Chromosome Transfer from F-lac\(^+\) Strains of 
*Escherichia coli* K-12 Mutant at 
recA, recB, or recC

BRIAN M. WILKINS
Radiobiology Laboratories, Yale University School of Medicine, New Haven, Connecticut 06510

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The frequency of chromosome transfer from various recombination-deficient F-lac\(^+\) donor strains was estimated by standardizing the yield of conjugants receiving a male chromosomal marker against the level of episome transfer in the mating mixture. The efficiency of chromosome transfer from newly formed F-lac\(^+\) cells carrying recB21 or recC22 was more than 50% of the wild-type value, although it was about 10 and 20%, respectively, if the male cell lines had become established. In contrast, recA13 donors transmitted the chromosome with less than 10\(^{-4}\) of the normal frequency. If chromosome transfer from F-lac\(^+\) strains reflects the cutting and subsequent joining of homologous single strands of episomal and chromosomal deoxyribonucleic acid by recombination, these results imply that the completed unions are not made in recA cells, but can be effected with more than 50% of normal efficiency in newly formed partial diploids mutant at either recB or recC. Thus, the defective stage in recA mutants may precede strand joining, whereas the deficiency in recB or recC cells may involve a later step in recombinant formation.

Mutations leading to recombination deficiency in *Escherichia coli* K-12 are currently known to map at three loci: recA, which lies between cysC and pheA (21), and recB and recC, which are both cotransducible with thyA (6; Willetts and Mount, *in preparation*). Recombination-deficient (Rec\(^-\)) recipient strains accept deoxyribonucleic acid (DNA) normally during mating but form abnormally few conjugants, or progeny inheriting characters of both parents (3), in crosses with Hfr bacteria (3, 4, 12, 13). Although such conjugants are generally partial diploids when derived from recA recipients, those formed in crosses with females mutant at either recB or recC are true haploid recombinants carrying an almost normal distribution of unselected male markers (14).

The nature of the defects in these three types of Rec\(^-\) mutants is not yet understood. However, the observation that abnormally few recombinant colonies are recovered in crosses with Rec\(^-\) female strains does not preclude the possibility that some of these mutants can still effect certain reactions in recombination involving the cutting and joining of DNA strands. Thus, it would be of interest to examine further the recombination properties of these mutants in a system which does not demand the formation of viable recombinant colonies. For this reason, the ability of various Rec\(^-\) F\(^+\) strains to transfer chromosomal markers during mating was investigated.

F\(^+\) donor strains transfer the sex factor very efficiently during mating but can also donate chromosomal DNA to recipient cells with moderate frequency (1). Scaife and Gross (19) postulated that this process of chromosome mobilization results from the joining of the F genote to the chromosome by a recombination event between regions of homology, so that the DNA of the sex factor becomes linked to the bacterial chromosome.

In the experiments reported in this paper, the frequency of chromosome mobilization by F-lac\(^+\) was measured in both established and newly formed F\(^+\) strains mutant at recA, recB, or recC. The results show that F-lac\(^+\) donor strains carrying recB or recC can mobilize the chromosome with efficiencies ranging from about 10\(^{-6}\) to more than 50% of that the wild type value, depending on the age of the male cell line. In contrast, the level of chromosome transfer from donors carrying recA is reduced to less than 10\(^{-4}\) of normal.

MATERIALS AND METHODS

**Bacteria.** The relevant strains of *E. coli* K-12 are described in Table 1. NH4104 was derived from
AB1115, an ancestor of AB1157, NH4011, NH4021, and NH4024 were derived as Thy+ transductants of NH4010 thyA, with the latter two strains carrying recC22 and recB21, respectively, as a cotransduced marker. NH4023 was selected as a Thy+ recombinant in a 40-min mating of NH4010 thyA with AB3113 Hfr313 recA13.

**Media and mating.** The media and procedure for mating were based on those of Adelberg and Burns (1). Bacteria were grown and mated in YET broth (0.5% Difco yeast extract, 1% Difco tryptone, 1% NaCl, pH adjusted to 7.0) supplemented with 0.1% glucose. YET agar contained YET broth solidified with 2% agar. Conjugants were selected on minimal medium 56 supplemented appropriately with 0.2% of either glucose or lactose, thiamine, and necessary amino acids. Interruption of mating by phage T6 was performed by mixing equal volumes of the mating mixture and a phage T6 suspension containing 2 × 10^6 particles/ml and incubating this for 10 min at 37 C. Interruption and contraselection of males was also achieved by violently agitating the mating mixture on a vortex mixer for 2 min in the presence of streptomycin and plating on media containing streptomycin (200 μg/ml).

**RESULTS**

Transfer of chromosomal markers from established F-lac+ donor cells. Chromosome mobilization by F-lac+ in Rec− donor strains was first examined with established donor cells which had descended through at least 50 generations since the original birth of the merodiploid strain. In these experiments, the donor cells were mated with the multiply auxotrophic recipient strain AB1157 for 80 min. The numbers of conjugants inheriting either the donor episomal lac+ or the male chromosomal proA+ or leu+ marker were then determined. These yields and the numbers of male cells and colony-forming units added to each mating mixture are shown in Table 2.

To compare the frequency of chromosome mobilization in different crosses, the yield of conjugants for a male chromosomal marker must be standardized against the number of genetic donors in the mating mixture. Possible standards are either the input number of male colony-forming units or some measure of the efficiency of DNA transfer between the mating cells, such as the yield of conjugants having an episomal marker. The results in Table 2 suggest that the latter measure more accurately describes the number of donors in a Rec− F+ culture. Whereas the numbers of input male cells and Lac+ SmR conjugants were approximately constant in all four experiments, the yield of Lac+ SmR conjugants per 10^8 input male colony-forming units was increased by up to fourfold if the males were Rec− rather than wild type. Assuming that the efficiency of an F+ cell to donate the sex factor during mating is not improved by a mutation at one of the rec loci, this increase implies that the number of colony-forming units in a Rec− F+ culture underestimate the number of genetic donors, and that a large fraction of the cells which fail to proliferate on YET agar can still donate the F genotype.

The frequency of chromosome mobilization is therefore shown in Table 2 as 100 times the ratio of the number of conjugants inheriting a male chromosomal marker (C) to the number of conjugants inheriting the episomal lac+ gene (E) of the donor; the units are percentages. A comparison of these data shows that, whereas the efficiency of mobilization by F-lac+ is reduced by more than 10^4 if the males carry recA, established recB21 and recC22 donors can mobilize the chromosome with about 10 and 19% of the wild type frequency, respectively.

Leu+ SmR cells were consistently produced with a lower frequency than Pro+ SmR conjugants in these crosses, presumably because chromosome
CHROMOSOME TRANSFER FROM Rec− F′ CELLS

Table 2. Transfer of the proA+ and leu+ chromosomal markers from established Rec+ and Rec− F-lac+ strains

<table>
<thead>
<tr>
<th>Genotype of F-lac+ donor</th>
<th>No./ml of mating mixture</th>
<th>No. of conjugates per ml</th>
<th>Chromosome mobilization frequency (%)</th>
<th>Mean efficiency index, Rec−/Rec+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male colony-forming units</td>
<td>Male cells</td>
<td>Lac+ SmR</td>
<td>Pro+ SmR</td>
</tr>
<tr>
<td>rec+ (NH4111)</td>
<td>2.8 × 10^7</td>
<td>3.0 × 10^7</td>
<td>6.3 × 10^7</td>
<td>2.5 × 10^4</td>
</tr>
<tr>
<td>recA13 (NH4123)</td>
<td>1.2 × 10^7</td>
<td>2.8 × 10^7</td>
<td>6.9 × 10^7</td>
<td>1.5 × 10^4</td>
</tr>
<tr>
<td>recB21 (NH4124)</td>
<td>5.5 × 10^4</td>
<td>2.8 × 10^7</td>
<td>4.9 × 10^7</td>
<td>1.9 × 10^4</td>
</tr>
<tr>
<td>recC22 (NH4121)</td>
<td>1.1 × 10^7</td>
<td>2.9 × 10^7</td>
<td>5.3 × 10^7</td>
<td>4.0 × 10^4</td>
</tr>
</tbody>
</table>

* A 0.3-ml amount of each F-lac+ donor was mixed with 2.7 ml of AB1157 and kept at 37 °C for 80 min without shaking. Mating was then interrupted and the numbers of Lac+ Met+ SmR, Pro+ Met+ SmR, and Leu+ Met+ SmR cells were assayed. The input numbers of male cells and colony-forming units were respectively determined by counting the cells in a Petroff-Hausser chamber and scoring the colonies formed on YET agar. Each number is the mean of the values obtained in two replicate experiments.

Transfer of the chromosome from newly formed F-lac+ donors. Chromosome mobilization was also measured in male cells containing the newly immigrant F-lac+ episome, to investigate the rate at which the newly formed males become donors and to determine whether the mobilization efficiency varies with the age of the male cell line.

The primary F-lac+ donor NH4104 was mated for 30 min with a recipient strain carrying rec+ (NH4011), recA13 (NH4023), recB21 (NH4024), or recC22 (NH4021), to allow transfer of the episome and the subsequent conversion of these intermediate cells into F-lac+ donors. NH4104 was then killed by the addition of phage T6. The mixture was next added to an exponentially growing culture of a second recipient strain, AB1157. At various times after the start of this second mating, the extent of genetic transfer from the intermediate strain was determined by assaying the numbers of Lac+ SmR, Pro+ SmR, and Leu+ SmR conjugants on appropriate selective media with streptomycin present to select against the newly formed donors. The results are shown in Fig. 1.

Pro+ SmR and Leu+ SmR conjugants can only be formed by transfer of chromosomal DNA...
FIG. 1. Kinetics of the formation of conjugants carrying either the episomal lac+ or chromosomal proA+ or leu+ marker in matings involving newly formed Rec+ and Rec- F-lac+ donors. At $t = -40$ min, 2 ml of NH4104 F-lac+ at $2.4 \times 10^8$/ml was mixed with 4 ml of one of the intermediate strains, and the kinetics of the formation of Lac+ T6R Pro+ Thr+ Leu+ His+ (△) conjugants was determined by interrupting mating in samples of the mixture by the addition of phage T6. At zero time, 1 ml of the T6-interrupted mixture extracted at $t = -10$ min was added to 9 ml of AB1157, and this second mating mixture was gently shaken at 37 C for the rest of the experiment. Mating in samples of this second mixture was interrupted by violent agitation, and the numbers of Lac+ Met+ SmR (□), Pro+ Met+ SmR (●), and Leu+ Met+ SmR (○) conjugants were assayed. The initial concentration of Lac+ T6R colony-forming units in the second mating mixture is indicated by an arrow. The broken curve (■) in Fig. 1A shows the kinetics of Lac+ Met+ SmR cell formation in a control experiment in which heat-killed NH4011 cells were used as intermediate cells. These conjugants were derived from the transfer of F-lac+ from NH4104 donors surviving phage T6 treatment. The intermediate strains were: (A) NH4011 rec+, (B) NH4023 recA13, (C) NH4024 recB21, and (D) NH4021 recC22.
from the intermediate strain to AB1157, because both NH4104 and AB1157, derived from a common ancestor, carry the same mutations at proA and leu and yield no conjugants of these types when mated together. However, a small fraction of the Lac⁺ SmR conjugants formed in the second mating with AB1157 were derived from the transfer of F-lac⁺ from cells of NH4104 which survived treatment with phage T6. The number of Lac⁺
SmR cells derived from this source, determined in a control experiment in which heat-killed NH4011 cells were used as intermediate cells, is indicated in Fig. 1A.

It can be seen in Fig. 1 that the three Rec⁻ intermediate strains accept F-lac⁺ almost normally from NH4104, as measured by the formation of Lac⁺ T6R cells in the initial matings. Although each of the initial mating mixtures contained approximately 1.6 x 10⁸ recipient cells/ml, about 60% of the cells of the Rec⁻ intermediate strains failed to form colonies on YET agar. This may explain the lower yields of Lac⁺ T6R cells in the crosses involving these strains. Moreover, there was no significant difference between the times at which the various intermediate strains become F-lac⁺ donors, as determined by the kinetics of the formation of Lac⁺ SmR cells.

The frequency of chromosome mobilization in the experiment with NH4023 recA13 was very low, the C/E index for pro⁺ and leu⁺ being 0.00021 and 0.00014%, respectively, after 140 min of mating. These values agree with those obtained in matings involving established F-lac⁺ donor cells carrying recA13 (Table 2).

In the experiments with NH4021 recC22 and NH4024 recB21, the ratio of Pro⁺ SmR or Leu⁺ SmR to Lac⁺ SmR cells dropped as the duration of the second mating increased; no equivalent decrease was detected in the control experiment involving the Rec⁺ intermediate strain. These effects were examined in greater detail by determining the C/E index for pro⁺ at various times after the start of the test mating of the newly synthesized F-lac⁺ donors with AB1157. The results, summarized in Table 4, confirm the trend observed in Fig. 1.

The principal difference between the data in Tables 3 and 4 is that, at the earlier sampling times (27.5 and 80 min), the C/E index for the newly formed F⁺Rec⁻ cells was higher than that observed when the corresponding established strains were used. Thus, the ratios of the Rec⁻ to Rec⁺ indices in Table 4 decreased progressively with the length of mating. After 27.5 min, the newly synthesized Rec⁻ males had donated the pro⁺ marker with more than 50% of the wild-type frequency, whereas after 140 min the C/E index for the recB and recC cells had dropped to about

<table>
<thead>
<tr>
<th>Time after start of mating with</th>
<th>Genotype of intermediate strain</th>
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<tbody>
<tr>
<td></td>
<td>rec⁺</td>
</tr>
<tr>
<td>27.5</td>
<td>6.3</td>
</tr>
<tr>
<td>50</td>
<td>3.5</td>
</tr>
<tr>
<td>80</td>
<td>3.9</td>
</tr>
<tr>
<td>110</td>
<td>3.7</td>
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<tr>
<td>140</td>
<td>3.4</td>
</tr>
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</table>

*Experimental procedures were as described in Fig. 1. Results are given as the C/E index of chromosome mobilization, determined by multiplying 100 times the ratio of the number of Pro⁺ SmR to Lac⁺ SmR conjugants formed per milliliter of mating mixture. Results given are the means of values obtained in four replicate experiments.*

16 and 25% of the control value, respectively. These results show that the efficiency of chromosome transfer from newly formed recB21 and recC22 F⁺ cells is greater than that from the equivalent established strains; presumably, the efficiency decreases as the newly formed F⁺ cells are allowed to multiply and the male cell lines thus become established.

**DISCUSSION**

Although F⁺ cells donate the F genote very efficiently during mating, structural exchanges can occur randomly within the partially diploid region of these cells so that the chromosome is transferred to the recipient behind the leading end of the F genote (16). These exchanges may reflect the linkage of the DNA of the chromosome and episome by recombination within homologous segments of the two structures (2, 19). This model is supported by the evidence that the frequency of chromosome transfer or mobilization is much reduced if the F⁺ cells either carry a mutation at recA (5), and are thus deficient in recombination, or have suffered a deletion of the chromosomal region corresponding to the F genote (17).

The above model for chromosome mobilization implies that, if a Rec⁻ F⁺ cell can donate chromosomal markers during mating, some form of recombination must have occurred in the male to link at least homologous single strands of episomal and chromosomal DNA. The results of experiments involving DNA transfer between mating cells pertain to the properties of the intact preexisting donor DNA which is transferred as a single strand to the recipient (11, 15, 18).

The experiments show that the frequency of chromosome transmission from F-lac⁺ cells is less
than 10^-4 of normal if the donors are mutant at recA. In contrast, the male chromosome is transferred from newly formed F-lac+ cells carrying recB21 or recC22 with more than 50% of the wild-type frequency. Presumably, the efficiency of chromosome transfer from these cells drops as the age of the male cell lines increases, because the corresponding established strains donate the chromosome with about 10 and 20% of the Rec+ frequency, respectively.

These frequencies of chromosome transfer are derived by standardizing the yield of conjugants for a chromosomal marker against the extent of F-lac+ transmission in the mating mixture. However, a large proportion of the established Rec- F-lac+ cells which are unable to proliferate can still donate the F-genote during mating (Table 2). If these nonviable bacteria fail to transfer chromosomal DNA as efficiently as the F genote, the above estimates of the frequency of chromosome mobilization in Rec- strains will tend to be as much as four times too low.

If it is assumed that the cutting and subsequent stable joining of homologous, single strands of DNA is a prerequisite for chromosome mobilization, the results presented above imply that recB and recC mutants can perform these steps, whereas recA cells fail either to initiate or to complete the necessary recombination. It is noteworthy that cells carrying recA13 can join DNA in other processes. This is evidenced by the polynucleotide ligase activity in extracts of these mutants (9, 10) and by their ability both to form deletion mutants with the normal frequency (7) and, when held in suitable media, to repair ultraviolet-induced photoproducts by the excision process (8). Thus, if recombination is a multistage process, the defective step in recA mutants may precede strand-joining, whereas the deficiency in recB and recC cells may involve some later stage in recombinant formation.

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LITERATURE CITED