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GENETIC RECOMBINATION IN *ESCHERICHIA COLI*

IV. ISOLATION AND CHARACTERIZATION OF RECOMBINATION-DEFICIENT MUTANTS OF *ESCHERICHIA COLI* K12

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SUMMARY

19 independent recombination-deficient mutants were isolated. 7 carried mutations that mapped near or in the *recB* and *recC* genes between *thyA* and *argA*. 10 mutants carried mutations cotransducible with *pheA* and exhibited no complementation with *recA* in temporary zygotic diploids.

Two new genes controlling recombination have been identified. Strain PC 0297 carried a mutation designated *recG162*, and was UV- and X-ray-sensitive. It was located between *pyrE* and *ilvA*. Strain PC 1250 was only slightly UV-sensitive, was X-ray-resistant and carried the mutation designated *recH166* which was located between *pheA* and *cysQ*.

INTRODUCTION

Rec$^-$ mutants have been isolated by several investigators. On this and other bases the following genes are now recognized: (*1*) *recA* (located[3,15] near *pheA*); (*2*) *recB* (located[7] between *thyA* and *argA*); (*3*) *recC* (located[5] between *thyA* and *recB*); (*4*) *recD*, reserved for *rec34* which is not cotransducible with *thyA*, *argA* (HOEKSTRA, unpublished data), or *cysC* (CLARK, personal communication), although its *rec*$^+$ allele is early injected by Hfr Kl16; (*5*) *recE*; and (*6*) *recF*. The last two genes are probably involved in recombination pathways alternative to that involving *recB* and *recC* (CLARK, paper submitted for publication to the 10th International Congress for Microbiology).

Many Rec$^-$ mutants have been isolated by selection for radiation sensitivity. The method of CLARK AND MARGULIES[3], however, is preferable as it directly screens for recombination deficiency by replicating recipient cells on a lawn of Hfr cells, which has the advantage that radiation-resistant Rec$^-$ mutants are not excluded.

Abbreviation: NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

The intention of this investigation was to isolate Rec⁻ mutants, and our special interest concerned the possible existence of radiation-resistant Rec⁻ mutants and mutants that are temperature-sensitive in recombination ability.

MATERIALS AND METHODS

Strains

The strains and their relevant markers are listed in Table I. The origin and the direction of transfer of the Hfr strains are given in Fig. 1.

TABLE I

BACTERIAL STRAINS

Phabagen collection	*Sex*	*Relevant markers*	*Remarks*
PC 0294	F^-	*thr, leu, proA, purA, mtl, xyl, strA*	
PC 0317	F^-	*thr, leu, proA, tyrA, purA, mtl, xyl, strA*	derived from PC 0294
PC 0455	F^-	*thi, thyA, pyr, strA*	KMBL 132
PC 0908	F^-	*his, trp, thyA, (lam), recA13, strA, spcA*	JC 5483
PC 0031	Hfr R4	prototroph	
PC 0616	Hfr Kl16	*thi*	
PC 0612	Hfr Kl16	*thi, (lam)*	derived from PC 0616
PC 0009	Hfr H	*thi, (lam)*	
PC 0619	Hfr P4x	*metB, argA*	5B 171D1
PC 0615	Hfr Kl19	*(lam)*	
PC 0611	Hfr Kl98-2	*leu, (lam)*	
PC 0544	Hfr P72	*thi, metB, (lam)*	
PC 0617	Hfr Cavalli	*metB, pyrE*	AT 2243
PC 0647	F′	F′8 *gal*⁺/*gal, met, lam (lam)*	
PC 0658	F′	F′15 *thy*⁺/*thr, leu, thi, pyrF, thyA*	

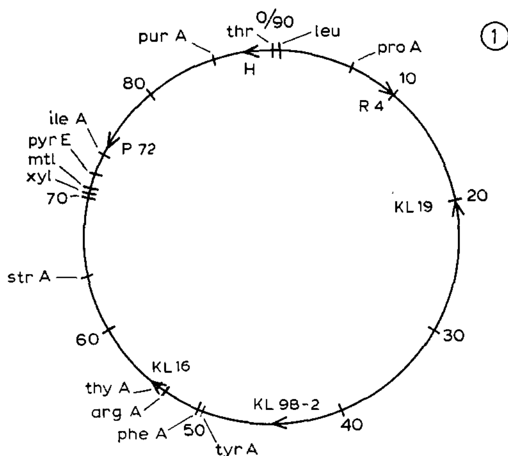

Fig. 1. Origin and direction of transfer of the Hfr strains used.

Media

Peptone – yeast extract medium was used as complete medium. The minimal

medium of WINKLER AND DE HAAN[16] was used. Both media were solidified with 1.25% Davis agar. When required, amino acids, vitamins, purines and pyrimidines were added in the following concentrations (μg/ml): arginine 40, histidine 20, leucine 60, methionine 20, phenylalanine 40, proline 60, serine 50, threonine 100, tyrosine 20, thiamine 5, adenine 20, thymine 20. For counter selection in crosses with *strA*$^+$ donors 100 μg streptomycin per ml was added; in crosses with *strA*$^-$ donors 120 μg rifampicine per ml was used. (Rifampicine (Lepetit) was a gift from the Ned. Farm. Handelmij., Amsterdam). In these crosses rifampicine-resistant recipients were used.

Induction and selection of mutants

Rec$^-$ mutations were induced into the strains PC 0294 and PC 0317 with NG as described by STOUTHAMER *et al.*[11]. Immediately after the NG treatment the cells were washed and divided into several portions. These portions were treated separately. From each sample not more than one Rec$^-$ mutant was isolated so that all were of different mutational origin. The mutagen-treated cells were incubated in broth for phenotypic expression.

After appropriate dilutions had been streaked on minimal agar, colonies were allowed to grow and then replicated in duplicate on a lawn of Hfr cells spread on minimal agar selective for recombinants. With strain PC 0294 the donor strain Hfr R4 was used and *thr*$^+$*leu*$^+$*strA*$^-$ recombinants were selected, whereas in the experiments with strain PC 0317 the Hfr Kl16 was used and *tyr*$^+$ *strA*$^-$ recombinants were selected. Plates were incubated at 30° as well as at 42° to detect thermosensitive recombination-deficient mutants. Colonies that did not form recombinants were taken from the master plate, purified and grown in nutrient broth, washed once in saline, and spotted on (*1*) a lawn of Hfr cells, either R4 or Kl16, and (*2*) a lawn of F'*gal*$^+$ donor cells.

Of the mutants suspected to be deficient in recombination, the following properties were examined; (*1*) frequency of recombinant formation per Hfr cell; (*2*) frequency of sexduction compared with strain PC 0294; (*3*) frequency of zygotic induction compared with strain PC 0294; (*4*) sensitivity to UV irradiation; and (*5*) kinetics of prophage λ induction in lysogenic derivatives.

Auxotrophic mutants were enriched with the help of penicillin according to the modified KAUFMAN AND GORINI[4] method. Thy$^-$ mutants were enriched by growing them in the presence of aminopterin or trimethoprim[2].

Determination of radiation sensitivity

Sensitivity of the mutants to UV irradiation was determined by irradiating log-phase cells suspended in saline. In open petri dishes 3-ml aliquots were irradiated at a distance of 30 cm from a 40-W Philips TUV tube with an output of 24 $erg \cdot sec^{-1} \cdot mm^{-2}$ at this distance. During the irradiation the dishes were gently shaken. Recombinants and transductants were screened for UV sensitivity by streaking them on nutrient broth agar and exposing them to suitable doses of UV irradiation.

Sensitivity to X-rays was determined by irradiating with an ENRAF Diffractor 601 X-ray source (Nonius, Delft). Log-phase cells suspended in saline were irradiated in 0.5-ml cuvettes. At 7 cm focal distance the dose rate was about 2.2 krad/min.

Crosses

Bacterial matings were performed as described by VERHOEF AND DE HAAN[13].

A recombination-deficient Hfr strain was isolated by crossing a *serA*⁻ derivative of a *rec171* F⁻ strain with the donor Hfr Kl16. *SerA*⁺ recombinants were selected after 120 min transfer time, and a UV-sensitive donor strain was isolated from the *SerA*⁺ colonies. This donor strain was of the Kl16 type.

Transductional crosses with phages 363 and P1 *vir* were carried out as described by LENNOX[8]. The phage lysates were prepared by the confluent lysis method.

Prophage induction

Induction curves for the lysogenic strains were made by irradiating log-phase bacteria in saline. After being centrifuged the cells were suspended in broth and incubated for 2 h. Bacterial cells were killed with chloroform and the number of plaque-forming units was determined by titration on a sensitive indicator strain.

RESULTS

After NG treatment of strains PC 0294 and PC 0317 nineteen independent Rec⁻ mutants were isolated. Two donor strains were used in the selection procedure, Hfr R4 and Hfr Kl16, to avoid the possibility that a particular Rec mutation was not detected because its Rec⁺ allele was injected early by one of the Hfr types (see Fig. 1). 18 mutants were found with Hfr R4 and only one, PC 0304 (carrying *rec169*), with Hfr Kl16 as donor.

For all mutants the frequencies of *thr*⁺*leu*⁺*strA*⁻ recombinants and of *gal*⁺/*gal*⁻ merodiploid formation and of zygotic induction of prophage λ with Hfr H (*lam*) as donor were determined at temperatures of 30 and 42°. All strains showed a lower

TABLE II

RECOMBINATION FREQUENCIES DETERMINED IN A CROSS WITH Hfr R4 AS DONOR BY CALCULATING THE NUMBER OF *thr*⁺ *leu*⁺ *strA*⁻ RECOMBINANTS PER DONOR CELL

Strain	*Genotype*	*Frequency of thr⁺ leu⁺ strA⁻ recombinant formation at 42°, relative to that of PC 0294*
PC 0294	*rec*⁺	1
PC 0295	*rec160*	$2 \cdot 10^{-1}$
PC 0296	*rec161*	$2 \cdot 10^{-1}$
PC 0297	*rec162*	$4 \cdot 10^{-2}$
PC 0298	*rec163*	$4 \cdot 10^{-3}$
PC 0299	*rec164*	$1 \cdot 10^{-2}$
PC 0300	*rec165*	$2 \cdot 10^{-2}$
PC 0301	*rec166*	$4 \cdot 10^{-4}$
PC 0302	*rec167*	$2 \cdot 10^{-2}$
PC 0303	*rec168*	$3 \cdot 10^{-2}$
PC 0304	*rec169*	$< 10^{-5}$
PC 0305	*rec170*	$< 10^{-5}$
PC 0306	*rec171*	$< 10^{-5}$
PC 0307	*rec172*	$< 10^{-5}$
PC 0308	*rec173*	$< 10^{-5}$
PC 0309	*rec174*	$< 10^{-5}$
PC 0310	*rec175*	$< 10^{-5}$
PC 0311	*rec176*	$< 10^{-5}$
PC 0312	*rec177*	$< 10^{-5}$
PC 0313	*rec178*	$< 10^{-5}$

frequency of recombination at both temperatures, while sexduction and zygotic induction were quite normal.

Table II shows that there are at least two classes of Rec⁻ mutants. The mutants of the first class are characterized by the fact that they are still able to give rise to recombinants, albeit with low frequencies. The mutants of the second class gave no recombinants at all in our test. It was calculated that the recombination frequency for these mutants was lower than 10^{-5}.

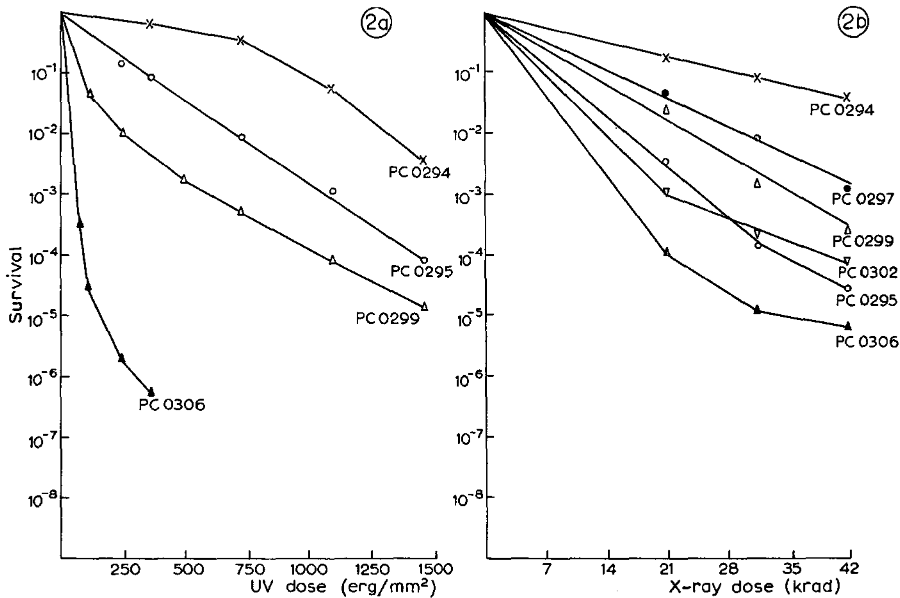

Fig. 2. (a) Survival curves for UV-irradiated bacteria. (b) Survival curves for X-ray-irradiated bacteria (see MATERIALS AND METHODS).

UV sensitivity

Of all strains listed in Table II UV survival curves were made. Three types of sensitivity were found. All strains of the second class of Rec⁻ mutants had exactly the same extreme UV sensitivity. The least sensitive mutants were those of class 1 which showed the highest recombination frequencies, whereas the mutants of class 1 with intermediate sensitivity had lower recombination frequencies. Examples of the three types of sensitivity and of the sensitivity of the wild-type strain are given in Fig. 2a.

Sensitivity to X-rays

In Fig. 2b survival curves for X-rays are given for all types of sensitivity. PC 0297 was only slightly X-ray-sensitive. All other mutants of class 1 had about the same survival curve, while the mutants of class 2 were extremely X-ray-sensitive.

Prophage induction

In general, *recA* mutants, which show high deficiency of recombination and high UV sensitivity, cannot[1,6], when lysogenized, produce λ. Rec⁻ mutants with inter-

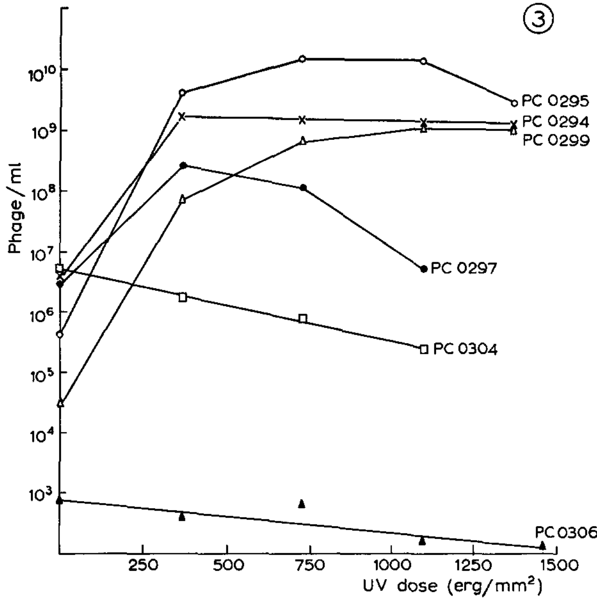

Fig. 3. Phage induction patterns after UV irradiation (see MATERIALS AND METHODS).

mediate recombination deficiencies and UV sensitivities, *recB* and *recC*, showed normal prophage induction. Most of our mutants gave normal induction curves (PC 0295 and PC 0299 in Fig. 3). It appears, however, that some of our Rec$^-$ mutants showed unexpected induction curves. PC 0297, carrying *rec162*, liberated normal amounts of phage spontaneously, and after irradiation with low UV-doses there was an increase, but at higher doses the level obtained with *recB* or *recC* mutants was not reached; on the contrary, a slightly decreasing number of plaque-forming units was found. The other mutants of class 1 behaved like PC 0294.

The mutants of class 2 were not inducible by UV and, except PC 0304, released (spontaneously) very low amounts of phage. Strain PC 0304 liberated normal amounts of phage spontaneously but could not be induced by UV irradiation.

Location

As all the properties of the class-2 mutants are similar to those described for *recA* mutants, we investigated whether the wild-type allele of each mutation responsible for UV sensitivity could be cotransduced with *pheA*. In several experiments recombination deficiency and UV sensitivity were always coinherited. Therefore, UV-resistant transductants were considered to be Rec$^+$. Results of the transductions are given in Table III. All class-2 mutations were cotransducible with *pheA*.

In order to detect the possibility of more than one complementation group among the isolated mutants, a recombination-deficient Hfr strain was constructed by crossing Hfr Kl16 and PC 0306 *serA*$^-$. From the *serA*$^+$ *strA*$^-$ colonies a UV-sensitive Kl16 type Hfr strain was isolated. This Hfr strain appeared to be a good donor both to Rec$^+$ and Rec$^-$ recipients (HOEKSTRA, unpublished results). As almost normal yields of recombinants were obtained by mating Hfr Kl16 with each of these mutants and

TABLE III

COTRANSDUCTION OF THE Rec^- MUTATIONS

Determined by P1 transduction with lysates grown on the Rec mutants, to the *thyA*-deficient strain PC 0455. As only low titres of P1 were obtained on the mutants of class 2 in these cases cotransduction of the wild-type for UV resistance (presumably $recA^+$) and $pheA^+$ was determined.

Donor genotype		*Recipient genotype*		*Cotransduction of rec⁺ and thyA⁺ (%)*	*Cotransduction of rec⁺ and pheA⁺ (%)*
rec160	$thyA^+$	rec^+	$thyA^-$	39 (77/200)	
rec161	$thyA^+$	rec^+	$thyA^-$	44 (88/200)	
rec162	$thyA^+$	rec^+	$thyA^-$	0 (0/200)	
rec163	$thyA^+$	rec^+	$thyA^-$	58 (116/199)	
rec164	$thyA^+$	rec^+	$thyA^-$	41 (82/200)	
rec165	$thyA^+$	rec^+	$thyA^-$	49 (97/200)	
rec166	$thyA^+$	rec^+	$thyA^-$	0 (0/200)	
rec167	$thyA^+$	rec^+	$thyA^-$	50 (100/200)	
rec168	$thyA^+$	rec^+	$thyA^-$	59 (118/200)	
rec^+	$pheA^+$	*rec169*	$pheA^-$		2[a] (2/72)
rec^+	$pheA^+$	*rec170*	$pheA^-$		25 (19/77)
rec^+	$pheA^+$	*rec171*	$pheA^-$		11 (22/200)
rec^+	$pheA^+$	*rec172*	$pheA^-$		32 (112/350)
rec^+	$pheA^+$	*rec173*	$pheA^-$		2[a] (2/134)
rec^+	$pheA^+$	*rec174*	$pheA^-$		15 (30/198)
rec^+	$pheA^+$	*rec175*	$pheA^-$		12 (3/23)
rec^+	$pheA^+$	*rec176*	$pheA^-$		16 (41/237)
rec^+	$pheA^+$	*rec177*	$pheA^-$		35 (73/210)
rec^+	$pheA^+$	*rec178*	$pheA^-$		20 (13/64)

[a] These percentages are lower than expected, because some $pheA^+$ revertants on the plates decreased the percentage of Rec^+ transductants.

the original *recA13* mutant in turn, while no recombinants were obtained with the Hfr strain carrying a *rec171* mutation, it appears that neither one of these mutants nor the *recA13* mutant complemented *rec171* (see also WILLETTS AND MOUNT[14]). In view of this lack of evidence for zygotic complementation, and the difficulty of developing a more rigorous test, we provisionally assign the mutant alleles *rec169–rec178* to the *recA* gene. The mutants of class 1 had most properties of *recB* and *recC* mutants and the mutations of these strains appeared to be cotransducible with *thyA* (Table III) except *rec162* and *rec166*. The markers cotransducible with *thyA* were also cotransducible with *argA*.

In order to locate the mutations *rec162* and *rec166*, mating experiments were performed with several donor strains. In a cross Hfr R4 × PC 0297 $thyA^-$ chromosome transfer was allowed for 90 min and $purA^+$, $thyA^+$, $strA^-$ recombinants were selected. Analysis of the recombinants for recombination proficiency showed that the linkage of the Rec^+ character with the two selected donor markers was 44%. As the donor markers xyl^+ and mtl^+ were inherited to the extent of 33 and 36% respectively, the *rec162* mutation was concluded to be located between *mtl* and *purA*. Because Hfr P72 did not transfer the rec^+ allele early, we looked for cotransduction of *rec162* and one of the markers in the 71–75-min region. The transductional cross P1 . $ilvA^+$, rec^+ × $ilvA^-$, *rec162* showed 40% cotransduction of recombination proficiency with $ilvA^+$; the cross P1 . *rec162*, $pyrE^+$ × rec^+, $pyrE^-$ showed 55% cotransduction of *rec162* and $pyrE^+$. These results indicate that *rec162* is located between *ilvA* and *pyrE*.

To locate the *rec166* mutation similar experiments were performed. In a cross

Hfr R4×PC 0301, *purA*$^+$ *strA*$^-$ recombinants were selected after 70-min transfer time. It appeared, however, that in the recombinants UV resistance and recombination proficiency were not always coinherited. This result led to the conclusion that PC 0301 carried two mutations, one responsible for recombination deficiency, and one determining high radiation sensitivity. From a cross Hfr AB 313×PC 0301 *thyA*$^-$, Rif^R, a number of *thyA*$^+$ Rif^R recombinants were analyzed. It appeared that besides the two parental types, two types of recombinant were formed, each probably carrying only one of the mutations. A recombination-deficient and almost UV-resistant recombinant was examined further. Whereas in a cross with Hfr Kl16 almost normal amounts of recombinants, and in a cross with Hfr Kl96 very few recombinants, were formed, the *rec166* mutation was concluded to be between the origins of both Hfr donors.

For further location a P1 lysate was grown on PC 0301 and transduction experiments were performed with several recipients. It appeared that *rec166* was cotransducible with *pheA* to about 32% and with *cysQ* to about 6%. The properties of one of the transductants, PC 1250, appeared to be identical to those of the investigated recombinant.

DISCUSSION

Most of the Rec$^-$ mutants presented here were of the two types already described by other authors. The Rec$^-$ mutants PC 0304 up to and including PC 0313 appeared to carry *recA*. All these mutations were cotransducible with *pheA* and extremely UV-sensitive and "reckless" (STORM, unpublished). They exhibited no zygotic complementation with an Hfr-carrying *rec171*. We have no indication that more than one gene was responsible for the phenotype of these mutants. There was one mutant, PC 0304, carrying *rec169*, with an exceptional phenotype; λ lysogens of the strain PC 0304 could not be induced by UV irradiation, but showed a higher than normal level of spontaneous induction.

Seven mutations were located between *thyA* and *argA* and had most properties in common with the *recB21* and *recC22* mutants. When we tried to divide the mutants into the complementation groups B and C (ref. 14), we discovered that the situation in this region is rather complicated. The results of these experiments will be published later.

Two of the mutations, *rec160* and *rec161*, caused less deficiency in recombination and lower UV sensitivity than the other mutations in this class. Like *recB* and *recC* mutants, they lack the ATP-dependent exonuclease[10]. Transfer of *rec160* and *rec161* by transduction to a *rec*$^+$ *thyA*$^-$ recipient yielded strains with exactly the same properties as PC 0295 and PC 0296. Possibly, an unknown kind of suppression results in a higher but still undetectable level of ATP-dependent exonuclease in these mutants.

The *rec162* mutation appears to be a new radiation-sensitive Rec$^-$ mutation located between *ilvA* and *pyrE* for which the notation *recG* is proposed. In our first experiments it seemed to be not very deficient but this was due to the fact that Hfr R4, which was used as donor, transferred the *rec*$^+$ allele early. In crosses with Hfr Kl16 this mutant turned out to be 10 times more deficient. Furthermore it showed abnormal λ induction.

The *rec166* mutation also appears to be a new mutation. It is as deficient in recombination as *recB* and *recC* mutants but its radiation sensitivity is of quite another type. It is only slightly UV-sensitive and fully X-ray-resistant. The location of this mutation, for which the notation *recH* is proposed, is between *pheA* and *cysQ*.

Based on the idea that recombination and radiation repair have several steps in common[7], *rec162* might be a new mutation in one of these common steps. Mutation *rec166* is rather peculiar in this aspect. In strains carrying *rec166*, UV sensitivity is only slightly increased, although they are X-ray-resistant. Perhaps the *rec166* gene is involved in a typical recombination step.

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