lac$^+$/10^5 cfu: UV-irradiated λ*plac*	EU/10^9 cfu: Non-UV-irradiated λ*plac*	EU/10^9 cfu: UV-irradiated λ*plac*
KL765	F$^-$ *lacZ*$^-$	0.120 (0.026)	3.50 (1.34)	0.018 (0.006)	1.03 (0.54)
RDP172	F$^-$ *lacZ*$^-$ *recB21*	0.034 (0.011)	2.10 (0.64)	0.048 (0.003)	4.54 (1.06)
KL771	F42 *lacZ*$^-$/Δ(*lac*)	2.26 (0.56)	4.95 (1.40)	0.833 (0.171)	3.04 (1.16)
RDP128	F42 *lacZ*$^-$ *traJ90*/Δ(*lac*)	0.442 (0.110)	5.08 (1.51)	0.097 (0.034)	1.36 (0.11)
RDP174	F42 *lacZ*$^-$/Δ(*lac*)*recB21*	0.058 (0.019)	1.02 (0.26)	0.340 (0.136)	10.8 (3.90)
RDP175	F42 *lacZ*$^-$ *traJ90*/ Δ(*lac*)*recB21*	0.050 (0.014)	1.06 (0.32)	0.322 (0.110)	12.7 (2.80)
EU/10^9 cfu corrected for relative EU/cfu obtained with λ*placZ*$^+$ in *recB* vs. Rec$^+$ strains:[b]					
RDP172	F$^-$ *lacZ*$^-$ *recB21*			0.022	2.05
RDP174	F42 *lacZ*$^-$/Δ(*lac*)*recB21*			0.068	2.15
RDP175	F42 *lacZ*$^-$ *traJ90*/ Δ(*lac*)*recB21*			0.046	1.83

[a] The indicated strains were infected with λcI857 *Sam7 placZ118* from KL551 at an MOI of five, and transductants and EU of β-galactosidase were determined as described in MATERIALS AND METHODS. All numbers have been normalized to cfu to facilitate comparisons. The UV-irradiated phage preparations were irradiated with 60 J/m^2 of 254 nm light from a germicidal lamp. Control infections of an F$^-$Δ(*lac*) strain with the tranducing phage preparations yield less than 1 Lac$^+$ colony/10^8 cfu and about 0.0006 EU/10^9 cfu. Uninfected cultures of the strains assayed showed less than 0.005 EU/10^9 cfu. The values shown represent averages from at least three experiments. The standard deviation for each value is shown in parentheses.

[b] The EU/cfu value of λ*placZ*$^+$ control infections of each *recB* strain was divided by the λ*placZ*$^+$ EU/cfu values for KL765 and KL771 (data not shown) to provide a correction factor for strain viability. The EU/cfu values for RDP172, RDP174 and RDP175 shown in the top half of the table were divided by the appropriate correction value to yield the EU/cfu values shown in the bottom half of the table.

tion factor, the EU/10^9 cfu values shown in the bottom half of Table 2 are obtained for the *recB* strains. When this correction has been made, it can be seen that EU/10^9 cfu is essentially the same for a chromosomal *lac* times λ*plac5* cross in a Rec$^+$ or a *recB* strain. The corrected values for the F42*lac* derivatives in a *recB* strain are only two- to threefold higher than those obtained for chromosomal *lac* times λ*plac5* in either Rec$^+$ or *recB* strains. This remaining two- to threefold difference may be largely attributable to F42*lac* copy number (PORTER 1981, 1982).

In each experiment, a portion of the culture for each strain is infected with λ*plac5* that has been subjected to UV irradiation. This UV irradiation of the transducing phage largely equalizes the levels of recombination obtained with chromosomal *lac* and F42*lac* in a *uvrA*-independent fashion (PORTER, MCLAUGHLIN and LOW 1978) and can be used as an indication that *lac* genes are present in the cell and available for recombination (PORTER 1981, 1982). In these experiments, the results with UV-irradiated λ*plac5* indicate that recombination initiation as indicated by EU/10^9 cfu is similar in Rec$^+$ and *recB* strains for all recipient *lac* allele configurations. The Lac$^+$/cfu data with UV-irradiated λ*plac5* shows that *recB* strains are capable of carrying a significant fraction (20–

60%) of these initiation events through to the point where a viable Lac^+ recombinant is obtained.

Recombination enhancement in RecE and RecF recombination pathway strains

In one previous study, it was found that the RecE and RecF recombination pathways were more effective than the RecBC (Rec^+) pathway for chromosomal *lac* times λ*plac5* recombination, but less effective than the RecBC pathway for F42*lac* times λ*plac5* recombination (PORTER, MCLAUGHLIN and LOW 1978). In these experiments, we wanted to determine whether or not the constitutive expression of the *tra* regulon on F42*lac* played any role in its recombination with λ*plac5* in strains utilizing the RecE or RecF recombination pathways. F42*lac* and F42*lac traJ90* were introduced into RecE and RecF pathway strains containing a deletion of their chromosomal *lac* region. A series of recombination assays was run on each group of strains as described in MATERIALS AND METHODS with appropriate chromosomal *lac* and RecBC pathway strains run in parallel. The results for the RecE pathway strains are shown in Table 3, and the results for the RecF pathway strains are shown in Table 4. The EU/cfu valves obtained with control infections of λ*placZ*$^+$ did not differ significantly for any of the strains in Tables 3 or 4 (data not shown). It was, therefore, not necessary to correct the EU/cfu data for viability problems with the RecE and RecF pathway strains as was required in Table 2 for the *recB* strains.

The comparison of EU/10^9 cfu and Lac^+/10^3 cfu for RDP176 and RDP177 in Table 3 shows that the fertility functions of F42*lac* play little or no role in recombination with λ*plac5* in a RecE pathway strain. The transcribable intermediate production obtained with these strains is three- to fourfold greater than with RDP128, whereas the Lac^+ transductants are only about 50% greater. A similar comparison is observed with LK765 *vs.* KL798 which are the chromosomal *lac* versions of RecBC and RecE pathway strains, respectively. Therefore, it appears that RecE pathway strains initiate recombination more frequently than RecBC pathway strains (as indicated by transcribable intermediate levels) but carry fewer of these initiation events through to the point where a Lac^+ transductant is obtained.

The comparison of EU/10^9 cfu and Lac^+/10^3 cfu for RDP180 and RDP181 in Table 4 shows that the fertility functions of F42*lac* also play little or no role in recombination with λ*plac5* in a RecF pathway strain. These strains show recombination initiation at a greater level than a RecBC strain containing F42*lac* (KL771) but yield about ninefold fewer Lac^+ transductants per cfu than an F42*lac*-containing RecBC strain. These results appear to be somewhat in conflict with a previous report involving similar strains (PORTER, MCLAUGHLIN and LOW 1978), but in this case the EU and Lac^+ colonies have been normalized to cfu and, therefore, the comparisons between strains are presumably more valid. The results obtained with RDP178 are perhaps the most surprising results in this set of experiments. With this chromosomal *lac* RecF pathway strain, the viable Lac^+ colonies per cfu are about six- or sevenfold greater than they are for the F42*lac* derivatives in RecF pathway strains (RDP180 and RDP181), even though the EU per cfu are two- to threefold lower in the

TABLE 3

Recombination in RecE pathway strains

Strain	Relevant properties	Lac$^+$/10^8 cfu		EU/10^9 cfu	
		Non-UV-irradiated λ*plac*	UV-irradiated λ*plac*	Non-UV-irradiated λ*plac*	UV-irradiated λ*plac*
KL765	F$^-$ *lacZ*$^-$	0.120 (0.026)	3.50 (1.34)	0.018 (0.006)	1.83 (0.54)
KL798	F$^-$ *lacZ*$^-$ *recB21 sbcA8*	0.176 (0.035)	5.26 (1.24)	0.105 (0.034)	4.41 (1.13)
KL771	F42 *lacZ*$^-$/Δ(*lac*)	2.26 (0.56)	4.95 (1.40)	0.833 (0.171)	3.04 (1.16)
RDP128	F42 *lacZ*$^-$ *traJ90*/Δ(*lac*)	0.442 (0.110)	5.08 (1.51)	0.097 (0.034)	1.36 (0.11)
RDP176	F42 *lacZ*$^-$/Δ(*lac*)*recB21 sbcA8*	0.698 (0.141)	11.1 (1.9)	0.397 (0.102)	2.11 (0.69)
RDP177	F42 *lacZ*$^-$ *traJ90*/ Δ(*lac*)*recB21 sbcA8*	0.568 (0.134)	13.2 (2.5)	0.275 (0.091)	2.06 (0.29)

Recombination assays with the indicated strains were performed as described in the legend to Table 2. The data shown here came from the same series of experiments reported in Table 2; hence, the values for KL765, KL771 and RDP128 are the same in both tables.

chromosomal *lac* case. We currently have no explanation as to why the RecF pathway is more efficient in generating viable Lac$^+$ transductants during recombination of λ*plac5* with chromosomal *lac* than with F42*lac*. These results with RecE and RecF pathway strains again demonstrate that the F42*lac* fertility-dependent enhancement of recombination is totally *recB* dependent (PORTER, WELLIVER and WITKOWSKI 1982).

The nature of the recombination end-product in RecE and RecF pathway strains

When Δ(*gal-att*λ-*bio*) recipient strains are used with N$^-$N$^-$P$^-$ genetically disabled λ*plac5* phage derivatives, all of the transductants observed are the result of *recA*-dependent general recombination (PORTER, LARK and LOW 1981). We have previously examined transductants obtained in such a system to determine whether they were addition or substitution transductants by determining their sensitivity to λ*c*71 phage (PORTER, WELLIVER and WITKOWSKI 1982). An addition transductant results from the addition of the entire λ*plac5* DNA molecule to the *lac* region in the recipient cell by a *recA*-dependent recombination event which is somewhat analogous to the λ integration reaction. These addition transductants contain λ DNA sequences which include the λ*c*I gene and are, therefore, resistant to superinfection by the λ*c*71 clear plaque phage. Substitution transductants result when *lac* times *lac* recombination occurs in such a fashion that the λ DNA sequences are not incorporated into the DNA of the recipient cell. These substitution transductants are sensitive to λ*c*71 infection. It should be noted that this experimental system differs considerably from those described in the preceding sections. In the preceding sections, the lytic functions of the incoming λ*plac5* are repressed by the λ*ind* prophage of the recipient strain. In this system, the chromosomal *att*λ site of the recipient strains is deleted to prevent site-specific recombination, and the replication and gene expression of the incoming λ*plac5* derivatives is prevented by multiple λ mutations. The lack of the λ*ind* prophage in these strains allows the distinction

TABLE 4

Recombination in RecF pathway strains

Strain	Relevant properties	Lac$^+$/10^3 cfu		EU/10^9 cfu	
		Non-UV-irradiated λ*plac*	UV-irradiated λ*plac*	Non-UV-irradiated λ*plac*	UV-irradiated λ*plac*
KL765	F$^-$ *lacZ*$^-$	0.138 (0.013)	6.62 (0.23)	0.021 (0.006)	3.66 (0.87)
RDP178	F$^-$ *lacZ*$^-$ *recB21 sbcB15*	2.00 (0.57)	38.6 (13.7)	1.20 (0.10)	4.87 (1.10)
KL771	F42 *lacZ*$^-$/Δ(*lac*)	2.70 (1.04)	8.64 (2.84)	1.28 (0.38)	6.15 (0.78)
RDP180	F42 *lacZ*$^-$/Δ(*lac*)*recB21 sbcB15*	0.319 (0.098)	2.41 (0.68)	1.99 (0.09)	4.20 (0.76)
RDP181	F42 *lacZ*$^-$ *traJ90*/ Δ(*lac*)*recB21 sbcB15*	0.282 (0.081)	3.09 (0.74)	3.20 (0.82)	7.95 (1.21)

Recombination assays with the indicated strains were performed as described in the legend to Table 2.

between an addition or substitution transductant to be made by examining the Lac$^+$ transductants for the presence of a λ genetic function.

RecE and RecF pathway strains were constructed with a *gal-att*λ*-bio* deletion in their genome and the appropriate *lac* alleles present either in their normal chromosomal location or on F42*lac*. These strains were transduced with *lacZ*$^+$ and *lacZ*$^-$ versions of genetically disabled λ*plac5* phage, and the transductants were assayed for addition *vs.* substitution as previously described (PORTER, WELLIVER and WITKOWSKI 1982). The results of these experiments are shown in Table 5. The values for the percentage of addition transductants obtained with RDP112 and RDP114 are from PORTER, WELLIVER and WITKOWSKI (1982) and are shown here again in Table 5 to enable direct comparisons with the RecE and RecF pathway strains.

The proportions of addition and substitution transductants obtained with RecE and RecF pathway strains show obvious differences from the results obtained with a RecBC pathway strain. These results are in agreement with a study involving λ *gal-bio* transduction which demonstrated a reduced level of addition transductants in a RecF pathway strain as compared with a Rec$^+$ or *recB* strain (WACKERNAGEL and RADDING 1974). It has previously been shown that the percentage of addition transductants with chromosomal *lac* is the same in Rec$^+$ and *recB* strains (PORTER, WELLIVER and WITKOWSKI 1982). The results shown here indicate that there are differences in recombination mechanism between RecE or RecF pathway strains and Rec$^+$ or *recB* strains that are reflected in the nature of the recombination end product.

DISCUSSION

The work presented in this paper has primarily involved two questions regarding λ*plac5* transduction in strains utilizing the RecE or RecF recombination pathways. The first question was whether or not the expression of the *tra* regulon on F42*lac* played any role in recombination with λ*plac5* in a RecE or RecF pathway strain. The approach to this question involved examining the recombination between λ*plac5* and *traJ*$^+$ and *traJ90* versions of F42*lac* in a

TABLE 5

Recombination end product in RecE and RecF pathway strains

Strain	Relevant properties	Phage	Lac$^+$/cfu	λ*c*71^r	% addition
RDP112	F$^-$ *lacZ*$^-$	λ*placZ*$^+$	2.6×10^{-5}	638/1069	64.2
		λ*placZ*$^-$	8.4×10^{-6}	396/1350	29.3
RDP114	F42 *lacZ*$^-$/Δ(*lac*)	λ*placZ*$^+$	3.2×10^{-4}	723/1061	68.1
		λ*placZ*$^-$	2.0×10^{-4}	586/1050	55.8
RDP182	F$^-$ *lacZ*$^-$ *recB21 sbcA8*	λ*placZ*$^+$	1.2×10^{-5}	414/1077	38.4
		λ*placZ*$^-$	5.3×10^{-6}	236/1175	20.1
RDP183	F42 *lacZ*$^-$/Δ(*lac*) *recB21 sbcA8*	λ*placZ*$^+$	6.6×10^{-6}	531/1103	48.1
		λ*placZ*$^-$	3.8×10^{-6}	287/1116	25.7
RDP184	F$^-$ *lacZ*$^-$ *recB21 sbcB15*	λ*placZ*$^+$	6.2×10^{-5}	259/1090	23.8
		λ*placZ*$^-$	3.0×10^{-5}	59/1092	5.4
RDP185	F42 *lacZ*$^-$/Δ(*lac*) *recB21 sbcB15*	λ*placZ*$^+$	3.5×10^{-5}	192/1292	14.9
		λ*placZ*$^-$	1.3×10^{-5}	140/1096	12.8

Transductions were carried out using the indicated Δ(*gal-att*λ-*bio*) strains and either λ*c*I857 *Nam*7 *Nam*53 *Pam*80 *placZ*$^+$ from KL759 or λ*c*I857 *Nam*7 *Nam*53 *Pam*80 *placZ118* from KL760 at an MOI of 0.1. The values shown for Lac$^+$/cfu are averages from at least four experiments for each transduction. There were fewer than 3 Lac$^+$ colonies/ml in uninfected control cultures for all of the strains used. The transductants were tested for addition (λ*c*71^r) *vs.* substitution (λ*c*71^s) as previously described (PORTER, WELLIVER and WITKOWSKI 1982). The numbers for λ*c*71^r are addition transductants per total transductants tested. The values given for λ*c*71^r and % addition for RDP112 and RDP114 are from PORTER, WELLIVER and WITKOWSKI (1982) and are repeated here for comparison purposes.

recB strain as well as in RecE and RecF pathway strains. The results shown in Table 2 confirm a previous result indicating that the *tra*-dependent enhancement of recombination between F42*lac* and λ*plac5* does not occur in a *recB* strain (PORTER, WELLIVER and WITKOWSKI 1982). The results shown in Tables 3 and 4 demonstrate that *tra* expression plays no role in recombination between F42*lac* and λ*plac5* in either a RecE or RecF pathway strain. This observation demonstrates that the *tra*-dependent enhancement of recombination between F42*lac* and λ*plac5* seen in a RecBC pathway strain (PORTER 1981) is specific in its requirement for the product of the *recB* gene. In the case of F42*lac* times λ*plac5* recombination, both RecE and RecF strains give more transcribable intermediate per viable Lac$^+$ colony produced than a RecBC strain. This same phenomenon is observed for chromosomal *lac* times λ*plac5* recombination in a RecE strain but not in a RecF strain. This differential between the transcribable intermediate to lac$^+$ colony ratio for chromosomal *lac* and F42*lac* in a RecF strain was unexpected, and we have no explanation for it at this time.

The second question was whether or not the nature of the recombination end product from λ*plac5* transduction in a RecE or RecF pathway strain differed from that observed in a RecBC pathway or *recB* strain. The testing of transductants for addition *vs.* substitution revealed that the percentages of addition transductants do vary in RecE and RecF pathway strains from that which is observed in RecBC pathway or *recB* strains. It was previously observed that the percentage of addition or substitution transductants is *recB* independent for chromosomal *lac* times λ*plac5* recombination (PORTER, WELLIVER and WITKOWSKI 1982). It was also shown that the *recB*-dependent, *tra*-dependent

enhancement of recombination between F42*lac* and λ*plac5* did change the percentages of addition and substitution transductants, presumably reflecting a change in mechanism (PORTER, WELLIVER and WITKOWSKI 1982). Hence, in the absence of recombination enhancement, the *recB* gene product plays no role in the mechanistic steps that determine whether an addition or a substitution transductant is produced. Although no direct conclusions about actual mechanism can be drawn from the data presented here, it can be concluded that changes in recombination mechanism that are reflected in the nature of the recombination end product do occur when *sbcA* or *sbcB* is introduced into a *recB* strain.

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LITERATURE CITED

ACHTMAN, M., N. WILLETTS and A. J. CLARK, 1971 Beginning a genetic analysis of conjugational transfer determined by the F factor in *Escherichia coli* by isolation and characterization of transfer-deficient mutants. J. Bacteriol. **106:** 529–538.

BIRGE, E. A. and K. B. LOW, 1974 Detection of transcribable recombination products following conjugation in Rec$^+$, RecB$^-$, and RecC$^-$ strains of *Escherichia coli* K-12. J. Mol. Biol. **83:** 447–457.

CAPALDO, F. N., G. RAMSEY and S. D. BARBOUR, 1974 Analysis of the growth of recombination-deficient strains of *Escherichia coli* K-12. J. Bacteriol. **118:** 242–249.

GILLEN, J. R., D. K. WILLIS and A. J. CLARK, 1981 Genetic analysis of the RecE pathway of genetic recombination in *Escherichia coli* K-12. J. Bacteriol. **145:** 521–532.

HORII, Z.-I. and A. J. CLARK, 1973 Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K-12: isolation and characterization of mutants. J. Mol. Biol. **80:** 327–344.

LOW, B., 1973 Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K-12. J. Bacteriol. **113:** 798–812.

MILLER, J. H., 1972 *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

PORTER, R. D., 1981 Enhanced recombination between F42*lac* and λ*plac5*: dependence on F42*lac* fertility functions. Mol. Gen. Genet. **184:** 355–358.

PORTER, R. D., 1982 Recombination properties of P1 *dlac*. J. Bacteriol. **152:** 345–350.

PORTER, R. D., M. W. LARK and K. B. LOW, 1981 Specialized transduction with λ*plac5*: dependence on *recA* and on configuration of *lac* and *att*λ. J. Virol. **38:** 497–503.

PORTER, R. D., T. MCLAUGHLIN and B. LOW, 1978 Transduction versus "conjuduction": evidence for multiple roles for exonuclease V in genetic recombination in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **43:** 1043–1047.

PORTER, R. D., R. A. WELLIVER and T. A. WITKOWSKI, 1982 Specialized transduction with λ*plac5*: dependence on *recB*. J. Bacteriol. **150:** 1485–1488.

WACKERNAGEL, W. and C. M. RADDING, 1974 Transformation and transduction of *Escherichia coli*: the nature of recombinants formed by Rec, RecF, and λ Red. pp. 111–122. In: *Mechanisms in Recombination*, Edited by R. F. GRELL. Plenum Publishing Corporation, New York.

WILLETS, N., 1977 The transcriptional control of fertility in F-like plasmids. J. Mol. Biol. **112:** 141–148.

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