RECOMBINATION DEFICIENT MUTANTS OF *ESCHERICHIA COLI* K12 THAT MAP BETWEEN *thyA* AND *argA*

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R ECOMBINATION deficient mutants of *E. coli* K12 have been described that degrade their DNA spontaneously during growth and undergo much greater degradation in their DNA than wild-type cells when they are incubated following exposure to either ultraviolet (UV) or X-irradiation (CLARK and MARGULIES 1965; HOWARD-FLANDERS and THERIOT 1966; CLARK, CHAMBERLIN, BOYCE and HOWARD-FLANDERS 1966; CLARK 1967). A number of these strains has been shown to carry a mutation at a locus, *recA*, which maps in the region between *cysC* and *pheA* (LOW, WILLETS and CLARK, in preparation). Lysogens carrying prophage λ and *recA* exhibit negligible spontaneous or UV-induced release of phage λ (TAKANO, 1966; BROOKS and CLARK 1967).

Other recombination deficient mutants have been obtained that differ from those mapping at the *recA* locus in that they exhibit less postirradiation degradation in their DNA than wild-type cells (HOWARD-FLANDERS and BOYCE 1967), induce almost normally as λ lysogens (BROOKS and CLARK 1967) and map at a locus which is cotransducible with *thyA* (EMMERSON and HOWARD-FLANDERS 1967).

This paper describes some phenotypic properties and the genetic mapping of three independently isolated mutants of the latter class. It will be shown that the mutant loci, *recB21*, *rec-22* and *rec-23* of these strains lie between *thyA* and *argA* in the sequence *lysA-thyA-recB-argA*.

MATERIALS AND METHODS

Strains: The genetic properties of the bacterial strains are presented in Table 1. AB2470 *recB21* (HOWARD-FLANDERS and THERIOT 1966) and AB3109 *rec-23* (isolated by DRS. R. P. BOYCE and M. TEPPER of this laboratory) were nitrosoguanidine-induced mutants of AB1157. AB3022 *rec-22* was a nitrosoguanidine-induced mutant of AB2495.

AB1181 *argA*, obtained from DR. A. L. TAYLOR, is defective in the enzyme N-acetyl glutamate synthetase. AB3046 was a low thymine requiring derivative of AB1181 isolated by the method of STACEY and SIMSON (1965), and mutant at both the *thyA* and *thyR* loci (OKADA 1966; ALIKHANIAN et al. 1966). AB3057 was a *thy+* transductant of AB3022, from a wild-type donor.

AB3080 *rec-22 argA* was a *rec (thy+)* transductant of AB3046, donor AB3057. Similarly, AB3082 *recB21 argA* was a *rec (thy+)* transductant of AB3046, donor AB2470 and AB3084 *rec-23 argA* was a *rec (thy+)* transductant of AB3046, donor AB3109.

PA 106-7 *lysA*, obtained from DR. B. Low, is defective in the enzyme diaminopimelic acid decarboxylase. AB3081 was a low thymine requiring derivative of PA 106-7.

TABLE 1

Description of the E. coli K12 strains

Strain	Derived from	Mutant loci and mutation sites*																						Sex
		rec	thr	leu	proA	his	metB	thi	arg	thyA	thyR	ilvD	trp	lysA	cys	lac	gal	ara	xyl	mtl	mal	tsx	str	
AB259	......	+	+	+	+	+	+	12	+	+	:	+	+	+	+	+	+	+	+	+	+	+	+	Hfr H
AB2383	......	+	+	+	+	+	1	+	+	2	:	+	+	+	+	+	+	+	+	+	+	+	+	Hfr J2
AB2528	......	+	+	6	+	+	+	1	+	+	:	132	+	+	+	4	+	+	+	+	+	+	8	Hfr 313
W4520	......	+	+	+	+	+	+	:	+	+	:	+	+	+	+	:	+/4	+	+	:	:	:	8	F-gal+
AB1157	......	+	4	8	2	3	+	1	3	+	:	+	+	+	+	1	2	14	5	1	+	33	31	F-
AB2495	AB1157	+	4	8	2	4	+	1	3	—	—	+	—	+	+	1	2	14	5	1	+	33	31	F-
AB2470	AB1157	(B)21	4	8	2	4	+	1	3	+	:	+	+	+	+	1	2	14	5	1	+	33	31	F-
AB3022	AB2495	22	4	8	2	4	+	1	3	—	—	+	—	+	+	1	2	14	5	1	+	33	31	F-
AB3109	AB1157	23	4	8	2	4	+	1	3	+	:	+	+	+	+	1	2	14	5	1	+	33	31	F-
AB1181	......	+	+	+	+	+	+	+	(A)21	+	—	+	+	+	+	+	+	+	+	+	+	+	+	F-
AB3046	AB1181	+	+	+	+	+	+	+	(A)21	—	:	+	+	+	+	+	+	+	+	+	+	+	+	F-
AB3057	AB3022	22	4	8	2	4	+	1	3	+	:	+	—	+	+	1	2	14	5	1	+	33	31	F-
AB3080	AB3046	22	+	+	+	+	+	+	(A)21	+	:	+	+	+	+	+	+	+	+	+	+	+	+	F-
PA106-7	......	+	—	—	+	+	+	—	+	+	:	+	+	7	—	—	:	:	—	—	—	+	+	F-
AB3081	PA106-7	+	—	—	+	+	+	—	(A)21	—	—	+	+	7	—	—	:	:	—	—	—	+	+	F-
AB3082	AB3046	(B)21	+	+	+	+	+	+	(A)21	+	:	+	+	+	+	+	+	+	+	+	+	+	+	F-
AB3084	AB3046	23	+	+	+	+	+	+	3	+	:	+	+	+	+	+	+	+	+	+	+	+	+	F-
AB2497	AB1157	+	4	8	2	4	+	1	3	—	—	+	+	+	+	1	2	14	5	1	+	33	31	F-
AB3058	AB2497	22	4	8	2	4	+	1	3	+	:	+	+	+	+	1	2	14	5	1	+	33	31	F-

* Gene symbol nomenclature follows the proposal of DEMEREC et al. (1966).
The Hfr strains were obtained from DRS. E. A. ADELBERG and D. G. EGGERTSSON. Their origins and direction of transfer are shown in Figure 1.

AB3058 was a *rec-22* (*thy+*) transductant of AB2497, a low thymine requiring derivative of AB1157. The donor strain was AB3057. AB3058 was constructed in order to facilitate comparison of the phenotypes of the *recB21*, *rec-22* and *rec-23* mutations in a similar genetic background.

Media: YET broth contained 5 g yeast extract, 10 g tryptone, 10 g NaCl, 120 mg NaOH per liter of water, and was supplemented with 1 g glucose for YET glucose medium. The YET agar contained YET broth with 2% agar. The selective media employed in the genetic crosses follow those described by ADELBERG and BURNS (1960). M9 medium contained 1 g NH_4Cl, 11 g Na_2 $HPO_4.7H_2O$, 3 g KH_2PO_4, 5 g NaCl, 4 g glucose, 120 mg $MgSO_4$, 10 mg $CaCl_2$ and water to one liter. Enriched M9 (EM9) consisted of M9 with 2.5 g casamino acids and 0.1 mg thiamine added per liter. Buffered saline (pH 6.8) contained 2.7 g $Na_2HPO_4.7H_2O$, 1.4 g KH_2PO_4, 7.6 g NaCl per liter of water.

Isolation of mutants: Cells were treated with the mutagen nitrosoguanidine, survivors were screened for sensitivity to 6 Mev electrons and those that were radiation sensitive were then screened further for ability to produce recombinants. These procedures have been described previously (HOWARD-FLANDERS and THERIOT 1966).

Labeling of bacteria: To study postirradiation DNA degradation, the bacteria were labeled in their DNA by growth in the presence of tritiated thymidine. Stationary phase cultures of bacteria in EM9 were diluted at least 30-fold into EM9 supplemented with 250 μg/ml deoxy-adenosine and 6 μc/ml of H^3-thymidine and incubated with aeration to late log phase. They were centrifuged, washed twice with M9, resuspended in M9 and incubated with aeration for 30 minutes to reduce the radioactivity in the acid soluble pool. After washing once more with M9, the bacteria were suspended in either buffered saline for X irradiation, or M9 for UV irradiation.

Irradiation: The X ray source operated at 250 kv and 15 ma giving a mean dose rate to the sample, measured by ferrous sulfate dosimetry, of 10 kilorads per min. The bacteria were irradiated in 25 mm tubes surrounded by crushed ice. They were bubbled with oxygen before and during the irradiation.

A 6 Mev linear accelerator was used to irradiate bacteria spread on agar plates, with high energy electrons. The dose rate at approximately 2 m from the electron window was 5×10^4 rads per min.

The UV source was a 15 watt low-pressure mercury germicidal lamp giving a dose rate at the sample. some 50 cm from the lamp, of ten ergs/mm² per sec. A General Electric Germicidal Light Meter was used for the UV dosimetry. Previously chilled samples of bacteria were irradiated at room temperature, in subdued light, in Petri dishes which were gently shaken on a rotary shaker. The cell concentration and thickness of the suspension was such that the absorption by the culture was negligible. After UV irradiation, the cultures were immediately chilled on ice.

Postirradiation DNA degradation: Following irradiation, the cultures were aerated at 37°C. Aliquots (0.5 ml) were withdrawn at suitable times, an equal volume of ice-cold 10% TCA was added, and then the mixture was stirred in an ice bath for 30 min. Serum albumin (0.05 ml at 1%) was added to increase the bulk of the precipitate and then the mixture was centrifuged at 10,000 rpm for ten minutes. An aliquot (0.1 ml) of the supernatant was removed and its radioactivity determined using a scintillation counter. This activity represents the total acid soluble material in the cell and surrounding medium. The TCA-insoluble radioactivity was determined by heating the remainder at 95°C for 30 min, centrifuging to remove cell debris and then counting 0.1 ml of the supernatant in the liquid scintillation counter. DNA degradation was expressed as the percent of the DNA rendered soluble in ice cold 5% TCA.

Survival curves: Bacteria were grown to late log phase in EM9, washed once in M9 and resuspended in either buffered saline or M9. After various doses of UV or X rays, aliquots were withdrawn. suitably diluted in buffered saline and spread on YET agar plates. Colonies were counted after one or two days growth at 37°C.

Genetic crosses: Log phase cultures of donor and recipient bacteria in YET glucose medium were mixed in Erlenmeyer flasks at a titer, based on colony forming ability, of about 10⁷ donors/ml and 10⁸ recipients/ml. They were incubated without shaking at 37°C. At suitable times, samples were withdrawn, diluted tenfold in buffered saline and violently agitated on a

"vortex" mixer. Further dilutions were made in buffered saline and the samples were spread on selective plates. Usually streptomycin (200 μg/ml) was used to kill the donors, but where appropriate for streptomycin resistant donors, a distal auxotrophic requirement of the donors was omitted from the plates. Recombinant colonies were counted after two to three days incubation at 37°C.

Transduction: Transductions were performed using P1v1, a virulent derivative of P1kc (IKEDA and TOMIZAWA 1965), provided by DR. TOMIZAWA. Phage stocks were obtained by infecting log phase cells of the donor strain in YET medium supplemented with 5×10^{-3} M $CaCl_2$ with P1v1 at a multiplicity of infection (m. o. i.) of 0.1 and then growing them in a layer of soft agar on top of YET agar for several hours or overnight at 37°C. Then the upper layer was transferred to a centrifuge tube, treated with 0.1 ml of chloroform and centrifuged at 6000 rpm for 10 min. The supernatant was decanted and freed from chloroform by aeration. Phage titers were usually approximately 10^{10} per ml.

The method of LENNOX (1955) was used in the transduction experiments. To log phase cells of the recipient strain in YET medium supplemented with 5×10^{-3} M $CaCl_2$ were added P1 phage to give a m. o. i. of approximately 0.01 to 0.1 and the mixture was incubated for 25 min at 37°C. After centrifugation, the cells were washed, resuspended in YET broth and spread on selective agar. After incubation for two to three days, at least 100 colonies were picked, purified

TABLE 2

Ratios of the number of recombinant colonies and sexductant colonies produced in crosses between Hfr and F' donor cells and wild-type cells to those produced in recombination deficient cells.

Donor (viable cells/ml)	Recipient	Selection	Number of recombinants/ml	Ratio of recombinant formation
AB259 (4.7 × 10⁷) Hfr H	AB2495 *rec+*	Pro+ Str^R	9.1 × 10⁵	306
	AB3022 *rec-22*		2.9 × 10³	
	AB2495 rec+	His+ Str^R	2.2 × 10⁴	2.3
	AB3022 *rec-22*		9.3 × 10³	
AB2383 (4.8 × 10⁷) Hfr J2	AB2495 *rec+*	Pro+ Str^R	3.4 × 10⁶	263
	AB3022 *rec-22*		1.3 × 10⁴	
AB2528 (6.5 × 10⁷) Hfr 313	AB2495 *rec+*	Thy+ Ilv+	1.5 × 10⁶	3.5
	AB3022 *rec-22*		4.2 × 10⁵	
	AB2495 *rec+*	His+ Ilv+	2.2 × 10⁵	2.0
	AB3022 *rec-22*		1.1 × 10⁵	
			Number of Gal+ recipients	Ratio of merodiploid formation
W4520 (5.0 × 10⁷) F-*gal+*	AB2495 *rec+*	Gal+ Str^R	2.7 × 10⁷	1.2
	AB3022 *rec-22*		2.2 × 10⁷	

AB2495 *rec+* (2.3 × 10⁸ viable cells/ml) and AB3022 *rec-22* (1.0 × 10⁸ viable cells/ml) were mated with the Hfr donor strains, AB259 Hfr H, AB2383 Hfr J2, and AB2528 Hfr 313 for 2 hrs and with the F-*gal+* donor strain W4520 for 1 hr. Comparison of the number of viable cells/ml in a culture of AB3022 *rec-22*, determined by plating on YET agar, with the total number of cells/ml determined by counting under the microscope indicated that only about 25% of the cells were viable.

by streaking to single colonies, and tested for unselected markers. To test the transductants for the presence of the *rec* gene, their X-ray sensitivity was determined. They were grown overnight in YET medium and inoculated in streaks of YET agar plates with wild-type and recombination deficient (Rec⁻) controls. Half of each plate was irradiated with about 30 krads of 6 Mev electrons. After overnight incubation at 37°C, the strains carrying *rec* failed to grow on the irradiated portion of the plates.

<div align="center">RESULTS</div>

The results of typical crosses between one of the Rec⁻ strains and several Hfr donor strains are presented in Table 2. The origins and direction of transfer of the chromosome by these Hfr strains are shown by arrowheads on the genetic map in Figure 1. In the cross with AB259 (Hfr H), when recombinants carrying the early marker *proA*⁺ are selected, there is a marked difference in the number of recombinants produced by the wild type and by the Rec⁻ strains. Approximately 300 times fewer Pro⁺ (Str^R) recombinants are produced in the cross with AB3022 *rec-22* than in the cross with AB2495 *rec*⁺. If, however, recombinants carrying *his*⁺, a more distant marker, are selected, only about a twofold difference is observed. Proline selection in the cross with AB2383 (Hfr J2) also leads to pronounced differences in yields of recombinants. The ratio of the number of
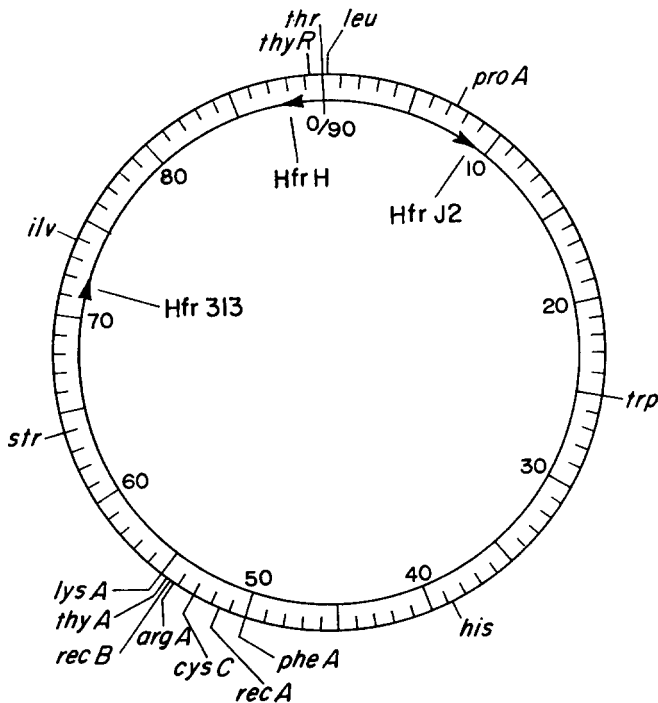

FIGURE 1.—Genetic map of *E. coli* from TAYLOR and TROTTER (1967) showing the positions of relevant genes and the origins and direction of transfer of the Hfr strains. The positions of *recB*, deduced from the present experiments, and *recA* (LOW, WILLETS, and CLARK, in preparation), are also shown.

PETER T. EMMERSON

Pro$^+$ (Str^R) recombinants produced in this cross from AB2495 *rec$^+$* to that produced from AB3022 *rec-22* is about 260. Little difference is observed in the yields of His$^+$(Ilv$^+$) or Thy$^+$ (Ilv$^+$) recombinants from AB2495 *rec$^+$* and AB3022 *rec-22* when the donor AB2528 (Hfr 313) is used, since *rec-22$^+$* enters at approximately the same time as *thyA$^+$* (EMMERSON and HOWARD-FLANDERS 1967). Practically no difference is observed in the ability of AB3022 *rec-22* and AB2495 *rec$^+$* to accept the episome F-*gal$^+$* from the donor strain W 4520. Similar results have been obtained when AB259 (Hfr H), AB2383 (Hfr J2), AB2528 (Hfr 313) and W4520 have been crossed with AB2470 *recB21* and AB3109 *rec-23* (HOWARD-FLANDERS 1967; BOYCE and TEPPER, unpublished results).

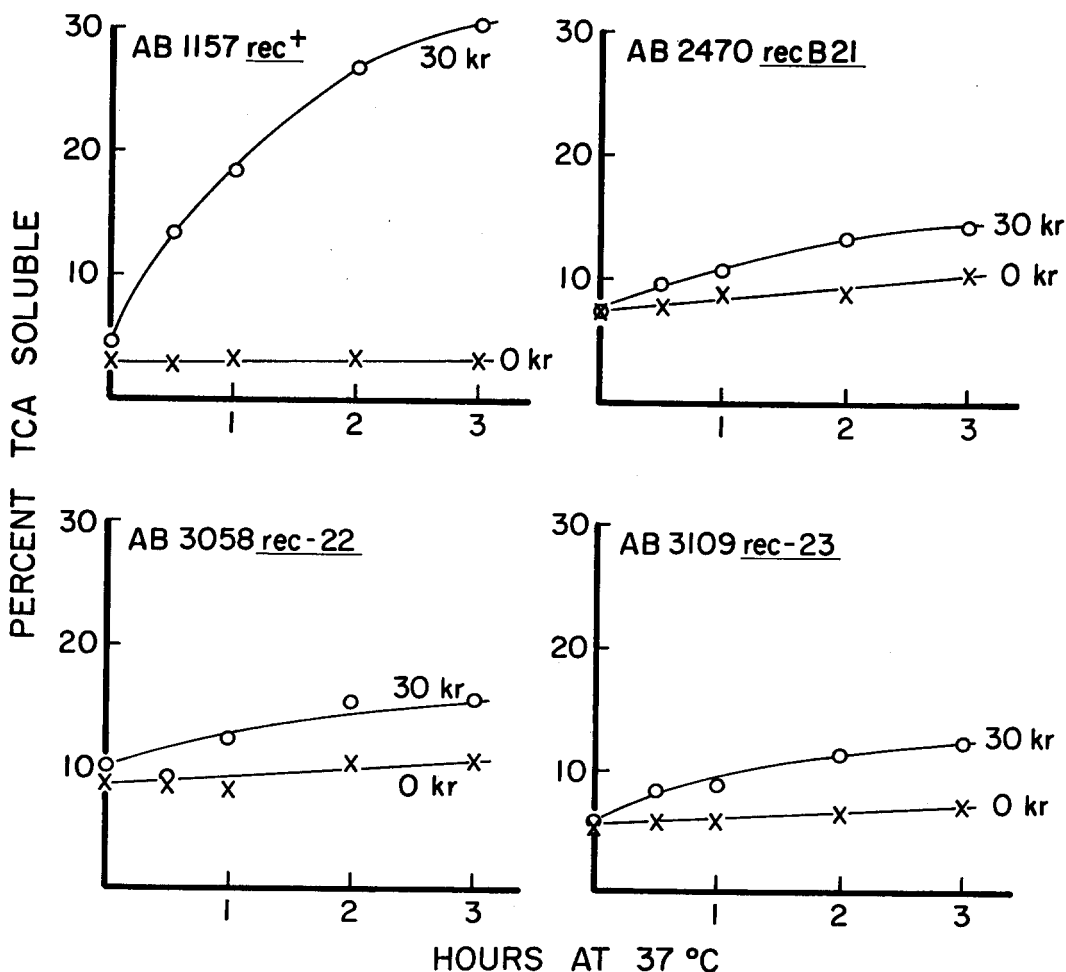Figure 2 shows the results of an experiment in which the strains AB2470

FIGURE 2.—The percentage of H^3 radioactivity in the cells and surrounding medium which is soluble in 5% TCA at 4°C is plotted as a function of time of incubation with aeration at 37°C following a dose of 30 kilorads of X rays. Unirradiated controls are shown for comparison. The cells were irradiated and incubated in buffered saline.
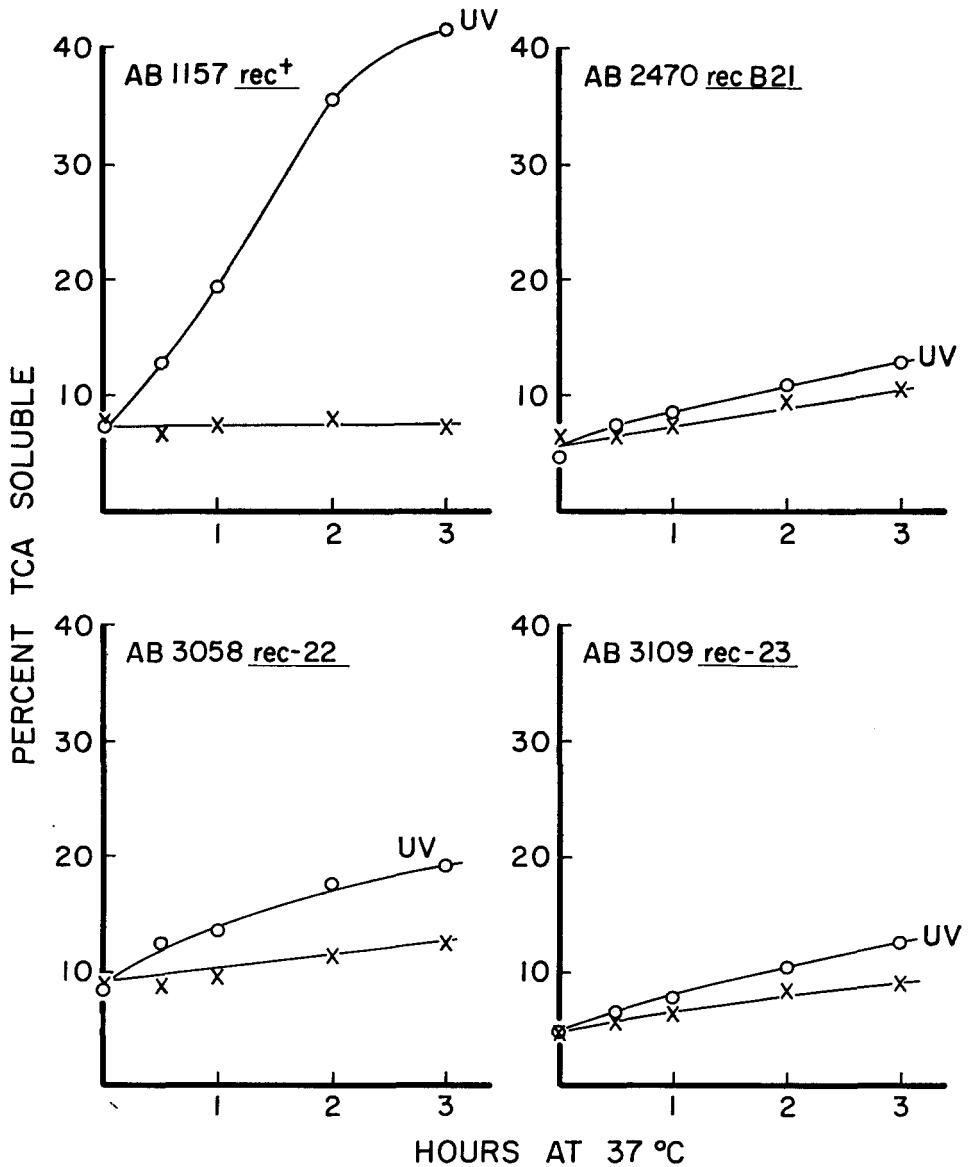
FIGURE 3.—The percentage of H³ radioactivity in the cells and surrounding medium which is soluble in 5% TCA at 4°C is plotted as a function of time of incubation with aeration at 37°C following a dose of 1000 ergs per mm² of UV irradiation (upper curves). Unirradiated controls, the lower curves, are shown for comparison. The cells were irradiated and incubated in M9 medium.

recB21, AB3058 rec-22, AB3109 rec-23 and AB1157 rec⁺ were examined for ability to degrade their DNA following X-irradiation. All three of the mutant strains exhibit a reduced capacity to degrade their DNA following a dose of 30 krads of X rays. Within the experimental error the three Rec⁻ strains behave
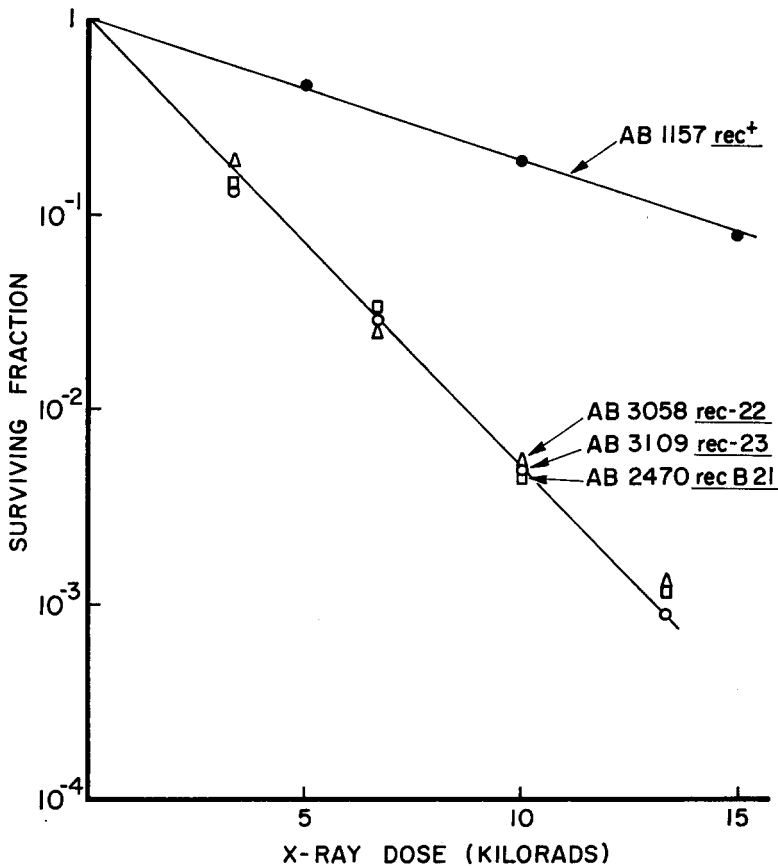
FIGURE 4.—The fraction of bacteria which retain the ability to form colonies on complete media, plotted as a function of X-ray dose.

identically, the postirradiation induced degradation being approximately 20% as much as in the wild-type strain.

Figure 3 shows the results of a similar experiment following a dose of 1000 ergs/mm² of UV irradiation. Again, all three of the Rec⁻ strains exhibit similar postirradiation degradation characteristics and the extent of the degradation is much less than in the wild-type strain. This large difference between the extent of the degradation in the Rec⁻ and Rec⁺ strains was also found in experiments in which the UV dose was 200 and 300 ergs/mm².

X-ray survival curves of the three Rec⁻ strains and the Rec⁺ strain are presented in Figure 4. No significant differences could be found in the sensitivity of the three Rec⁻ strains which were all approximately three and a half times more sensitive than wild-type cells. In this experiment, the cells were grown to log phase in EM9 medium prior to irradiation. In other experiments in which survival curves were determined for cultures grown in YET broth the Rec⁻ strains were approximately five times more sensitive than the Rec⁺ strains.

Figure 5 shows that the Rec⁻ strains also gave identical UV survival curves.

Earlier experiments had shown that *rec-22* was cotransducible with *thy* (EMMERSON and HOWARD-FLANDERS 1967). Further work was carried out to map this and the *recB21* and *rec-23* alleles more accurately. The results of transducing AB3046 *thyA argA* with P1 phage from AB3057 *rec-22* and selecting for Arg⁺ and Thy⁺ transductants are shown in Table 3. When the Arg⁺ transductants were tested for the unselected markers *thyA* and *rec*, it was found that 76% were Thy⁺ and 84% were Rec⁻. Only 1% were Thy⁺ and Rec⁺. This is consistent with the sequence *thyA–rec-22–argA* since the production of an Arg⁺ Rec⁺ Thy⁺ transductant would require a double genetic exchange. This sequence is also supported by the results of testing the Thy⁺ transductants for the unselected markers *arg* and *rec*. It was found that 43% were Arg⁺ and 64% were Rec⁻. The least frequently occurring combination of markers in the Thy⁺ transductants was found to be Thy⁺ Arg⁺ Rec⁺ which is also consistent with the sequence *thyA–rec-22–argA*.
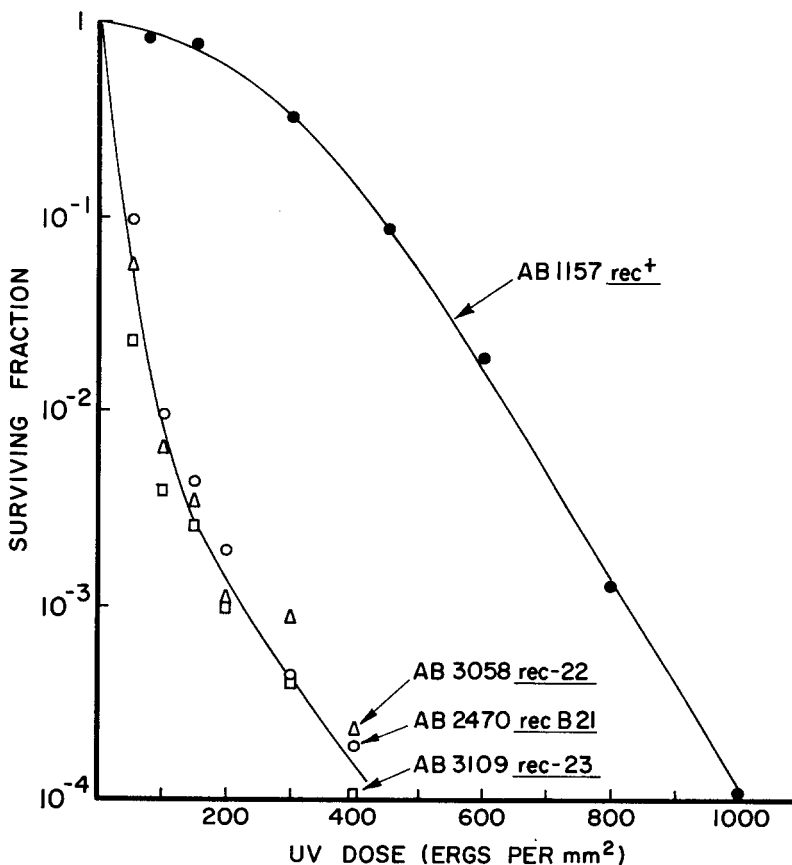

FIGURE 5.—The fraction of bacteria which retain the ability to form colonies on complete media, plotted as a function of UV dose.

In order to obtain more evidence bearing on the sequence of these genes, AB3081 *lysA thyA* was transduced by P1 phage from AB3080 *rec-22 argA* and Lys$^+$ and Thy$^+$ transductants selected. An analysis of the unselected markers of these transductants is presented in column I of Table 4. All of the results are consistent with the sequence *lysA–thyA–rec-22–argA*. The cotransduction frequencies of *thyA$^+$*, *rec-22* and *argA* with *lysA$^+$* were found to be 56%, 22% and 9%, respectively. All combinations of the three genes that appeared less frequently than 8% in the Lys$^+$ transductants required double exchanges on the basis of the above sequence. Analysis of the Thy$^+$ transductants revealed the cotransduction frequencies of the *thyA$^+$* allele with *lysA$^+$*, *rec-22* and *argA* to be 60%, 58% and 38%, respectively. Only two combinations of the three unselected markers would result from double exchanges and both appeared in low yield. Only 5% of the Thy$^+$ transductants were Rec$^+$ Arg$^-$ Lys$^+$ and only 1% were Rec$^+$ Arg$^-$ Lys$^-$. Table 4 also contains the results of two similar experiments in which the donor strain carried *recB21* (in column II) and *rec-23* (in column III). The results clearly indicate that these mutant alleles are also between *thyA* and *argA*.

DISCUSSION

The three independently isolated recombination deficient mutants could not be distinguished from one another by their sensitivity to UV or X irradiation or by the way in which they degrade their DNA following UV or X irradiation. Moreover, the three mutant alleles were found to map in the same region of the

TABLE 3

Transduction of AB3046 thyA argA *with P1v1, donor AB3057 rec-22*

	Unselected characteristics		
Selection	Thy	Rec	Percent
	+	±	76
	±	—	84
Arg$^+$	+	—	75
	+	+	1
	—	+	15
	—	—	9
	Arg	Rec	.
	+	±	43
	±	—	64
Thy$^+$	+	—	34
	+	+	9
	—	+	27
	—	—	30

± signifies "plus or minus" and does not imply an intermediate phenotype.

TABLE 4

Transduction of AB3081 lysA thyA with P1v1

Selection	Unselected characteristics			rec allele of donor		
	Rec	Arg	Thy	I rec-22	II recB21	III rec-23
					Percent	
	—	±	±	22	4	10
	±	—	±	9	0.4	5
	±	±	+	56	60	83
	+	+	+	34	56.5	72
	—	+	+	14	3	6
Lys+	+	—	+	0	0	1
	+	+	—	43	39.5	17
	+	—	—	1	0	0
	—	+	—	0	0.5	0
	—	—	+	8	0.5	4
	—	—	—	0	0	0
	Rec	Arg	Lys			
	—	±	±	58	14.5	24
	±	—	±	38	5.5	15
	±	±	+	60	62	61
	+	+	+	23	55	52
	—	+	+	16	5	6
Thy+	+	—	+	5	0	0
	+	+	—	13	30.5	24
	+	—	—	1	0	0
	—	+	—	10	4	3
	—	—	+	16	2	3
	—	—	—	16	3.5	12

Donor Strains: I: AB3080 *rec-22 argA*; II: AB3082 *recB21 argA*; III AB3084 *rec-23 argA*.
± signifies "plus or minus" and does not imply an intermediate phenotype.

chromosome, between *thyA* and *argA*. However, N. WILLETTS and D. MOUNT (personal communication) have recently demonstrated complementation between *recB21* and *rec-22*. On the basis of this result and complementation and mapping data of eighteen other Rec⁻ strains mapping near *thyA*, they have concluded that *rec-22* lies in a new gene, *recC*, mapping between *thyA* and *recB*.

SUMMARY

Three nitrosoguanidine-induced, radiation sensitive, recombination deficient mutants of *E. coli* K12 were found to degrade their DNA less than wild-type cells when incubated following either UV or X irradiation. In this respect, they differ from recombination deficient mutants defective at the *recA* locus, which have been shown to degrade their DNA to a much greater extent than wild-type

cells following irradiation. The three mutant alleles, two of which have been designated *recB* and *recC*, were shown to map between *thyA* and *argA*.

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