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Characteristics of Some Multiply Recombination-Deficient Strains of *Escherichia coli*

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Strains of *Escherichia coli* have been made carrying lesions in more than one gene determining recombination. The following genotypes were constructed and verified: *recC22 recB21 recA*$^+$, *recC22 recB21 recA13*, *recC22 recB*$^+$ *recA13*, and *recC*$^+$ *recB21 recA13*. All multiple *rec*$^-$ strains carrying *recA13* were similar to AB2463, which carries *recA13* alone, in their UV sensitivities, recombination deficiencies, and inabilities to induce lambda phage in a lysogen. However, whereas AB2463 shows a high rate of ultraviolet (UV)-induced deoxyribonucleic acid (DNA) breakdown, the multiple *rec*$^-$ strains showed the low level characteristic of strains carrying *recC22* or *recB21* alone. The strain carrying both *recC22* and *recB21* was similar in all properties to the single mutants, suggesting that both gene products act in the same part of the recombination and UV repair pathways. It is concluded that in a Rec$^+$ strain, the *recA*$^+$ product acts to inhibit DNA breakdown determined by the *recC*$^+$ and *recB*$^+$ products.

Mutations in at least three different genes can give rise to recombination deficiency in *Escherichia coli*. Mutations in the gene denoted *recA* (4, 6, 11) give strains which are highly recombination-deficient, very UV-sensitive, and whose lambda lysogens show a very low level of spontaneous or UV-induced phage production (2, 4, 9). A high rate of UV-induced deoxyribonucleic acid (DNA) breakdown is observed in these strains (5, 11). Such mutations have been shown to map between *cysC* and *pheA* (14). Mutations giving rise to a different phenotype have been separated into two complementation groups, one comprising mutations in *recB*, the other mutations in *recC* (Willetts and Mount, *unpublished data*). Strains carrying mutations in these genes are much less recombination-deficient and UV-sensitive than *recA* mutants, and their lambda lysogens show a normal level of spontaneous or UV-induced phage production (2, 4; Willetts and Mount, *unpublished data*). UV-induced DNA breakdown after UV irradiation is less than normal in these strains (7, 11). Both *recB* and *recC* map between *thyA* and *argA* (7; Willetts and Mount, *unpublished data*).

Strains carrying mutations in any two or all three of these loci have been constructed by conjugation and P1 transduction. Their properties are described, and some deductions are made as to the possible functions of the three loci in the process of recombination.

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MATERIALS AND METHODS

Bacterial strains are described in Table 1.

Media have previously been described by Adelberg and Burns (1). L medium was the standard complex medium, and minimal medium was a 56/2 salts mixture to which organic supplements were added.

Procedures. Matings in liquid medium were carried out by the standard procedure of Adelberg and Burns (1). P1 transduction was performed as described by Willetts, Clark, and Low (14). Plate tests for UV sensitivity and recombination ability were performed as described by Clark and Margulies (6).

UV survival curves. To determine survival of different strains after UV irradiation, cultures were first grown at 35 C, with shaking in L broth, to a density of about 2×10^8 cells/ml. They were then diluted 10^{-2} in 56/2 phosphate buffer at 0 C, and 5-ml samples were pipetted into glass petri dishes (9 cm in diameter), giving a layer less than 1 mm thick. These were irradiated with light from a 15-w G.E. germicidal lamp placed 45 cm from the dish. The intensity of the light was reduced when required by interposing sheets of Saran Wrap. The output of the lamp was monitored both before and after the experiment with a photocell standardized by R. Latarjet coupled with a Weston d-c microammeter; the average was used to calculate the dose given. Samples were withdrawn after various times; they were then diluted and plated in duplicate on L plates. These were incubated in the dark for 1 to 2

TABLE 1. *Bacterial strains used*[a]

Strain no.	Sex	*rec*	Sm	*lac*	*thy*	*his*	Other characteristics
KL98	Hfr	+	S	+	+	+	
JC2598	Hfr	+	S	+	+	+	(λ ind$^-$)
JC5029	Hfr	+	S	+	+	+	*thr300, ilv318, spc-300*
JC5088	Hfr	*recA56*	S	+	+	+	*thr300, ilv318, spc-300*
JC5412	Hfr	*recB21*	S	+	+	+	*thr300, ilv318, spc-300*
JC5426	Hfr	*recC22*	S	+	+	+	*thr300, ilv318, spc-300*
E3	F′	+	S	+	+	+	F*lac*$^+$/*lac*$^-$
AB1157	F$^-$	+	R	*lac-1*	+	*his-4*	*thr-4, leu-8, proA2, arg-3, thi*$^-$
AB2463	F$^-$	*recA13*	R	*lac-1*	+	*his-4*	AB1157 UV^{S}Rec$^-$
AB2470	F$^-$	*recB21*	R	*lac-1*	+	*his-4*	AB1157 UV^{S}Rec$^-$
JC2915	F$^-$	+	R	*lac-1*	+	*his-4*	*cysC39*
JC5421	F$^-$	*recA13*	R	*lac-1*	*thyA326*	*his-4*	
JC5474	F$^-$	*recC22*	R	*lac-1*	+	*his-4*	
JC5408	F$^-$	*recB21*	R	*lac-1*	*thyA333*	*his-4*	
JC5422	F$^-$	+	R	*lac-1*	*thyA325*	*his-4*	
CP154	F$^-$	+	R	*lac-?*	+	*his-?*	*argA321*

[a] Derivations of strains are given by Clark (4) and Willetts and Mount (*in preparation*), except for that of JC2915 which is a *thy*$^+$*cysC39* transductant of JC5422 using P1 grown on AT714 (*from* A. L. Taylor). The gene symbols used are those recommended by Taylor and Trotter (13). Abbreviations used are the following: Sm, streptomycin; Rec, recombination; Thy, thymine; His, histidine; Lac, lactose; +, proficient, independent, or utilizing; −, deficient, dependent, or nonutilizing; S, sensitive; R, resistant.

days and then counted. Two separate determinations of the survival curve of each strain were made, using different cultures on different days, and the average was taken. The results given combine separate experiments, some done with the attenuated source measuring the effects of doses up to 50 ergs/mm^2 and others done with the unattenuated source measuring the effects of high doses up to 500 ergs/mm^2.

DNA degradation. The method for labeling DNA with ^{3}H-thymidine and measuring the release of acid-soluble radioactivity after UV irradiation was essentially that described by Clark, et al. (5). A dose of 570 ergs/mm^2 was routinely used.

Lambda lysogenization. The method used to measure the frequency of lysogenization by lambda phage was that described by Brooks and Clark (2).

Spontaneous and UV-induced production of lambda phage. The method used to measure the spontaneous and UV-induced production of lambda by its lysogens has been described by Brooks and Clark (2).

Zygotic induction. The method described by Clark and Margulies (6) was used.

RESULTS

Construction of the multiple rec$^-$ mutant strains. Construction of the multiple *rec*$^-$ mutants depended on three things: (i) the recessiveness of all the mutations involved, (ii) the ability to transfer the mutations conveniently by conjugation and transduction, and (iii) the ability to differentiate multiple *rec*$^-$ mutants from single *rec*$^-$ mutants. We chose to use the following recessive mutations as representative of each *rec* gene: *recC22*, *recB21*, and *recA13* (4; Willetts and Mount, *unpublished data*). Transfer of the mutations and the mode of detecting the multiple *rec*$^-$ mutants are described below.

Construction of an F$^-$ strain carrying recC22 and recB21. An F$^-$ strain carrying *recC22* and *recB21* was constructed by transducing JC5408 (*thyA333 recC*$^+$ *recB21*) with P1 grown on JC5474 carrying *thyA*$^+$ *recC22 recB*$^+$, selecting Thy$^+$ transductants. Since both *recC* and *recB* are very close to *thyA*, many of the *thyA*$^+$ transducing fragments should carry both *recC22* and *recB*$^+$. Those transductional zygotes that receive such fragments will then be heterozygous not only for *thyA* (*thyA*$^+$/*thyA333*) but also for *recC* (*recC22*/*recC*$^+$) and for *recB* (*recB*$^+$/*recB21*); hence, they will be Rec$^+$. Two cross-overs at the appropriate places should then give the double *recB21 recC22* recombinant (Fig. 1A). One hundred Thy$^+$ transductants were patched onto minimal media (i.e., without thymine), and were replica-plated onto medium selective for His$^+$[Sm^R] recombinants which bore separately the Hfr strains JC5029 (*recC*$^+$ *recB*$^+$), JC5412 (*recC*$^+$ *recB21*), and JC5426 (*recC22 recB*$^+$). JC5426 and JC5412 are both derived from JC5029. All three transfer the three *rec* genes prior to the transfer of the *his* operon. In crosses with these Hfr strains, a normal yield of His$^+$[Sm^R] recombinants is formed only if the temporary conjugational zygotes are pheno-

typically Rec⁺. Thus a transductant clone giving a normal yield of His⁺[Sm^R] recombinants with JC5029, but not with either JC5426 or JC5412, should carry both *recC22* and *recB21*. One such clone, JC5519, was purified and tested as indicated below to confirm its genotype.

Construction of an Hfr strain carrying recC22 and recB21. To construct a triple *rec* mutant, i.e., one carrying *recC22 recB21* and *recA13*, a donor strain carrying *recC22* and *recB21* was first made. P1 grown on JC5519 (*thyA*⁺ *recC22 recB21*) was used to transduce a Thy⁻ derivative of the Hfr strain JC5029. Thy⁺ transductants which had inherited both *recC22* and *recB21* were expected to be formed by the cross-overs shown in Fig. 1B and were detected by a technique similar to that used to detect the double *rec*⁻ mutant, JC5519, described above. Patches of transductant clones were replica plated to selective medium bearing separately the F⁻ strains AB1157 (*recC*⁺ *recB*⁺), AB2470 (*recC*⁺ *recB21*), and JC5474 (*recC22 recB*⁺). Again, His⁺[Sm^R] recombinants were selected. A transductant clone, JC5491, which sired a normal number of recombinants with AB1157 but a reduced number with both JC5474 and AB2470, was purified and retested. It was subsequently crossed with both Rec⁻ F⁻ strains in liquid medium and was found to sire 1/80 and 1/200 as many His⁺[Sm^R] recombinants with AB2470 and JC5474, respectively, as it sired with AB1157.

Construction of an F⁻ strain carrying recC22 recB21 and recA13. In the second step of the construction of a triple *rec*⁻ mutant, the *thyA*⁺ *recC22 recB21 recA*⁺ Hfr, JC5491, was mated with the F⁻ JC5421 which carries *thyA326 recC*⁺ *recB*⁺ and *recA13*. This cross produces zygotes heterozygous for *thyA recC recB* and *recA*, and because of the recessiveness of the mutant *rec* alleles these zygotes will be Rec⁺. The mating was interrupted after 15 min by vortexing to reduce transfer and inheritance of *his*⁺. Thy⁺[Sm^R] recombinants were selected and were tested for the hoped-for triple *rec* mutant expected to be formed by two cross-overs such as those indicated in Fig. 1C. The test involved patching the recombinants on minimal medium (i.e., without thymine) and replica plating onto cultures of the following four different Hfr strains spread on medium selective for His⁺[Sm^R] recombinants: JC5029 (*recC*⁺ *recB*⁺ *recA*⁺), JC5412 (*recC*⁺ *recB21 recA*⁺), JC5426 (*recC22 recB*⁺ *recA*⁺), and JC5088 (*recC*⁺ *recB*⁺ *recA56*). [See reference 4 for the derivation of this last strain.] All of the Thy⁺ conjugational recombinants, being His⁻ Sm^R, were expected to mother large numbers of His⁺[Sm^R] recombinants when crossed with

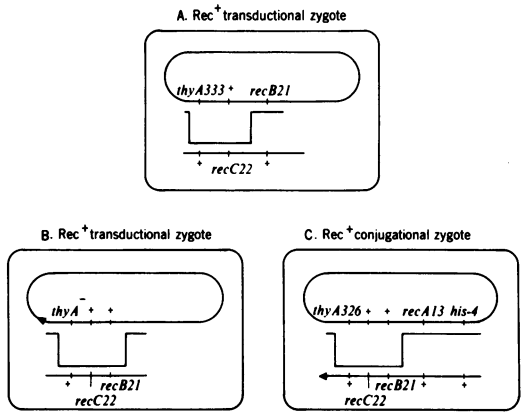

FIG. 1. *Schematic representation of zygotes from which multiple rec⁻ strains are derived. Recipient genomes are represented as closed curves and exogenotes as straight lines. An arrowhead represents the origin of conjugational transfer. Lines between recipient genomes and exogenotes represent cross-overs required to produce multiple rec⁻ progeny.*

the Rec⁺ Hfr strain JC5029; however, any Thy⁺ recombinant which had inherited all three *rec* mutations would be expected to mother reduced numbers of His⁺[Sm^R] recombinants when crossed with each of the three Rec⁻ Hfr strains. One such strain, JC5547, was purified and tested further to confirm its genotype.

Construction of an F⁻ strain carrying recB21 and recA13. To construct a strain carrying *recB21* and *recA13*, a method similar to that used to construct the triple *rec*⁻ mutant was used. The Hfr strain JC5412 which carries *thyA*⁺ *recC*⁺ *recB21* and *recA*⁺ was crossed with F⁻ strain JC5421 which carries *thyA326 recC*⁺ *recB*⁺ and *recA13*. Thy⁺[Sm^R] recombinants were selected after a 15-min interrupted mating period under the expectation that they would arise from zygotes heterozygous not only for *thyA* (*thyA*⁺/*thyA326*) but also for *recB* (*recB21*/*recB*⁺) and *recA* (*recA*⁺/*recA13*). Among 100 Thy⁺[Sm^R] recombinants, those inheriting *recB21* and *recA13* were detected by replica-plate crosses with the following three Hfr strains: JC5029 (*recB*⁺ *recA*⁺), JC5412 (*recB21 recA*⁺), and JC5088 (*recB*⁺ *recA56*). The double *rec* mutants were detected as those producing fewer His⁺[Sm^R] recombinants when crossed with the Rec⁻ Hfr strains than when crossed with the Rec⁺ Hfr. One such strain, JC5495, was purified for further testing.

Construction of an F⁻ strain carrying recC22 and recA13. To construct a double *rec* mutant carrying *recC22* and *recA13*, a procedure similar to that just described was used. The Hfr strain

JC5426, carrying *thyA*$^+$ *recC22* and *recA*$^+$, was used as the donor and the F$^-$ strain JC5421, carrying *thyA326* *recC*$^+$ and *recA13*, was used as recipient. Thy$^+$[Sm^R] recombinants inheriting both mutant *rec* alleles were detected by selecting for His$^+$[Sm^R] recombinants in crosses with three Hfr strains: JC5029 (*recC*$^+$ *recA*$^+$), JC5426 (*recC22* *recA*$^+$), and JC5088 (*recC*$^+$ *recA56*). One double *rec*$^-$ mutant, JC5544, was purified for further testing.

Actually, three clones were found to have inherited *recC22* and *recA13*. All three were unusual in being sensitive to a "step-down" in growth conditions. Thus, most L broth-grown cells were unable to grow when plated on minimal medium but were enabled to grow when this medium was supplemented by adding 0.1 ml of L broth to each plate. In subsequent crosses using JC5544 as recipient, the selective medium was always supplemented with this small amount of L broth to avoid anomalies. Neither JC5426 nor JC5421, the immediate progenitors of JC5544, nor any other multiple *rec*$^-$ strain showed this sensitivity to "step-down" growth conditions.

Verification of the genotypes of the multiply deficient strains. Conjugational complementation tests had been used to detect the presence of the mutant *rec* alleles in the multiply deficient strains, but quantitatively significant results had not been obtained. These crosses were repeated in liquid medium so that differences in recombination frequency could be accurately measured (Table 2). In general, the results are what would be expected on the basis of the recessiveness of the mutations involved. No recombinants were found when JC5088 *rec-56* was mated with any singly or multiply deficient strain carrying *recA13*; such an absence of complementation indicates that *rec-56* should be written *recA56*.

Advantage was then taken of the cotransduction of *recA* with *cysC* (14), and of *recC* and *recB* with *thyA* and *argA* (7, 8; Willetts and Mount, *unpublished data*) to demonstrate the presence of each of the *rec*$^-$ mutations. P1 was grown on the multiple *rec*$^-$ strain and used to transduce JC5422 (*thyA325*), CP154 (*argA321*), and JC2915 (*cysC39*), selecting transductants prototrophic for these markers. Among these, Rec$^-$ strains were recognized by their UV sensitivity and reduced ability to mother His$^+$[Sm^R] recombinants in replica-plate crosses with the Hfr KL98, which transfers all *rec*$^+$ alleles to very few zygotes because of their distance from the origin of transfer. Transductants inheriting *recC22* or *recB21* were distinguished by replica plating onto selective media spread with the Hfr strains JC5029, JC5412, and JC5426, as described previously.

The frequencies of cotransduction of the *rec* mutations with the prototrophic markers from the multiple *rec*$^-$ strains were similar to those found using singly mutant *rec*$^-$ strains (Table 3). Transductants carrying separately each mutation presumed to have been in the multiple *rec*$^-$ mutant donor were purified, and broth crosses between these and KL98 confirmed that they had the expected level of recombination deficiency characteristic of the parental single *rec*$^-$ mutant strains of known genotype.

Properties of the multiple rec$^-$ strains: sensitivity to UV radiation. The survival of the multiple *rec*$^-$ strains to different doses of UV irradiation

TABLE 2. *Conjugational complementation tests*

Recipient	Hfr donor mutant *rec* alleles	Deficiency indices obtained in cross with Hfr strains[a]				
		JC5029 *rec*$^+$	JC5412 *recB21*	JC5426 *recC22*	JC5491 *recB21 recC22*	JC5088 *recA56*
AB1157	*rec*$^+$	1	1	1	1	1
AB2470	*recB21*	4	1×10^2	4	8×10^1	1×10^1
JC5474	*recC22*	6	7	1×10^2	2×10^2	6
AB2463	*recA13*	5	9	8	6	1×10^4
JC5519	*recB21 recC22*	6	8×10^2	6×10^1	6×10^1	1×10^1
JC5495	*recB21 recA13*	3	6×10^2	7	1×10^2	$>4 \times 10^4$
JC5544	*recC22 recA13*	4	3	1×10^{2b}	2×10^{2b}	$>4 \times 10^4$
JC5547	*recB21 recC22 recA13*	7	9×10^2	6×10^1	9×10^1	$>4 \times 10^4$

[a] Deficiency indices represent the number of recombinants mothered by a Rec$^+$F$^-$ strain divided by the number mothered by a Rec$^-$F$^-$ strain. They are directly proportional to the infertility of a cross involving a Rec$^-$ mutant.

[b] These values were calculated on the basis of results obtained by plating 0.1 ml of a 10^{-1} dilution of the mating mixture of the two Rec$^-$ strains. The number of colonies observed when 0.1 ml of a $10 \times$ dilution was plated was less than 10 times the number observed at the $10 \times$ higher dilution. No explanation for this can be offered at present.

is compared with that of the single *rec*⁻ counterparts in Fig. 2. The UV sensitivity of JC5519 (*recB21 recC22*) was intermediate between that of AB2470 (*recC*⁺ *recB21*) and JC5474 (*recC22 recB*⁺). The strains carrying *recC22* or *recB21*, or both, as well as *recA13* were more sensitive to small doses of UV than AB2463 (*recA13 recB*⁺ *recC*⁺), but were somewhat more resistant to larger doses. All strains carrying *recA13* showed a characteristic concave survival curve. There were fewer than 1% *recA*⁺ revertants among survivors of 200 ergs/mm².

Recombination deficiency. The ability of the multiple *rec* mutant strains to act as recipients was tested in three different ways (Table 4). Their abilities to mother His⁺[Sm^R] recombinants with JC5029, which transfers the *rec*⁺ alleles close to the origin of transfer, were similar to those of the singly deficient strains, as were their abilities to mother F*lac*⁺ merodiploids. Also, they were good recipients as measured by zygotic induction, which showed that both Hfr transfer into and lambda induction and growth in these strains were normal.

Crosses with KL98, which transfers all the *rec*⁺ alleles as distal markers, selecting His⁺[Sm^R] recombinants, showed that JC5519 (*recC22 recB21*) was deficient to an extent similar to both AB2470 (*recB21*) and JC5474 (*recC22*), and that the strains carrying *recC22* or *recB21*, or both, in addition to *recA13* were as highly recombination-deficient as AB2463 (*recA13*) itself.

Construction and induction of lambda lysogens. Lambda lysogens of the multiply deficient strains were constructed by using two different multiplicities of lambda phages, and the frequency of lysogenization was measured (Table 5). No abnormality was apparent. Measurements were then made of the levels of spontaneous induction of lambda, and of induction by both high and low doses of UV (Table 5). JC5519 (*recC22 recB21*) showed normal levels of induction, whereas those for all strains carrying *recA13* were very low. In addition, most of the plaques observed in the latter cases were clear, as has previously been found for the lysogen of AB2463 (*recA13*) itself (2).

UV-induced DNA degradation. Degradation of DNA after UV irradiation was measured by using a UV dose which gave 10% survival of the Rec⁺ parent AB1157 (570 ergs/mm²). JC5519 (*recC22 recB21*) showed a low rate of UV-induced breakdown, similar to that of either AB2470 (*recB21*) or JC5474 (*recC22*) (Fig. 3, 4). The strains carrying *recC22* or *recB21*, or both, in addition to *recA13* also showed this "cautious" breakdown, rather than the "reckless" breakdown exhibited by AB2463 [(*recA13*) Fig. 3, 4].

TABLE 3. *Transductional separation of the rec mutations present in the multiple rec⁻ strains*

Donor strain	Mutations (*rec*)	Rec⁻ among Thy⁺ [a]	Rec⁻ among Arg⁺ [a]	Rec⁻ among Cys⁺ [a]
		%	%	%
AB2470	*recB21*	41	76	0.6
JC5474	*recC22*	65	52	
AB2463	*recA13*	<0.5	<0.5	4
JC5519	*recC22 recB21*	77[b]	72[c]	
JC5495	*recB21 recA13*	46		3
JC5544	*recC22 recA13*	63		4.5
JC5547	*recC22 recB21 recA13*	65[d]	81[e]	2

[a] JC5422 was the Thy⁻ recipient; CP154, the Arg⁻ recipient; JC2915, the Cys⁻ recipient. Two hundred transductants were tested for their recombination ability in each case.

[b] Of the Thy⁺ transductants, 15% were *recC*⁻ *recB*⁺ and 62% were *recC*⁻ *recB*⁻.

[c] Of the Arg⁺ transductants, 16% were *recC*⁺ *recB*⁻ and 56% were *recC*⁻ *recB*⁻.

[d] Of the Thy⁺ transductants, 16% were *recC*⁻ *recB*⁺ and 49% were *recC*⁻ *recB*⁻.

[e] Of the Arg⁺ transductants, 19% were *recC*⁺ *recB*⁻ and 72% were *recC*⁻ *recB*⁻.

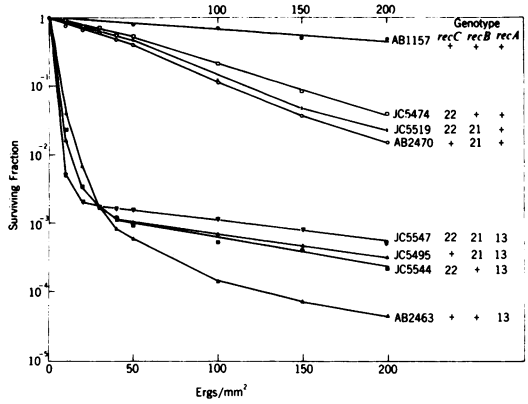

FIG. 2. *Survival of Rec⁺ and Rec⁻ strains after various doses of UV irradiation.*

The "cautious" UV-induced degradation of strains carrying *recC22* or *recB21*, or both, as well as *recA13* made it necessary to check that *recA13* could be separated from the second *rec* mutation in such strains and then confer the expected "reckless" UV-induced breakdown. This was done for JC5495 (*recB21 recA13*). By transduction into *cysC39* strain JC2915, the strain JC2966 (*recA13 cysC*⁺) was isolated, and this was shown to have the UV sensitivity,

TABLE 4. *Recipient ability and recombination deficiency of multiple rec⁻ strains*

Recipient	Mutant *rec* alleles	Deficiency indices obtained in crosses with donor strains[a]			
		JC5029[b]	E3[c]	JC2598	KL98[d]
AB1157	*rec*⁺	1	1	1	1
AB2470	*recB21*	4	6	2	30
JC5474	*recC22*	6	30	5	90
AB2463	*recA13*	5	2	1	4×10^4
JC5519	*recB21 recC22*	6	10	2	30
JC5495	*recB21 recA13*	3	10	2	3×10^4
JC5544	*recC22 recA13*	4	8	1	2×10^4
JC5547	*recB21 recC22 recA13*	7	15	2	4×10^4

[a] Deficiency index is a ratio of the number of progeny obtained from a Rec⁺ recipient to the number of progeny obtained from a Rec⁻ recipient. It is a number directly proportional to the infertility of a cross involving a Rec⁻ recipient.
[b] His⁺ [Sm^R Ilv⁺] recombination deficiency indices.
[c] Lac⁺[Sm^R] conjugation deficiency indices (F*lac*⁺ inheritance).
[d] Lambda zygotic induction deficiency indices.

recombination deficiency, and "reckless" DNA breakdown found in the original strain carrying *recA13*, AB2463. In addition JC2967, a Rec⁻Thy⁺ clone obtained by transduction of JC5422 (Rec⁺-Thy⁻) with P1 grown on JC5495, exhibited the recombination deficiency, UV sensitivity, and "cautious" DNA breakdown characteristic of AB2470, the original mutant carrying *recB21*.

DISCUSSION

By taking advantage of complementation between mutations in different *rec* genes, it has proven possible to isolate bacterial strains which carry mutations in more than one of them. A summary of the characteristics of these strains is shown in Table 6. Perhaps the most startling characteristic is the "cautious" UV-induced breakdown of DNA shown by those strains which carry a *recC* or a *recB* mutation, or both, in addition to a *recA* mutation. Care was taken to ascertain genetically that one of these strains, JC5495, did carry both the *recB* and *recA* mutations and that the latter when separated from the *recB* mutation again gave rise to the characteristic high rate of UV-induced DNA breakdown. Our conclusion, therefore, is that *recC* and *recB* mutations prevent the high rate of UV-induced breakdown produced by *recA* mutations. The most straightforward interpretation of this finding is that *recC* and *recB* determine proteins which are necessary for the sequence of reactions leading to DNA breakdown after UV irradiation; for example, one or both products may be nucleases. Some evidence has been obtained in cell-free systems that both the *recC* and *recB* products are necessary for nuclease action (Barbour and Clark, *unpublished data*). The DNA breakdown produced at high temperature in a mutant strain which is temperature-sensitive for DNA synthesis was also reduced on introduction of the *recB21* mutation into the strain (3). Similarly, the high rate of spontaneous DNA breakdown so characteristic of *recA*⁻ strains (5, 11) is prevented by the *recB* or *recC* mutations.

The other characteristics of the strains carrying *recC22* or *recB21*, or both, in addition to *recA13*, however, are similar to those of AB2463, which carries *recA13* alone. Thus, these strains are all very sensitive to UV irradiation and incapable of mothering His⁺[Sm^R] recombinants with KL98 as donor; their lysogens show little spontaneous induction of prophage lambda. Therefore, these properties are not correlated with the extent of UV-induced breakdown, and hypotheses which explain the recombination deficiency and UV sensitivity of *recA* mutants on the basis of uncontrolled or extensive breakdown of DNA (5) may be considered untenable. Similarly, a high rate of DNA breakdown is not responsible per se for the inability to induce a lambda lysogen of a *recA* strain by UV irradiation.

The *recC*⁻ *recB*⁻ mutant has a UV sensitivity and recombination ability similar to those of the *recC*⁻ *recB*⁺ and *recC*⁺ *recB*⁻ strains, showing that the *recC* and *recB* gene products probably act in the same biochemical pathway. The products may be the subunits of an enzyme complex, or perhaps two enzymes acting consecutively. In either case, both products are necessary for that part of the process leading to both recombination and repair which is manifested in *recA* mutants as DNA degradation after UV irradiation.

TABLE 5. *Construction and induction of lambda lysogens*

Strain	Mutant *rec* alleles	Frequency of lysogenization[a]		Lysogen strain no.	Spontaneous induction		Low dose of UV				High dose of UV (570 ergs/mm²)		
		M = 0.2	M = 10		Amt[b]	Clear plaques	Dose (ergs/mm²)	Survival	Amt[b]	Clear plaques	Survival	Amt[b]	Clear plaques
						%		%		%	%		%
AB1157	*rec*⁺	84	98	JC2941	4.6×10^6	<1					20	1.5×10^8	<1
AB2470	*recB21*	62	97	JC2943	1.4×10^6	<1	100	7	4.4×10^6	<1	0.2	1.2×10^8	<1
JC5474	*recC22*	33	95	JC2949	1.5×10^6	<1	140	26	8.5×10^5	<1	1.1	2.9×10^7	<1
AB2463	*recA13*	73	92	JC2945	5.9×10^2	87	10	19	5.1×10^2	100	2×10^{-4}	7.8×10^1	80
JC5519	*recB21 recC22*	78	96	JC2955	1.1×10^6	<1	110	3	8.1×10^6	<1	0.4	1.1×10^8	<1
JC5495	*recB21 recA13*	59	93	JC2953	4.7×10^2	93	10	5	3.0×10^2	94	7×10^{-4}	8.5×10^1	64
JC5544	*recC22 recA13*	76	93	JC2951	3.0×10^2	98	10	22	9.5×10^2	81	3×10^{-3}	1.5×10^2	65
JC5547	*recB21 recC22 recA13*	69	82	JC2959	3.8×10^2	99	10	3	2.9×10^2	95	5×10^{-4}	4.3×10^2	35

[a] M = multiplicity of infection.
[b] Expressed as plaque-forming units per milliliter.

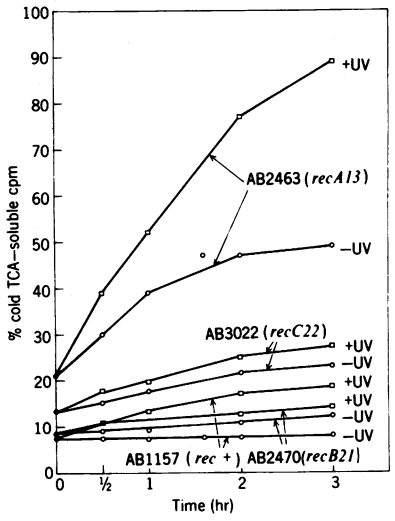

FIG. 3. *Breakdown of ^{3}H-thymidine-labeled DNA after UV irradiation of Rec$^+$ and single rec$^-$ strains. The dose of UV was 570 ergs/mm^2.*

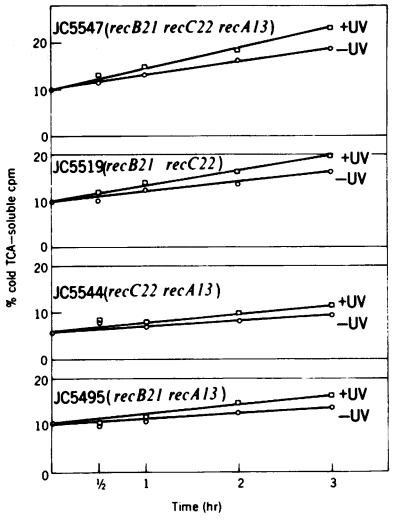

FIG. 4. *Breakdown of ^{3}H-thymidine-labeled DNA after UV irradiation of multiple rec- strains. The dose of UV was 570 ergs/mm^2.*

TABLE 6. *Summary of properties of Rec$^-$ strains*

Strains with the following mutant *rec* alleles	UV-induced DNA breakdown	UV sensitivity	Recombination in a cross with KL98	Spontaneous production of lambda phage
None	Normal	Low (resistant)	High	Normal
recA13	Reckless	High	None	Low
recB21	Cautious	Moderate	Low	Normal
recC22	Cautious	Moderate	Low	Normal
recA13 recB21	Cautious	High	None	Low
recA13 recC22	Cautious	High	None	Low
recB21 recC22	Cautious	Moderate	Low	Normal
recA13 recB21 recC22	Cautious	High	None	Low

The similarity of the UV sensitivities and recombination deficiencies of all the multiple *rec*$^-$ strains carrying *recA13* to that of the *recA13* single mutant AB2463 may be taken as indicative that the gene products of all three *rec* genes act in the same pathway of recombination and repair. This is not to say, however, that there is only one pathway for each process. Indeed, it has been suggested (10, 11*a*) that an examination of a *uvrA6 recA13* strain reveals two pathways of dark repair. Likewise, it may be suggested that the residual recombination characteristic of *recB*$^-$ mutants (12) and *recC*$^-$ mutants (7) indicates that there are two pathways of recombination in *E. coli*.

In summary, we conclude that the wild-type *recC* and *recB* products lead to nuclease activity in cells of *E. coli*. Furthermore, the wild-type *recA* product reduces the amount of this nuclease activity manifested by UV-irradiated or unirradiated cells.

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