JOURNAL OF BACTERIOLOGY, Jan. 1981, p. 521-532
0021-9193/81/010521-12$02.00/0
Vol. 145, No. 1

Genetic Analysis of the RecE Pathway of Genetic Recombination in *Escherichia coli* K-12

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The RecE pathway of genetic recombination in *Escherichia coli* K-12 was defined to be the pathway that is utilized in deoxyribonucleic acid exonuclease V (ExoV)-defective cells which express constitutively *recE*$^+$, the structural gene for deoxyribonucleic acid exonuclease VIII. Dependence on ExoVIII was shown by the occurrence in a *recB21 recC22 sbcA23* strain of recombination deficiency mutations in *recE*, the structural gene for ExoVIII. Point mutations in *recE* were found as well as deletion mutations in which the entire Rac prophage, carrying *recE*, was lost. In addition, strain construction and mutagenesis revealed the dependence of the RecE pathway on *recA*$^+$ and on *recF*$^+$. Dependence on a fourth gene was shown by a mutation (*rec-77*) which does not map near the other genes. The problem of distinguishing the RecE pathway from that previously called RecF is discussed.

Genetic recombination of bacterial DNA in *Escherichia coli* K-12 has been hypothesized to occur by several alternative pathways, each of which utilizes at least one biochemical function not shared by the others (6, 7). The major pathway operating in wild-type cells is called the RecBC pathway and requires exonuclease V (ExoV), the product of genes *recB* and *recC*, and possibly the functions of several other genes. A second pathway, the RecF pathway, plays a major role in the special situation in which the RecBC pathway is blocked by mutation of *recB* or *recC* or both and exonuclease I (ExoI) is absent owing to mutation of gene *sbcB*. The RecF pathway has been analyzed genetically, and at least one gene has been identified that is not involved in the RecBC pathway, namely, *recF* (20). Both the RecBC and the RecF pathways absolutely require *recA*$^+$, whose function may be to facilitate interaction of DNA molecules (11, 30, 32, 39) to regulate recombination enzymes.

A third pathway has been postulated—the RecE pathway. Like the RecF pathway, the RecE pathway is ordinarily detected only when there are mutations in *recB* and or *recC* or both and in another gene, in this case called *sbcA*. Like *sbcB* mutations, *sbcA* mutations restore to *recB21 recC22* strains recombination proficiency and resistance to UV irradiation and mitomycin (3). Unlike *sbcB* mutations, they do not cotransduce with *his* (36); they map near *trp* (25) and are cotransducible with *ksgD* (S. D. Barbour, personal communication). Mutants that are genotypically *recB21 recC22 sbcA* lack ExoV but have a high level of ATP-independent DNase activity on duplex DNA (3). This new activity has been called exonuclease VIII (ExoVIII) and has been purified and further characterized (16, 23).

We have previously proposed that the structural gene for ExoVIII is a gene, *recE*, that is regulated by gene *sbcA* in such a way that mutation of *sbcA* is required for its expression (3). We have also suggested that *recE* may determine activities other than ExoVIII (17). For example, the *recE* cistron may code for a "Ral" activity responsible for alleviation of restriction of unmodified λ phage, another activity associated with *sbcA* mutants (33). Such an activity has been identified in λ by P. Toothman (personal communication), and the responsible *ral* gene has been mapped between cIII and *N* on the lambda chromosome (14). When the region containing *ral* is present in the host cell before infection by unmodified phage, the plating efficiency of the unmodified phage increases about 100-fold.

In this publication we examined two matters: the dependence of the RecE pathway on ExoVIII and the relationship of the RecE and RecF pathways. For this purpose, we constructed strains carrying different combinations of *rec* and *sbc* mutations and isolated a set of new recombination-deficient (Rec$^-$) mutants. A preliminary report of some of these results has appeared (9).

MATERIALS AND METHODS

Bacterial strains. The strains of *Escherichia coli* K-12 used in this study and their relevant genetic characteristics are listed in Tables 1 and 2 or are

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described elsewhere in the paper. The nomenclature system recommended by Demerec et al. (12) is employed. Gene symbols are those used by Bachmann and Low (2). Phenotype abbreviations are generally self-evident because of their similarity to genotype designations. The ones which differ substantially or for which no corresponding genotype designations are used follow: Mit, mitomycin; str, streptomycin; Spc, spectinomycin; Ura, uracil; IV, isoleucine and valine; TL, threonine and leucine; UV, ultraviolet radiation; Sup, nonsense suppressor; Mod, modification; and Res, restriction. The superscripts used with phenotype abbreviations include: +, independent when used with the abbreviation of an amino acid or pyrimidine, utilizing when used with the abbreviation of a sugar, and proficient when used with Rec; −, requiring, nonutilizing, or deficient, respectively; r, resistant; and s, sensitive. See reference 20 for further explanation of the nomenclature involving *rec* and Rec.

Most of the bacterial strains used in this study fall into one of three main genetic backgrounds: "AB1157," standing for a line derived from the multiply auxotrophic F⁻ AB1157; "Sup⁻," standing for a line (derived from JC4688) carrying amber *leu* and *trp* mutations in the absence of amber-suppressing alleles; and "KL16," standing for a line derived from Hfr strain KL16. See the article by Bachmann (1) for the relationships among these genetic lines and for the pedi-

TABLE 1. *Basic bacterial strains*

Basic strain	Sex	PO[a]	Mutant genotype[b]	Reference or source
AB261	Hfr	3	*metB1 rel-1* (Hfr P4x-6)	(13)
AB1157	F⁻		*thr-1 leuB6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31 tsx-33 supE44*	(1)
AB3311	Hfr	19	*metB1 thi-1 rel-1* (Hfr Reeves 1)	(1)
AT2092	F⁻		*his-1 purF1 pheA2*::Mu *argH1 thi-1 lacY1* or *lacZ4 malA1 xyl-7 mtl-2 rpsL8*, *-9*, or *-14 tonA2* or *-14 tsx-23* or *-25 supE44* λ⁻(?)	(33)
JC158	Hfr	1	*serA6* (Hfr H)	(4)
JC5029	Hfr	45	*thr-300 ilv-318 rpsE300* (from KL16)	(5)
JC7221	pJC536		F *thr⁺ leu⁺/thr⁺ leu-6 recA56 pyrB66 tonA2 tsx-315 argG6 rpsL104 malA1 xyl-7 mtl-2*	M. Guyer[c]
KL14	Hfr	68	*thi-1 rel-1*	(1)
KL96	Hfr	44	*thi-1 rel-1*	(1)
KL701	F123	43	*trp⁺/trp-45 pyrD34 thi-1 his-68 recA1 mtl-2 xyl-7 malA1 galK35 rpsL118*	B. Bachmann[d]
PK38	Hfr	202	*thr-1 leu-6 thi-1 supE44 lacY1 lonA21 azi-15*[e]	(21)

[a] PO, Point of transfer origin of an Hfr strain (see Fig. 1 and reference 1).

[b] All strains are λ⁻ unless otherwise indicated. The symbol *rpsL* replaces *strA*, and *rpsE* replaces *spcA* (2); strains carrying mutations in these genes are resistant to streptomycin and spectinomycin, respectively. Strains carrying *malA1* are resistant to lambda.

[c] Leu⁺ [Str^r] transconjugant from the cross of donor JC7105 (19) by recipient JC7133 (19).

[d] Previously called KLF23/KL181. Probably made as a Trp⁺ [Str^r] transconjugant from a cross of B7 by KL181 (26, 27).

[e] Phenotypically λ⁻ and colicin E1 and colicin V resistant. Locus designations for the two mutations conferring the latter two have not been assigned (B. Bachmann, personal communication).

TABLE 2. *Derivatives of basic strains*

Derivative of basic strain	Basic strain ancestor	Mutant differences from basic strain	Reference or source
JC4729	AB1157	*recB21 recC22 sbcB15 metE46*	L. J. Margossian[a]
JC5519	AB1157	*recB21 recC22*	(40)
JC7609	JC4693	*recB21 sbcA20*	A. Templin[b]
JC7661	JC5029	*recB21 recC22 sbcA23*	A. Templin[b]
JC8101	AB1157	*recB21 recC22 sbcB15 ilv-334*	(20)
JC8563	AB261	*ilv-331*	(20)
JC8566	AB261	*recL152* (λ?)	(20)
JC9239	AB1157	*recF143*	(20)
JC9248	AB261	*recF143*	(20)

[a] IV⁺ Met⁻ transductant of JC8101 by P1·AB1976 (15).

[b] Mit^r revertants produced after ethyl methane sulfonate treatment of Mit^s Rec⁻ strains: JC7609 from JC4695 (37) and JC7661 from JC5491. JC5491 is a Thy⁺ Rec⁻ (*recB21 recC22*) transductant from JC5401 by P1·JC5519 (40). JC5401 is a spontaneous *thy-294* mutant of JC5029 (9) selected in the presence of trimethoprim (35).

grees of most of the other strains listed in Tables 1 and 2.

See Figure 1 for the map locations of the genes mentioned, the points of transfer origin of Hfr strains, and the genetic distances spanned by F′ plasmids.

Media. L broth was routinely used for growing bacterial cultures. 56/2 buffer was used for diluting bacterial suspensions. L broth, 56/2 buffer, and 56/2 minimal medium have been described previously by Willetts et al. (41). For solid L or minimal medium, 2% agar was added.

The following media were used for phage stock preparation and phage titration: LCTG agar, L broth supplemented with 4 ml of 0.25% thymidine, 2.5 ml of 40% glucose, and 4 ml of 0.5 M $CaCl_2$ per liter and solidified with 1% agar; L top agar, L broth solidified with 0.7% agar; T (tryptone) broth, 10 g of tryptone (Difco Laboratories) and 5 g of NaCl per liter; T (tryptone) agar, T broth containing 1.0 to 1.2% agar; and T top agar, T broth with 0.7% agar.

General genetic methods. Earlier articles have described the method used for bacterial matings in liquid medium (41), P1 transduction, and replica plate tests for recombination proficiency and UV sensitivity (10). Sensitivity to mitomycin was determined by using L agar plates containing mitomycin C (1 μg/ml).

Exonucleases. ExoVIII activity was assayed as described by Kushner et al. (23), and the enzyme was purified through the fraction V stage as described by Gillen et al. (17). The ExoV assay was identical to that for ExoVIII except that the reaction mixture contained 0.2 mM ATP. The ExoI assay has been given by Kushner et al. (24).

Construction of JC8679, an ExoVIII$^+$ derivative of AB1157. Although ExoVIII$^+$ mutants (i.e., strains that are *sbcA* mutants) have never been isolated in the AB1157 genetic background (36), we were able to construct an ExoVIII$^+$ derivative of AB1157 by conjugation. A rec$^+$ UV^r ExoVIII$^+$ strain, JC8675, was isolated from the His$^+$ [Str^r IV$^+$] progeny of a mating between Hfr strain JC7661 (*recB21 recC22 sbcA23*, PO45) and JC5519 (*recB21 recC22* derivative of AB1157). The *his-328* marker was introduced by conjugation with DS13A, a derivative of KL96, and the resulting strain was called JC8679. The ExoV$^-$ ExoI$^+$ ExoVIII$^+$ phenotype was checked by assays performed upon crude extracts. The presence of ExoVIII was further confirmed by partial purification and characterization of the enzyme (17). The enzyme was indistinguishable from the ExoVIII isolated from a different bacterial strain by Kushner et al. (23).

Isolation of Rec$^-$ mutants from JC8679. A culture of JC8679 was grown in L broth to log phase. The cells were collected by centrifugation, washed twice with 10-ml portions of 0.1 M sodium citrate buffer (pH 5.5), and suspended in 10 ml of NTG solution (50 g of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine per ml in 1.0 M sodium citrate; pH 5.5). The mixture was incubated at 37°C for 15 or 30 min (to give 90 to 95% killing). The cells were then washed twice with 56/2, and a 56/2 suspension was diluted fivefold into L broth and distributed in 1-ml portions to 10 large test tubes. The 10 cultures were grown at 37°C, with shaking, for about 5 h to allow for recovery, segregation of mutated chromosomes, and expression of mutated genes. The

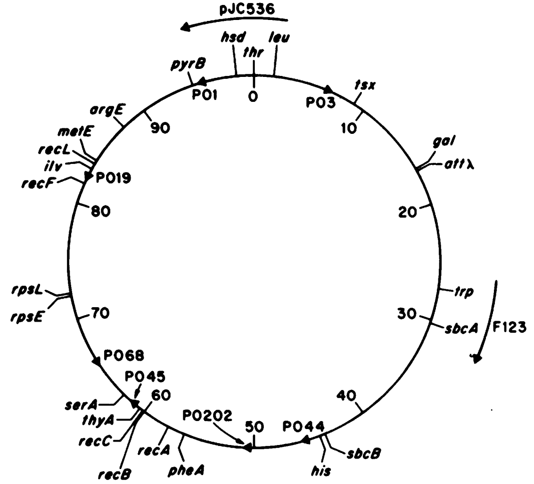

FIG. 1. *Genetic map of E. coli adapted from that of Bachmann and Low (2). Map units in minutes of conjugational transfer time are indicated inside the circle, and genes relevant to the work in this paper are indicated outside. On the circle are indicated points of origin of conjugational transfer of several Hfr strains. These are numbered as recommended by Bachmann (1). The direction of transfer is determined by using the arrowhead as the lead marker transferred point first. Outside the circle are two arcs representing the extent to which F′ plasmids carry the chromosomal genes.*

cultures were then diluted and plated on minimal medium so as to obtain 100 to 200 colonies per plate. For the isolation of Rec$^-$ mutants, colonies were usually picked, patched, and tested by replica plating for recombination ability with two Hfr strains: JC158 and either PK38 or AB3311. Selections were made for TL$^+$ [Ser$^+$ Str^r], His$^+$ [Str^r], and ArgE$^+$ [Str^r] transconjugants, respectively. The use of two Hfr strains was intended to reveal mutants carrying recessive *rec* mutations located near the origin of transfer of one but not the other Hfr. The colonies were also tested for their sensitivity to UV irradiation by replica plating. In one experiment, picking and patching were circumvented by the use of an apparatus designed to dispense droplets, containing zero or one cell each, directly from a cell suspension onto agar plates, forming a grid pattern (courtesy of D. Glaser and the members of his group); the plates were incubated until each cell grew into a colony and then used directly for replica plating.

Test for restriction of unmodified λ. The test for restriction of unmodified λ was patterned after that used by Simmon and Lederberg (33). Cultures of the bacterial strains to be tested were grown to a turbidity of 50 to 120 Klett units in 10 ml of broth (8 ml of T broth, 2 ml of L broth, and 0.06 ml of 20% maltose). The cells were centrifuged, and the pellets were suspended in 7-ml portions of 0.85% saline containing 0.01 M $MgSO_4$ and shaken for 20 to 30 min at 37°C. A 0.2-ml volume of the bacterial suspension was combined with 0.1 ml of λ phage (diluted in T broth) in a small test tube and allowed to stand for 15 min at room

temperature before being plated with 2.5 ml of melted T top agar on a T plate. Plaques were counted after overnight incubation of the plates at 37°C. The unmodified phage used was λ *vir* that had been grown through (at least) two cycles on the *hsd-15* mutant bacterial strain JC9646 (formerly "342-1"); the modified phage used as a control was λ *vir* that had been grown on the hsd^+ strain JC9645 (formerly "342"). In every experiment, restricting strain C600 and nonrestricting (nonmodifying) mutant strain C600.4 were used as controls. JC9645 and JC9646 are *met-161 pur-70 thy-145* derivatives of C600. JC9646 is an *hsd-15* mutant of JC9645 produced by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (S. Lederberg, personal communication). Strains C600, C600.4, JC9645, and JC9646 were obtained from V. Simmon (33).

Southern blotting technique. To identify restriction nuclease digestion products of the *E. coli* chromosome hybrizable to phage DNAs, the Southern blotting technique (34) was used as modified by many investigators. The procedures used in each step of the technique are described below.

(i) Isolation of bacterial and phage DNA. Bacterial DNA was isolated from 25-ml cultures grown at 37°C with aeration in Luria broth. The cells were harvested in the log phase and washed with 0.15 M NaCl, and the pellet was suspended in 2.5 ml of 25% sucrose in 50 mM Tris-hydrochloride (pH 7.4). All subsequent operations were performed at approximately 4°C (except where otherwise noted). Next, 0.5 ml of lysozyme (10.0 mg/ml in 0.25 M Tris-hydrochloride, pH 8.0) was added, and the solution was mixed gently and incubated for 5 min. EDTA (1.0 ml, 0.25 M, pH 8.0) was added and mixed gently, and the solution was incubated for 5 min. The cells were lysed by adding 80.0 μl of 25% sodium dodecyl sulfate and mixing gently. This resulted in a clear, very viscous lysate. The RNA was removed from the lysate by adding 0.4 ml of heat-treated RNase (5.0 mg/ml in 50 mM sodium acetate, pH 5.0) and incubating at 37°C for 20 min. The lysate was extracted three times with 4.0 ml of phenol (equilibrated with 50 mM Tris-hydrochloride, pH 7.4) plus 2.0 ml of chloroform-isoamyl alcohol (24:1, vol/vol) by inverting the mixture several times in a 15-ml screw-cap tube. The phases were separated at 5,000 rpm (Sorvall GLC-1 centrifuge) for 10 min. The aqueous phase was then extracted once with 4.0 ml of chloroform-isoamyl alcohol (24:1), and the phases were separated as before. The aqueous phase was then dialyzed extensively against TE buffer (1 mM Tris-hydrochloride [pH 7.4], 1 mM EDTA; pH 8.0). The DNA concentration was measured by absorbance at a wavelength of 260 nm. A typical yield was 2.0 ml of solution containing 250 μg of DNA per ml.

DNA was isolated from purified phage particles essentially as described by Wu et al. (43). Phage λ *c*I857 *S7* was obtained by lytic growth in *E. coli* strain C600, and phage λrev *c*I857 *S7* was obtained by thermal induction of lysogen N2262(λrev *c*I857 *S7*). Cell lysis was brought about by the addition of chloroform to the culture since the *S7* mutation blocks lysis by the phage. DNA was released from purified phage particles by treatment with distilled phenol equilibrated with 50 mM Tris (pH 7.4). Excess phenol was removed by extracting once with chloroform-isoamyl alcohol (24:1). The resulting aqueous phase was dialyzed extensively against TE buffer.

(ii) Endonuclease digestions and gel electrophoresis. Reaction conditions for restriction endonuclease digestions have been described previously (42). Restriction endonucleases *Eco*RI, *Hin*dIII, and *Bam*HI were purchased from New England Biolabs. In a typical reaction, 7.0 μg of bacterial DNA was incubated with 15.0 U of *Eco*RI in a total volume of 40 μl at 37°C for 2 h. The reaction mixture was then heated to 65°C for 10 min and cooled to 4°C.

DNA fragments were separated by electrophoresis in 0.7% (wt/vol) agarose gel in TEB buffer (0.09 M Tris, 2.8 mM EDTA, 0.09 M boric acid) on a horizontal gel apparatus (29). The running gel on this apparatus had the following dimensions: 13.3 by 21.5 by 0.6 cm. The samples (20 μl) containing 3.5 μg of bacterial DNA were mixed with 10 μl of loading solution (0.05% bromothymol blue, 10% Ficoll) and applied to wells in the gel. Electrophoresis was carried out at 3.7 V/cm until the dye front was 19.5 cm from the wells (ca. 15 h at 20°C). The gels were calibrated by two standards added at 17 ng to a well in the gel: λ *c*I857 *S7* DNA digested with *Hin*dIII and with *Hin*dIII plus *Bam*HI.

(iii) In vitro labeling of phage DNA. Phage DNA was labeled in vitro with ^{32}P by a "nick translation" procedure modified from that of Rigby et al. (31). Phage DNA (1 μg) was mixed with 125 pmol of two α-^{32}P-labeled deoxynucleoside triphosphates (Amersham Corp.; 400 Ci/mmol) and 1 nmol of each of the other two unlabeled deoxynucleoside triphosphates in 100 μl of 50 mM Tris-hydrochloride (pH 7.5)–10 mM $MgCl_2$–1 mM dithiothreitol–50 μg of bovine serum albumin per ml. The reaction was started by the addition of 50 pg of DNase I (Sigma Chemical Co.) and 6 U of DNA polymerase I (Boeringer Mannheim Corp.), incubated at 15°C for 1 h, and terminated by the addition of EDTA to a final concentration of 20 mM. These conditions resulted in the incorporation of 30 to 60% of the input label into acid-insoluble material. Labeled phage DNA probes were denatured by heating at 100°C for 10 min before use.

(iv) Transfer of DNA from agarose gels to nitrocellulose and hybridization with ^{32}P labeled phage DNA probes. Transfer of DNA fragments separated by electrophoresis in agarose gel was by the method of Wahl et al. (38) with the following modifications. The optimum conditions for depurination were found to be 8 min in 250 ml of 0.25 M HCl for gels of the dimensions used in this study. After elution, the filters were washed in 125 ml of 2× SSC (SSC = 0.15 M NaCl plus 0.015 M sodium citrate) for 5 min and 70% ethanol for 5 min and baked overnight at 65°C. The nitrocellulose filters were cut in half, and each half was hybridized with 10^6 cpm of ^{32}P labeled phage DNA for 12 to 16 h at 42°C sealed in plastic bags (38). The filters were then washed in 2× SSC–0.1% sodium dodecyl sulfate (three times, 5 min each) and 0.1× SSC–0.1% sodium dodecyl sulfate (two times, 15 min each at 42°C) and rinsed in 70% ethanol for 5 min. After drying at 65°C, the filters were autoradi-

ographed with presensitized X-ray film, covered by a phosphotungstate intensifying screen, and exposed at −70°C for 2 days to 2 weeks.

RESULTS

Effect of *recA*, *recF*, and *recL* mutations on bacterial recombination via the RecE pathway. To determine the roles in the RecE pathway played by several genes associated with recombination, strains were constructed, in various genetic backgrounds, that carried mutations in *recA*, *recF*, or *recL* in addition to mutations in *recB* or *recC* or both and *sbcA*. The abilities of these multiple mutants to carry out conjugational recombination are summarized in Table 3.

(i) *recA*. A *recA56* derivative of JC8679 (*recB21 recC22 sbcA23*) was constructed by crossing JC8679 with the *recA56 recB21* KL16 Hfr, JC7505, and selecting His$^+$ [Str^r IV$^+$] progeny. About 10% (14/124) of the recombinants were UV^s Rec$^-$ in patch tests, hence putative *recA56 recB21* (*recC22?*) *sbcA23* strains. This genotype was confirmed for one recombinant, JC9604, by assay for ExoVIII activity and by cotransduction of the *recA56* allele with *pheA*. Strain JC9604 had two characteristics of *recA* single mutants (Table 3): extreme deficiency in the ability to produce recombinants in conjugation with Hfr strains and proficiency in the ability to produce merodiploid transconjugants after conjugation with F′ strains. (A *recA* mutant derivative of JC8679 made by nitrosoguanidine mutagenesis showed similar behavior [see Table 4].) Thus, it appears that the *recA* function is essential for bacterial recombination via the RecE pathway, as it is for recombination via the RecBC and RecF pathways.

A strain of genotype *recA56 recB21 sbcA20* was constructed in the Sup$^-$ background. The *recA56 recB21* Hfr JC7505 was crossed with JC8661, a *thyA254* derivative of the *recB21*

TABLE 3. *Recombination and repliconation proficiencies of recB sbcA strains carrying a recA, recF, or recL mutation*

Genetic line	Strain	Genotype					Deficiency indexes[a]	
		recA	*recB*	*recF*	*recL*	*sbcA*	Hfr crosses	F′ crosses
AB1157	AB1157	+	+	+	+	?[b]	≡1[c]	≡1[c]
	JC8679	+	*21*	+	+	*23*	1	2
	JC9604	*56*	*21*	+	+	*23*	$>10^5$	10
	JC9610	+	*21*	*143*	+	*23*	10^2	10
	JC9613	+	*21*	+	+	*23*	1	2
	JC7871	+	*21*	+	*152*	*23*	1	1
Su$^-$	JC4693	+	+	+	+	+	≡1[d]	≡1[d]
	JC7609	+	*21*	+	+	*20*	1	1
	JC8672	*56*	*21*	+	+	*20*	10^4	5
	JC8631	+	*21*	+	+	*20*	≡1[e]	≡1[e]
	JC8630	+	*21*	*143*	+	*20*	35	1
KL16	JC5029	+	+	+	+	+	≡1[f]	ND[f]
	JC7661	+	*21*	+	+	*23*	2	ND
	JC8633	+	*21*	*143*	+	*23*	25	ND
	JC8638	+	*21*	+	+	*23*	2	ND
	JC8651	+	*21*	+	*152*	*23*	1	ND
	JC8648	+	*21*	+	+	*23*	1	ND

[a] A deficiency index is calculated by dividing the recombinant (or merodiploid) frequency obtained with a Rec$^+$ control strain by the recombinant (or merodiploid) frequency obtained with the strain in question. Interrupted matings of 60 min were performed in liquid medium. JC158 was the donor in all Hfr crosses; the selections are given in footnotes *b* through *e*. In most F′ crosses, JC7221 was the F′ donor, and the merodiploids selected were TL$^+$ [Ura$^+$] or Leu$^+$ [Ura$^+$]; the exception is noted in footnote *d*. The recombinant and merodiploid frequencies for the Rec$^+$ control strains ranged from 5 to 35%, with one exception (noted in footnote *d*).

[b] The *sbcA* genotype of AB1157 is unknown because this strain does not carry the prophage on which *recE* and *ral* genes are located and whose expression is required for the detection of *sbcA* mutations (22).

[c] For Hfr JC158, the selection was TL$^+$ [Ser$^+$ Str^r].

[d] For Hfr JC158, the selection was Trp$^+$ [Ser$^+$ Str^r]. Trp$^+$ recombinants were obtained at a frequency of only 0.2%. A Leu$^+$ selection could not be used here because JC8631 and JC8630 were already Leu$^+$. The F′ donor was KL701, and the selection was for Trp$^+$ [Str^r].

[e] For Hfr JC158, the selection was for Thr$^+$ [Ser$^+$ Spc^r].

[f] Since the strains derived from KL16 were Hfr strains, the repliconation test with F′ plasmid donors was omitted.

sbcA20 Sup$^-$ strain JC7609, and Thy$^+$ [IV$^+$ Thr$^+$] recombinants were selected. Of 132 recombinants tested, one was UV^s Rec$^-$ ExoVIII$^+$, JC8672. Like JC9604, JC8672 was competent in merodiploid formation but extremely deficient in recombinant formation (Table 3).

(ii) *recF*. Gene *recF* plays a major role in the RecF pathway but is completely dispensible for the RecBC pathway. To determine whether or not it is involved in the RecE pathway of bacterial recombination, suitable strains were constructed in three different genetic lines: AB1157, Sup$^-$, and KL16.

To construct a *recF143 recB21 recC22 sbcA23* strain in the AB1157 line, an *ilv-331* derivative of JC8679, JC9606, was transduced to IV$^+$ with a P1 lysate made on JC9239, an *ilv$^+$ recF143* strain. About 2.5% of the transductants would be expected to have inherited *recF143* (20). Indeed, about 2% (4/158) of the IV$^+$ transductants were found to be both UV^s and Rec$^-$ in patch tests. To establish that these IV$^+$ UV^s Rec$^-$ strains were really *recF* mutants, P1 was grown on one of them, JC9610, as well as on an IV$^+$ UV^r Rec$^+$ strain produced in the same experiment, JC9613, and used to transduce an *ilv-334 recB21 recC22 sbcB15* (i.e., RecF pathway) strain, JC8101, to IV$^+$. In the case of the JC9610 donor, about 2% (17/773) of the IV$^+$ transductants of JC8101 were UV^s Rec$^-$; in the case of the JC9613 donor, none (0/475) was UV^s or Rec$^-$. ExoVIII was present at normal *sbcA* mutant levels in both JC9610 and JC9613. Thus, a mutation in *recF* impairs the proficiency of the RecE pathway of recombination as well as makes the strain UV sensitive.

Since the Sup$^-$ strains do not carry any genetic markers cotransducible with the *recF* locus, conjugation was used to introduce *recF143* into this genetic background. *recF143* Hfr strain JC9248 was crossed with Sup$^-$ *recB21 sbcA20* strain JC7609, and Leu$^+$ [Str^r] recombinants were selected. Of several hundred recombinants, one, JC8630, was UV^s and partially Rec$^-$ in patch tests. Since JC8630 is *leu$^+$*, the *leu* marker could not be used to test recombination proficiency in the standard way with JC158. Instead, the *trp* marker was used with JC158, and JC8630 was found to have a deficiency index of about 35 compared to JC7609 (Table 3); JC8631, a UV^r strain generated in the same cross as JC8630, gave the same frequency of recombinants as JC7609 (Table 3). To prove that JC8630, despite its relatively low recombination deficiency, was really a *recF* mutant, the *recF143* allele was transduced with *ilv$^+$* out of JC8630 into JC8101, at a cotransduction frequency of 3% (9/300). The resulting UV^s Rec$^-$ transductants, which now carried *recF143* in a *recB21 recC22 sbcB15* background, had deficiency indexes characteristic of a block in the *recF* step of the RecF pathway, i.e., 2×10^3 to 6×10^3 in a standard cross with JC158.

A *recF143 recB21 recC22 sbcA23* strain, JC8633, was constructed in the KL16 genetic background by using stationary-phase (F$^-$ phenocopy) cells of the *recB21 recC22 sbcA23* strain, JC7661, as a recipient in a cross with JC9248 and selecting for IV$^+$ [Spc^r] recombinants. Of these recombinants, 30% (40/120) were UV^s, and when several were made F$^-$ phenocopies and tested in a cross with JC158, with selection for Thr$^+$ [Spc^r] recombinants, they had deficiency indexes of 10 to 35. A typical UV^r recombinant, JC8638, had a deficiency index of 2. The mutant *recF* gene was transduced with *ilv$^+$* out of the UV^s recombinant JC8633 into JC8101 at a cotransduction frequency of 2% (6/300), and the transductants tested had the predicted deficiency indexes ($\geq 10^3$) in test crosses.

(iii) *recL*. Since *recL*, like *recF*, maps close to *ilv*, a method similar to that used for *recF* was used to introduce *recL152* into the KL16 genetic background. The cross was done by using as the donor the *recL152* Hfr JC8566 and as the recipients the *recB21 recC22 sbcA23* strain JC7661 and, as a control, the *recB$^+$ recC$^+$ sbcA$^+$* strain JC5029. It was known that a *recL* mutation in the latter genetic background confers UV sensitivity, although not recombination deficiency (20). Of the IV$^+$ [Spc^r] progeny from the cross with JC5029, approximately 39% (31/80) were UV^s; of the IV$^+$ [Spc^r] progeny from the cross with JC7611, a similar percentage, about 29% (48/163), were UV^s. These results suggest that a *recL* mutation confers UV sensitivity in a *sbcA23* strain as well as in *sbcA$^+$* strains.

Seven IV$^+$ UV^s and one IV$^+$ UV^r recombinants from JC7661 were purified, as were UV^s and UV^r recombinants from JC5029. All were then used as recipients in test crosses with JC158. The same numbers of Thr$^+$ [Spc^r] recombinants were produced in all cases. The conclusion is that *recL* is not involved in the RecE pathway of bacterial recombination. To confirm the *recB21 recC22 sbcA23 recL152* genotype, assays for ExoV and ExoVIII were carried out, and the *recL152* allele was transduced with *metE$^+$* into a RecF pathway strain, JC4729 (*recB21 recC22 sbcB15 metE46*).

K. McLoed, A. Templin, and A. J. Clark (unpublished data) have constructed a *recL152* derivative of the *recB21 recC22 sbcA23* AB1157 strain, JC8679. This new strain, JC7871, is UV^s but Rec$^+$, just like the strains similar in the KL16 genetic background. JC7871 has been shown to be ExoVIII$^+$.

Isolation and preliminary characteriza-

tion of Rec⁻ mutants from the *recB21 recC22* ExoVIII⁺ strain, JC8679. An analysis of phenotypically Rec⁻ mutants derived from a *recB21 recC22 sbcA23* strain should reveal the steps involved in the RecE pathway of recombination. Among the mutations leading to recombination deficiency in such a strain, one would expect to find mutations in genes (such as *recA* and *recF*) shared by the RecE and other pathways as well as in genes unique to the RecE pathway. The latter class of mutation is expected to include mutations to $sbcA^+$ (from *sbcA23*) and mutations to $recE^-$ (from $recE^+$); all such mutations should eliminate ExoVIII activity. One would also expect to find mutations in RecE pathway genes not affecting ExoVIII.

Cultures of JC8679 were treated with nitrosoguanidine, and survivors were screened by standard replica-plating methods for recombination deficiency and sensitivity to UV radiation. Potential mutants were tested for recombination ability by crossing with the Hfr JC158 in liquid medium and selecting for TL^+ [Ser^+ Str^r] transconjugants. To make certain that this reduction reflected inability to perform recombination rather than inability to act as conjugal recipients, we crossed the strains with a donor carrying thr^+ leu^+ on the F′ plasmid pJC536. TL^+ [Ura^+ Str^r] transconjugants were selected, and the deficiency index relative to JC8679 was calculated (column 3, Table 4). Some reduced ability to inherit pJC536 was found for all the mutant strains. Those included in Table 4 showed more than 10 times greater deficiency in the cross with the Hfr than in the cross with the F′ strain. A total of 7,800 colonies were screened, and six mutants were found. They were subdivided into *recA*, ExoVIII⁻, and other mutants as indicated below.

(i) *recA* mutants. *recA* mutants were recognized by their behavior with JC5029 and JC5088, which are Hfr strains transferring *recA* early. The former is $recA^+$; the latter is *recA56*. Since His^+ [IV^+ Str^r] transconjugants are formed from zygotes into which the *recA* allele has been transferred, *recA* mutant recipients will be recombination proficient with the $recA^+$ donor and deficient with the *recA56* donor. In confirmation of the *recA* mutant genotype, the mutation in JC8682 was cotransduced with $pheA^+$ into AT2092 at a frequency characteristic of a *recA* mutation, 1.8% (9/500). The partial deficiency of JC8682 with JC5029 indicated that *recA76* was partially dominant under these conditions (5; L. N. Csonka, A. Templin, and A. J. Clark, unpublished data). Although cotransduction of *rec-158* with *pheA* was not done, the evidence in Table 4 suggests that it is recessive.

TABLE 4. *Properties of primary Rec⁻ mutants as compared to their parent, JC8679*[a]

Strain	Deficiency index				ExoVIII sp act	UV	Suspected mutational difference from JC8679
	JC158 TL^+	JC7221 F′ TL^+	His^+				
			rec^+ JC5029	*recA* JC5088			
Parental strains							
JC8679	1 (8%)	1 (16%)	1 (2%)	1 (4%)	28	R	None
JC5519	50–500	7	8	4	19	S	$sbcA^+$
recA mutants							
JC8682	$\geq 10^4$	5	150	$>10^4$	25	VS	*recA76*
JC9636	10^4	10	11	>1,700	24	VS	*recA158*
ExoVIII mutants							
JC8689	140	3	3	3	17	S	$sbcA^+$
JC8690	120	3	3	3	20	S	$sbcA^+$
JC8691	150	2	4	3	17	S	*recE159*
Other mutants							
JC8683	700	17	12	20	25	S	*rec-77*

[a] Deficiency index is defined in footnote *a* of Table 3. See Tables 1 and 2 and Fig. 1 for points of origin and direction of transfer of Hfr and F′ strains. Selections are indicated for each cross. Contraselections were as follows: with JC158, Ser^+ Str^r; with JC7221, Ura^+ Str^r; with the KL16 Hfr strains JC5029 and JC5088, IV^+ Str^r. Deficiency indexes and recombination or merodiploid frequencies (in parentheses) represent averages of two or more experiments. Symbols used to indicate response to UV are: R, resistant; S, sensitive; VS, very sensitive. ExoVIII assays were performed on crude extracts. Specific activity is defined as units of enzyme activity per milligram protein, where 1 U of activity is defined as the amount of protein catalyzing the release of 1 nmol of nucleotide in 30 min.

Assays on crude extracts of JC8682 and JC9636 cultures showed that ExoVIII activity was present in both strains.

(ii) ExoVIII⁻ mutants. Mutant strains JC8689, JC8690, and JC8691 were indistinguishable from JC5519, their *recB21 recC22* ExoVIII⁻ ancestor, in their behavior in crosses with various *rec*⁺ Hfr strains. These four strains also showed similar deficiencies (deficiency indexes, 30 to 70) with JC7661, a *recB21 recC22 sbcA23* Hfr with the same point of origin as JC5029. These results showed that the recombination occurring in the crosses with the *recB*⁺ *recC*⁺ Hfr strains JC5029 and JC5088 was due to early introduction of the *recB*⁺ *recC*⁺ alleles into the zygotes. On the basis of preliminary data, four other mutants (JC9634, JC9635, JC9637, and JC9638, isolated by M. Kotowicz) appeared very similar to JC5519. Like JC5519, all these strains had reduced levels of ExoVIII activity detectable in assays of crude lysates.

Because the results from assays of crude lysates were somewhat ambiguous, we carried out the ExoVIII purification procedure (23) on mutant strains JC8689, JC8690, and JC8691, as well as on JC8679 and JC5519. The new mutant strains behaved identically to the ExoVIII⁻ ancestor, JC5519, in that fraction V contained less than 3% of the ExoVIII activity shown by fraction V of JC8679 and polyacrylamide gel electrophoresis revealed no band with the mobility characteristic of ExoVIII polypeptide. (See reference 17 for data for JC8679 and JC5519.)

The genotypes of the new ExoVIII⁻ mutants can be predicted from our hypothesis of the *sbcA23 recE*⁺ genotype of JC8679, the ExoVIII⁺ immediate ancestor of these ExoVIII⁻ mutants. If this hypothesis is correct, there are two possible ways in which JC8679 could lose ExoVIII in a single step: by regaining *sbcA*⁺ function (e.g., by *sbcA23* → *sbcA*⁺), resulting in the repression of ExoVIII production, or by losing the wild-type function of *recE*, the postulated ExoVIII structural gene, by point mutation, insertion, or deletion. To examine this, we made use of the restriction pattern of the chromosome spanning *recE*, recently obtained by Kaiser and Murray (22). By using the technique of Southern blotting (see Materials and Methods), these workers have supported the hypothesis that *recE* is carried by a cryptic lambdoid prophage known as Rac (28, 36). λrev is a derivative of λ carrying 8.8 kilobases (kb) of the Rac prophage substituted for 12.7 kb of lambda DNA (18). The substitution includes *recE* (17), and Kaiser and Murray (22) have hypothesized that *recE* is carried on a 7.6-kb *Hin*dIII fragment of the *E. coli* chromosome. In Fig. 2 is shown an *Eco*RI

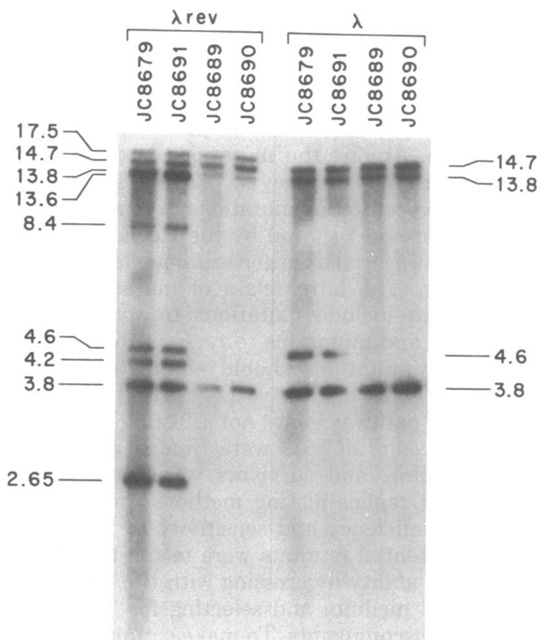

FIG. 2. *An autoradiograph showing EcoRI fragments of ExoVIII⁺ strain JC8679 and its ExoVIII⁻ derivatives, JC8691, JC8689, and JC8690, homologous to ³²P-labeled λrev (λrev cI857 S7) or λ (λ cI857 S7) DNA. Identical samples were applied to each half of a 0.7% agarose gel and electrophoresed. The DNA fragments were transferred to a nitrocellulose filter and cut in half before hybridization with the probe indicated (see Materials and Methods). Molecular weights were determined by the addition of 34 ng of a mixture of λ cI1857 S7 DNA cut with HindIII and HindIII plus BamHI as markers (not shown). Five of the EcoRI bands which share homology with the λrev probe (13.6, 8.4, 4.6, 4.2, and 2.65 kb) are associated with the Rac cryptic prophage (22). These bands are present in the ExoVIII⁺ strain, JC8679, and its ExoVIII⁻ derivative, JC8691, but are not found in the ExoVIII⁻ derivatives, JC8689 and JC8690. The four EcoRI bands (17.5, 14.7, 13.8, and 3.8 kb), which hybridize to λ DNA or λrev DNA or both, are due to homology present in the JC8679 background and are not associated with Rac cryptic prophage (22).*

restriction analysis of DNA from JC8679 and the three ExoVIII mutants. JC8679 DNA contained the four *Eco*RI bands of the Rac prophage which hybridize specifically to λrev (13.6, 8.4, 4.2, and 2.65 kb) including the three (13.6, 8.4, and 2.65 kb) thought to include *recE* (22). DNA from one of the three ExoVIII⁻ mutants (JC8691) showed the same pattern. By contrast, DNA from the other two mutants not only showed none of the Rac prophage bands specifically hybridizing to λrev but also lacked the 4.6-kb band which is part of Rac hybridizable to λ as well as λrev (22). This evidence seems best

explained by the hypothesis that these two ExoVIII⁻ mutants are deleted for the entire Rac prophage, whereas JC8691 is a point mutant either to $sbcA^+$ or $recE^-$. That the latter hypothesis is more likely suggested by examining restriction of unmodified lambda (Table 5). As expected if they were Rac prophage deletion mutants, JC8689 and JC8690 showed no alleviation of restriction; i.e., they were Ral^-. On the other hand, JC8691 was Ral^+, although it alleviated restriction to a lesser degree than did JC8679. This makes it seem likely that JC8691 carries a *recE* mutation rather than an $sbcA^+$ back mutation. The alternative that there is a mutation in another gene polar on *recE* but not on *ral* can not be eliminated at present.

(iii) Other mutants. Three other mutants with interesting properties were isolated. Two of these showed greater deficiency with some Hfr strains than with others. One was UV^s and one was UV^r. Since these strains were relatively recombination proficient in our standard cross with JC158 and since we have not mapped their mutations, we have omitted them from Table 4. We have included the third strain, JC8683, because its recombination deficiency pattern in Hfr crosses (data not shown) suggested that it carried a mutation lying between *lysA* and *argG*. This has recently been confirmed (S. Lovett and A. J. Clark, unpublished data). That this mutation did not repress *recE* or directly inactivate ExoVIII was shown by an assay of crude extracts (Table 4). ExoVIII activity was purified to the fraction V stage (17, 23), and a band with the characteristic mobility of ExoVIII polypeptide was visualized on an sodium dodecyl sulfate-polyacrylamide gel.

DISCUSSION

Recombination in *sbcA* derivatives of *recB* or *recC* mutant strains has been postulated to occur via a RecE pathway (6, 7). The name was intended to indicate the hypothesized dependence of this pathway on the *recE* gene, which was defined to be the structural gene for ExoVIII. To support the hypothesis of separate *sbcA* and *recE* genes, we isolated a mutant in which ExoVIII activity was reduced without elimination of the restriction alleviation activity caused by *sbcA* mutations. The mutation, *rec-159*, is thought to be a point mutation in *recE* because Southern blot analysis of restriction fragments, using the *recE* transducing phage λrev as a probe, revealed no chromosome abnormalities in the *recE159* mutant. In contrast, two mutants lacking both ExoVIII activity and restriction alleviation were shown to be deleted for the chromosomal segment identified as the Rac prophage, which contains *recE* (22). This loss corresponds to the unrecorded loss of Rac in the pedigree of AB1157 (28). Experiments to map *recE159* and another similar mutation are in progress.

To explore the relationship of the RecE pathway to the RecF pathway, we constructed several *recB21 recF143 sbcA* mutant strains. Compared with the *recB21* $recF^+$ *sbcA* parent strains, these strains showed a decrease in conjugational recombinants of more than 10-fold. This result seems to indicate that the RecE

TABLE 5. *Efficiency of plating of modified and unmodified λ vir on ExoVIII⁻ and ExoVIII⁺ hosts*

Host	Host genotype				ExoVIII	EOP of *vir*[a]	
	recB	*recC*	*sbcA*[b]	Other		Modified	Unmodified
C600	+	+	+		−	≡1.0	3×10^{-4}
C600.4	+	+	+	*hsd-15*	−	1.0	≡1.0
AB1157	+	+	−[c]		−	0.9	2×10^{-5}
JC5519	*21*	*22*	−[c]		−	1.0	2×10^{-5}
JC8679	*21*	*22*	*23*		+	1.0	9×10^{-3}
JC8680	*21*	*22*	*23*	*rec-157*	+	0.9	8×10^{-3}
JC8682	*21*	*22*	*23*	*recA76*	+	0.7	1×10^{-2}
JC8683	*21*	*22*	*23*	*rec-77*	+	0.8	2×10^{-2}
JC9604	*21*	*22*	*23*	*recA56*	+	0.6	4×10^{-3}
JC8689	*21*	*22*	−[c]		−	0.7	2×10^{-5}
JC8690	*21*	*22*	−[c]		−	0.6	2×10^{-5}
JC8691	*21*	*22*	*23*		−	0.6	2×10^{-3}

[a] The procedures used to prepare modified and unmodified phage and to determine the efficiency of plating (EOP) are given in Materials and Methods.

[b] The *sbcA* mutant strains carry the *sbcA23* allele.

[c] AB1157, JC5519, JC8689, and JC8690 lack the Rac prophage and hence are deleted for *recE* and may be deleted for *sbcA*.

pathway is actually a branch of the RecF pathway, defined as the ExoV-independent pathway whose effectiveness is inhibited by ExoI (20). Perhaps ExoVIII acts to overcome this inhibition. A diagram invented to illustrate such an hypothesis has already been published (9). Figure 3 is a similar diagram, which illustrates the configuration of both RecE and RecF pathways in the presence of *recF143* and accounts qualitatively for the new experimental results presented here. The figure does not explain, however, why the *recF143* mutation had a much less inhibitory effect on conjugational recombinant production in *recB21 sbcA* mutants than in *recB21 sbcB15* strains, where it reduces recombinant production a 1,000-fold (20). One possibility is that ExoVIII can substitute for the *recF* product but does so only poorly. We know that ExoVIII is not required for the RecF pathway because this pathway was originally discovered in a derivative of AB1157, which has been shown to be deleted for *recE* (22).

The close relationship of the RecE and RecF pathways was also shown by the phenotypes of strains carrying the *rec-77* mutation. This mutation has been mapped near *serA* and shown to reduce conjugational recombinant formation in *recB21 sbcB15* as well as *recB21 sbcA* mutant strains (S. Lovett and A. J. Clark, unpublished data). Like *recF143*, *rec-77* does not reduce recombination in $recB^+$ $recC^+$ strains and so does not affect a gene required for the recBC pathway (Lovett and Clark, unpublished data).

The activity of *recA* is required by the RecE pathway as well as by the RecF (20) and RecBC (8) pathways. Recent work in vitro shows that *recA* protein (RecAp) is active in renaturing single-stranded DNA (39), forming a complex between homologous or nonhomologous single- and double-stranded DNAs (11), and producing D-loops between homologous single- and double-stranded DNAs (30, 32). It is most likely in this role as a synapsis protein that RecAp is required by all three pathways (9). Nonetheless, *recA* has been omitted from the pathway diagram (Fig. 3) because it does not seem appropriate here to discuss whether *recE*, *recF*, *recB*, and *recC* act presynaptically, postsynaptically, or both. It is even possible that the products of one or more of these genes act to catalyze synapsis in concert with RecAp. Clark (*in* B. Alberts and C. F. Fox, ed., *Mechanistic Studies of DNA Replication and Genetic Recombination, ICN-UCLA Symposia on Molecular and Cellular Biology XIX*, in press) has speculated on these matters elsewhere.

ACKNOWLEDGMENTS

We gratefully acknowledge the advice of L. Margossian concerning the experiments with *recL152* and the assistance of E. Blackburn in mastering the techniques concerned with the Southern blotting procedure. M. Kotewicz provided unpublished data on independently isolated Rec^- derivatives of

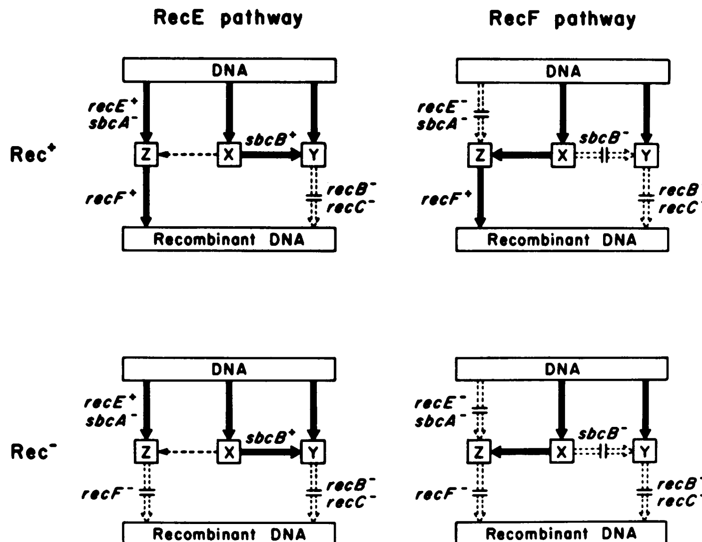

FIG. 3. *Formal diagram showing dependence of the RecE and RecF pathways of recombination on recF. Hypothetical branch point intermediates are symbolized as X, Y, and Z. Arrows are not intended to indicate the number of steps. Solid and single dashed-line arrows indicate that a portion of the pathway is operative at high or low effectiveness, respectively. A double dashed line arrow crossed by two lines indicates a genetic block. Genotype is indicated above arrows representing known genes, and the corresponding phenotype as determined by Hfr with* F^- *crosses is stated at the far left.*

JC8679, and K. Kaiser and N. Murray provided unpublished data on prophage Rac, for which we are also grateful.

This work was supported, in part, by Public Health Service research grant AI05371 from the National Institute of Allergy and Infectious Diseases. During much of this investigation, J. R. Gillen was a Predoctoral Trainee, supported by Public Health Service training grant AI00120 from the National Institute of Allergy and Infectious Diseases. D. K. Willis was a Predoctoral Trainee, supported by Public Health Service training grant GM07232 from the National Institute of General Medical Science. During a portion of this study, A. J. Clark was a Miller Research Professor at the University of California, Berkeley.

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