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Genetic Analysis of Recombination-Deficient Mutants of *Escherichia coli* K-12 Carrying *rec* Mutations Cotransducible with *thyA*

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The *rec* mutations carried by 20 strains of *Escherichia coli* K-12 which are defective in genetic recombination and sensitive to ultraviolet light and X rays, and whose λ lysogens show spontaneous phage production, have been mapped near *thyA*. In 15 of the strains, the *rec* mutation fails to complement *recB21* but complements *rec-22*. The other five strains carry a *rec* mutation which complements *recB21* but not *rec-22*. These mutations map closer to *thyA* than those which fail to complement *recB21*. They therefore appear to be defective in a different recombination gene, denoted *recC*. The order of *recB* and *recC* on the linkage map of *E. coli* K-12 is *thyA-recC-recB-argA*.

One group of recombination-deficient (Rec⁻) mutants of *Escherichia coli* K-12 consists of strains which have intermediate recombination deficiencies and sensitivities to ultraviolet light (UV), and which, when lysogenic for phage λ, show a normal level of spontaneous λ production. They belong to the first phenotypic class, Rec_1, described by Clark (2). They differ from strains carrying a mutation in *recA*, which are extremely UV-sensitive and do not yield any detectable genetic recombinants (13), and whose lysogens do not produce λ phage spontaneously (1, 2). The mutations carried by two Rec_1 strains (AB2470 and AB3022) are cotransduced at a high frequency with *thyA* (5, 6), whereas *recA* mutations are located elsewhere on the genetic map between *cysC* and *pheA* (18).

This paper describes a genetic analysis of 20 strains which have the Rec_1 phenotype. The mutations carried by all of these strains were cotransducible with *thyA*. Complementation tests performed by three different methods separated the mutations into two distinct complementation groups, *recB* and *recC*. More precise mapping experiments established the gene order *thyA-recC-recB-argA* on the *E. coli* K-12 linkage map (Fig. 1).

MATERIALS AND METHODS

Bacterial strains. The properties of the bacterial strains used are described in Tables 1 and 2.

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The Hfr strains JC5412 and JC5426 carry *recB21* and *recC22*, respectively, and were obtained by cotransducing the *rec* mutation with *thyA*⁺ into a Thy⁻ derivative of JC5029 (JC5401) which had in turn been derived by mutation of KL16 (2). Both strains originally showed a characteristically low viability, but, in the course of subculturing, JC5412 became a better growing, UV-resistant type by an as yet unidentified mutational event. That both strains carried the original *rec* mutations was confirmed by using them as transductional donors to the Thy⁻ Rec⁺ recipient JC5422: *recB21* in JC5412 was cotransduced with *thyA*⁺ at a frequency of 42% (compared with 41% when AB2470 was used as donor), and *recC22* in JC5426 was cotransduced with *thyA*⁺ at a frequency of 64% (compared with 65% when JC5474 was used as donor). The transductants were both UV-sensitive and recombination-deficient.

JC5467 is a Thy⁻ derivative of CP154 (obtained from N. Fiil) and JC5475 is a Thy⁺ Arg⁻ transductant of a Thy⁻ derivative of AT724 (16) obtained with CP154 as donor.

Derivatives of F15 (which carries the *recB*⁺ and *recC*⁺ alleles, as is shown later) carrying *recB21* and *recC22* were obtained as follows. F15 was transferred from W4580 (8) to the Rec⁻ Thy⁻ strains JC5408 (*recB21*) and AB3022 (*recC22*), selecting Thy⁺ [Str^R] merodiploids. Several hundred single colonies of each purified merodiploid derivative were tested (in the case of the AB3022 derivative, prior UV irradiation was used to increase the recombination frequency) in order to find Thy⁺ UV^s clones still capable of transferring *thyA*⁺ to the Thy⁻ RecA⁻ Spc^R Str^s stain JC5483. These clones were assumed to be homozygous for the *rec* mutation. The presumed F15 *recB21* and F15*recC22* episomes were transferred to JC5483 selecting Thy⁺ [Spc^R] clones, and, after purification, the resultant derivatives were used as

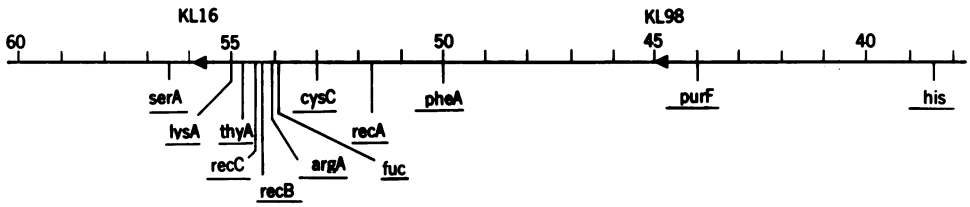

FIG. 1. *Part of the linkage map of E. coli K-12 as given by Taylor and Trotter (13).*

TABLE 1. *Bacterial strains*[a]

Strain no.	*rec* allele	Sex	*str*	*spc*	*arg*	*lac*	*his*	*thr*	*thy*	*leu*	*met*	*ilv*	*thi*	Other markers	Source
AB1157	*rec*$^+$	F$^-$	31	S	3	1	4	4	+	8	+	+	1	*proA2*	E. A. Adelberg
JC1557	*rec*$^+$	F$^-$	309	S	G6	1, 4	1	+	+	7	B1	+	+		A. J. Clark
AB2495	*rec*$^+$	F$^-$	31	S	3	1	4	4	−	8	+	+	1	*proA2, trp*	P. Howard-Flanders
KL98	*rec*$^+$	Hfr	S	S	+	+	+	+	+	+	+	+	−		K. B. Low
KL16	*rec*$^+$	Hfr	S	S	+	+	+	+	+	+	+	+	−		K. B. Low
JC5029	*rec*$^+$	Hfr	S	300	+	+	+	300	+	+	+	318	+		A. J. Clark
E3	*rec*$^+$	F*lac*$^+$	S	S	+	+	+	+	+	+	+	+	−		A. J. Clark
W4580	*rec*$^+$	F15	S	S	+	+	+	+	+	+	−	+	+		Y. Hirota
JC5412	*recB21*	Hfr	S	300	+	+	+	300	+	+	+	318	+		This paper
JC5426	*recC22*	Hfr	S	300	+	+	+	300	+	+	+	318	+		This paper
JC5483	*recA56*	F$^-$	S	R	+	−	−	+	A323	+	+	+	?	*trp*	This paper
JC5467	*rec*$^+$	F$^-$	R	S	A	−	−	+	A324	+	+	+	?		This paper
JC5475	*rec*$^+$	F$^-$	A1	S	A	+	1	+	+	+	B1	+	?	*lysA10, fuc-1*	This paper

[a] The nomenclature used is that recommended by Demerec et al. (4) and Taylor and Trotter (16).

donors to transfer the episome to Thy$^-$ Str^R strains carrying *rec*$^+$, *recB21*, or *recC22* (Table 7). Proof of the presence of the mutant *rec* alleles on the transferred episomes was obtained by using the strains JC5534 (F *thyA*$^+$ *recB21/thyA323 recB*$^+$) and JC5537 (F *thyA*$^+$ *recC22/thyA323 recC*$^+$) as transductional donors and the Thy$^-$ Rec$^+$ strain JC5422 as recipient, selecting Thy$^+$ transductants: cotransduction of *thyA*$^+$ and *recB21* or *recC22* could result only from the incorporation of episomal deoxyribonucleic acid (DNA) into the transducing particle. The cotransduction frequencies of *recB21* and *recC22* with *thyA*$^+$ were 46% and 80%, respectively, both similar to the frequencies found with the original derivatives AB2470 and JC5474 themselves, confirming the presence of the *rec* mutations on F15.

Cured F$^-$ derivatives of the nine strains carrying different arrangements of *rec*$^+$, *recB21*, and *recC22* alleles on episome and chromosome were analyzed, and in each case the chromosome retained the original *rec* and *thy* mutations. It proved impossible to cure AB3022 (*recC22*) derivatives of their F15, and therefore to prove that the chromosome carried the original alleles. In addition, these strains mothered many more recombinants than F15 derivatives of JC5422 or JC5408. Therefore, F15 merodiploids of a derivative strain, JC5790 (Table 2 and below), also Thy$^-$ and carrying *recC22*, were used to confirm these complementation results.

Isolation of mutant strains. JC4535, JC4536, and JC4456 were isolated by Clark (2) on the basis of their inability to mother recombinants in crosses with Hfr strains. AB2470, AB3022, and AB3109 were isolated by Howard-Flanders and Theriot (10), Emmerson and Howard-Flanders (6), and by Emmerson (5) on the basis of their X-ray sensitivity. KMBL279 was derived as a UV-sensitive strain by van de Putte, Zwenk, and Rorsch (17).

The remaining strains were isolated as follows: a log-phase culture started from a single-colony isolate of strain AB1157 was centrifuged and the cells were resuspended at their original concentration in a solution of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1 mg/ml) in 0.1 M sodium acetate buffer, *p*H 5.0. After 30 min of incubation at 37 C, during which more than 50% of the cells remained viable, they were washed three times in broth by centrifugation. The culture was then resuspended in its original volume in broth, incubated overnight, diluted, and spread on complex medium. Clones which were exceptionally radiation-sensitive were detected by the method of Hunt and Borsa (11). These were purified by three single-colony isolations. Survival of the purified isolate was measured after exposure to a series of increasing X-ray doses. Only one isolate was retained from each mutagen-treated culture.

Two strains, AB3022 and JC4456, carried secondary mutations affecting their growth or ability to form F' merodiploids. Therefore, AB3022 was transduced with P1 grown on AB1157, and a Thy$^+$ Rec$^-$ transductant, JC5474, was isolated. This was used as the transductional donor to the Thy$^-$ Rec$^+$ strain JC5422, and a Rec$^-$ Thy$^+$ transductant, JC5489, was selected. Similarly, JC4456 was used as donor to transduce

TABLE 2. *Properties of the Rec⁻ strains*[a]

Strain no.	*rec* allele	Deficiency indices[b]			UV survival	Cotransduction of *rec* mutant allele with *thyA*⁺[c] (%)	Strain no. and *thy* mutation no. of Thy⁻ derivative	Deficiency index, Thy [Str^R] merodiploids with W4580	Corrected deficiency indices, Thy⁺ [Str^R] recombinants with	
		Lac⁺ [Str^R] merodiploids with E3	His⁺ [Str^R] recombinants with KL16	His⁺ [Str^R] recombinants with KL98					JC5412 (*recB21*)	JC5426 (*recC22*)
AB1157	+	1	1	1	0.50	—	JC5422 *thyA325*	1	1	1
AB2463	*recA13*	2	5	4×10^4	4×10^{-5}	<0.5	JC5421 *thyA326*	—	—	—
JC4536[d]	*rec-61*	4	1	90	0.01	34 ± 8[f]	JC4641 *thyA329*	4	130	3
JC5721	*rec-94*	3	4	50	0.004	38 ± 9	JC5722 *thyA345*	6	600	0.2
JC4535[d]	*rec-60*	5	1	90	0.01	39 ± 9	JC4645 *thyA330*	5	140	2
JC5707	*rec-85*	6	4	40	0.01	39 ± 9	JC5708 *thyA339*	3	300	3
AB2470	*recB21*	6	4	30	0.02	41 ± 9	JC5408 *thyA333*	5	400	2
JC5723	*rec-95*	2	3	40	0.004	41 ± 9	JC5724 *thyA346*	4	170	2
JC4457	*rec-58*	4	4	20	0.002	42 ± 9	JC4639 *thyA327*	4	140	3
JC5715	*rec-91*	4	4	40	0.003	45 ± 9	JC4654 *thyA331*	4	500	1
JC5717	*rec-92*	5	8	40	0.004	45 ± 9	JC5718 *thyA343*	5	90	1
AB3109	*rec-23*	6	3	20	0.004	46 ± 10	JC5531 *thyA335*	3	200	1
JC5713	*rec-90*	8	4	40	0.006	46 ± 10	JC5714 *thyA342*	6	300	2
JC5719	*rec-93*	5	4	30	0.005	46 ± 10	JC5720 *thyA344*	6	90	2
JC5711	*rec-89*	4	3	110	0.003	48 ± 10	JC5712 *thyA341*	7	500	1
JC5709	*rec-88*	5	5	50	0.003	49 ± 10	JC5710 *thyA340*	3	170	1
JC5701	*rec-81*	2	4	30	0.02	50 ± 10	JC5702 *thyA336*	3	140	2
JC5474	*rec-22*	32[e]	5[e]	60[e]	0.04	65 ± 11	AB3022[d] *thyA?*	8	1	40
JC5489	*rec-22*	4	5	20	0.01	—	JC5790 *thyA347*	—	—	—
JC5726	*rec-38*	6[e]	3[e]	40[e]	—	—	KMBL279[d] *thyA?*	—	—	—
JC5725	*rec-38*	3	2	40	0.01	74 ± 12	JC5520 *thyA334*	4	1	13
JC4456	*rec-73*	300	6	80	—	80 ± 13	JC4640 *thyA328*	6	—	—
JC2787[d]	*rec-73*	7	2	20	0.005	—	JC4662 *thyA332*	3	1	21
JC5705	*rec-83*	6	5	20	0.004	81 ± 13	JC5706 *thyA338*	2	2	60
JC5703	*rec-82*	2	4	40	0.003	86 ± 13	JC5704 *thyA337*	4	2	30

[a] The procedures used for transduction, and for the measurements of radiation sensitivity and of the frequency of conjugant formation, are described in the text. A UV dose of 200 ergs/mm² was used. All matings lasted for 120 min at 37 C and were plated directly without being interrupted.

[b] The deficiency index has been defined by Clark (2) as the ratio of the number of recombinants obtained with the *Rec*⁺ parent as recipient, divided by the number obtained with the mutant strain as recipient. The "corrected" deficiency index (see text) was calculated to allow for changes in the recipient ability of any particular mutant.

[c] The recipient used was JC5422 carrying *thyA325*. Two hundred *Thy*⁺ transductants were picked and their UV sensitivity and recombination ability were tested as described in Materials and Methods.

[d] JC4535, JC4536 and derivatives of JC1557; AB3022 is a derivative of AB2495; KMBL279 is a derivative of KMBL146. All other strains are derivatives of AB1157.

[e] These values were obtained with the original Thy⁻ derivatives.

[f] The 95% confidence interval.

JC5422, giving the Rec⁻ Thy⁺ strain JC2787. One other Rec⁻ Thy⁻ strain, KMBL279, had a completely different genetic background from AB1157; for ease of comparison, a Thy⁺ Rec⁻ transductant JC5726 was derived by transduction with AB1157 as donor, and this was used as the donor to transduce JC5422, giving the Thy⁺ Rec⁻ transductant JC5725. These three derived strains had recombination deficiencies and UV sensitivities similar to those of the original strains, but did not carry the unwanted mutations (Table 1).

Media. The complex and synthetic media described by Willetts, Clark, and Low (18) were used.

Transduction and mating procedures. The procedures used for transduction, for liquid culture matings, and for plate matings as a test for recombination ability were those described by Clark and Margulies (3) and Willetts, Clark, and Low (18). The Hfr strain KL98 was routinely used in testing recombination ability by plate matings.

When F15 derivatives were used as recipients in crosses performed in liquid media, they were converted to the recipient phenocopy state by incubating a culture overnight in L broth with shaking. The cultures were diluted immediately prior to mating in fresh L broth to the normal cell density used for recipients. The proportion of the population which retained F15 at the time of mating was checked, these

being Thy$^+$ clones capable of giving Thy$^+$ [Spc^R] conjugants with the Thy$^-$ RecA$^-$ Spc^R strain JC5483; >90% F15-carrying clones were always found.

Radiation sensitivity. For the determination of UV sensitivity, cultures in exponential phase at about 10^8/ml were diluted 100-fold in phosphate buffer, *p*H 7.0. A 5-ml amount of this diluted suspension was transferred to a glass petri dish, giving a layer less than 1 mm thick. The suspension was gently agitated during irradiation for the required time with UV light from a GE 15-w germicidal lamp placed 43 cm away. A calibrated photocell was used to measure the light intensity. Samples were taken both before and immediately after irradiation, diluted, and plated on complex medium; the plates were incubated overnight in the dark.

Plate tests for UV sensitivity were carried out as described by Clark and Margulies (3). UV sensitivity was invariably associated with recombination deficiency in the several thousand conjugant or transductant clones tested.

For determination of X-ray sensitivity, a log-phase culture grown at 37 C to 2 × 10^8 colony-forming units per ml was cooled in ice water, and 3 ml was placed in a circular plastic petri dish (60 × 15 mm) along with a sterile stainless-steel pin for magnetic stirring. The lid was replaced and the culture was irradiated, the base of the dish being 15.2 cm from the target of an unfiltered berryllium window X-ray tube (Philips FA-100) operating at 100 kv, 20 ma. During irradiation, the culture was stirred continuously. The approximate dose rate was 14,000 r/min as measured by a calibrated Victoreen Radocon model 602 ionization chamber irradiated in air 15.2 cm from the unfiltered tube. Samples were diluted and plated on complex medium both before and after irradiation.

Curing of F15. L broth adjusted to *p*H 7.8 to 7.9 with NaOH, and containing 25 mg of acridine orange and 50 mg of thymine per ml, was used. A 0.1-ml amount of a 10^{-2} dilution of a standing overnight culture of the merodiploid strain was added to 2 ml of the broth in a sterile tube covered with aluminum foil to exclude light. The culture was shaken overnight, and dilutions were plated on to L plates containing 50 μg of thymine per ml.

Isolation of Thy$^-$ derivatives. The selection method was that of Stacey and Simson (15), in which trimethoprim is used. Nonreverting Thy$^-$ clones were chosen.

RESULTS

Characteristics of the mutant strains. The properties of the 20 recombination-deficient strains under consideration are listed in Table 2. Some of the properties of AB2470 (*recB21*), AB3109 (*rec-23*), AB3022 (*rec-22*), JC4535 (*rec-60*), and JC4536 (*rec-61*) have been described previously (2, 5, 6, 9). The fifth column of Table 2 shows that, when crossed with Hfr strain KL98, the mutant strains yield fewer recombinants than the parental Rec$^+$ strain. This difference is expressed as the deficiency index, which is inversely proportional to the number of recombinants obtained with a particular Rec$^-$ mutant (2). The deficiency indices vary over a 10-fold range, but are much smaller than that of a *recA* mutant, AB2463, which is included for comparison.

That this deficiency in recombination is not due solely to poor recipient ability in these strains is shown in the third and fourth columns of Table 2. Crosses with the F*lac*$^+$ donor E3 yielded almost normal numbers of Lac$^+$ merodiploids, for whose formation the Rec$^+$ phenotype is therefore not required. Those strains yielding less than the normal number of merodiploids carried a second mutation producing this effect, as described above in Materials and Methods. In addition, high yields of His$^+$ [Str^R] recombinants were obtained with the donor strain KL16, which not only confirmed that the mutant strains are good recipients, but also suggested that KL16 transfers the *rec*$^+$ allele of the *rec* mutations early during conjugation. KL98 presumably does not transfer the *rec*$^+$ alleles as early markers. These results suggested that all of the mutations are in genes lying between the origins of KL16 and KL98, as are mutations such as *recA13* (2).

The strains are all more sensitive to ultraviolet light and to X rays than the Rec$^+$ parents, although they are less UV sensitive than the RecA$^-$ strain AB2463. Some representative UV and X-ray survival curves are shown in Fig. 2, and the fraction of cells surviving exposure to a single dose of 200 ergs/mm^2 of UV for all of the mutant strains is listed in Table 2.

Lambda lysogens of most of the strains have been made. All are inducible by UV and show normal spontaneous induction of lambda (Mount, *unpublished data*), as found previously for some of the strains (1). RecA$^-$ strains such as AB2463 differ in showing abnormally low lambda induction.

Two of the strains listed in Table 2, AB2470 and JC4535, and another strain carrying *rec-38* (KMBL243) show, along with RecA$^-$ strains, the property of spontaneous lethal sectoring (7). Although the frequency of spontaneous lethal sectoring of the other strains in Table 2 has not been measured, all of these grow more slowly than their Rec$^+$ parents, and have a greatly reduced viable count per culture density unit, indicating that they all have this property.

One other property characteristic of the strains carrying *recB21*, *rec-22*, and *rec-23* is that, after UV irradiation, they degrade their DNA to cold trichloroacetic acid-soluble material at a slower rate than the *rec*$^+$ parent (5, 9). This reduction in DNA breakdown also serves to distinguish such strains from those having a high

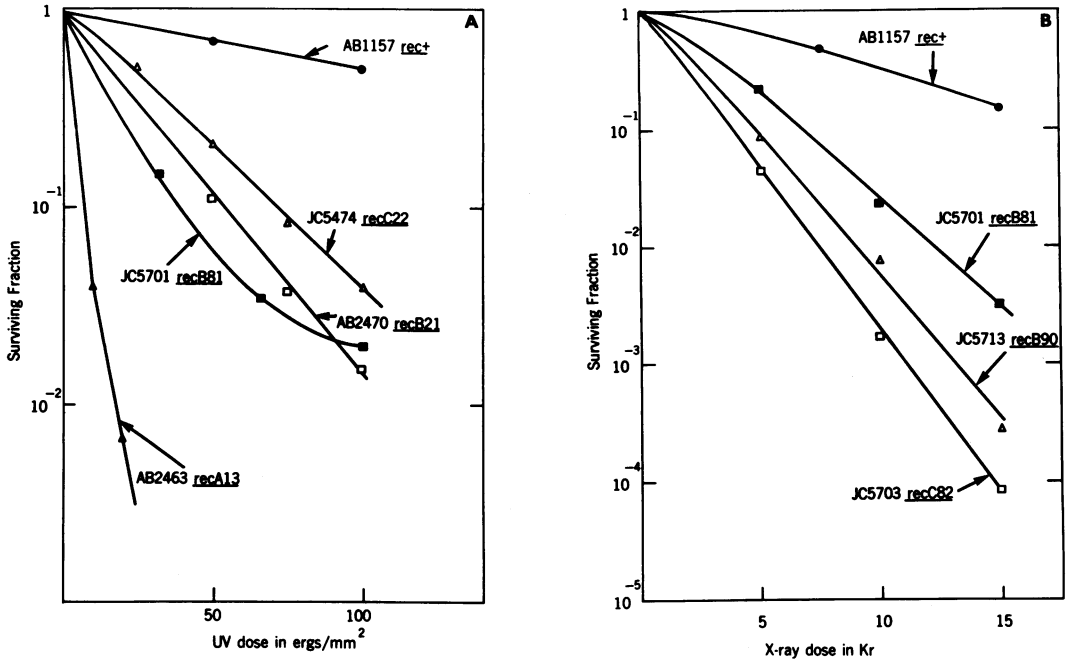

FIG. 2. *Survival curves for Rec⁺ and Rec⁻ strains. (A) Ultraviolet light survivals:* ●, *AB1157 (rec⁺);* △ *JC5474 (recC22);* □, *AB2470 (recB21);* ■, *JC5701 (recB81);* ▲ *AB2463 (recA13). (B) X-ray survivals:* ● *AB1157 (rec⁺);* ■, *JC5701 (recB81);* △, *JC5713 (recB90);* □, *JC5703 (recC82).*

level of induced degradation such as AB2463, carrying *recA13*. However, these measurements have not so far been made on any other of the strains discussed here.

Cotransduction with thyA. It is shown above that all of the mutations appear to be in genes located between the origins of KL16 and KL98. Emmerson and Howard-Flanders (6) and Emmerson (5) found that three of the mutations, *recB21*, *rec-22*, and *rec-23* are cotransducible with *thyA*. Therefore, attempts were made to cotransduce all the mutations with *thyA*.

One unusual feature associated with such mapping of *rec* mutations is that the zygote, in this case the temporary transductional zygote, must be Rec⁺, to allow a normal level of recombinant formation. If both donor and recipient carry the same *rec⁻* mutation, the yield of Thy⁺ transductants is about 1% of that obtained with a Rec⁺ recipient (Tables 5 and 6). In a cross between a Rec⁺ donor and a Rec⁻ recipient strain, only those recipient cells which have received the *rec⁺* gene as well as the wild-type allele of the selected marker can become transductants, leading to an overestimate of the cotransduction frequency of *rec⁺* and the selected marker. Table 3 shows the frequencies obtained with three different *rec* mutations when present in either donor or recipient. When the recipient is Rec⁻, the frequency of cotransduction is always higher, as predicted by the above argument. As expected, the discrepancy is greatest for *recB21* which maps farthest from *thyA*, since the proportion of *thyA⁺* transducing particles carrying the *rec⁺* allele should be smallest in this case.

The *rec* mutations carried by all the strains are cotransducible with *thyA⁺* at frequencies varying from 34 to 86%, and the strains can be divided into two groups on this basis: those with cotrans-

TABLE 3. *Cotransduction frequencies with Rec⁺ or Rec⁻ strains as recipients*[a]

rec allele of Thy⁺ donor	*rec* allele of Thy⁻ recipient	Cotransduction of *thyA* and *rec*
		%
recB21	*rec⁺*	41
rec⁺	*recB21*	82
recC22	*rec⁺*	65
rec⁺	*recC22*	84
recC82	*rec⁺*	86
rec⁺	*recC82*	90

[a] Transduction and the analysis of the transductants was carried out as described in Materials and Methods. P1 grown on the Thy⁺ strain carrying the *rec* allele specified in the first column was used to transduce a Thy⁻ strain carrying the *rec* allele given in the second column.

duction frequencies in the range 34 to 50%, and those with frequencies in the range 74 to 86% (Table 2). The *rec-22* mutation carried by JC5474, with a frequency of cotransduction of 65%, cannot be clearly assigned to either group, but complementation studies described below show that it belongs to the group with higher cotransduction frequencies.

Location of recB21 and rec-73 with respect to genes near thyA. These two mutations were chosen as representatives of the two groups with different cotransduction frequencies with *thyA*. The Rec$^+$ LysA$^-$ ThyA$^-$ ArgA$^-$ His$^-$ Str^R strain JC5467 was used as recipient. The order *lysA-thyA-argA* (Fig. 1) was confirmed by analysis of the unselected marker frequencies with Rec$^+$ AB1157 as transductional donor for the wild-type alleles, and, from the same crosses, the cotransduction frequencies of these markers were obtained (Fig. 3a).

AB2470, carrying *recB21*, and JC4456, carrying *rec-73*, were then used as transductional donors, and Lys$^+$, Thy$^+$, and Arg$^+$ transductants were separately selected. Among these, inheritance of the unselected auxotrophic markers was analyzed by a replica-plating technique, and inheritance of the *rec* mutation was analyzed by UV sensitivity and recombination deficiency as described in Materials and Methods. The cotransduction frequencies of the auxotrophic markers were similar to those found when AB1157 was used as donor (Fig. 3b and c). It was clear that *recB21* and *rec-73* were to the right of *thyA* because their frequencies of cotransduction with *lysA* were much smaller than either those of *thyA* with *lysA*, or their own cotransduction frequencies with *thyA*. It also appeared that they were to the left of *argA* but this was difficult to decide from the cotransduction frequencies alone because of the large difference obtained for the cotransduction frequency of *thyA* and *argA* when Thy$^+$ or Arg$^+$ transductants were selected. That they are indeed to the left of *argA* was shown by an analysis of the different classes of transductants obtained (Table 4A), after pooling the Lys$^+$ and Lys$^-$ classes in each case.

The larger fraction of Rec$^-$ transductants obtained among the Arg$^+$ class compared to the

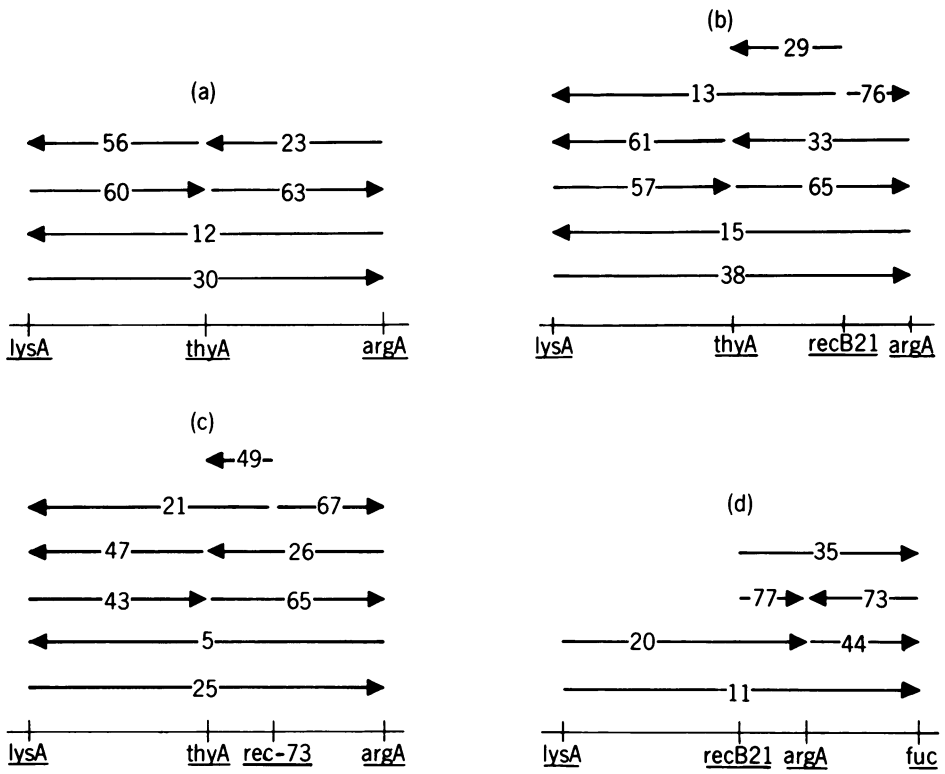

FIG. 3. *Cotransduction frequencies obtained in the transductional crosses described in the text and Table 4. The arrow in each case points toward the marker selected. (a) Donor AB1157 (rec$^+$), recipient JC5467. (b) Donor AB2470 (recB21), recipient JC5467. (c) Donor JC4456 (recB73), recipient JC5467. (d) Donor AB2470 (recB21), recipient JC5475.*

TABLE 4. *Location of recB21 and rec-73 with respect to thyA, argA, and fuc*

Expt	Selected marker	Unselected marker	Fractions which inherit unselected *rec*⁻ marker	
			recB21 donor (AB2470)	*rec-73* donor (JC4456)
A[a]	Thy⁺	Arg⁺	82/128 (66%)	88/104 (85%)
	Thy⁺	Arg⁻	32/272 (12%)	108/296 (37%)
	Arg⁺	Thy⁺	228/260 (88%)	240/260 (92%)
	Arg⁺	Thy⁻	76/140 (54%)	28/140 (20%)
B[b]	Arg⁺	Fuc⁺	124/146 (85%)	—
	Arg⁺	Fuc⁻	34/54 (63%)	—
	Fuc⁺	Arg⁺	68/88 (77%)	—
	Fuc⁺	Arg⁻	2/112 (2%)	—

[a] P1 grown on AB2470 or JC4456 was used to transduce JC5467; Thy⁺ or Arg⁺ transductants were selected, and approximately 400 of each were analyzed for unselected markers as described in the text. The results for Lys⁺ and Lys⁻ transductants are pooled. The fractions are the number of Rec⁻ colonies divided by the total number (Rec⁺ plus Rec⁻) obtained in each class.

[b] P1 grown on AB2470 was used to transduce JC5475: 200 each of Arg⁺ and Fuc⁺ transductants were analyzed; the results are presented as in A.

Arg⁻ class with Thy⁺ selection, and the Thy⁺ class compared to the Thy⁻ class with Arg⁺ selection, for both the *recB21* and the *rec-73* donors, showed that *recB21* and *rec-73* lay between *thyA* and *argA*. The larger fraction of *recB21* transductants in the Arg⁺ Thy⁻ class than in the Thy⁺ Arg⁻ class suggested further that *recB21* is more closely linked to *argA* than to *thyA*. The reciprocal result obtained with the *rec-73* transductants suggested that *rec-73* is more closely linked to *thyA* than to *argA*. The deduced order is therefore *thyA-rec-73-recB21-argA*. It will be confirmed later that *rec-73* is in fact closer to *thyA* than to *recB21*.

The location of *recB21* between *thyA* and *argA* was confirmed by using the Rec⁺ LysA⁻ ArgA⁻ Fuc⁻ His⁻ Str^R strain JC5475 as recipient, *fuc* being to the right of *argA*. Fuc⁺ or Arg⁺ transductants were selected, and the unselected marker frequencies were measured. The lower cotransduction frequency of *recB21* with *fuc* than with *argA*, together with the high cotransduction frequency of *argA* and *fuc*, showed *recB21* to be to the left of *argA* (Fig. 3d). This result was also given by analysis of the different transductant classes (Table 4B). With Arg⁺ selection, there was very little difference in the frequency of Rec⁻ in the Arg⁺ Fuc⁺ and Arg⁺ Fuc⁻ classes, suggesting that *recB21* is not between *argA* and *fuc*. That it is to the left of *argA* was revealed by the fact that, when Fuc⁺ was selected, very few of the Fuc⁺ Arg⁻, but most of the Fuc⁺ Arg⁺, were Rec⁻. These results also suggested, as did earlier ones, that *recB21* and *argA* are closely linked.

One from each of the different auxotrophic classes of Rec⁻ transductants of JC5467 obtained with JC4456 as donor were tested for their ability to form F*lac*⁺ merodiploids. All were normal, confirming that the mutation leading to the inability of JC4456 to form merodiploids was separable from the *rec* mutation and located elsewhere on the chromosome.

Presence of the rec⁺ alleles on F15. Since *recB21* and *rec-73* both map between *thyA* and *argA*, and it is known that F15 carries these two genes (12), it seemed likely that F15 might carry the *rec*⁺ alleles of all the mutations cotransducible with *thyA*. Thy⁻ derivatives of all of the mutant strains were therefore isolated, and F15 derivatives of these made by crossing with W4580 (Table 2). Twenty-five Thy⁺ conjugants from each cross were patched on to minimal medium and analyzed for recombination ability, UV sensitivity, and ability to donate *thyA*⁺ to the RecA⁻ ThyA⁻ Spc^R strain JC5483, as described in Materials and Methods. Between 23 of 25 and 25 of 25 of the Thy⁺ conjugants for all strains were found to be UV^R Rec⁺ and able to donate *thyA*⁺. A few exceptional colonies were found which were UV-sensitive, recombination-deficient, or nondonors of *thyA*⁺, or which had a combination of these characteristics. These appeared to have arisen by a recombination event between the chromosome and the episome. The results confirmed the map location of the *rec* mutations obtained from the cotransduction studies, and showed that F15 carries dominant *rec*⁺ alleles of all of the *rec* mutations tested.

Complementation by Hfr × F⁻ crosses. This test is based upon the observation that a Rec⁻ recipient yielded almost as many recombinants with the Hfr strain KL16 as a Rec⁺ recipient. It was deduced that this Hfr must transfer *rec*⁺ as a proximal marker, and cotransduction of the *rec* mutations with *thyA* confirmed this, since *thyA* is one of the first markers transferred by this Hfr. Therefore, derivatives of KL16, called JC5412 and JC5426 and carrying *recB21* and *rec-22*, respectively, were constructed as described in Materials and Methods. These two mutations were chosen on the basis of preliminary evidence suggesting complementation between them, obtained by P. Howard-Flanders (*see* 3). In crosses between either of these Hfr strains and a Rec⁻ F⁻ strain, a reduced yield of Thy⁺ [Str^R] recombinants was expected if Hfr and F⁻ mutations were in the same gene.

The results of such crosses are shown in Table

2. The deficiency indices were calculated as usual, but in addition were corrected for changes in the recipient ability of the mutant by dividing by the deficiency index found in a cross between the same mutant culture and the Rec$^+$ parental Hfr, JC5029. A value near one indicates that the conjugational zygote was Rec$^+$ and that the two *rec* mutations complemented; a greater value indicates that the zygote was Rec$^-$ and thus that the two *rec* mutations did not complement. Two complementation groups are apparent: 15 strains carry mutations which complement *rec-22* carried by JC5426 but not *recB21* carried by JC5412, and 5 strains carry mutations which fail to complement *rec-22* but do complement *recB21*. These two groups were identical to those deduced from the frequencies of cotransduction with *thyA*. Thus, *rec-22* appears to be a mutation in a new gene, which from here on will be referred to as *recC*.

The number of recombinants obtained in crosses between the Hfr *recB21* donor and a *recB*$^-$ recipient in the above crosses was about two- to threefold lower than that observed between Hfr *recC22* and a *recC*$^-$ recipient. This result might be explained in terms of a greater leakiness of the *recC22* mutation compared with the *recB21* mutation.

Complementation by transduction. Two requirements for the formation of a Thy$^+$ transductant of a Rec$^-$ Thy$^-$ strain are that the transducing fragment carry the *thyA*$^+$ allele and that the transductional zygote be Rec$^+$. Advantage was taken of the fact that *thyA* and *recB* or *recC* are cotransduced at high frequencies, and thus that the transducing fragment often carries *thyA*, *recB*, and *recC*, to perform an unusual complementation test by transduction.

For this test, a Rec$^-$ Thy$^+$ strain was used as the transductional donor to a Rec$^-$ Thy$^-$ recipient. If the *rec* mutation carried by the donor is in the same gene as that carried by the recipient, there should be no complementation; the temporary transductional zygotes should all be Rec$^-$, and very few Thy$^+$ transductants should be formed. If, however, the mutations complement, then some of the transductional zygotes should be Rec$^+$ and, because *thyA*$^+$ is often carried by the same transducing fragment, Thy$^+$ transductants should be produced. These transductants might also receive one or the other of the *rec* alleles carried by the transducing fragment, depending upon where the crossover events occurred. One advantage of transduction over conjugation in these complementation tests was that in the case of transduction it was easy to perform reciprocal crosses.

In these experiments, complementation by *recC22* of five mutations classified as *recB* mutations was tested; reciprocal transductions were performed, with the *recB* mutation in either the donor Thy$^+$ strain or the recipient Thy$^-$ strain. Also, controls were done with strains carrying *recC22* or another *recC* mutation to confirm that no Thy$^+$ transductants were formed under these circumstances. The results are shown in Table 5. The measured frequencies were corrected for (i) the reduced yield per infective particle (10 to 80%) of transducing phage carrying *thyA*$^+$ in lysates of Rec$^-$ strains compared with the yield from Rec$^+$ strains, and (ii) the lower yield of transductants obtained when a Rec$^+$ donor was used with a Rec$^-$ rather than a Rec$^+$ recipient. The reason for the first result is unknown. The basis for the

TABLE 5. *Frequency of formation of Thy$^+$ transductants with recC22*

A[a]

rec allele in Thy$^-$ recipient	Donor AB1157 (*rec*$^+$) measured frequency/ 10^6 P1	Donor JC5474 (*recC22*)		
		Measured frequency/ 10^6 P1	Corrected frequency/ 10^6 P1	Per cent Rec$^+$
rec$^+$	40	9.0	40	35
recC22	3.5	0.01	0.4	—
recC73	13	<0.005	<0.2	—
recB21	6.1	2.5	70	3
recB58	3.6	2.2	70	5
recB93	7.2	2.2	50	4
recB88	2.9	2.0	100	8
recB81	2.7	1.6	120	3

B[b]

rec allele in Thy$^+$ donor	Recipient JC5422 (*rec*$^+$) measured frequency/ 10^6 P1	Recipient AB3022 (*rec C22*)		
		Measured frequency/ 10^6 P1	Corrected frequency/ 10^6 P1	Per cent Rec$^+$
rec$^+$	40	3.5	40	84
recC22	9.0	0.01	0.8	—
recC73	5.5	<0.002	<0.4	—
recB21	34	3	20	28
recB58	9.2	0.56	40	33
recB93	12	0.56	40	33
recB88	8.4	0.73	60	25
recB81	25	1.3	40	20

[a] P1 grown on Rec$^+$ Thy$^+$ AB1157 or RecC$^-$ Thy$^+$ JC5474 was used to transduce Thy$^-$ strains carrying the *rec* alleles given in the first column. The corrected frequency of formation of Thy$^+$ transductants using P1·JC5474 was derived from the measured frequency as described in the text.

[b] The reciprocal crosses. P1 grown on the Thy$^+$ strain carrying the *rec* allele given in the first column was used to transduce Rec$^+$ Thy$^-$ JC5422 or RecC$^-$ Thy$^-$ AB3022, and corrected frequencies of formation of Thy$^+$ transductants of AB3022 were derived as given in the text.

second correction is that, first, unless the transducing particles carry both *thyA*$^+$ and the necessary *rec*$^+$ allele, the chance of obtaining a Thy$^+$ transductant in a Rec$^-$ strain is considerably reduced (reference 5 and Tables 5 and 6), and, second, Rec$^-$ strains contain many inviable cells which may take up transducing phage without giving Thy$^+$ progeny (7). Thy$^+$ transductants were obtained at approximately the expected corrected frequencies with all strains carrying *recB*, whether these were used as donors or recipients. However, no or very few Thy$^+$ transductants were obtained when strains carrying the same or a different *recC* mutation were used. Although the corrections were often rather large, even the uncorrected frequencies demonstrated quite convincingly whether or not complementation had occurred in the transductional zygote. These experiments were extended to show that Thy$^+$ transductants were obtained by complementation of *recB21* with any one of five *recC* mutations, but not with *recB21* or another *recB* mutation (Table 6). The transductional complementation tests therefore confirmed the allocation of these mutations to the two complementation groups described in the previous section.

Complementation in F15 merodiploids. The discovery that F15 carried both *recB*$^+$ and *recC*$^+$ made possible a third type of complementation test employing stable heterozygotes. This test was more satisfactory than those with temporary zygotes described above, because it did not depend upon rapid expression of the *rec*$^+$ allele for recombinant formation. The construction of F15 episomes carrying *recB21* and *recC22*, and the transfer of these to Thy$^-$ strains of genotype *rec*$^+$, *recB21*, or *recC22* is described in Materials and Methods. By such means, a complete set of nine merodiploids was constructed. In addition, the F15 derivatives of a second strain carrying *recC22* were tested for reasons discussed in Materials and Methods.

The properties of the strains are shown in Table 7. All of them had the UV sensitivities or resistances which would be expected from the *rec* alleles present, and from the information obtained from the other types of complementation tests. In particular, *recB21/recC22* heterozygotes were UV-resistant. Their recombination ability was measured by converting them into the recipient phenocopy state by overnight incubation with shaking, and then crossing them with the donor strain KL98. His$^+$ Thy$^+$ [Str^R] recombinants were selected, thereby ensuring that these still carried F15. The recombinant frequencies were normalized to the frequency obtained with wild-type F15 in the same Thy$^-$ strain, since, in particular, F15 derivatives of AB3022 were unusually good recipients. Again, the expected results were obtained, confirming the dominance of the *rec*$^+$ alleles and the complementation of *recB21* by *recC22*.

Relative position of recB and recC. The relative positions of *thyA*, *recB*, and *recC* were obtained from the three-factor reciprocal transductional crosses previously used to demonstrate complementation between *recB* and *recC* mutations. This was done by analyzing the Thy$^+$ transductants in the manner previously described to discover what percentage of these was Rec$^+$. These values are given in the last columns of Tables 5 and 6. Reference to Fig. 4A will show

TABLE 6. *Frequency of formation of Thy$^+$ transductants with recB21*

A[a]

rec allele in Thy$^-$ recipient	Donor AB1157 (*rec*$^+$) measured frequency/ 10^6 P1	Donor AB2470 (*recB21*)		
		Measured frequency/ 10^6 P1	Corrected frequency/ 10^6 P1	Per cent Rec$^+$
rec$^+$	40	34	40	59
recB21	6.1	0.06	0.4	—
recB88	2.9	0.3	5	—
recC22	3.5	3.0	40	28
recC38	6.8	9.0	60	14
recC73	13	16	80	24
recC83	6.7	6.1	40	34
recC82	5.0	5.0	50	41

B[b]

rec allele in Thy$^+$ donor	Recipient JC5422 (*rec*$^+$) measured frequency/ 10^6 P1	Recipient JC5408 (*rec B21*)		
		Measured frequency/ 10^6 P1	Corrected frequency/ 10^6 P1	Per cent Rec$^+$
rec$^+$	40	6.1	40	82
recB21	34	0.06	0.4	—
recB88	8.4	0.02	0.8	—
recC22	9.0	3.4	70	3
recC38	18	5.2	80	4
recC73	5.5	0.8	40	4
recC83	34	1.5	10	3
recC82	11	1.4	30	1

[a] P1 grown on Rec$^+$ Thy$^+$ AB1157 or RecB$^-$ Thy$^+$ AB2470 was used to transduce Thy$^-$ strains carrying the *rec* alleles in the first column. The corrected frequency of formation of Thy$^+$ transductants using P1·AB2470 was derived from the measured frequency as described in the text.

[b] The reciprocal crosses. P1 grown on the Thy$^+$ strain carrying the *rec* allele given in the first column was used to transduce Rec$^+$ Thy$^-$ JC5422 or RecB$^-$ Thy$^-$ JC5408, and corrected frequencies of Thy$^+$ transductants of JC5408 were derived as given in the text.

TABLE 7. *Phenotype of various rec heterozygotes*[a]

Strain	Genotype		UV sensitivity	No. of His+ Thy+ [Str^R] recombinants with KL98 per 100 Hfr cells[b]	Relative no. of His+ Thy+ [Str^R] recombinants with KL98
	F15	chromosome			
Derivatives of JC5422					
JC5488	+	+	R	0.052	1
JC5534	*recB21*	+	R	0.026	2.0
JC5537	*recC22*	+	R	0.031	1.7
Derivatives of JC5408					
JC5486	+	*recB21*	R	0.076	1
JC5535	*recB21*	*recB21*	S	0.001	76
JC5538	*recC22*	*recB21*	R	0.041	1.9
Derivatives of AB3022					
JC5487	+	*recC22*	R	1.0	1
JC5536	*recB21*	*recC22*	R	0.90	1.1
JC5539	*recC22*	*recC22*	S	0.072	14
Derivatives of JC5790					
JC5825	+	*recC22*	R	0.10	1
JC5826	*recB21*	*recC22*	R	0.11	0.9
JC5827	*recC22*	*recC22*	S	0.0038	27

[a] These derivatives were constructed as described in Materials and Methods, and their UV sensitivities and recombination abilities were measured as described in the text.

[b] Frequency with AB1157 as recipient is 4%.

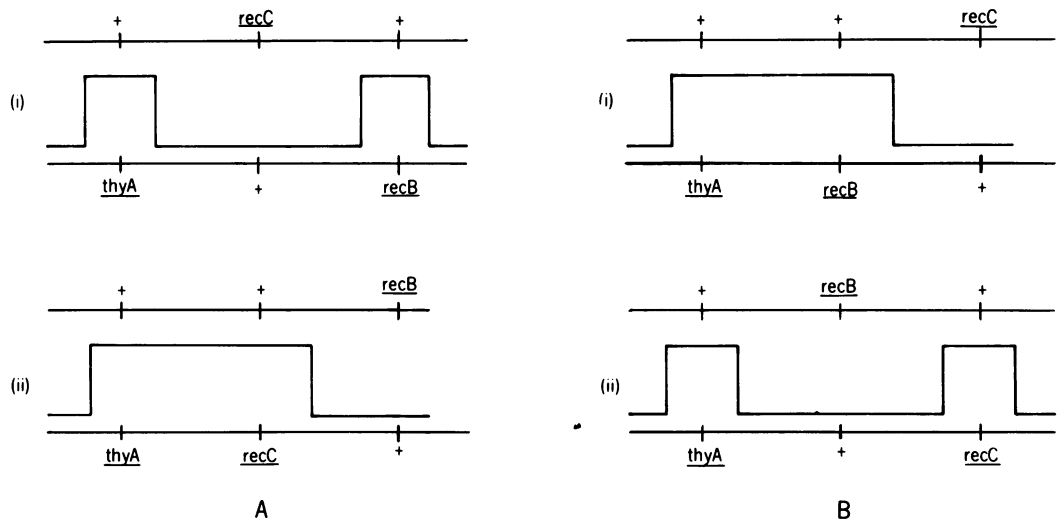

FIG. 4. *Two possible orders of thyA, recB, and recC. (A) thyA-recC-recB: (i) recC mutation in the donor, and recB mutation in the recipient: (ii) recB mutation in the donor, and recC mutation in the recipient. (B) thyA-recB-recC: (i) and (ii) as in A. The crossovers necessary to give Rec+ Thy+ transductants are indicated.*

that, if the order is *thy-recC-recB*, then, if *recC22* was in the donor and *recB21* in the recipient, a four-crossover event would be necessary to give Rec+ Thy+ transductants, and consequently many fewer would be obtained than when *recB21* was in the donor and *recC22* in the recipient. If the order were reversed, then so would be the results expected (Fig. 4B). The results showed that A (Fig. 4) was correct; the five *recB* mutations tested were all to the right of *recC22*, and the five *recC* mutations tested were all to the left of *recB21*. This was the order expected from the frequencies of cotransduction with *thyA* or *argA* previously described (Table 2 and Fig. 3).

DISCUSSION

The mutations in 20 Rec− strains which have intermediate recombination deficiencies and radiation sensitivities, and whose lysogens show normal spontaneous production of phage λ, are located near *thyA* on the *E. coli* K-12 linkage map.

Complementation tests by means of radiation sensitivity and recombination measurements of either temporary conjugational or transductional or permanent F′ merodiploid heterozygotes show that the mutations fall into two complementation groups. Fifteen of the mutations fail to complement *recB21* but complement *rec-22*; the remaining five mutations fail to complement *rec-22* but complement *recB21*. These five latter mutations are all cotransduced with *thyA* at a higher frequency than those which do not complement *recB21* and, by means of three-factor crosses, have been mapped closer to *thyA* than to *recB21*. These results suggest the existence of two genes required for recombination near *thyA*, denoted *recB* and *recC*.

The inability of the strains tested to produce genetic recombinants was not due to their poor recipient ability during conjugation. This was shown by the high yields of conjugants obtained in crosses either with an Hfr strain which transfers the rec^+ allele early, or with an F′ donor. A reduction of about fivefold compared to the Rec^+ parent was, however, usually observed in either case. This is most likely due to the inability of a large fraction of the recipient cells to form colonies, which has been generally observed for all $RecB^-$ and $RecC^-$ strains. Other explanations, such as a partial degradation of the transferred DNA, or a suboptimal level of expression of the newly injected rec^+ gene in the case of Hfr × F^- matings, seem less likely. Very little reduction in recombinant formation was observed in crosses in which the permanent F15 heterozygotes were used as recipients.

It may be significant that all 20 strains analyzed here produced about the same number of recombinants as *recB21* and *recC22* strains; they all produced 20- to 110-fold fewer recombinants than rec^+ strains when crossed with the Hfr donor strain KL98. In contrast, *recA* strains produce 10^3 to 10^5 fewer recombinants than rec^+ in similar crosses. Perhaps a second minor mechanism for making recombinants still functions in *recB* and *recC* mutants, in which case it should be possible to remove the residual recombination ability by introducing secondary *rec* mutations. Alternatively, it is possible that certain minimal levels of *recB* and *recC* gene products are essential for survival. This is suggested by the lethal sectoring associated with all of these mutations; i.e., a large fraction of the cells in their cultures are unable to form colonies. The similar degrees to which *recB* and *recC* mutant strains are able to form recombinants would then reflect the degree to which the activity of their product may be altered such that, on the one hand, a reduction in recombination ability or an increase in radiation sensitivity may be detected, while, on the other, cell survival is possible. Low (13 and *personal communication*) has shown that the few recombinants produced by either a *recB21* or *recC22* recipient when rec^+ is transferred late during conjugation are haploid and show a normal likage between selected and unselected markers.

Accurate transduction mapping of *recB21* and *recC73* has established the gene order *thyA-recC73-recB21-argA*, in agreement with Emmerson (5), who found that *recB21* and *recC22* were both located between *thyA* and *argA*. A peculiarity of the cotransduction frequencies measured was that the linkage between *lysA* or *thyA* and *argA* appeared to be much greater from analysis of the Arg^+ transductants for unselected markers than from a similar analysis of Lys^+ or Thy^+ transductants. Also, the frequency per phage of Arg^+ transductants was only one-quarter of that of Lys^+ or Thy^+ transductants. Similar nonreciprocity in other regions of the chromosome has been noted previously (14, 16).

One of the mutations (*recC38*) was positioned close to *thr* by van de Putte et al. (17) on the basis of time-of-entry experiments. This result is in discord with the observation reported here that *recC38* is cotransducible with *thyA*. Time-of-entry experiments may be misleading in strains carrying mutations that affect recombination. Moreover, Low (13) found a 400-fold variation in the yield of recombinants in a *recB21* recipient relative to rec^+ when it was mated with different Hfr strains under conditions not allowing transfer of rec^+. The yield of transductants in a *recC* recipient also varies by an order of magnitude depending upon the marker selected (6). These results suggest that determinants on the donor chromosome may modify the Rec^- phenotype of the recipient.

The complementation results and the finding that all 5 *recC* mutations map closer to *thyA* than the 15 *recB* mutations constitute rather strong evidence that there are two genes required for recombination in this region of the chromosome. A closer examination of the linkages of *recB* and *recC* to *thyA* revealed that these two genes are probably not contiguous. Application of the theoretical relationship between cotransduction frequency and map distance derived by Wu (19) showed that the *recC* mutations are about 0.2 min (10 genes), and the *recB* mutations about 0.7 min (30 genes), distant from *thyA325*. This result also argues against the interpretation that there is only one gene with intragenic complementation occurring between mutations in the two ends of the gene. However, one reservation about this interpretation is that it assumes that

recB⁻ and *recC*⁻ transductants have the same chance of producing a transductant clone, whereas, perhaps due to differences in the frequencies of lethal sectoring (7), this may not be the case.

Although the nature of the biochemical defect in *recB* and *recC* strains has yet to be determined, recent work by Barbour and Clark (*personal communication*) suggests that the *recB* and *recC* products together determine a deoxyribonuclease activity which may be involved in the process of genetic recombination. This is compatible with the known phenotype of these strains, especially their inability to degrade their own DNA after UV irradiation. In addition, if the deoxyribonuclease is composed of subunits specified by these genes, this would account for the close phenotypic similarities between *recB* and *recC* mutants.

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