FORMATION OF MERODIPLOIDS IN MATINGS WITH A CLASS OF REC⁻ RECIPIENT STRAINS OF ESCHERICHIA COLI K12*

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Recombination-deficient (Rec$^-$) recipient strains of *E. coli* K12 are characterized by their inability to produce conjugational recombinants with the usual high efficiency, even though the injection of F′ factors and Hfr donor DNA occurs normally.[1] Various Rec$^-$ mutants have been described that support recombination with frequencies ranging from 10^{-1} to less than 10^{-3} times that of the corresponding Rec$^+$ strains.[1–3] At least two general classes of Rec$^-$ mutations have been described to date in terms of their response to irradiation by ultraviolet light (UV). Those of the *rec*A class are characterized by a striking excess (compared to the wild type) of DNA breakdown following UV treatment[2, 4] and hence have been termed "reckless."[5] Similarly, the term "cautious" has been given to the other class, *rec*B, since mutants of this class degrade their DNA to a *less* than normal extent, following UV irradiation.[5] Both of these classes of Rec$^-$ mutants have a much lower ability to survive X rays or UV than does the wild type, although the system for excision and repair of UV-induced pyrimidine dimers seems normal.[4, 5] A revealing study of strains which are defective in dimer excision and/or genetic recombination has led Howard-Flanders and collaborators to the reasonable opinion that recombination between newly replicated sister strands in a cell efficiently serves to construct a viable chromosome even when many radiation-induced lesions are present on the individual sister strands; this reconstitution by exchange occurs regardless of whether or not the dimer excision and repair system of the cell is functional.[5, 6]

In an attempt to understand more clearly the nature of the Rec$^-$ defect(s), a study of the genetic properties of recombinants obtained from crosses of Hfr donor strains with several Rec$^-$ recipient strains has been carried out. The particular aim was to determine whether or not Rec$^-$ mutants can support normal recombinational events at all. This paper describes recombinants obtained with several of the presently available Rec$^-$ mutants. With two of these strains, both belonging to the *rec*A class, results indicate that no normal recombinants arise but that partial diploid progeny are formed. The yield of partial diploids, which in some cases comprise a variety of F′ strains, depends on the origin of chromosomal transfer of the Hfr strain used. In contrast to *rec*A, matings with a recipient of the *rec*B class were found to yield haploid recombinants with normal linkage between selected and unselected markers. This finding implicates the *rec*B defect in some step of the recombination process which either allows or prevents all of the normal crossovers in any given merozygote.

Materials and Methods.—The genotypes and derivation of the *E. coli* K12 strains used are listed in Table 1. The origins of chromosomal transfer of the Hfr strains are indicated as arrowheads on the genetic map shown in Figure 1 (modified from Taylor and Thoman[7]). Matings were carried out by growing donor and recipient strains in broth at 37° to a concentration of 1–2 × 10^8 cells/ml and then mixing them in a ratio of 1:10.

TABLE 1. *Description of E. coli K12 strains.*

Strain	Mating type	Description*	Source
AB259	Hfr (Hayes)	Thi$^-\lambda^-$	E. Adelberg
KL17	Hfr (Hayes)	Thi$^-$leu$^-$ λ^+	Pasteur Institute
R5	Hfr	Thi$^-$lac$^-$gal$^-$malA xyl$^-$ mtl$^-$	P. Reeves
P4X	Hfr	MetB λ^+	E. Adelberg
K10	Hfr (Cavalli)	T1^{r}T2^{r}T6^r	A. Garen
KL84	Hfr	Thi$^-\lambda^-$	AB259 → F^+ → KL84
KL19	Hfr	λ^+	UV treatment of F^+ strain KL23
B7	Hfr	Met$^-\lambda^-$	A. Rörsch
44	Hfr	Rarg$^-$argS1	P. Horn
KL96	Hfr	Thi$^-\lambda^-$	UV treatment of F^+ strain KL20
AB311	Hfr	Thi$^-$thr$^-$leu$^-$lacZ str^r	A. L. Taylor
KL98-2	Hfr	Leu-2 λ^+	UV treatment of F^+ strain KL23, then introduction of leu-2
KL16	Hfr	Thi$^-\lambda^-$	UV treatment of F^+ strain KL20
KL16-99	Hfr	Thi$^-\lambda^-$recA1	Recombinant from KL16 × JC1553
JC12	Hfr	Thi$^-$met$^-$purC1λ^-lac$^-$ xyl$^-$mtl$^-$	A. J. Clark
Hfr 1	Hfr	λ^+	A. Garen
KL25	Hfr	λ^-	UV treatment of F^+ strain W1485
Ra-2	Hfr	λ^-	UV treatment of Hfr strain Ra-1
JC182	Double male	λ^-Thi$^-$purC1; Hayes and JC12 origins of transfer	A. J. Clark
AB1157	F^-	Thi$^-$thr$^-$leu$^-$pro$^-$his$^-$argE str$^r\lambda^-$lac$^-$gal$^-$ara$^-$xyl$^-$mtl$^-$	P. Howard-Flanders
AB2463	F^-	As AB1157 but recA13	P. Howard-Flanders
AB2470	F^-	As AB1157 but recB21	P. Howard-Flanders
KL104	F^-	As AB1157 but leu$^+$	Recombinant from AB259 × AB1157
KL105	F^-	As AB2463 but leu$^+$	Spontaneous leu$^+$ revertant
JC411	F^-	Leu-2his-1argG6met-1str^r λ^-lacY1, 4malA1xyl$^-$mtl$^-$	A. J. Clark
JC1553	F^-	As JC411 but recA1	A. J. Clark

* Unlisted characters are assumed to be wild type. Symbols are explained in the caption to Fig. 1, with the following additions: requirements, thi (thiamine), purC (adenine or guanine); sugar utilization, mtl (mannitol), ara (arabinose); T1^{r}T2^{r}T6^r (resistance to bacteriophages T1, T2, and T6); λ^+, λ^- (presence or absence of λ prophage); Rarg (arginine regulation); argS (arginyl tRNA synthetase).

The cultures were shaken gently for aeration during matings and then chilled prior to being plated out onto media selective for recombinants. Matings were interrupted with the use of a vibratory blending device.[8] Genetic analysis of recombinant clones was carried out by the method of Lederberg and Lederberg.[9] The minimal medium of Davis and Mingioli[10] was used (supplemented where necessary with appropriate amino acids at concentrations of 100 μg/ml; adenosine, 40 μg/ml; thymine, 25 μg/ml; thiamine, 1 μg/ml; streptomycin, 100 μg/ml) for selection of recombinants and genetic analysis. Recombinant colonies were scored for their Rec phenotype by testing their UV sensitivity as described by Clark and Margulies.[1] UV irradiations were carried out with the use of a General Electric G15T8 15-watt germicidal lamp, which gave a dose rate of 43 ergs mm^{-2} sec^{-1} at a distance of 25 cm from the bulb.

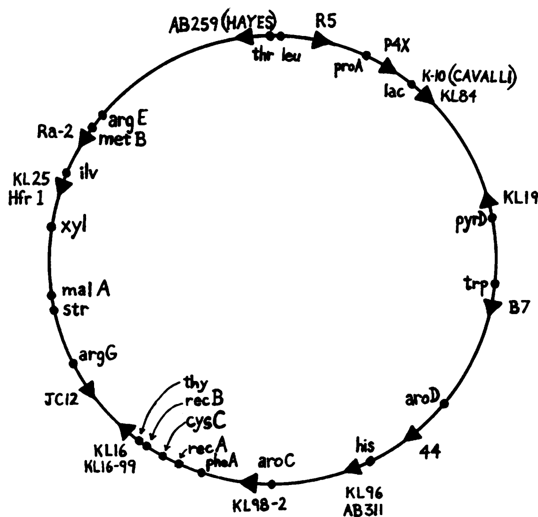

FIG. 1.—Genetic map of *E. coli* K12 showing Hfr points of origin. This modified version of the genetic map (from Taylor and Thoman[7] and Willetts *et al.*[11]) shows the relative position of mutant loci which result in auxotrophy for threonine (thr), leucine (leu), proline (proA), uracil (pyrD), tryptophan (trp), aromatic amino acids (aroC, aroD), histidine (his), phenylalanine (pheA), cysteine (cysC), thymine (thy), arginine (argE, argG), methionine (metB), isoleucine-valine (ilv); inability to utilize lactose (lac), maltose (malA), xylose (xyl); resistance to streptomycin (str); recombination deficiency (recA, recB).

The arrowheads indicate the origins and directions of transfer for the various Hfr strains. The use of one arrowhead for two Hfr strains indicates that the two origins are very close but do not necessarily coincide.

Results.—Dependence of recombination frequency on Hfr origin of transfer: Since the primary aim in these studies was the examination of recombinants formed in zygotes which are Rec^-, the various crosses described here were interrupted before transfer of the pertinent *rec*$^+$ genes from the Hfr donor into the Rec^- recipient strain. The "reckless" mutations (in strains JC1553(*rec*A1) and AB2463(*rec*A13)) are located between *cys*C and *phe*A on the genetic map[11] (Fig. 1). The "cautious" mutation (strain AB2470 (*rec*B21)) is cotransducible with *thy*[12] and does not lie between *cys*C and *phe*A.[11] Various Hfr strains were chosen which transfer the *thy*-*rec*B-*cys*C-*rec*A-*phe*A region late during conjugation, and each of these was used in parallel matings with Rec^+(AB1157) and Rec^- (AB2463 or AB2470) recipients which differ from each other only at the *rec* loci. All of the matings were allowed to proceed for 60 minutes before interruption except when the following Hfr's were used: B7 (75 min), Hfr 1 (80 min), KL96 (90 min), and KL98-2 (90 min). In all of the crosses, streptomycin was used for counterselection of the Hfr strain. With the *rec*$^+$ recipients, the recombination frequencies per input Hfr cell ranged from 15 to 60 per cent.

The results of these matings are given in Table 2, which shows a comparison of the frequencies of recombinant production in the Rec^- recipients and the frequencies with the Rec^+ recipients. It is evident from these data that both the *rec*A and the *rec*B mutations cause decreases in recombination frequency which vary greatly from cross to cross, depending on the Hfr donor.

Nature of recA recombinants: If the low numbers of recombinants obtained with Rec^- recipients were simply due to a lower than normal probability of completing a genetic exchange, independent of other exchanges, then the recombinants obtained with such recipients would be expected to show a higher linkage

TABLE 2. *Recombinant production in Rec⁻ females as compared to Rec⁺ females.*

Hfr	Selected donor marker	Ratios of Recombinant Production AB2463(*rec*A13) / AB1157(*rec*⁺)	AB2470(*rec*B21) / AB1157(*rec*⁺)
Hfr 1	*arg*⁺	<0.00001	0.0005
KL25	"	0.0002	0.003
Ra-2	"	0.002	0.02
AB259	*leu*⁺	0.0005	0.02
R5	"	0.00014	—*
P4X	*pro*⁺	0.002	—*
Cavalli	"	0.0006	0.0008
KL84	"	0.002	0.002
B7	"	<0.0001	0.2
KL96	*his*⁺	0.0013	—*
KL98-2	"	<0.00001	—*

* Not tested.

(i.e., fewer crossovers) between selected and unselected markers than Rec⁺ recombinants. To put this possibility to a test, *rec*A recombinants (100 or more per cross) were examined for the frequencies of inheritance of other donor markers which were transferred prior to the selected one. Results for the *rec*A13 recipient AB2463 are shown in Figure 2 together with corresponding data for the *rec*⁺ control (AB1157). Figure 2*A* shows that when Pro⁺Str^r recombinants are selected in matings with the Hayes Hfr donor AB259, the *rec*⁺ recombinants were 77 per cent Thr⁺ and 81 per cent Leu⁺; these percentages reflect a normal amount of

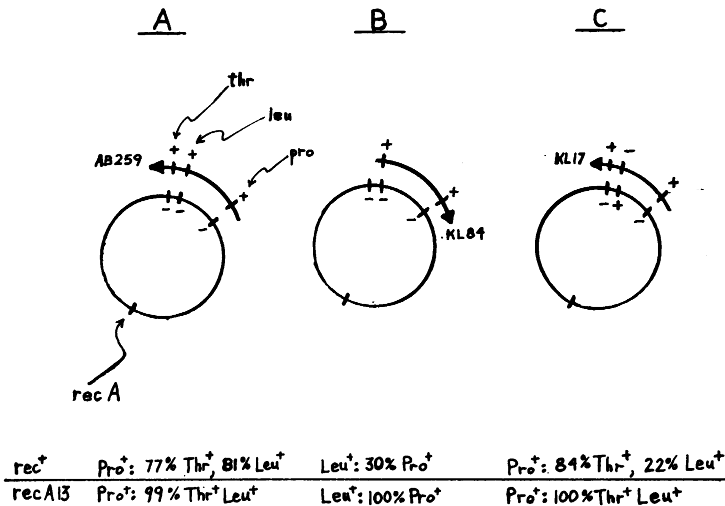

FIG. 2.—Genetic analysis of *rec*⁺ and *rec*A recombinants. The arrowhead arcs represent the portions of the male genome which must have been transferred in order that the selected male marker appear in recombinants. In all cases streptomycin was used to counterselect the male strain. In (*A*) and (*B*) the *rec*⁺ and *rec*A13 recipient strains were AB1157 and AB2463, respectively. In (*C*) the corresponding strains were KL104 and KL105. The matings were interrupted after 60 min of transfer in order to prevent the entry of the *rec*⁺ gene from the donors.

crossing-over in the formation of recombinants. The *rec*A recombinants, by contrast, almost always inherited Thr^+ and Leu^+ from the donor. Similarly, Figure 2*B* shows that when Hfr KL84 is used in the selection of Leu^+Str^r recombinants, virtually all of them inherit the earlier Pro^+ donor marker when the *rec*A female is used, as opposed to 30 per cent Pro^+ with the rec^+ control.

We next inquired whether or not the unusually high co-inheritance of selected and unselected donor markers in *rec*A recombinants was a consequence of a perpetuation of the genetic information of the entire chromosomal fragments injected from the donor, thus forming partially diploid cells (merodiploids). This question could be answered by mating a $Thr^+Leu^-Pro^+$ Hayes Hfr strain (KL17) with rec^+ and *rec*A recipients which were $Thr^-Leu^+Pro^-$ (KL104 and KL105). The analysis of Pro^+Str^r recombinants obtained from these crosses is shown in Figure 2*C*. The dominant leu^+ gene from the F^- parent was absent in most of the rec^+Pro^+ recombinants, a result that would be expected from exchange of donor and recipient DNA. By contrast, the *rec*A Pro^+ recombinants were both Leu^+ and Thr^+ in 100 per cent of the cases examined. The results shown in Figure 2 thus strongly suggest that the *rec*A "recombinants" are in fact merodiploids which carry both male and female genetic material in the region between the origin of transfer and the selected male marker.

In order to obtain further evidence for this merodiploidy, several *rec*A recombinants were examined for their ability to give rise to segregants, i.e., daughter cells which have lost one or more of the dominant genes in the region of presumed heterozygosity. Fresh isolates of three of the *rec*A recombinants from each of the *rec*A crosses described in Figure 2 were grown overnight in minimal medium containing neither threonine, leucine, nor proline, and then grown for about five generations in broth to allow for growth of segregants as well as merodiploid cells. These cultures were inoculated onto plates which included threonine, leucine, and proline. Genetic analysis of the resulting clones showed that in every case it was possible to find segregants which had become auxotrophic for one or more of the markers that were originally injected from the Hfr strain.

The patterns of segregation were found to vary markedly from merodiploid to merodiploid, and not enough data have as yet been collected to allow complete description of their structures. However, two classes of *rec*A merodiploids can clearly be distinguished and partially characterized on the basis of their response to male-specific and female-specific phages. The male-specific phage MS2 lyses cells which harbor the sex factor, e.g., Hfr, F′, and F^+ donor cells. The sex factor is presumably the genetic determinant for the F pili which are found on donor cells only and which serve as attachment sites for the male-specific phages;[13] F^- cells are thus not susceptible to male phage infection. The female-specific phage φII, by contrast, infects and lyses F^- cells but is inhibited in cells which carry an F factor.[14]

Many of the *rec*A merodiploids were found to be sensitive to MS2. When these were tested for their ability to act as donors, it was found that all were high-frequency donors of the markers that were derived from the original Hfr parent. Transfer of markers derived from the *recA* female parent was very low (less than 10^{-5} per merodiploid cell). Furthermore, most of the recombinants obtained

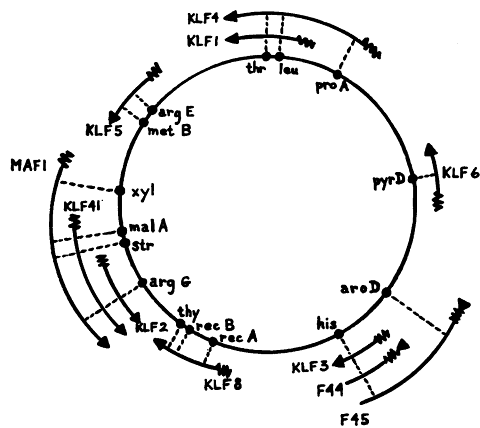

Fig. 3.—F′ factors obtained from Hfr × F⁻ *rec*A crosses. The genetic length of these F′ factors has been determined only as far as the markers shown. The F′ factors were derived from the following Hfr strains: KLF1 and KLF4 from AB259; KLF6 from KL19; KLF3 from KL96; F44 from Hfr 44 (by P. Horn); F45 from B7 (by P. Horn); KLF8 from KL16; KLF2 and KLF41 from JC12; MAF1 from JC182 (by W. K. Maas); KLF5 from Ra-2. The recipent strains used in the crosses were either AB2463 (*rec*A13) or JC1553 (*rec*A1), or derivatives which carry *rec*A1 or *rec*A13.

from transfer of Hfr-derived portion of the MS2-sensitive merodiploid into another recipient are in turn good donors of the same chromosomal segment. In short, all the MS2-sensitive *rec*A merodiploid recombinants examined so far have been indistinguishable from F′ strains; the F′ factors of these merodiploids are derived from the Hfr parent and are actually responsible for the "recombinant" phenotype. Figure 3 shows a series of F′ factors that have been isolated in this way.

The fraction of *rec*A merodiploid recombinants which are MS2-sensitive F′ strains depends markedly on which Hfr is used as the parental donor. For example, 16 out of 23 merodiploids obtained with the Hayes Hfr as shown in Figures 2*A* and *C* were MS2-sensitive. Ten out of 10 Leu$^+$Str^r merodiploids from Hfr KL84 (Fig. 2*B*) were MS2-resistant. Among 18 ArgG$^+$Str^r merodiploid recombinants from JC12 × JC1533, 12 were MS2-sensitive. None of the MS2-resistant merodiploids tested was found to be a donor. However, all of those tested were found to be resistant to the female-specific phage φII, as were the Hfr parents for each cross. (The parental F⁻ strains were φII-sensitive.) Thus it appears that all of the recombinants from Hfr × F⁻ *rec*A crosses examined thus far have been either F′ strains which carry a complete F factor or else nondonor merodiploids which appear to carry part of an F factor as indicated by resistance to φII.

Nature of recB recombinants: A similiar analysis was carried out with recombinants obtained from matings of Hfr strains with a *rec*B recipient. As with *rec*A, the question is asked whether the linkage between selected and unselected markers in *rec*B recombinants is normal or whether there are fewer crossovers per unit map distance because of a lower than normal probability for independent crossover events.

The answer to this question may be seen from the genetic analysis of *rec*$^+$ and *rec*B21 recombinants shown in Table 3. These recombinants were obtained from crosses of four Hfr strains with strains AB1157 (*rec*$^+$) and AB2470 (*rec*B21). The mating procedures were the same as those used in obtaining the results of Table 2, i.e., the matings were interrupted after 60 or 70 minutes in order to prevent the entry of *rec*$^+$ from the donors.

TABLE 3. *Genetic analysis of Hfr* × $\left\{\begin{matrix} F^- \ rec^+ \\ \text{or} \\ F^- \ recB21 \end{matrix}\right\}$ *recombinants.*

Hfr	Selected donor marker	Unselected proximal donor marker	Recipient Strain: AB1157(*rec*$^+$) (%)	AB2470(*rec*B21) (%)
AB259	*pro*$^+$	*thr*$^+$	77	85
		leu$^+$	81	87
		thr-leu c.o.*	12	8
Cavalli	*arg*$^+$	*pro*$^+$	54	23
		leu$^+$	54	41
		thr$^+$	58	44
		thr-leu c.o.*	10	16
B7	*arg*$^+$	*pro*$^+$	35	25
		leu$^+$	41	30
		thr$^+$	39	34
		thr-leu c.o.*	11	16
KL25	*pro*$^+$	*arg*$^+$	70	42
		thr$^+$	82	76
		leu$^+$	84	81
		thr-leu c.o.*	8	8

* These rows give the percentages of recombinants in which a crossover was observed between *thr* and *leu*, i.e., either *thr*$^+$*leu*$^-$ or *thr*$^-$*leu*$^+$.

Table 3 shows that, for the groups of recombinants examined (approximately 200 per cross), there was very little difference between *rec*B21 and *rec*$^+$ recombinants with regard to unselected donor markers. For example, the percentage of recombinants with crossovers in the *thr-leu* interval was approximately 11 per cent for both *rec*B21 and *rec*$^+$ recombinants.

In contrast to *rec*A, the *rec*B recombinants were almost always (95% of those examined) found to be resistant to the male-specific phage MS2 and sensitive to the female-specific phage φII. We conclude, therefore, that, although the *numbers* of recombinants obtained with the *rec*B21 recipient are generally much lower than with the *rec*$^+$ recipient (Table 2), the genetic makeup and F status of *rec*B21 recombinants appear to be almost identical to those observed in *rec*$^+$ recombinants. Another Rec$^-$ mutant, *rec*-22, which very likely belongs to the *rec*B class,[15] was also subjected to an analysis similar to the one shown in Table 3, and the results obtained were similar to those obtained with *rec*B21.

Discussion.—It is clear from the foregoing that the two types of Rec$^-$ mutants described so far, *rec*A and *rec*B, differ fundamentally in the type of recombinant formation which they support after mating with Hfr strains. None of the *rec*A recombinants studied here was found to be a true recombinant of the classical type, i.e., formed by exchange of genetic information between homologous regions of the two parental chromosomes to yield a recombinant chromosome. Instead, the *rec*A recombinant phenotypes were found to be due to the perpetuation and replication of the genetic material injected by the Hfr strain. These perpetuating fragments were found to be of two types: (*a*) F′ factors, which may have formed during the growth of the Hfr culture according to the model elaborated by Scaife,[16] and (*b*) fragments that can be replicated but cannot be transferred to other strains. The finding that the latter type of donor fragments confer φII-resistance upon the recombinants (but not MS2 sensitivity) suggest that they

carry the portion of the sex factor which enables F to replicate itself, even though they lack the determinants for F pili formation and DNA transfer.

Since the level of normal recombinant formation involving homologous crossing-over is reduced in *rec*A strains to practically zero ($<10^{-5}$ of the Rec^+ level), these strains should be especially useful for the isolation and manipulation of F′ factors. Heretofore, the isolation of F′ factors has usually involved selection for the entry of a very late Hfr marker in an Hfr × F^- cross which is interrupted after 30–60 minutes of mating.[17] In such a cross, the normal Hfr cells in the culture are prevented from transferring the very late marker because of the early interruption, but the few cells in which an appropriate F′ factor was formed (by a rare "loop-out" of the sex factor and attached distal end of the chromosome) are able to transfer the same late marker early as a part of the F′ factor. By using a *rec*A recipient, the normal recombinant formation for early Hfr markers is eliminated, and therefore it is possible to select for the transfer of F′ factors which carry early Hfr markers instead of (or as well as) late ones.

The *rec*B recombination deficiency is quite different from that observed with *rec*A. Even though *rec*B zygotes form recombinants at a much lower frequency than *rec*+ zygotes do, those few recombinants are found to be formed with normal probability of crossing-over per unit map length. Since the transfer of the DNA from the Hfr appears to occur normally,[3] it is concluded that some step in recombinant production, which either allows or prevents the formation of a finished recombinant cell with a normal number of genetic exchanges, is defective in *rec*B cells.

The results presented above are due in large part to the provocative suggestions and kind hospitality of W. K. Maas.

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