Genetics of Host-Controlled Restriction and Modification of Deoxyribonucleic Acid in *Escherichia coli*

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ABSTRACT

LEDERBERG, SEYMOUR (Brown University, Providence, R.I.). Genetics of host-controlled restriction and modification of deoxyribonucleic acid in *Escherichia coli*. J. Bacteriol. 91:1029–1036. 1966.—The locus for the host specific restriction and modification of deoxyribonucleic acid in *Escherichia coli* has been mapped by matings between mutants for these characters in strains K-12, C600, and B. Linkage analysis and kinetics of marker transfer indicate that a single or closely linked multiple chromosomal site located about 4 min counterclockwise to leucine is responsible for these activities. Secondary factors which affect the quantitative level of restriction also were detected. Wild-type recombinants were isolated in crosses between rm^- (restriction or modification, or both) mutants. The expression in zygotes of the restrictionless character of a rm^- donor is masked by a separate, physiological impairment of restriction, which results from mating and is independent of the modification state of the donor. The relevance of the restriction character to mating incompatibilities in these and other bacterial strains is considered.

Bacteriophage grown in a given bacterial strain acquire a host specificity characteristic of that strain, with the result that they may either be rejected or accepted upon infection of a new host (23). For coliphages T1, λ, and ϵ, rejection of phage is associated with the loss and degradation of the infecting phage deoxyribonucleic acid (DNA; 3, 12, 21, 22, 29). When a bacteriophage is accepted by a new host, a host specificity characteristic of that host is imparted to the progeny phage. Two host activities operate in this phenomenon: one is a restricting activity which prevents phage multiplication according to the host of origin of the phage; the other is a modifying activity which imparts a host specificity to phage. These activities also apply to the mating ability of one bacterial strain with another: the mating is relatively fertile or infertile according to the match of host restricting activity in the recipient strain with the host specificity of the genetic donor (5, 7, 8, 16, 17; Lederberg, *in press*).

The mating ability of restriction and/or modification mutants of strains K-12 and B of *Escherichia coli* parallels qualitatively the behavior of phage infections. If the recipient strain is impaired in its restricting activity toward phage λ, then the modifying ability of the donor strain is irrelevant to the mating efficiency. However, if the recipient strain is restrictive toward phage λ, then the donor strain needs the protective modifying ability for the mating to be fertile (Lederberg, *in press*).

The experiments reported here deal with the genetic properties of the loci for restriction and modification in some of these mutants.

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used: MNNG, 1-methyl-3-nitro-1-nitrosoguanidine; EMS, ethyl methane sulfonate; O, origin of transfer of the bacterial chromosome; B_1^+, arg^+, his^+, leu^+, met^+, pro^+, and thr^+, ability to synthesize thiamine, L-arginine, L-histidine, L-leucine, L-methionine, proline, or L-threonine, respectively; ara^+, gal^+, lac^+, mtl^+, and xyl^+, ability to use L-arabinose, D-galactose, lactose, D-mannitol, or D-xylose, respectively, as carbon source; *T6-r*, resistant to phage T6; *T1,5-r*, resistant to phages T1 and T5; *str-r*, resistant to streptomycin; *rm*, restriction and/or modification of DNA; rm_K, *rm* allele of K-12 strains; λ·host, λ with the host specificity of the given strain; λ·K indicates λ with K-12-type host specificity characteristic of strains K-12, AB259, P4X6, and C600, for example; λ·B indicates λ with B-type host specificity characteristic of

strain Bc251, and $\lambda \cdot C$ indicates λ with C-type host specificity characteristic of strains C and C600 rm_4.

Media. Broth was 1% trytone (Difco) with 0.5% NaCl. Tryptone-agar and soft tryptone-agar contained broth with 1% agar (Difco or Oxoid No. 3) and 0.6% agar (Difco), respectively. E M B Agar contained broth with 0.5% yeast extract (Difco), 0.04% Eosin Y, 0.0065% methylene blue, 0.2% K_2HPO_4, 1% sugar (lactose or L-arabinose), and 1.2% agar (Difco). PN medium contained 0.3% K_2HPO_4, 0.2% $(NH_4)_2SO_4$, 0.012% $MgSO_4$, and 0.03 to 0.05% carbon source (glucose, lactose, or L-arabinose). PN agar (PNA) was PN medium with 1% Noble agar (Difco). When necessary, PN medium or agar was supplemented with 0.004% of a given L-amino acid or 0.0005% thiamine hydrochloride. Streptomycin, when used, was present in concentrations of 0.01% (as streptomycin sulfate; E. R. Squibb & Sons, New York, N.Y.).

Bacteriophage. Variants of phage λ were employed: λ reference type (20); λ c_{26}, a clear plaque mutant (18); and λ h vir, a complex virulent host range mutant capable of plating on λ-lysogens. All variants of phage λ were obtained from M. Meselson. Routine assays of phage λ were made with cells grown to about 10^9 per milliliter in broth and resuspended or diluted into 0.01 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.3) plus 0.001 to 0.01 M $MgSO_4$. In other respects, the general methods used for handling phage were those summarized by Adams (1).

Bacteria. The strains of *E. coli* employed are described in Table 1. Strains AB259, P4X6, and AB1122 were obtained from S. E. Luria. Strains C600, C600 rm_4, C600 rm_5, Bc251, and C were obtained from M. Meselson.

Isolation of bacterial mutants resistant to phage or to streptomycin. Bacterial mutants resistant to phage were isolated by spreading onto tryptone-agar plates 10^7 to 10^9 cells from stationary-phase cultures together with about 10^8 phage T6 or phages T1 plus T5. Resistant colonies which appeared by 15 hr of incubation at 37 C were serially streaked three times on tryptone-agar to obtain pure clones. Bacterial mutants resistant to streptomycin were isolated by spreading about 10^{10} cells from stationary-phase cultures onto tryptone-agar with streptomycin. Resistant colonies which appeared by 60 hr of incubation at 37 C were serially streaked on tryptone-agar with streptomycin to obtain pure clones. Mutants were used whose phage and streptomycin resistance did not affect the host-specific characters of restriction and modification.

Isolation of restriction-modification mutants. Bacteria grown in broth to about 3×10^8 per milliliter were resuspended in 0.1 volume of 0.2 M potassium acetate at *p*H 5.0. The mutagen MNNG (26) was added to a final concentration of 50 to 100 mg per liter. The treated cells were maintained at 37 C for 30 to 120 min, then washed, diluted 1:40 into tryptone broth, incubated overnight to segregate mutants, and plated on tryptone-agar. Isolated colonies which formed were transferred to broth and grown to about 10^9 cells per milliliter. About 10^7 cells were deposited as spots on tryptone-agar plates which had been previously seeded with about 10^6 phage λ c_{26}. Cells which

restrict λ grown on a given host (for example, cells of strain C600 which restrict λ grown on strain C or on strain Bc251) produce from zero to three plaques per spot. Cells whose restriction is impaired produce proportionately more plaques or lyse the area. In this manner, mutants with varying degrees of impairment of restriction for phage λ were obtained. A number of these mutants also were impaired in their ability to modify λ. The phage restriction and modification properties of these mutants and their effect on bacterial recombination efficiency were reported in a previous paper (Lederberg, *in press*). For the present study on the mapping of the genetic determinants for host-specific restriction and modification, mutants were chosen from among those which had lost only their ability to restrict, and from those which had lost both the ability to restrict and to modify. In addition, two host-specificity mutants of strain C600 (C600 rm_4 and C600 rm_5) were studied. These mutants were derived by M. Meselson from strain C600 treated with EMS by selection for the loss of the ability to impart K-12 host modification specificity to phage λ. Both of these mutants lack the ability to restrict and to modify phage λ.

Isolation of nutritional mutants. Subcultures of cells treated with MNNG as above were plated on E M B Agar supplemented with lactose or L-arabinose. Presumptive nonfermenting mutants were further tested on minimal PNA supplemented with different sugars and serially restreaked to obtain pure clones. Mutants requiring amino acids for growth were detected by spotting broth cultures of mutagen-treated cells on plates of PNA, with and without amino acid supplements. About 10% of the clones examined had some nutritional defect, and about 0.5% were impaired in their host-specificity properties. Most of the latter still had the nutritional properties of their parent strains.

Bacterial matings. Matings for linkage analysis were carried out according to the method of de Haan and Gross (11). Matings were interrupted after 40 min, and recombinants for desired markers were selected on the appropriate minimal medium. Counterselection with T6 or streptomycin was used, depending on the characteristics of the parent strains. Purified recombinants were scored for nonselected markers on supplemented medium or by infection by bacteriophage.

In experiments on the kinetics of transfer of donor markers for restriction and modification, it was necessary to modify the usual mating procedure to permit nuclear segregation and phenotypic expression for these characters, and to obtain populations sufficiently dense for convenient infection by phage λ. About 10^9 donor cells and 10^9 recipient cells were mixed and immediately filtered onto a Millipore membrane (27). The cells on the membrane were incubated on a tryptone-agar plate for 2 to 6 min, then resuspended in broth, and diluted to a concentration of about 5×10^5 recipient cells per milliliter. Samples were withdrawn from this suspension at intervals and shaken vigorously to interrupt the mating. Residual donor cells were selected against the T6 or streptomycin, or both. The samples were then incubated until densities of 10^9 to 2×10^9 cells per milliliter were reached. All

TABLE 1. *Characteristics of bacterial strains employed*

Type[*]	Strain	Characteristic
1	AB259	Hfr Hayes: $B_1^- \lambda^-$; order of gene transfer: O thr^+ ara^+ leu^+ $T1$, 5-s lac^+ str-r (15)
	P4X6	Hfr: $met^- \lambda^+$; order of gene transfer: O pro^+ $T1$, 5-s leu^+ ara^+ thr^+ met^- str-r; from collection of F. Jacob
	P4X6 $T1$, 5-r	Spontaneous mutant of P4X6 selected with phages T1 and T5
	AB1122	F^-: pro^- arg^- his^- B_1^- lac^- gal^- xyl^- mtl^-; from collection of E. A. Adelberg
	AB1122 $T1$, 5-r $T6$-r	Derived from AB1122 by two separate spontaneous mutations by sequential exposure to phages T1 plus T5 and to phage T6
	C600	F^-: thr^- leu^- B_1^- lac^- (2)
	C600 $T6$-r	Spontaneous mutant of C600 selected with T6
2	Bc251	F^-: mal^+ λ-s prototroph derived by Arber and Lataste-Dorolle (4) from Bc, a strain of B cured of the prophage X (9)
	Bc251 $T6$-r	Spontaneous mutant of Bc251 selected with T6
	Bc251 $T6$-r str-r	Spontaneous mutant of Bc251 $T6$-r selected with streptomycin
	Bc251 $T6$-r leu^-	Isolated from Bc251 $T6$-r after mutagenesis with MNNG
3	C	F^-: prototroph (6)
	C600 rm_4	Isolated from C600 after mutagenesis with EMS (28)
	C600 rm_5	Isolated from C600 after mutagenesis with EMS (Meselson, *personal communication*).
	C600 rm_4 $T6$-r	Spontaneous mutant of C600 rm_4 selected with T6
	C600 rm_5 $T6$-r	Spontaneous mutant of C600 rm_5 selected with T6
	C600 rm_{23} $T6$-r	Isolated from C600 $T6$-r after mutagenesis with MNNG
	Bc251 $T6$-r str-r rm_{111}	Isolated from Bc251 $T6$-r str-r after mutagenesis with MNNG
	Bc251 $T6$-r str-r rm_{111} lac^-	Isolated from Bc251 $T6$-r str-r rm_{111} after mutagenesis with MNNG
	Bc251 $T6$-r str-r rm_{111} ara^-	Isolated from Bc251 $T6$-r str-r rm_{111} after mutagenesis with MNNG
	Bc251 $T6$-r str-r rm_{111} ara^- (λ)	Isolated from Bc251 $T6$-r str-r rm_{111} ara^- after infection with phage λ
4	AB259 rm_{11}	Isolated from AB259 after mutagenesis with MNNG
	AB259 rm_{11} $T1$, 5-r	Spontaneous mutant of AB259 rm_{11} selected with phages T1 and T5
	Bc251 $T6$-r leu^- rm_{124}	Isolated from Bc251 $T6^r$ leu^- after mutagenesis with MNNG

* Type 1: strains with the host specificity of *Escherichia coli* K-12 (these restrict $\lambda \cdot$B and $\lambda \cdot$C, and impart the host specificity of K-12 to λ). Type 2: strains with the host specificity of *E. coli* B (these restrict $\lambda \cdot$C and $\lambda \cdot$K, and impart the host specificity of B to λ). Type 3: strains with the host specificity of *E. coli* C (these neither restrict nor modify λ). Type 4: Strains which cannot restrict phage λ, but which still impart the host specificity of their parent strain.

of the above operations were carried out at 37 C. The *rm* character was assayed by resuspending cells from portions of the resulting mixed recombinant cultures in 0.01 M Tris buffer (pH 7.3) plus 0.001 to 0.01 M $MgSO_4$, and infecting them with λ c_{26} or λ h vir of C-type host specificity. Alternately, for examination of the modification character, a lysogenic recipient was employed in the mating. The resulting recombinant lysogenic culture was induced to lyse by ultra-

violet light, and the phage produced was assayed on strains of type K-12 and type C host specificity. The total number of cells infected or induced, multiplied by the efficiency of their infection by $\lambda \cdot$C or multiplied by the proportion of $\lambda \cdot$K in their lysates, was taken as the number of recombinant progeny having the appropriate *rm* genotype. Unmated cells of both parents provided reference values for restriction and modification efficiencies. The counterselection agents,

phage T6 or streptomycin, suppressed the residual donor population sufficiently to permit detection of several thousand-fold increases in the proportion of cells bearing nutritional or phage-resistance markers. A low background frequency for *rm* and nutritional and phage-resistance markers, and their abrupt in-increase during mating (corresponding to known entry times for the latter types), support the validity of this method. Selective growth pressures and random mating during the growth of the recombinant mixture were either absent or did not interfere.

RESULTS

Linkage relationships for restriction and modification loci. The inheritance of host-controlled restriction and modification was examined in recombinants selected for nutritional markers in matings. Crosses between Hfr K-12 strains and mutants of C600 or Bc251 revealed that donor markers for restriction and modification were unlinked to the locus for lactose fermentation, but were linked to the threonine-arabinose-leucine sector (Table 2). These results implicated a chromosomal site near the latter region. When the lactose marker distal to this region was selected, progressively greater linkage was found for donor alleles for *rm*, *thr*, *leu*, and *T1*, *5-r* in that order (Table 3). Therefore, the *rm*

site is bounded by the Hfr Hayes origin and the locus for threonine synthesis.

A secondary factor which improves the morphology of λ plaques and increases the efficiency of infection of rm_K cells by $\lambda \cdot C$ had a high frequency of joint inheritance with donor *T6-s* in *ara*+ recombinants selected from crosses between AB259 and Bc251 *T6-r str-r rm₁₁₁ara⁻*. About 55% of the recombinants with donor *rm* show this secondary effect. Of these, over 90% are also *T6-s*. When the secondary effect was not inherited with donor *rm*, *T6-s* was inherited in only 10% of the cases. The efficiency of infection by $\lambda \cdot K$ was normal for all recombinants. Therefore, either this second factor also contributes to host-specific control of DNA multiplication, or else this factor affects phage multiplication non-specifically, but is manifest only in those cases where restriction has reduced the probability of successful infection.

In purified *leu*+ recombinants from crosses between P4X6 and C600 *rm₄* or C600 *rm₅*, the modification specificity was either of the K type or the C type as in the parent strains. However, these recombinants exhibited either the restrictionless character of the *rm⁻* recipient, or a restrictionless character considerably less com-

TABLE 2. *Linkage between unselected donor rm and selected donor markers**

Cross		Frequency of unselected donor *rm* when selected donor marker is		
Donor	Recipient	*thr*+ and *leu*+	*ara*+	*lac*+
AB259	Bc251 *T6-r str-r rm₁₁₁ ara⁻*	—	84	—
AB259	Bc251 *T6-r str-r rm₁₁₁ lac⁻*	—	—	52
AB259	C600 *T6-r rm₂₃*	77	—	60
AB259 *rm₁₁*	C600 *T6-r rm₂₃*	74	—	65
AB259 *rm₁₁*	C600 *T6-r*	88	—	—

* Selection for recombinants for *thr*+*-leu*+, *ara*+, or *lac*+ was made in standard 40-min interrupted matings. Counterselection was by streptomycin for matings with derivatives of Bc251 *str-r*, and by T6 for matings with C600 *T6-r* derivatives. In each cross, 85 to 130 recombinants were studied. The frequency of unselected donor *rm* is given as the per cent of the recombinants for selected markers.

TABLE 3. *Linkage between unselected donor markers and selected donor lac⁺ marker**

Cross		Frequency of donor *lac*+ recombinants which have donor marker			
Donor	Recipient	*rm*	*thr*+	*leu*+	*T1*, *5-s*
AB259	C600 *T6-r rm₂₃*	60	75	83	84
AB259 *rm₁₁*	C600 *T6-r rm₂₃*	65	76	80	80

* Selection for *lac*+ was made in standard 40-min interrupted matings. Counterselection was carried out with phage T6, and 120 purified *lac*+ recombinants were then scored for nonselected markers. The frequency of unselected markers is given as the per cent of recombinants for donor *lac*+.

plete than that possessed by the K-12 donor parent (an efficiency of infection by $\lambda \cdot C$ of about 3×10^{-2} instead of 10^{-4}). Again, secondary factors appear to participate in the quantitative expression of restriction.

Location of the rm locus by kinetics of transfer. To learn more precisely the chromosomal location for the *rm* factor, the kinetics of transfer of donor markers was studied. In crosses between Hfr Hayes AB259 $rm_{11} \times$ C600 *T6-r*, rm_K, the rm_{11} gene which modifies but does not restrict, entered the recipient about 4 min before *leu*+ (Fig. 1). In crosses with the same donor and C600 *T6-r*, rm_5, the donor character for modification, likewise appeared about 4 min before *leu*+ (Fig. 2). Crosses with mutants of B as recipient gave similar results for the time of entry of the K-12 allele for the B modification site (Fig. 3). Crosses with donor P4X6, which transfers markers in a direction opposite to that of Hfr Hayes strains, confirmed the indicated location

FIG. 2. *Transfer of donor markers in cross Hfr AB259 rm₁₁ × F⁻ C600 rm₅ T6-r. The rm₁₁ allele in the donor imparts K modification, but does not restrict. The rm₅ allele in the recipient neither modifies nor restricts. The appearance of recombinants imparting K modification was determined by assays of lysates prepared on the progeny of the mated population.*

for *rm* (Fig. 4). Therefore, the chromosomal locations for the loci for host-controlled restriction and modification of K-12 and B specificities are coincident within 1 min of transfer by this technique, and lie about 4 min counterclockwise to *leu* on the chromosome map for K-12. This location is in agreement with the linkage relations described earlier, although negative interference makes it difficult to equate linkage values with distance (25).

Recombination within the rm region. The appearance of dual defects in restriction and modification in several of the *rm* mutants studied here may be ascribed to changes in a single genetic site common to both activities, or to polarity mutations in an operon composed of

FIG. 1. *Transfer of donor markers in cross Hfr AB259 rm₁₁ × F⁻ C600 T6-r. The rm₁₁ allele transferred imparts K modifications, but does not restrict. The rm_K allele in the recipient imparts K modification and restricts λ·C and λ·B. The appearance of recombinants with a restrictionless phenotype was measured by infection of the progeny of the mated population with λ c₂₆ of type C host specificity.*
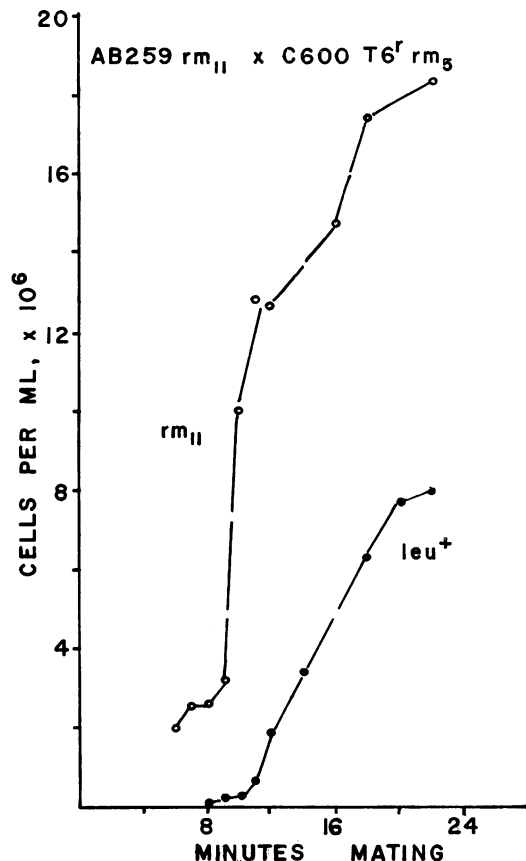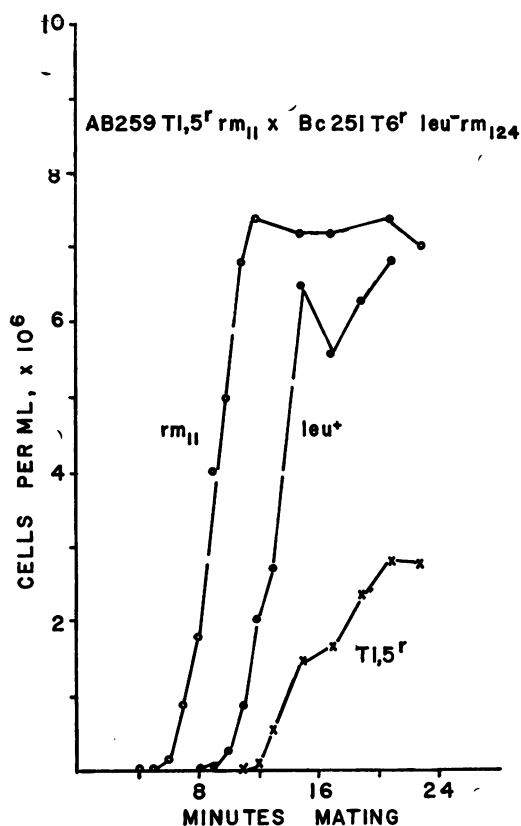
FIG. 3. Transfer of donor markers in cross Hfr AB259 rm_{11} × F⁻ Bc251 T6-r leu⁻ rm_{124}. The rm_{11} allele in the donor imparts K modification, but does not restrict. The rm_{124} allele in the recipient imparts B modification, but does not restrict. The appearance of recombinants imparting K modification was determined by assays of λ lysates prepared on the progeny of the mated population.

FIG. 4. Transfer of donor markers in cross Kfr P4X6 × F⁻ Bc251 T6-r str-r rm_{111} ara⁻ (λ). The rm_K allele in the donor imparts K-12 host specificity and restricts λ·C and λ·B. The rm_{111} allele in the recipient neither restricts nor modifies. The appearance of recombinants imparting K modification was determined by assays of λ lysates prepared from the progeny of mated populations by induction with ultraviolet light.

cistrons for restriction and modification, or to multiple mutations in linked, but uncoordinated, cistrons for restriction and modification. In crosses between rm mutants, both of which lacked restriction and modification ability, 1 stable, restricting and modifying recombinant was found among 217 purified recombinants for leu⁺. In crosses between other types of rm mutants, 1 stable rm_K recombinant was recovered among 1,047 recombinants for nearby nutritional markers. These rm_K recombinants each possessed other genetic markers from both parents. The nature and frequency of recombination did not permit an assignment of a single or dual cistron structure to the rm factor.

Expression of the restriction character. To determine the relative dominance of the rm alleles, a study was made of the rate of develop-
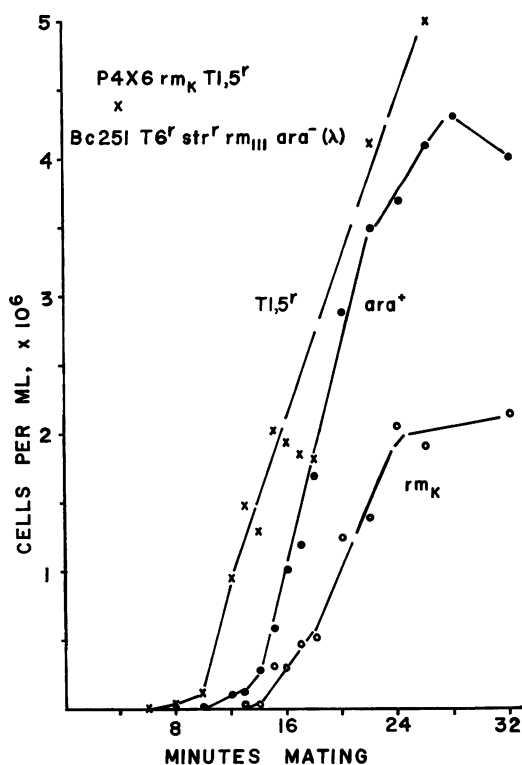
ment in zygotes of the phenotype of the transferred rm marker as compared with the rate of zygote growth and cell division. In crosses with AB259 rm_{11} × AB1122 T6-r, rm_K, the F⁻ and zygote population remaining after elimination of the donor cells with T6 promptly exhibited an impairment of restriction many times higher than that ascribable to transfer of the restrictionless rm_{11} gene. This physiological impairment was absent in purified rm_K recombinant clones, but sufficed to mask the early kinetics of expression of restrictionless genotypes. A similar breakdown of restriction during mating between K-12 and B has been reported (14). In the present case, the modification property of the donor cell makes it unlikely that the DNA of the donor cell competes with phage DNA as susceptible substrate for the restricting system. Reconstruction experiments with T6 lysates of donor cells of different rm genotypes produced only slight variations in the

restriction efficiency. Hence, the physiological impairment is due to some other factor attendant upon mating.

DISCUSSION

The present experiments indicate that a chromosomal site just counterclockwise to the *thr* locus is responsible for the primary restriction and modification of DNA in *E. coli*. This location agrees with the region responsible for restriction and modification of phage λ and for hybridization efficiency between parental strains K-12 and B (8, 17), and presumably represents the same site by virtue of the fertility of the restrictionless mutants.

The restrictionless mutants of strain B studied here were selected by their acceptance of phage λ. The coli-dysentery phage P2·*Shigella dysenteriae* which is restricted in parental strain B (6) can multiply in these restrictionless mutants (Lederberg, *unpublished data*). The selection of fertile mutants of strains whose incompatibilities are due to host-controlled restriction is, therefore, not limited to those strains which can adsorb phage λ.

One or more secondary factors affecting restriction but not modification were revealed in recombinants from crosses between restricting Hfr donor strains AB259 or P4X6 and restrictionless recipient mutants of C600 and Bc251. The secondary factor has a high frequency of joint inheritance with *T6-s* in the case of crosses with AB259 and with *ara* in the case of crosses with P4X6. These secondary effects cannot be attributed to the *X*-prophage which also exerts a restriction on λ (17) because the *X*-prophage lies in a distant part of the chromosome between *424*-prophage and *his* (10), which should require about 45 and 55 min for transfer by the Hfr strains AB259 and P4X6, respectively. Therefore, the *X*-prophage should be unlinked to the earlier markers *T6-s* or *ara* in the respective crosses.

In crosses between *E. coli* Hfr Hayes or Cavalli strains (*lac⁺*, *met⁻*) and a recipient strain of *Salmonella typhimurium* (*lac⁻*, *met⁺*), a locus which modifies *Salmonella* phage P22 was found (30). This locus (Mp) "seems to lie between T6 and *lac*, as some of the few progeny that did not obtain T6^coli did not obtain Mp^coli" when selection was made for donor *lac⁺* and recipient *met⁺* markers. A further analysis is needed to determine whether the loci for host specificity for phages λ and P22 share any genetic or physiological elements.

It is possible that restriction systems are operative in other mating incompatibilities between bacterial strains of different species. The in-

fertility between donor strains of *E. coli* and recipient cells of *S. typhimurium*, which can be overcome by mutation in the recipient strain (13), may be such an example. If the findings reported in the present paper extend directly to the *coli-typhimurium* system, then some *coli-typhimurium* fertility mutations should be located at the *rm* site for strains K-12, B, and C near *thr*, or at the P22 host specificity site near *T6-r*, or at both locations, and should affect transfer of phage and bacterial DNA.

In matings between *E. coli* and *S. typhosa*, hybrids containing *coli* genes proximal to the entry of chromosomes from Hfr Hayes and Hfr Cavalli strains are usually more fertile than the parent recipient when backcrossed with these *coli* donors (19). These authors proposed that the enhanced fertility was due to an artificially created chromosomal homology. It is possible that, in fact, the *coli-typhosa* matings also are regulated by phage restriction factors similar to those operative in the *coli* K-12 *coli* B matings. This alternative explanation takes into account the fact that the genetic sites made homologous in the *coli-typhosa* matings included the Hayes *origin-ara* and the Cavalli *origin–T6-r–lac* regions. In the *coli* system, these restriction factors are more complex than the genetic homology of genes at the chromosomal transfer origin *per se*, since crosses with P4X6 × Bc251 *rm₁₂₄* (see Fig. 3) locate the donor primary *rm* gene several minutes after the entry of other markers. Furthermore, the *coli rm* site affects the multiplication of certain phages (e.g., virulent and temperate forms of P2 and λ), as well as the mating efficiency of diverse *coli* strains. Therefore, the *rm* mutations appear to relate to the ability of host enzymes to deal with phage and bacterial DNA.

In a previous paper (Lederberg, *in press*), models were proposed for host restriction and modification. Modification was visualized as the protection conferred to DNA by a site-specific restriction-blocking activity. Alternatively, removal of a degradation-sensitizing activity could be involved. The behavior of phage λ indicated that K-12-type strains would possess the former protecting activity or lack the latter sensitizing activity, and vice versa for C type strains. An earlier report that zygotic induction of hybrids between *E. coli* Hfr Hayes (λ) and *Shigella flexneri* strains 2aA (a type C strain) produced phage half of which had K-12 specificity (24), suggests that a positive, protecting action is operative. This view is reinforced by preliminary observations that the *rm*_K modification character begins to be expressed by 20 min after its entry by mating into a modificationless recipient.

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