SOME GENETIC CONSEQUENCES OF CHANGES IN THE LEVEL OF *recA* GENE FUNCTION IN *ESCHERICHIA COLI* K-12

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ABSTRACT

Genetic recombination was studied in *E. coli* mutants that carry lesions in the *recA* gene but retain some capacity for generating recombinant progeny. We observed that recombination was detectable only at a very low level during the incubation of leaky *RecA*$^-$ merozygotes in broth. However, recombination appeared to occur at much higher frequencies when recombinant progeny were assayed by selection on minimal agar. Analysis of the recombinants obtained with Hfr donors revealed a deficiency of multiple exchanges per unit length of DNA in leaky *RecA*$^-$ strains. In many of these crosses recombinants that inherited donor alleles close to the transfer origin were much reduced in frequency, except when the recipient was also *RecB*$^-$.

WHEN Hfr and F$^-$ strains of *E. coli* K-12 are crossed, recombinants are generated in the merozygotes by some process that involves physical joining of donor and recipient DNA (CURTISS 1969; SIDDIQI and FOX 1973; PAUL and RILEY 1974). The molecular processes required for these recombination events are poorly understood although recovery of viable progeny from the zygotes depends on the activity of the *recA*$^+$ and *recB/C*$^+$ gene products (CLARK 1971, 1973). BIRGE and Low (1974) devised a method for detecting recombinant *lacZ*$^+$ DNA molecules in zygotes soon after mating by the assay of β-galactosidase activity. They did not detect any recombination in *RecA*$^-$ zygotes but in *RecB*$^-$ and *RecC*$^-$ zygotes the yield of β-galactosidase indicated that some kind of recombination event was able to generate transcribable *lacZ*$^+$ DNA as efficiently as in Rec$^+$ zygotes. We have been unable to detect recombination by this method in *RecA*$^-$*RecB*$^-$ zygotes even when the exogenote was an F-prime DNA molecule rather than a fragment of Hfr DNA (unpublished observations). Evidence of this nature has led to the proposal that recombination in *RecA*$^-$ zygotes is blocked at an early stage whereas in *RecB/C*$^-$ zygotes it proceeds as far as some covalent joining of donor and recipient DNA (HALL and HOWARD-FLANDERS 1972; BIRGE and Low 1974; BERGMANS, HOEKSTRA and ZUIDWEG, 1975). The fate of Hfr

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DNA in *RecA*$^-$ merozyyotes is not clear but it has been suggested that it is exposed to nuclease digestion and degraded, presumably as a consequence of its not being involved in recombination (DUBNAU and MAAS 1969; ITOH and TOMIZAWA 1971; BERGMANS, HOEKSTRA and ZUIDWEG 1975).

In this communication we first describe the properties of strains in which recombination is reduced, but not abolished, as a result of an atypical mutation in the *recA* gene. Using these strains and others in which recombination was made temperature-dependent by the *recA200* mutation described previously (LLOYD *et al.* 1974), we have monitored some recombinational consequences of changes in the level of *recA* gene function. From the results we conclude that leaky *RecA*$^-$ merozygotes which yield recombinant progeny after selection on minimal medium show very little evidence of recombination in mating cultures at times when merozygotes that carry *recA*$^+$ have clearly recombined. We also found that the progeny recovered from these *RecA*$^-$ merozygotes generally show an altered linkage between selected and unselected markers.

MATERIALS AND METHODS

Strains. The *E. coli* K-12 strains used are listed in Table 1. Figure 1 shows the genetic map

TABLE 1

E. coli *K-12 strains**

Strain	Genotype	Source or derivation
S491	F$^-$ *his*$^-$ *proA*$^-$ *galT22(?) lac*$^-$ *upp*$^-$ *tonA24* or *27*	Complex derivative of strain K12S (BACHMANN 1972; STACEY and LLOYD 1976).
R2881	F$^-$ as S491 but *recA255*	NG-induced mutant of S491, *Rec*$^-$ UV^S (STACEY and LLOYD 1976).
AB1157	F$^-$ *thr-1 leu-6 proA2 his-4 argE3 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 str-A31 sup-37*	BACHMANN 1972.
JC2915	F$^-$ as AB1157 but *cysC43*	BACHMANN 1972.
AB2487	F$^-$ as AB1157 but *thyA*$^-$ *recA13*	High thymine requiring mutant of AB2463 (BACHMANN 1972).
KL108	F$^-$ as AB1157 but *thyA*$^-$ *recA12*	BACHMANN 1972.
S541	F$^-$ as JC2915 but *cysC*$^+$	*P1vir* (R2881) × JC2915 → *Cys*$^+$.
S551	F$^-$ as JC2915 but *cysC*$^+$ *recA255*	*P1vir* (R2881) × JC2915 → *Cys*$^+$*Rec*$^-$.
PC1230	F$^-$ *thr*$^-$ *leu*$^-$ *trp*$^-$ *his*$^-$ *arg*$^-$ *cysI*$^-$*thi?* *mal*$^-$ *lac*$^-$ *gal*$^-$ *xyl*$^-$ *mtl*$^-$ *tonA*$^-$ *strA*$^-$	P. G. DEHAAN.
S561	F$^-$ as PC1230 but *cysI*$^+$	*P1vir* (R2881) × PC1230 → *Cys*$^+$.
S571	F$^-$ as PC1230 but *cysI*$^+$ *recA255*	*P1vir* (R2881) × PC1230 → *Cys*$^+$*Rec*$^-$.
KL398	F$^-$ *thi-1 metE70 leu-6 proC32 hisF860 thyA54 malA38 lacZ36 ara-14 mtl-1 xyl-5 str-109 spc-15*	(LLOYD *et al.* 1974). Carries an uncharacterized mutation near *his* which confers a *Mal*$^+$, λ^S phenotype.
KL399	F$^-$ as KL398 but *recA200*	LLOYD *et al.* 1974. $RecA_{ts}$.

TABLE 1—Continued

Strain	Genotype	Source or derivation
KL410	F⁻ as KL398 but *recB21 thyA*⁻	*P1vir* (S583 *recB21* (LLOYD 1974)) × KL398 → *Thy*+*Rec*⁻, then trimethoprim selection for *Thy*⁻.
KL411	F⁻ as KL399 but *recB21 thyA*⁻	*P1vir* (S583 × KL399 at 35° → *Thy*+ *Rec*⁻, then trimethoprim selection for *Thy*⁻.
KL424	F⁻ *thi-1 metE70 leu-6 proC32 thyA54 cysC43 lacI3 lacZ118 malA38 ara-14 mtl-1 xyl-5 str-109 spc-15 nalA*⁻ *supD*⁻	Derivative of strain KL266 (LLOYD *et al.* 1974). Carries an uncharacterized mutation near *his* which confers a *Mal*+ phenotype.
KL425	F⁻ as KL424 but *cysC*+	*P1vir* (KL399) × KL424 → *Cys*+.
KL426	F⁻ as KL424 but *cysC*+ *recA200*	*P1vir* (KL399) × KL424 → *Cys*+ and *Rec*⁻ UV^S and mitomycin C sensitive at 42°.
N1093	F⁻ as KL424 but *thyA*+ *cysC*+	*P1vir* (S583) × KL424 → *Thy*+, then × *P1* (S551) → *Cys*+.
N1094	F⁻ as KL424 but *thyA*+ *cysC*+ *recA255*	As for N1093 expect × *P1* (S551) → *Cys*+*Rec*⁻ and mitomycin C sensitive.
KL320	F⁻ *lacI3 lacZ118 pro-48 metE*⁻ *trpA605 his-29 str*⁻ *nalA*⁻	BIRGE and LOW 1974.
NH4104	F′ *lac*+ (F42)/*uvrA6 proA2 leu-8 thr-4 his*⁻ *thi*⁻ *ara-14 lac-1*	B. WILKINS.
KL340	F′ *lacI3 lacZ608*$_{amber}$/Δ(*lac-pro*)$_{XIII}$ *spc*⁻ *xyl*⁻ *mtl*⁻ *φ80dlacI*+ *tonB*⁻	Derived from X855 (from J. H. MILLER), F42(*lac*), and strains given in BIRGE and LOW (1974).
KL330	F′ *lacI3 lacZ118*/Δ(*lac-pro*)$_{XIII}$ *spc*⁻ *xyl*⁻ *mtl*⁻	
KL329	F′ *lacI3 lacZ36*/Δ(*lac-pro*)$_{XIII}$ *spc*⁻ *xyl*⁻ *mtl*⁻	
KL334	Hfr (Cavalli) *lacI3 lacZ118 lysA23*	BIRGE and LOW 1974.
KL335	Hfr (Cavalli) *lacI3 lacZ608*	BIRGE and LOW 1974.
KL336	Hfr (Cavalli) *lacI3 lacZ36 metB*⁻	BIRGE and LOW 1974.
KL16	Hfr *thi-1 rel-1*	LOW 1973a.
KL16–99	Hfr (KL16) *thi-1? rel-1? drm-3? recA1*	BACHMANN 1972.
JC5088	Hfr (KL16) *thr-300 ilv-318 spc-300 recA56 thi-1 rel-1*	BACHMANN 1972.
KL96	Hfr *thi-1 rel-1*	LOW 1973a.
KL397	Hfr (KL96) *hisF860*	*hisF860* allele from a strain from P. HARTMAN introduced into KL96.
KL98	Hfr λ+ λ^{R}MS2^R	BACHMANN 1972.
KL226	Hfr (Cavalli) *rel-1 tonA22* T2^R	LOW 1973a.
KL25	Hfr *supE42*	LOW 1973a.
3000	Hfr (Hayes, HfrH) *thi-1 rel-1*	BACHMANN 1972.
PK191	Hfr Δ(*proB-lac*)$_{XIII}$ *sup-56 thi-1* (ColV–ColV^{R}Hfr$_v$)	LOW 1973a.
P801	Hfr *ara*⁻ *lac*⁻ *xyl*⁻ *mtl*⁻	F. JACOB via B. J. BACHMANN

* All loci are assumed to be wild type except those listed here, and except for KL98 all strains are λ⁻. NG = N-methyl-N′-nitro-N-nitrosoguanidine. *supD*⁻ = Su-I+, i.e. the active suppressor form of the *supD* locus.

location of relevant markers and Hfr points of origin. The gene symbols used are those given by BACHMAN, Low and TAYLOR (1976). Phenotypic symbols are the non-italicized form of the gene symbols with the initial letter in capitals (DEMEREC et al. 1966), except that UV, with superscript R for resistant or S for sensitive, is used to denote the response to ultraviolet irradiation. A minus sign used either with gene symbols or phenotypic symbols denotes a mutant form as opposed to the wild-type form for which a plus sign is used.

Media. Low-salt Luria broth and agar (LLOYD *et al.* 1974) were used for routine growth of bacterial strains. 56/2 salts supplemented as described by Low (1973a) was used as a defined medium and contained 0.1% Luria broth for the selection of recombinant colonies (RILEY and PARDEE 1962). For radio-isotope labelling, strains were grown in enriched M9 (EM9) salts medium (LLOYD and BARBOUR 1974). Filtered Luria broth medium was prepared as described previously (BIRGE and Low 1974). Dilution buffer was unsupplemented 56/2 salts.

Growth conditions. Experimental cultures were routinely grown to mid-logarithmic phase (c. 2×10^8 cells per ml) in broth medium at 37°, unless stated otherwise. For experiments with temperature-sensitive strains, all media and glassware were pre-warmed to the required temperature. Cell density was determined by measuring the absorbance at 650nm (A_{650}) with a Bausch and Lomb Spectronic 20 spectrophotometer.

Matings and transductions. The procedures used for liquid and plate matings and for transduction with phage *P1vir* have been described (Low 1973a,b; Low *et al.* 1971). Separation of mating pairs in liquid matings was achieved by the blending method of Low and WOOD (Low and WOOD 1965; MILLER 1972). In all the crosses described in this paper, samples of mating mixtures were blended before plating for recombinants. When the experimental procedure involved a temperature shift-up at the end of mating, samples of the mating mixture were

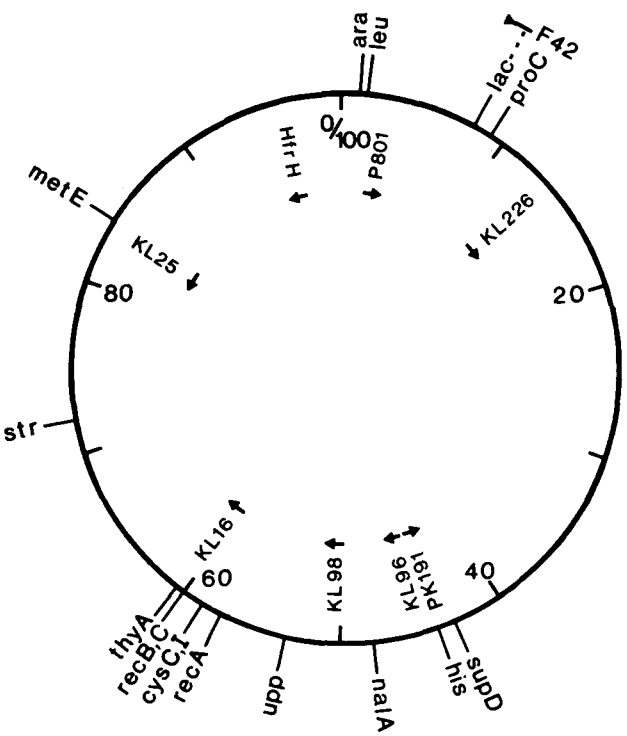

FIGURE 1.—*Escherichia coli* K-12 genetic linkage map modified from BACHMANN, Low and TAYLOR (1976). Hfr origins and directions of chromosome transfer are indicated by arrowheads.

pipetted into tubes containing 3 ml of molten soft agar kept at 42°. After separation of mating pairs, the soft agar was poured over the surface of appropriate agar plates pre-warmed to 42°. Solidification of the soft agar at 42° was achieved by incubating the plates with their lids removed for 15–30 minutes.

Analysis of mutant and recombinant phenotypes. The replica-plating method (LEDERBERG and LEDERBERG 1952) was used to score the phenotype of large numbers of colonies as described previously (LLOYD *et al.* 1974). Strains that carried *recA*⁻ or *recB*⁻ mutations were usually identified by their failure to grow on Luria agar medium containing 0.2 or 0.5 μg of mitomycin C (Sigma Chemical Co.) per ml respectively, and sometimes by replica-plating tests for ultraviolet sensitivity and recombination proficiency (Low 1973a). Temperature-sensitive strains were scored for these phenotypes at 35° and 42°. Estimates of the recombination proficiency of *Rec*⁻ mutants were based on at least two (usually more) crosses with the exception of those shown in Tables 4b, 5b and 5c.

Radiation survival. Sensitivity to ultraviolet and gamma radiation was measured by the methods described previously (LLOYD and BARBOUR 1974).

DNA breakdown. The release of trichloroacetic acid-soluble material from cells labelled with ^{3}H-thymidine (methyl-^{3}H, specific activity 18.4 Ci/m mol; Amersham, England) was measured by the procedure of HOWARD-FLANDERS, THERIOT and STEDEFORD (1969).

Detection of molecular recombination in zygotes. The method used was simplified from the procedures described by BIRGE and Low (1974). Recipient strains that carried *lacZ118* and a *supD*⁻ mutation were crossed with donors (either Hfr or F-prime) of $lacZ608_{amber}$, *lacZ118* or *lacZ36*, in parallel matings. The strains were grown in filtered broth to an A_{650} of 0.3 and mixed in a ratio of one part donor to two parts recipient and the mating mixture was incubated at the required temperature for 2½ to 3 hours without dilution. Samples were removed at intervals and assayed for β-galactosidase activity, viable recipient cells and *Lac*+ progeny as described previously (BIRGE and Low 1974). The amount of β-galactosidase obtained in the crosses with a donor of *lacZ36* was corrected for that detected in the parallel cross with the donor of *lacZ118*. This correction was significant only for the first 30 minutes of mating when the recipient carried *recA*+. The remaining β-galactosidase was taken as a measure of recombination in the *lacZ36/lacZ118* partial zygotes that yielded *lacZ*+ DNA. Both donor and recipient strains carried the *lacI3* mutation so that β-galactosidase synthesis was constitutive. When donors of *lacZ608* were used, the *supD*⁻ mutation in the recipients allowed expression of the Lac+ phenotype without prior recombination. β-galactosidase activity in these crosses was corrected for that obtained in a parallel culture of the donor strains diluted with two volumes of filtered broth that had been conditioned (BIRGE and Low 1974) by prior growth of this same strain. The yield of Lac+ progeny and the β-galactosidase activity measured in these crosses were used as indices of DNA transfer and '*lacZ*+' gene expression respectively for the recipient strain. In all the crosses the yield of β-galactosidase was finally expressed as enzyme units (E.U.) per 10^8 recipient cells in the mating mixture (one E.U. equals that amount of enzyme which hydrolyzes 1 nmol of *o*-nitrophenyl-β-D-galactopyranoside per minute at 28°). Levels of β-galactosidase activity as low as 1% of the maximal level obtained when the recipient carried *recA*+ could be reliably detected by this method. Since only 60% of the cells observed microscopically in cultures of *RecA*⁻ mutants were able to make colonies on Luria agar, the total cell count in the *RecA*⁻ recipient population was obtained by multiplying the viable cell count by 1.67.

RESULTS

Genetic and phenotypic characterization of a "leaky" recA⁻ mutation. STACEY and LLOYD (1976) reported the isolation of a Rec⁻ mutant (R2881) that retained a significant residual capacity for recombination. Apart from being less extreme, the phenotype of the strain resembled that of known *RecA*⁻ *mutants* (Table 2a, Figure 2) A mutant allele (*rec-255*) responsible for the Rec⁻ phenotype of R2881 was found to be co-transducible with *cysC*. When *P1* phage grown on R2881 was

TABLE 2

Recombination proficiency of strains carrying rec-255

	Cross*	Recombinant class selected†	Recombination deficiency index (R.D.I.)‡
a)	HfrH × R2881	Leu+	0.02
	KL98 × R2881	His+	0.02
	KL16 × R2881	His+	0.59
	KL226 × R2881	Pro+	0.04
b)	HfrH × S551	Leu+	0.05
	KL226 × S551	Pro+	0.045
	KL96 × S551	His+	0.025
	KL397 × S551	His+	0.03
	JC5088(*recA56*) × S551	His+	0.025
	KL16-99(*recA1*) × S551	His+	0.05
	NH4104(*Flac*+) × S551	Lac+	0.60
	KL16 × S551	His+	0.33
c)	KL98 × S571	His+	0.10
	HfrH × S571	Leu+	0.16
	JC5088(*recA56*) × S571	His+	0.10
	KL16 × S571	His+	0.63
	KL226 × S571	Pro+	0.07
d)	HfrH × N1094	Leu+	0.01
	KL25 × N1094	Met+	0.02
	KL226 × N1094	Pro+	0.14
	P801 × N1094	Leu+	0.07
	KL16 × N1094	His+	0.72

* The Rec+ strains used as control for R2881, S551, S571 and N1094 were S491, S541, S561 and N1093, respectively.

† Counter-selection of donor strains was achieved by incorporating 100 μg streptomycin sulphate per ml in the plate agar except for strains S491 and R2881 when 150 μg 6-azauracil per ml was used.

‡ The numbers refer to the ratios of (Rec− recombinant yield)/(Rec+ recombinant yield). Matings were interrupted after 40 min with NH4104; 60 min with HfrH, KL226, KL25, KL96, KL397 and P801; 70 min with KL98; and 90 min with KL16, KL16-99 and JC5088. With the Rec+ recipients, the yields of recombinants per Hfr cell were 5–20% except with KL16-99, KL397 and JC5088 which gave approximately 0.5%.

used to transduce JC2915 (*cysC43*) and PC1230 (*cysI*−), 5% (43/870) and 10% (21/209) respectively of the Cys+ transductants were found to be Rec− in plate matings. Since R2881 was isolated as a result of extensive mutagenesis, mutations other than *rec-255* might be contributing to the observed phenotype. Two of the transductant strains that carried *rec-255*, S551 and S571 derived from JC2915 and PC1230 respectively, were examined for some of their properties. The results obtained are shown in Table 2 (b and c) and Figure 2.

Previous results had shown that in a cross between R2881 and Hfr KL16 there was no segregation of the Rec− and UV^S phenotypes among 200 His+ progeny examined. The intermediate level of UV sensitivity in transductant strain S571 and S551 (Figure 2a) suggested that R2881 might carry a second mutation that is responsible for its UV^S phenotype and which was separable from *rec-255* by *P1* transduction. However, when *rec-255* was transferred from the Rec− (UV^R?)

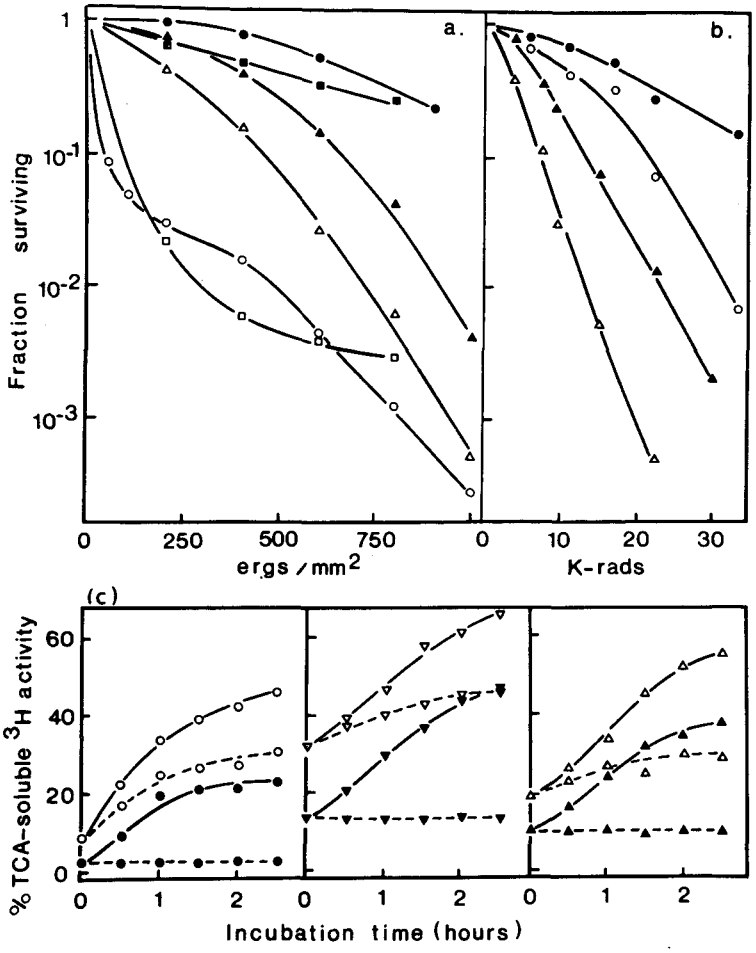

FIGURE 2.—DNA repair properties of strains carrying *rec-255*, (a) UV survival; (b) gamma ray survival; (c) DNA breakdown, both spontaneously (broken lines) and after exposure to 600 ergs UV light (continuous lines). ●, S491; ○, R2881; ▲, S561; △, S571: ■, N1093; □, N1094; ▼, S541; ▽, S551. The UV survival of S541 and S551 were similar to S561 and S571 respectively.

strain S551 to a new strain that differed from JC2915 in its derivation from the ancestral stock of *E. coli* K12 (personal communication from BARBARA BACHMANN), it then conferred UV sensitivity in addition to the expected Rec$^-$ phenotype (Table 2d). The UV survival of this new strain (N1094) proved similar to that of the original mutant, R2881 (Figure 2a). It was concluded that the *rec-255* mutation alone was responsible for the Rec$^-$UV^s phenotype of R2881 and that the weak expression of the UV^s phenotype in the JC2915 and PC1230 genetics backgrounds depended on other (unidentified) mutation(s).

In several other respects, strains carrying *rec-255* were qualitatively similar. Thus, compared with isogenic strains carrying *rec*$^+$, they were deficient in

recombination (Table 2), sensitive to gamma irradiation (Figure 2b) and degraded their DNA spontaneously and after UV irradiation (Figure 2c). So far, we have been unable to explain the considerable variation in these phenotypes caused by *rec-255* in the different genetic backgrounds used. All the *rec-255* strains were unable to grow on Luria agar plates containing 0.2 μg of mitomycin C per ml. When crossed with Hfr KL16, the early transfer of the *rec*$^+$ allele to these strains allowed recovery of recombinants (His$^+$) at much higher frequencies. This indicated that *rec-255* is recessive to *rec*$^+$. However, if derivatives of Hfr KL16 carrying *recA56* (JC5088) or *recA1* (KL16–99) were used as donors, the yield of *His*$^+$ progeny was reduced compared with control crosses with Rec$^+$ recipients (Table 2). Furthermore, when λ*bio-1* phage (obtained from C. RADDING) was assayed on λ-sensitive strains carrying *rec-255* the yield of plaques was much lower ($< 10^{-4}$) than on*Rec*$^+$ strains. We concluded from these results that the *rec-255* mutation is an allele of the *recA* locus which leads to only a partial loss of *recA* gene activity, and as such is quite unusual in that all the *recA* mutations reported so far have rather similar (extremely recombination deficient and UV sensitive) phenotypes (CLARK 1971, 1973).

Recombination under conditions of limited recA *gene activity.* LLOYD *et al.* (1974) showed that in crosses with Hfr donors the formation of recombinant progeny in recipient strains carrying *recA200* is temperature-dependent. Compared with crosses with Rec$^+$ recipients the yield of progeny at 35° is approximately 50%, but is reduced to 0.2% or less at 42° except when the *recA*$^+$ allele is transferred to the zygotes. In the previous section we showed (Table 2) that, in the absence of *recA*$^+$ transfer, the *recA255* allele reduces recombinant formation to an average of 5% (1–16%) of that in control crosses with Rec$^+$ recipients, depending on the strains used and the markers selected. Since this variation was seen in recipient strains that differed from those used as controls only in the allele carried at the *recA* locus we concluded that it reflected differences in the level of *recA* gene activity, i.e. *recA* gene activity in *recA200* strains at 35° seemed greater than that in *recA255* strains which in turn seemed to retain more *recA* activity than *recA200* strains at 42°. A reduction in *recA* gene activity might cause changes in the recombination process that could be reflected in the genotype of the recombinants recovered at the reduced level. Further crosses between these *RecA*$^-$ recipients and various Hfr donors were therefore analyzed for both the yield and genotype of the recombinants obtained.

The result of these and of control crosses with Rec$^+$ recipients are summarized in Table 3. When Hfr donors that transfer their chromosome in the clockwise direction with regard to the genetic map (Hfrs Hayes and KL25) were used, recombinant formation in the Rec$^-$ recipients differed from that in the Rec$^+$ controls in three ways: (a) excessive recombination deficiency was noted for certain donor markers near the transfer origin; (b) there was a deficiency of progeny that inherited unselected proximal Hfr markers close to the transfer origin; (c) when two or more unselected markers could be scored in the progeny, clones showing multiple exchanges were less frequent. These effects were more significant when the recipient carried *recA255* even though recipients carrying

TABLE 3

Analysis of recombination in recipient strains carrying recA+, recA255 *or* recA200

Cross*	Recombinant class selected (Str^R)	R.D.I.†	Number tested	% carrying various donor markers					% showing§ multiple crossovers
				Met+	Ara+	Leu+	Lac+	Pro+	
(a)									
KL226 × N1093	Leu+	...	300	...	88.7	(100)	67.0	69.0	4.6
KL226 × N1094	Pro+	0.148							
KL226 × N1094	Leu+	0.20	300		88.7	(100)	69.7	66.0	2.0
KL226 × N1094	Met+	0.109							
P801 × N1093	Met+		300	(100)		58.0			
P801 × N1094	Met+	0.056	300	(100)		43.3			
P801 × N1094	Leu+	0.072							
HfrH × N1093	Pro+		300		64.7	65.7	86.7	(100)	5.7
HfrH × N1094	Pro+	0.051	400		13.8	16.0	82.0	(100)	0.25
HfrH × N1094	Leu+	0.011							
KL25 × N1093	Leu+		300	40.7	94.7	(100)			0.67
KL25 × N1094	Leu+	0.158	300	5.3	95.3	(100)			0
KL25 × N1093	Pro+		300	38.7	68.3	69.3	83.7	(100)	18.0
KL25 × N1094	Pro+	0.136	300	7.3	73.7	76.3	93.3	(100)	1.0
KL25 × N1094	Met+	0.023							
(b)									
KL226 × KL398	Leu+		600		83.8	(100)	69.7	68.7	2.5
KL226 × KL399	Leu+	0.001	500		89.6	(100)	57.0	49.0	1.8
KL226 × KL399	Pro+	0.002							
KL226 × KL398‡	Met+		200	(100)	31.0	30.5	23.0	27.0	16.5
KL226 × KL399‡	Met+	0.0012	200	(100)	43.0	44.5	38.5	38.5	16.5
HfrH × KL398	Pro+		200		72.0	79.0	90.5	(100)	5.0
HfrH × KL399	Pro+	0.0016	200		48.0	52.5	90.5	(100)	3.0
HfrH × KL399	Leu+	0.0009							

* Recipient strains were grown in Luria broth at 37° (N1093 (*rec*+) and N1094 (*recA255*)) or 42° (KL398 (*rec*+) and KL399 (*recA200*)), and donor strains at 37°, to an A_{650} of 0.3 (c. 2×10^8 cells/ml). Donor and recipients were mixed in a ratio of 1:10 (a); or 1:4 (b). Mating was at 37° for 60 min with KL226 and HfrH, 70 min with P801, and 80 min with KL25, in (a) or at 42° for 60 min with KL226, and 50 min with HfrH, in (b), before interruption. Plates were incubated at 37°, (a); and 42°, (b).

† R.D.I. = recombination deficiency index, calculated as in Table 2.

‡ Not the same mating as that in which Pro+ and Leu+ were selected.

§ Based on the total number of recombinants tested.

recA200 (at 42°) were much more recombination deficient. When Hfrs that transfer their chromosome in the opposite direction (KL226 and P801) were used as donors, these differences were much less apparent or even absent.

It seems, therefore, that recombination in these leaky Rec⁻ mutants occurs not only with a reduced efficiency but also in a way that produces abnormal progeny in the sense that linkage relationships are altered. Since there is normally some variability in the types of recombinants produced even when the recipient carries *recA*+, the differences seen in the progeny of the Rec⁻ zygotes might be explained if the *rec*⁻ mutations allowed recombinants to be recovered only from a certain unrepresentative fraction of the zygotes. Experiments were therefore designed to try and recover as many recombinants as possible from

RecA⁻ zygotes by increasing *recA* gene activity at some time after the start of the cross. The linkage relationship of unselected Hfr markers in the progeny obtained might give some further indication of the events that were in progress when *RecA* activity was limited. Recovery of recombinants was carried out in two ways: (a) a recipient carrying *recA200* was grown and mated at 42° (Rec⁻ conditions) and the zygotes plated at 35° for the selection of progeny; (b) *RecA*⁻ recipients were mated with Hfr donors and selection made for progeny carrying a donor marker transferred to the zygotes after the *recA*+ allele.

These procedures did indeed allow a greater recovery of viable progeny than was obtained when crosses were conducted at 42° throughout (when the recipient carried *recA200*) or when *recA*+ transfer from the Hfr was kept to a minimum by selecting fairly early Hfr markers and interrupting mating at the appropriate time. However, the yields of progeny recovered never reached that obtained in control crosses with recipients carrying *recA*+ (Tables 4 and 5). The temperature shift-down experiment (Table 4a) showed a relatively greater recovery of recombinants for the later of two independently selected donor markers. When progeny carrying the more distal allele were selected the less efficient recovery of a proximal donor allele was reflected in the appearance of fewer progeny

TABLE 4

Recovery of recombinants from zygotes carrying recA200

(a) by temperature shift from 42° to 35° at the end of mating

Cross*	Temp. of mating (°C)	Recombinant selection at (°C)	Recombinant class selected (*Str*^{R})	R.D.I.	Number tested	% carrying donor marker				% showing multiple crossovers†
						Ara+	Leu+	Lac+	Pro+	
KL226 × KL398	42	35	Leu+		200	89.0	(100)	79.0	81.0	3.0
KL226 × KL399	42	35	Leu+	0.095	200	90.0	(100)	38.5	35.5	1.0
KL226 × KL399	42	35	Pro+	0.046						
KL226 × KL398	35	35	Leu+		200	87.5	(100)	70.5	72.5	4.0
KL226 × KL399	35	35	Leu+	0.64	200	88.5	(100)	86.5	84.5	0.5
HfrH × KL398	42	35	Pro+		300	63.0	70.0	87.3	(100)	3.3
HfrH × KL399	42	35	Pro+	0.106	300	18.3	23.7	83.7	(100)	0.33
HfrH × KL399	42	35	Leu+	0.071						

(b) by transfer of the Hfr *recA*+ allele during mating at 42°

Cross*	Recombinant class selected (*Str*^{R})	R.D.I.	Number tested	% carrying unselected donor markers							% showing multiple crossovers†
				Rec+	His	Pro	Lac	Leu	Ara	Met	
HfrH × KL398	Thy+		100		37	38	31	42	36		47
HfrH × KL399	Thy+	0.0166	100	70	44	24	21	9	6		12
KL25 × KL398	Thy+		100		28	48	46	42	39	41	68
KL25 × KL399	Thy+	0.0115	100	81	49	33	32	30	28	19	17

* Strains were grown in Luria broth at 35° or 42° (recipients) or 37° (donors) to an A_{650} of 0.3. Donors and recipients were mixed in a ratio of 1:5 and the mating mixtures incubated for 70 min at 35°, or 50 min at 42°, (a); or at 42° for 110 min (× HfrH), or 125 min (× KL25), (b). Matings were interrupted and plated on prewarmed medium (42° incubation), (b); or chilled and plated on cold plates, (a).

† See Table 3.

TABLE 5

Recovery of Thy+ recombinants in matings with Hfr PK191

	Recipient strain	Temp (°C)	R.D.I.	His+/Thy+ %
(a)	KL398 *rec+*	35	...	57.5 (200)
	KL398 *rec+*	42		48.5 (200)
	KL399 *recA200*	35	0.57	23.0 (200)
	KL399 *recA200*	42	0.25	2.25(400)
	KL410 *recB21*	35	0.65	68.0 (200)
	KL410 *recB21*	42	0.66	53.5 (200)
	KL411 *recA200 recB21*	35	0.28	78.5 (200)
	KL411 *recA200 recB21*	42	0.26	34.5 (200)
(b)	AB1157 *rec+**			20.5 (200)
	AB2487 *recA13*		0.05	2.5 (200)
	KL108 *recA12*		0.00085	13.5 (200)
	S541 *thy⁻ rec+*			14.8 (250)
	S551 *thy⁻ recA255*		0.21	5.2 (250)
(c)	S561 *thy⁻ rec+*			41.2 (250)
	S571 *thy⁻ recA255*		0.29	14.8 (250)

Hfr PK191 was grown in broth to an A_{650} of 0.3 at 37°. Recipient strains were grown at 35° or 42°, (a); or 37°, (b) and (c). Donor to recipient ratio was 1:10 and mating was for 70 min at 35°, or 50 min at 42°, (a); or 60 min at 37°, (b) and (c). Matings were then interrupted. Thy+ (Str^R) progeny were scored after incubation at the indicated temperature and then tested for the His phenotype. The numbers in brackets refer to the total of Thy+ recombinants tested.

* A *thyA⁻* version was used.

inheriting both markers, particularly when the unselected marker was very close to the origin. This was true whether the Hfr transferred the chromosome with a clockwise (Hfr Hayes) or counterclockwise (Hfr KL226) orientation with regard to the genetic map. Again, we noticed that the selected progeny showed less evidence of multiple exchanges between donor and recipient DNA in the merozygotes, but we were surprized that multiple exchanges appeared least evident in the cross conducted at 35° throughout.

In the crosses with Hfr PK191 the *recA+* allele can be transferred fairly early to the zygotes and in most cases good recovery of Thy+ recombinants was achieved (Table 5). For each *RecA⁻* recipient strain the number of Thy+ progeny that inherited the unselected donor *his+* allele was significantly reduced. This effect was most apparent when the yield of Thy+ progeny reflected a very large recovery of recombinants as seen at 42° with a strain carrying *recA200*. In this case only 2.25% of the Thy+ recombinants inherited *his+* as opposed to 23% at 35° and approximately 50% when the recipient carried *recA+*. However, when the recipient carried both *recA200* and *recB21* the number of Thy+ progeny that were also His+ was increased to nearly 35% at 42°, as opposed to 78.5% at 35° and 53.5% and 68% respectively in the *RecB⁻* single mutant. For some reason *recA12* prevented efficient recovery of Thy+ progeny, and in this case the inheritance of *his+* was only slightly reduced (Table 5b).

Analysis of the Thy+ recombinants recovered in crosses with Hfrs Hayes and KL25 again indicated that fewer multiple exchanges occurred in those mero-

zygotes that produced recombinants (Table 4b). In the Rec$^+$ recipient the unselected proximal markers appeared with fairly uniform frequency and the majority of the Thy$^+$ progeny inherited one or more of these in a pattern that in most cases was the result of multiple exchanges. By contrast, there was a distinct polarity in the genotype of Thy$^+$ recombinants obtained with the Rec$^-$ recipient. The majority fell into two categories, those that inherited none of the proximal markers, and those that inherited one or more of the proximal markers but showed no multiple exchanges. The remainder showed evidence of at least one multiple exchange.

Kinetics of recombinant formation in strains carrying recA200 *or* recA255. If the activity of the *recA* gene product is reduced but not abolished in the leaky *RecA*$^-$ strains, then the rate of recombinant formation could be lower than in strains carrying *recA*$^+$. We therefore attempted to measure the kinetics of recombinant formation in merozygotes carrying *recA200* or *recA255*.

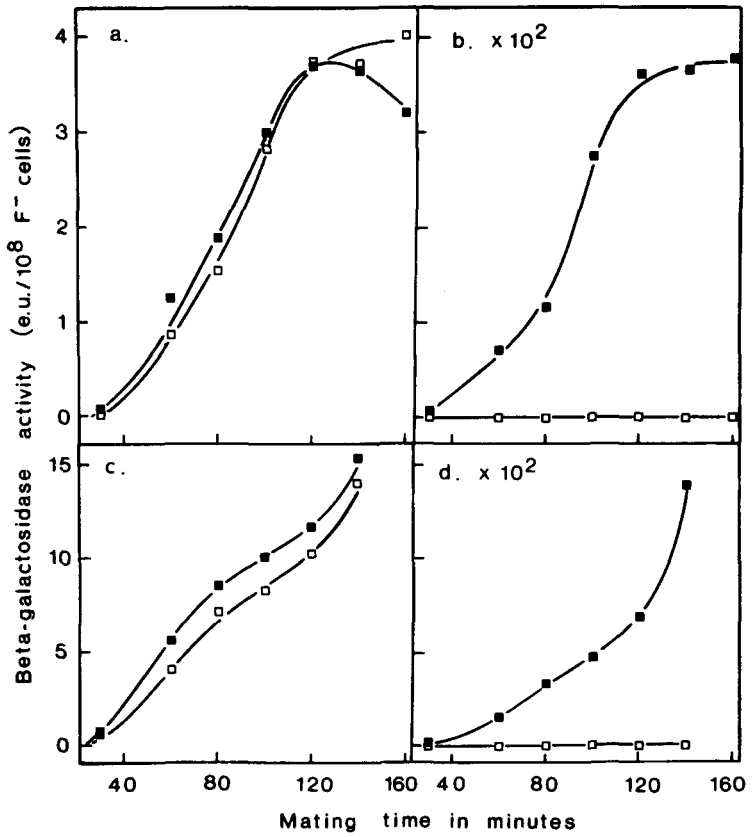

FIGURE 3.—β-galactosidase synthesis in zygotes after mating with donors of *lacZ608*$_{amber}$ (a and c) or *lacZ36* (b and d). (a) and (b), Hfr donors; (c) and (d), F'*lacZ*$^-$ donors. Mating and sampling procedures were as described in the METHODS section. Samples taken at time 0 and 15 minutes showed no β-galactosidase activity. The recipient strains were N1093*recA*+(■) and N1094*recA255*(□).

Two independent studies (BIRGE and LOW 1974; BERMANS, HOEKSTRA and ZUIDWEG 1975) have shown that in crosses between donor and recipient strains that carry different *lacZ*$^-$ mutations the amount of β-galactosidase activity detectable in the merozygotes is a reliable measure of recombination events, presumably intragenic, that generate transcribable *lacZ*$^+$ DNA molecules. With Rec$^+$ cells, 150 minutes is ample time for expression of the expected number of *lacZ*$^+$ genes (BIRGE and LOW 1974). Recipient strains that carried *lacZ118* were therefore crossed with donor strains carrying *lacZ36* and the formation of transcribable *lacZ*$^+$ DNA molecules was monitored by the assay of β-galactosidase synthesis. Depending on the donor strain used, the *lacZ*$^-$ allele on the exogenote was carried either by a fragment of Hfr chromosome or by an F-prime. Samples of the same matings were also assayed for viable Lac$^+$ progeny on minimal lactose agar. The results obtained are shown in Fig. 3 and Tables 6 and 7. In the Rec$^+$ receipient strains, enzyme activity was detected as early as 50 minutes after the onset of mating and, in the Hfr cross, reached a maximum level after approximately 120 minutes. (A maximum level of enzyme activity would not have been reached in crosses with F-prime donors until every recipient cell inherited the episome as a result of secondary matings.) In the *recA255* strain, however, no β-galactosidase was detectable even after 160 minutes (Hfr donor) or 140 minutes (F-prime donor) of mating, even though the yield of Lac$^+$ colonies on selective plates was 12% and 60% respectively, compared with crosses using Rec$^+$ recipients (Table 6). In the cross with the F-prime donor we

TABLE 6

Viable Lac+ recombinant formation in zygotes carrying recA+ *or* recA255

Cross*	Lac$^+$(Str^R) recombinants/ml†
(a) Hfr donors	
KL336(*lacZ36*) × N1093	1.67×10^6
KL336(*lacZ36*) × N1094	2.10×10^5
KL334(*lacZ118*) × N1093	0
KL334(*lacZ118*) × N1094	6
KL335(*lacZ608*) × N1093	3.4×10^7
KL335(*lacZ608*) × N1094	4.3×10^6
(b) F-prime donors	
KL329(*lacZ36*) × N1093	7.43×10^8
KL329(*lacZ36*) × N1094	4.48×10^8‡
KL330(*lacZ118*) × N1093	40
KL330(*lacZ118*) × N1094	80
KL340(*lacZ608*) × N1093	1.09×10^9
KL340(*lacZ608*) × N1094	6.26×10^8

* Mating procedure as described in legend to Figure 3.

† After 160 min, (a); or 145 min, (b), of mating.

‡ This number was seen after 72 hours incubation of the selection plates. At 48 hours, the colonies on the plates were too small to score in this cross but not in the other crosses.

Viable cell counts per ml at the end of matings were 2×10^9 (N1093), and 6×10^8 (N1094) in (a); and 1.6×10^9 (N1093), and 9×10^8 (N1094) in (b).

In the crosses involving donors of *lacZ118* the Lac$^+$ colonies observed were assumed to be revertants.

TABLE 7

β-galactosidase synthesis in zygotes carrying recA+ *or* recA200

Cross	Temp (°C)	E.U. per 10^8 cells
KL329(*lacZ36*) × KL425	35	0.148
KL329(*lacZ36*) × KL426	35	0.0058
KL329(*lacZ36*) × KL425	31	0.117
KL329(*lacZ36*) × KL426	31	0.012
KL340(*lacZ608*) × KL425	35	18.4
KL340(*lacZ608*) × KL426	35	18.0
KL340(*lacZ608*) × KL425	31	14.0
KL340(*lacZ608*) × KL426	31	11.8

Recipient strains were grown and mated at the indicated temperature. The donor strains (F'*lacI3 lacZ36* and F'*lacI3 lacZ608*) were grown at 37° before mating. Mating was for 150 min at 35°, or for 180 min at 31.

noticed that after 40 hours of incubation only about 0.6% of the Rec+ control number of large colonies were visible on the plates though a large number of micro-colonies were discernible. After a further period of 18–24 hours incubation these micro-colonies grew into normal size colonies. Several of these "late" Lac+ recombinants were purified to single colonies and tested for their growth rate on minimal lactose medium and for β-galactosidase production in cultures grown in broth medium. No differences were detected between these Lac+ recombinants and those obtained from crosses with the Rec+ recipient. With the F-*lacZ608* donor, expression of the Lac+ phenotype did not require recombination (see MATERIALS AND METHODS) and Lac+ colonies appeared on the selective plates at the same time in both the Rec+ and Rec− crosses.

Similar results were obtained with a recipient carrying *recA200*. Although at 35° this strain yielded 50% or more of the Rec+ control number of Lac+ viable progeny in *lacZ36* × *lacZ118* crosses, when β-galactosidase was measured in a mating with an F-*lacZ36* donor the enzyme yield after 150 minutes reached only 3.9% of the Rec+ level at 35° and 10% at 31° (Table 7). No enzyme activity was detected in matings at 42°.

Using the *recA200* allele, we could also monitor the recovery of recombination function after shifting from high to low temperatures. An F*lac*+ merodiploid strain carrying *recA200* was constructed, purified and maintained at 42°. Recombination between the F-prime and the chromosome to generate temporary Hfrs was then monitored by mating with an F− recipient either at 42° or after various periods of growth at 35°. The results are shown in Figure 4. In all the matings transfer of F*lac*+ itself to the recipient was 35–95% as efficient as from the Rec+control strain. No mobilization of the chromosome could be detected at 42°. Furthermore, the rate of mobilization from a culture of this strain appeared to be very low even after several generations of growth at 35° after its initial isolation at 42°. If recombination proficiency was rapidly restored after the shift-down to 35° then mobilization should be fairly efficient (compared with the control carrying *recA*+) unless it takes considerable time for autonomous

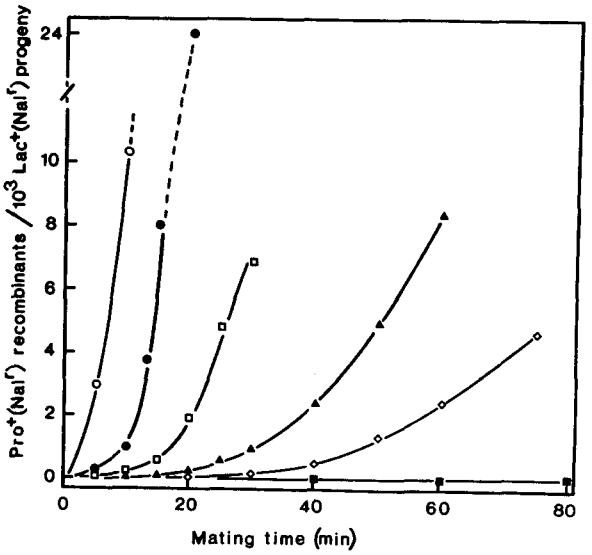

FIGURE 4.—Chromosome mobilization from F'*lac*+ derivatives of KL398 (*recA*+) and KL399 (*recA200*). Strain KL320 was grown to an A_{650} of 0.3 at 37°. Donor strains KL398 F'*lac*+ and KL399 F*lac*+ were grown in Luria broth at 42° to an A_{650} of 0.3. Samples of the donor strains were then diluted to varying degrees into fresh broth at 35° and incubation continued at 35° until the A_{650} again reached 0.3. 2 ml portions of KL320 were then mixed with 0.2 ml KL398 F'*lac*+ or 0.5 ml of KL399 F'*lac*+, grown under various conditions, and the mating mixtures incubated either at 42° or 35°. Samples were removed at intervals, and mating was interrupted before plating for *Pro*+*(Nal*^{R}*)* recombinants. After 30 minutes mating a sample was also removed for assay of *Lac*+*(Nal*^{R}*)* progeny as a measure of F'*lac*+ transfer.

○, KL398 F'*lac*+ grown and mated at 42°;

●, KL398 F'*lac*+ grown at 42° and mated at 35°;

■, KL399 F'*lac*+ grown and mated at 42°;

◇, KL399 F'*lac*+ grown at 42° and mated at 35°;

▲, □, KL399 F'*lac*+ grown first at 42° and then at 35° for 50 minutes and 4 hours respectively, before mating at 35°.

F-primes to form temporary Hfrs even in strains that are Rec+. Since the results obtained by HALL and HOWARD-FLANDERS (1972) indicate that an F-prime transferred to a strain carrying *recA*+ can mobilize the chromosome efficiently within a short period of time we considered the latter possibility unlikely or at least only partly responsible. Therefore we conclude that mobilization of the chromosome at 35° from strains carrying *recA200* is inefficient either because recovery of recombination proficiency in the majority of the cells required more than four hours of growth after the shift-down or because even at 35° the rate of recombination to generate temporary Hfrs is very low.

With the *recA200* allele we also attempted to measure the length of time required at 35° before *RecA* function could be dispensed with by shifting to 42°. Samples of matings at 35° were removed at intervals and mating pairs separated by blending. Recombinants were then assayed by plating at 35° and 42°. In this temperature shift-up experiment only a very small fraction of the merozygotes

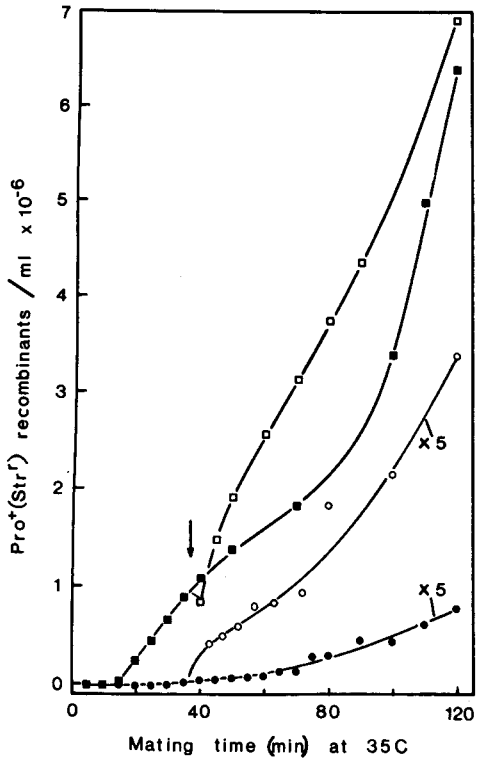

FIGURE 5.—Effect of temperature shift-up on recombinant yield from zygotes carrying *recA200*. Strain KL399 (*recA200*) was grown to an A_{650} of 0.3 in Luria broth at 35°. 10 ml of this culture was mixed with 2 ml of Hfr KL226 grown to an A_{650} of 0.3 at 37°. The mating mixture was incubated at 35° and samples removed at intervals were interrupted and plated immediately on selective medium either at 35° (■) or 42° (●) for *Pro+(Str^R)* recombinants. At 37 minutes (shown by the arrow) a portion of the mating mixture was removed and shaken vigorously to separate more mating pairs before continuing with incubation at 35°. Samples of the interrupted mixture were also assayed at 35° (□) and 42° (○) for recombinants. These samples were blended again immediately before plating.

that were made at 35° and which went on to generate viable progeny at this temperature did so when the final selection was made at 42°, even after mating had been allowed to proceed at 35° for 120 minutes before the shift-up (Figure 5). If in recipients that are *RecA+* recombination is completed in the majority of the merozygotes within 120–160 minutes of mating with an Hfr donor (see Figure 3b) and if recombination proceeded at a similar rate in a recipient carrying *recA200* (at 35°), one might have expected more of the merozygotes to yield progeny when plated at 42°. The number of recombinants at 42° did increase from about 0.15% after 20 minutes of mating to 2.5% after 120 minutes, which suggested that some event was slowly taking place at 35° that allowed progeny to be recovered when *recA* gene activity was subsequently reduced by raising the temperature of incubation. The continued increase in the numbers of recombinants obtained at 35° suggests that initiation of new matings occurred during the

incubation. In order to test the possibility that the creation of a free distal end of the Hfr DNA by spontaneous breakage allowed recombination to be completed in the Rec⁻ strain at 35°, a portion of the mating pairs were separated after 37 minutes of mating and the zygotes incubated for further periods of up to 80 minutes at 35° before interrupting again and plating samples at 42° (see Figure 5). In this case the yield of recombinants at 42° was indeed found to increase by 3- or 4-fold, although the absolute level was still only 10% of that obtained at 35°. Similar increases were seen when mating was interrupted after 28 or 52 minutes of mating followed by incubation at 35°. Since recipient cells were in excess, the formation of new mating pairs could also have lead to some increase in progeny but this would have been seen only after further incubation to allow transfer of the selected donor allele (15–20 minutes in this case). After interruption at 37 minutes, however, the increase observed at 42° was almost immediate, though secondary mating pair formation might have contributed to the increased yield of progeny observed at later times.

A common feature of these kinetic experiments was that the recombination event assayed had to occur within a specified period of growth in rich medium. Though open to further interpretation, the results obtained indicate that the recombination deficiency of strains carrying *recA200* (at 35°) or *recA255* is much greater than is indicated from the numbers of colonies seen after selection of recombinants on minimal agar plates. Thus the recombination deficiency index as expressed in Tables 3–5 may not be an accurate representation of the extent of the defect in recombination in *RecA*⁻ recipients carrying *recA255* or indeed in strains carrying *recA200* which we have previously described as having a Rec⁺ phenotype at 35° (LLOYD *et al.* 1974).

DISCUSSION

Recent studies have identified a cluster of closely linked mutations in the *recA* genetic region that modify the phenotype of *RecA*⁻ strains or alter physiological processes thought to be related to *recA* gene activity (DEVORET and BLANCO 1970; CASTELLAZZI, GEORGE and BUTTIN 1972; CLARK, personal communication). We feel that further study of atypical *recA* mutations is likely to provide valuable information towards a definition of *recA* gene activity and the recombination process itself. With this in mind the studies reported in this communication were initiated. In particular we were interested in analyzing for the first time recombination under partial *RecA*⁻ conditions.

STACEY and LLOYD (1976) reported the isolation of several mutant (Rec⁻) strains that retained a significant residual capacity for recombination. The *rec*⁻ mutation in one of these strains was localized on the genetic map and then transferred into other strains for further study. From the results we concluded that the phenotype of the original mutant was due to a mutation in the *recA* gene because (a) the mutation was linked to *cysC* to an extent similar to other *recA* mutation in one of these strains was localized on the genetic map and then trans-when the exogenote carried *recA*⁺ than when it carried *recA56* or *recA1*, and

(c) λ-sensitive strains that carried this mutation failed to support the growth of a phage λ mutant (*λbio-1*) deleted for *int*, *red* and *gam* genes, a feature that as far as we know is characteristic of only *RecA*⁻ among recombination-deficient mutants (MANLY, SIGNER and RADDING 1969; ZISSLER, SIGNER and SCHAEFER 1971). However, the mutant allele, *recA255*, differs from other *recA* mutations that have been described (CLARK 1973). Thus, in Hfr × F⁻ (*recA255*) crosses it allowed haploid progeny to be recovered, though at a reduced level (Table 2), in contrast to crosses with recipients carrying *recA1* or *recA13* which yield progeny at extremely low frequencies that are almost always stable merodiploids (Low 1968). The *recA255* mutation did not confer the extreme UV sensitivity usually associated with strains carrying *recA*⁻ mutations; indeed some strains carrying *recA255* were only very slightly sensitive compared with control strains carrying *recA*⁺ (Fig. 2a). Finally, although *recA255* caused strains to degrade their DNA spontaneously, the additional degradation observed after UV irradiation was no more than that seen after irradiating Rec⁺ strains (Figure 2c). Other *recA*⁻ mutations are notable for causing excessive DNA breakdown under the conditions that were used (CLARK *et al.* 1966; HOWARD-FLANDERS, THERIOT and STEDEFORD 1969). We attributed the somewhat variable phenotype caused by *recA255* in different genetic backgrounds to other (unidentified) mutations since the control (*recA*⁺) strains also showed considerable variation (Figure 2a and b).

In this paper we have described some of the consequences for recombinant formation in leaky *RecA*⁻ strains. We assumed on the basis of values for the recombination deficiency index that the *recA200* and *recA255* alleles reduce but do not abolish *recA* gene activity and that the residual activity in strains carrying *recA200*, at 35°, was greater than in those carrying *recA255* which in turn retain more activity than strains carrying *recA200* at 42°. Although our experiments did show (Figure 3, Table 7) that the rate of formation of recombinant *lacZ*⁺ DNA was reduced more by *recA255* than by *recA200* (at 35°), in both cases the rate was much lower than expected from other measures of recombination proficiency (Tables 2 and 3). This kind of apparent anomaly was even more striking when the exogenote was an F-prime (F*lacZ36*), rather than a fragment of Hfr DNA, since the yield of recombinant progeny on plates (in relation to the control strain carrying *recA*⁺) appeared to be almost normal (60% of the control) even when the recipient carried *recA255*. In this case the persistence of the F-prime factor in cells within micro-colonies produced after plating would allow recombination to occur at later times and give rise to colonies under the conditions used. Indeed we did observe a delayed appearance of Lac⁺ colonies on the selection plates (Table 6). We surmise that the relatively high yields of recombinants in many of the Hfr × F⁻ crosses (Tables 2 and 3) could be due to the persistence of some merozygotes for a time on the selective plates. Thus we may be working with *RecA* mutants that possess a finite but very small capacity for recombination at all times. This would be consistent with experiments in which the *RecA* function in cells carrying *recA200* was modulated by temperature shifts. Thus, cells in which F-prime-chromosome recombination could be detected were accumulated at a low rate after shifting F′ merodiploids from 42° to 35° (Figure 4). In addition, the rate at which merozygotes carrying

recA200 became independent of *RecA* function was also very low at 35° (Figure 5). An alternative explanation for the apparently high yield of recombinants obtained after plating (Tables 2, 3 and 6) is that the nutritional shift-down in some way allows more of the merozygotes to form recombinants.

Our crosses with multiply marked recipient strains allowed some measure of the genotype of the recombinants selected. In general, the presence of *recA200* or *recA255* in the recipient caused a marked decrease in the observed number of multiple crossovers. The frequencies of multiple exchanges shown in Tables 3 and 4 do not take into account the fact that in many of the crosses the incorporation of markers near the Hfr origin was low. This effect on early markers reduces the numbers of recombinants which inherit enough donor markers to allow detection of multiple exchanges. Nevertheless, when this is taken into account there is still in most cases a 3- to 4-fold reduction in multiple crossovers among the recombinant from leaky *RecA*$^-$ recipients. This effect was seen even under conditions when the *recA200* allele allowed high numbers (64% of Rec$^+$) of recombinants to be produced (Table 4a). The only case where these effects were not seen was that of an extreme Rec$^-$ phenotype, i.e. *recA200* at 42° (Table 3), where the few recombinants obtained may have included some fraction of F-prime merodiploids whose phenotypes could have distorted the linkage analysis (Low 1968).

One conceivable explanation for the low recovery of recombinants carrying markers close to the Hfr point of origin in most of the crosses using leaky *RecA*$^-$ recipients is that Hfr DNA is subjected to limited degradation near the proximal end. The experiment in which the activity of exonuclease-V was eliminated by *recB*$^-$ mutation tends to support this possibility since we observed in this case a high incorporation of an early Hfr marker (Table 5a). Another interpretation of this effect is that in the leaky *RecA*$^-$ strains, a decreased frequency of crossovers per unit length of chromosome leads to the exclusion of longer regions from the proximal end of the transferred Hfr fragment, except when the recipient is also *recB*$^-$.

This analysis of the recombinants generated in leaky *RecA*$^-$ strains demonstrates in particular a new component to be included in the overall phenotype associated with mutations in *recA*. We observed that a limitation of *RecA* activity causes a marked decrease in the frequency of crossovers per unit length of chromosome. It is possible therefore that the *recA* gene product is involved in the initiation of crossover events after conjugation.

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