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GENETIC CONTROL OF RESTRICTION AND MODIFICATION IN *ESCHERICHIA COLI*[1]

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ABSTRACT

BOYER, HERBERT (Yale University, New Haven, Conn.). Genetic control of restriction and modification in *Escherichia coli*. J. Bacteriol. **88**:1652–1660. 1964.—Bacterial crosses with K-12 strains of *Escherichia coli* as Hfr donors (Hfr Hayes, Hfr Cavalli, and Hfr P4X-6) and B/r strains of *E. coli* as F⁻ recipients were found to differ from crosses between K-12 Hfr donors and K-12 F⁻ recipients in two ways: (i) recombinants (*leu*, *pro*, *lac*, and *gal*) did not appear at discrete time intervals but did appear simultaneously 30 min after matings were initiated, and (ii) the linkage of unselected markers to selected markers was reduced. Integration of a genetic region linked to the threonine locus of K-12 into the B/r genome resulted in a hybrid which no longer gave anomalous results in conjugation experiments. A similar region of the B strain was introduced into the K-12 strain, which then behaved as a typical B F⁻ recipient. These observations are interpreted as the manifestation of host-controlled modification and restriction on the *E. coli* chromosome. This was verified by experiments on the restriction and modification of the bacteriophage lambda, F-*lac*, F-*gal*, and sex-factor, F_1. It was found that the genetic region that controlled the mating responses of the K-12 and B/r strains also controlled the modification and restriction properties of these two strains. The genes responsible for the restricting and modifying properties of the K-12 and B strains of *E. coli* were found to be allelic, linked to each other, and linked to the threonine locus.

This investigation was initiated when an attempt was made to extend the *Escherichia coli* K-12 conjugation system (for a recent review, see Clark and Adelberg, 1962) to *E. coli* B/r. The B/r strain was found to act as an F⁻ recipient for Hfr derivatives of K-12 as reported previously (deHaan, 1954; Maas and Maas, 1962), but the frequency of recombinant formation was measurably lower than in comparable crosses with a K-12 F⁻ recipient. Some of the recombinants from a K-12 Hfr × B/r F⁻ cross, when backcrossed to the parent Hfr, yielded frequencies of recombinants equal to those of comparable crosses where the recipient was a K-12 F⁻ strain.

Interrupted mating experiments were employed to investigate the differences between the K-12 F⁻, B/r F⁻, and hybrid F⁻ strains. These indicated that the chromosome of the male donor was being transferred at a normal rate in K-12 × B/r crosses, but no recombinants were observed until a rather large fragment of the male's genome was deposited in the recipient cell. Furthermore, the linkage of unselected markers to selected markers was much lower than in the controls.

These anomalies were not always found when the hybrid strains were backcrossed to the parental Hfr; rather, some responded as typical K-12 F⁻ strains. It was subsequently found that the Hfr parental strain could be any of three different types, and the resulting recombinant hybrids would mate normally when backcrossed with any of the Hfr strains. By introducing an F-*lac* merogenote into a B/r strain, the latter was made a genetic donor; it was then possible to introduce a part of the B/r genome into a K-12 F⁻ strain and select for recombinants (Maas and Maas, 1962). It was found that some of these hybrid recombinants, when backcrossed to K-12 Hfr strains, no longer responded as K-12 F⁻ strains, but rather as B/r F⁻ strains. The genetic region responsible for these two mating phenotypes was found to be allelic in K-12 and B/r and linked to the threonine locus.

These mating phenotypes are explained as the manifestation of host-controlled modification and restriction on the *E. coli* chromosome (Arber and Dussoix, 1962; Dussoix and Arber, 1962). This explanation was verified when it was found that the host-controlled modification and restriction of deoxyribonucleic acid (DNA) elements (the

[1] A preliminary report of this paper was presented at the 64th Annual Meeting of the American Society for Microbiology in Washington, D.C.

bacteriophage lambda, F-*lac*, F-*gal*, and F_1) by these hybrid strains were exactly as expected if the genetic region controlling the mating phenotype also controlled the modification and restriction properties of these strains.

MATERIALS AND METHODS

Organisms. The strains of bacteria used in this investigation are described in Table 1. Wild-type bacteriophage lambda was obtained from a ultraviolet-treated lysogenic culture. The male specific bacteriophage was MS. 2.

Media and culture methods. The media and culture methods were those described by Adelberg and Burns (1960).

Mating conditions. The conditions under which recombination experiments were done were the same as those described by Adelberg and Burns (1960), with the exceptions described later. In all crosses the male strains were contra-selected by streptomycin. The bacteriophage techniques were the same as those described by Arber and Dussoix (1962).

F^+ phenotype. The test employed for assaying the presence of F^+ consisted of plating a suspension of the strain to be tested with 200 to 300 particles of a male specific bacteriophage, MS. 2.

RESULTS

Efficiency of recombinant formation. A *thr*$^-$ *ara*$^-$ *leu*$^-$ *sm*^{r} derivative of *E. coli* B/r was used as an F^- recipient in matings with Hfr Hayes and Hfr P4X-6 male donors of *E. coli* K-12. Selection was made for *leu*$^+$ recombinants after 40 min of

TABLE 1. *List of bacterial strains*[a]

Strain no.	Origin and reference	Prototrophic characters				Carbohydrate utilization			Streptomycin	Sex	Other markers	Remarks[b]
		thi	*thr*	*leu*	*pro*	*ara*	*lac*	*gal*				
AC2511	*E. coli* B/r	+	+	+	+	+	+	+	s	B/r F$^-$		
AC2512	AC2511[c]	+	−	−	+	−	+	+	r	B/r F$^-$		
AC2513	AC2512	+	−	−	−	−	−	−	r	B/r F$^-$		1
AC2514		+	+	−	−	−	−	−	r	K-12 F$^-$		2
AB2601		−	+	−	−	−	−	−	r	B/r F$^-$		3
AC2515	*S. typhosa*[d]	+	+	+	+	+	−	+	r	F-*lac*$^+$	*cys*$^-$ *try*$^-$	4
AC2516	AC2517	+	+	+	+	+	−	+	s	F-*lac*$^+$		5
AC2517	AC2511	+	+	+	+	+	−	+	s	B/r F$^-$		
AB2604[e]		+	+	+	+	+	+	−	s	F-*gal*$^+$		
AB259		−	+	+	+	+	+	+	s	Hfr Hayes		
AB261		−	+	+	+	+	+	+	s	Hfr P4X-6	*met*$^-$	
AB257		+	+	+	+	+	+	+	s	Hfr Cavalli		
AC2518		+	−	−	+	13	+	+	r	B/r F$^-$	*arg*$^-$ *pil*$^-$	
AB2602		−	+	+	+	A15	+	+	s	Hfr P4X-6	*met*$^-$ *pil*$^+$	
AB2603		−	+	+	+	H9	+	+	s	Hfr P4X-6	*met*$^-$ *pil*$^+$	
AB266		−	−	−	−	−	−	−	r	K-12 F$^-$		
AB264		−	+	+	+	+	+	+	s	F$^+$		

[a] Strain numbers prefixed with AB are K-12 strains; strain numbers prefixed with AC are non-K-12 strains. All AC numbers listed here, with the exception of AC2515, are B/r strains of *E. coli*. The F^- strains are designated as B/r or K-12 F^- sexual types on the basis of their mating response with Hfr derivatives of strain K-12; thus a K-12–(B/r) hybrid may be classified as a B/r F^-, etc. The following abbreviations are used in the table and the text; *thi*, thiamine; *thr*, threonine; *leu*, leucine; *pro*, proline; *cys*, cysteine; *try*, tryptophan; *met*, methionine; *arg*, arginine; *pil*, pilli; *ara*, arabinose; *lac*, lactose; *gal*, galactose; *sm*, streptomycin; r, resistant; s, sensitive. Numbers refer to allele numbers.

[b] Remarks: (1) Additional markers of the B/r strain were induced with 2-aminopurine. (2) Derived from crosses between AB259 and AC2513 in which selection was made for *thr*$^+$. (3) Derived from crosses between AC2516 and AB266 in which selection was made for *thr*$^+$. (4) This F-*lac* element originated from *E. coli* K-12. (5) F-*lac* introduced to AC2517 from AC2515.

[c] Gross and Englesberg, 1959.

[d] Johnson et al., 1964.

[e] Echols, 1962.

TABLE 2. *Efficiency of recombinant formation**

Cross	Hfr	F⁻	*leu*⁺/input male
			%
1	P4X-6	K-12	7.0
2	P4X-6	B/r	0.5
3	P4X-6	*thr*⁺ *ara*⁻ *leu*⁻ #1	0.7
4	P4X-6	#2	6.8
5	P4X-6	#3	6.7
6	P4X-6	#4	5.6
7	P4X-6	#5	0.7
8	P4X-6	#6	5.6
9	P4X-6	#7	6.4
10	P4X-6	#8	9.0
11	P4X-6	#9	0.6
12	P4X-6	#10	0.6
13	Hfr Hayes	K-12	31
14	Hfr Hayes	B/r	2
15	Hfr Hayes	*thr*⁺ *ara*⁻ *leu*⁻ #1	26
16	Hfr Hayes	#2	2
17	Hfr Hayes	#3	35
18	Hfr Hayes	#4	4
19	Hfr Hayes	#5	2
20	Hfr Hayes	#6	4
21	Hfr Hayes	#7	27
22	Hfr Hayes	#8	34
23	Hfr Hayes	#9	29
24	Hfr Hayes	#10	33

* All crosses were carried out in an enriched nutrient medium for 40 min without interruption; dilutions were made in buffer and samples were plated on selective media. The Hfr P4X-6 donor was AB261; the K-12 F⁻ recipient was AB266; and the B/r F⁻ recipient was AC2512. Additional samples were taken from crosses 2 and 14 and selection was made for *thr*⁺. Ten of these recombinants from each cross that were also *ara*⁻ *leu*⁻ were purified and used as F⁻ recipients in crosses 3 through 12 and crosses 15 through 24, respectively.

uninterrupted mating. The results of these crosses were compared with similar crosses where the F⁻ recipient was a *thr*⁻ *ara*⁻ *leu*⁻ *pro*⁻ *lac*⁻ *gal*⁻ B_1⁻ *sm*ʳ derivative of *E. coli* K-12. These results are summarized in Table 2 (crosses 1, 2, 13, and 14). The B/r strain acted as a competent recipient, but the recombinant frequencies were always measurably less than in comparable crosses with a K-12 F⁻ recipient.

About 60% of the *thr*⁺ *ara*⁻ *leu*⁻ recombinants from K-12 Hfr × B/r F⁻ crosses, when purified and backcrossed to the parental Hfr, were found to behave as typical K-12 F⁻ recipients with respect to the efficiency of recombinant formation; the other 40% behaved as typical B/r F⁻ recipients (Table 2, crosses 3 through 12 and crosses 15 through 24). These results eliminated the possibility that F⁻ mutations were being selected from a non-F⁻ population.

The threonine locus of K-12 was introduced into the B/r F⁻ strain by three donors, Hfr Hayes, Hfr Cavalli, and Hfr P4X-6. In all cases, about 60% of the *thr*⁺ *ara*⁻ *leu*⁻ recombinants responded as typical K-12 F⁻ recipients when backcrossed to any of these Hfr male donors. These results suggested that the mating behavior of the B/r and K-12 F⁻ strains was under the genetic control of a region near the *thr* locus, at least in a region between the leading points for transfer (origins) of Hfr Hayes and Hfr P4X-6.

Interrupted mating experiments. Interrupted mating experiments similar to those described by deHaan and Gross (1962) were employed to compare the kinetics of recombinant formation in K-12–B/r and K-12–K-12 zygotes. Two Hfr derivatives of *E. coli* K-12 (Hfr Hayes and Hfr Cavalli) were used as male donors in crosses in which the F⁻ recipient was either the B/r or K-12 strain of *E. coli*. The Hfr to F⁻ ratio was 1:20, and the cell density was sufficient to insure maximal cell contact. Specific pair formation was allowed to occur for 5 min, and the cell density was reduced by gentle dilution so that no new specific pairs were formed for at least an additional 50 min. Dilutions were made into enriched nutrient broth or minimal medium. Samples were removed at 5-min intervals, and conjugating cell pairs were disrupted by agitation with a Vortex mixer. Duplicates were then plated on appropriate selective media.

The results of interrupted mating experiments in K-12 × K-12 and K-12 × B/r crosses are presented in Fig. 1 and 2. It can be seen in both the Hfr Hayes × B/r F⁻ and Hfr Cavalli × B/r F⁻ crosses (Fig. 1B and 2B) that the appearance of recombinants is delayed until approximately one-third of the male's genome is deposited in the B/r F⁻ cell, all of the markers having a common time of entry. Since *gal*⁺ recombinants appeared after 30 min of mating in both the Hfr Hayes × K-12 F⁻ (Fig. 1A) and Hfr Hayes × B/r F⁻ crosses, it is clear that in both of these crosses the male's chromosome is being transferred at the same rate. It would appear, then, that the proc-

esses involved in bacterial conjugation of K-12 Hfr strains with K-12 and B/r F^- strains are identical prior to the recombination of genetic material.

When each class of selected recombinants from each of the crosses with the B/r F^- strain was scored for linkage of unselected markers, it was found that such linkage was reduced about one-half when compared with the K-12 Hfr × K-12 F^- crosses (Tables 3 and 4).

When a B/r–(K-12) F^- hybrid, which yielded recombinants at frequencies identical to a K-12 F^- strain, was used as a recipient in interrupted mating experiments, these anomalies were not present; rather, the results were identical to normal K-12 × K-12 crosses (Fig. 1C and 2C; Tables 3 and 4). [The recombinant hybrids from K-12 Hfr × B/r F^- crosses are referred to as B/r–(K-12) hybrids. The parenthetical notation indicates that only a small part of the K-12 genome is present in the hybrid. Likewise, recombinant hybrids from F-*lac* B/r × K-12 F^- crosses are referred to as K-12–(B/r) hybrids.]

Thus, the typical K-12 F^- mating response can be imposed upon the B/r strain by introduction of a region near the *thr* locus. The following experiments were performed to map this region more extensively. Two crosses were made with two strains of Hfr P4X-6 (these strains differed only by their *ara*$^-$ alleles) and a B/r F^- strain. The genotypes of these strains and the relative order of their mutant alleles are presented in Fig. 3. Selection was made for *ara*$^+$ recombinants, and then various hybrid recombinant classes were tested for the K-12 and B/r F^- mating response. The K-12 F^- mating response segregated with the *thr* locus rather than the *leu* locus (Table 5).

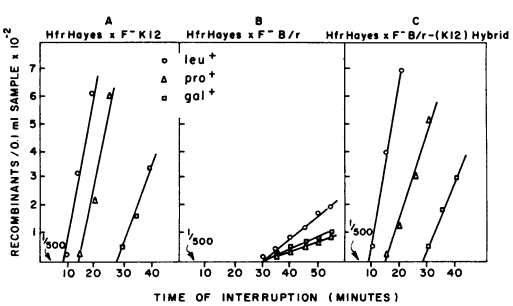

FIG. 1. *Interrupted mating experiments. In each of the crosses the male donor was Hfr Hayes (AB259). In A, the F^- recipient strain was AB266; in B, AC2513; in C, AC2514.*

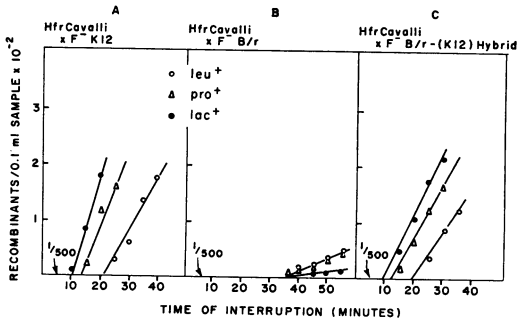

FIG. 2. *Interrupted mating experiments. In each of the crosses the male donor was Hfr Cavalli (AB257). In A, the F^- recipient was AB266; in B, AC2513; in C, AC2514.*

Since the *leu* locus segregates with the *thr* locus in these crosses with a frequency of about 90%, and the K-12 F^- mating response segregates with the *thr* locus about 60% of the time, it would appear that the K-12 F^- mating response must be controlled by a region between the *thr* locus and the origin of Hfr Hayes. The linkage of the *pil* locus to the *thr* locus in these crosses was about 10 to 20%, indicating that the K-12 F^- mating response is controlled by a region bounded by the *thr* and *pil* loci.

These experiments eliminated the possibility that the increased fecundity of the B/r–(K-12) strain was the result of an artificially constructed chromosomal homology in the region between the *thr* and *pil* loci (Johnson, Falkow, and Baron, 1964). If this explanation were correct, then one would not expect to have the B/r–(K-12) hybrid mate as a typical K-12 F^- strain in crosses with Hfr Cavalli. But the appearance of *lac*$^+$ recombinants during interrupted mating experiments with Hfr Cavalli and the B/r–(K-12) hybrid F^- was normal; therefore, recombination occurs without requiring the presence of the region between the *thr* and *pil* loci in the exogenote.

Introduction of the B/r mating response into K-12. The F-*lac* element conducts the B chromosome into K-12 (Maas and Maas, 1962); a mechanism for this type of transfer has been proposed by Scaife and Gross (1963) and elaborated upon by Pittard and Adelberg (1964). Chromosome transfer by an F-merogenote can be adequately explained on the basis of crossovers between the F-merogenote and the host chromosome, which means that only one point of origin will be observed in such crosses. The F-*lac* element was

TABLE 3. *Linkage analysis**

Unselected marker	Selected marker								
	leu+			*pro*+			*gal*+		
	K-12 F−	B/r F−	B/r−(K-12) F−	K-12 F−	B/r F−	B/r−(K-12) F−	K-12 F−	B/r F−	B/r−(K-12) F−
leu+ (%)	—	—	—	96	46	90	71	8	83
pro+ (%)	75	26	70	—	—	—	74	8	74
gal+ (%)	26	10	16	22	14	25	—	—	—

* Matings were carried out in enriched nutrient medium for 60 min, interrupted with a Vortex mixer, and appropriate dilutions were plated on selective media. The donor was Hfr Hayes (AB259) in each cross; the K-12 F− was AB266; the B/r F− was AC2513; the B/r-(K-12)F− was AC2514. One hundred colonies of each selected class were streaked onto homologous medium, incubated overnight at 37 C, and then replicated to appropriate media to score for unselected markers.

TABLE 4. *Linkage analysis**

Unselected marker	Selected marker								
	lac+			*pro*+			*leu*+		
	K-12 F−	B/r F−	B/r−(K-12) F−	K-12 F−	B/r F−	B/r−(K-12) F−	K-12 F−	B/r F−	B/r−(K-12) F−
lac+ (%)	—	—	—	85	40	87	60	26	54
pro+ (%)	80	30	93	—	—	—	75	28	69
leu+ (%)	55	22	84	43	22	61	—	—	—

* The F− strains are identical to those in Table 3. The male donor was Hfr Cavalli (AB257).

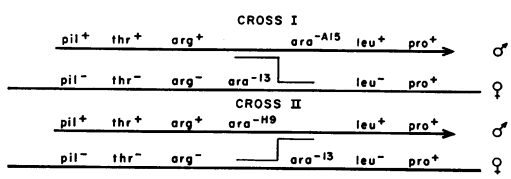

FIG. 3. *Order of mutant alleles. The F− recipient in crosses I and II was a B/r F− strain (AC2518). The male donor in cross I was AB2602 and in cross II, AB2603. These two strains differed only by the ara− and were derivatives of AB261.*

introduced into a *lac*− *sm*^{s} B/r F− strain (AC2517) by mating with a *Salmonella typhosa* 643 W strain (AC2515), which contained the *E. coli* F-*lac* element. The F-*lac*+/*lac*− B/r diploid strain (AC2516) was then used as a male donor in a cross with the K-12 F− strain (AB266), and selection was made for *thr*+ recombinants. About 50% of these recombinants exhibited the mating response of the B/r F− strain when mated to Hfr Hayes and Hfr Cavalli; i.e., they yielded low frequencies of recombinants per input male. Several of these K-12–(B/r) hybrids which responded as B/r F− strains were used as recipients in interrupted mating experiments with Hfr Hayes and Hfr Cavalli. These strains again exhibited the mating response of the B/r F− strain in that the appearance of recombinants was delayed until about one-third of the male's genome

TABLE 5. *Mapping experiment**

Cross	Recombinant class	No. tested	K-12 F−	Per cent
I	*ara*+ *thr*+ *arg*+	69	42	61
	ara+ *thr*− *arg*−	57	0	<2
II	*ara*+ *thr*− *arg*− *leu*−	10	0	<10
	ara+ *thr*− *arg*− *leu*+	10	0	<10

* A schematic representation of the strains used in crosses I and II is presented in Fig. 3. In each cross selection was made for *ara*+ recombinants. A number of these were then purified and separated into various recombinant classes. They were then backcrossed to Hfr Hayes (AB259) and scored as either K-12 F− or B/r F− on the basis of the efficiency of recombinant formation.

was deposited in the female cell (Fig. 4), and the linkage of unselected markers to selected markers was reduced (Tables 6 and 7). Thus, it appeared that the K-12 F^- and B/r F^- mating responses were under the genetic control of a region which could be transferred with the *thr* locus about 60% of the time.

Restriction and modification of DNA elements. These observations are compatible with the model proposed by Arber and Dussoix (1962) and Dussoix and Arber (1962) for the host-controlled modification and restriction of the bacteriophage lambda. They demonstrated that the DNA of the bacteriophage lambda, which was prepared on one strain of *E. coli*, was hydrolyzed when it infected another strain of *E. coli*. The host upon which the lysate was prepared apparently modified the DNA of the bacteriophage so that it was recognized as "foreign" DNA when it gained entrance to the other strain. These two mechanisms have been termed host-controlled modification and restriction, and these authors generalized that these phenomena would affect all DNA structures, not just the DNA of some bacteriophages.

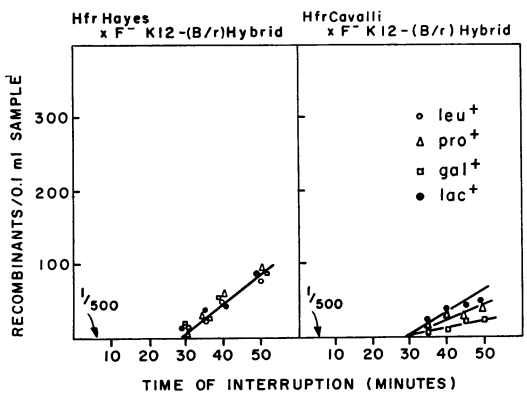

FIG. 4. *Interrupted mating experiments. In each cross the F^- recipient was a K-12-(B/r) hybrid F^- strain (AB2601). In A, the male donor was Hfr Hayes (AB259); in B, Hfr Cavalli (AB257).*

TABLE 7. *Linkage analysis**

Unselected marker	Selected marker					
	lac⁺		*pro*⁺		*leu*⁺	
	K-12 F⁻	K-12-(B/r)F⁻	K-12 F⁻	K-12-(B/r)F⁻	K-12 F⁻	K-12-(B/r)F⁻
lac⁺ (%)	—	—	43	17	60	20
pro⁺ (%)	80	38	—	—	75	32
leu⁺ (%)	55	12	85	24	—	—

* The male donor was Hfr Cavalli (AB257); the F^- strains are the same as in Table 6.

Little is known about the biochemical nature of these phenomena, but it is generally thought that the restrictive process must be the result of a nuclease capable of recognizing "foreign" DNA but not capable of recognizing the DNA of the cell in which it was synthesized. The recognition of DNA as "foreign" or not is thought to be dependent upon chemical modification(s) of DNA controlled by the cell. There is some evidence that one mechanism of host-controlled modification might reside in the glucosylation of DNA in certain bacteriophages, although there are probably numerous biochemical modifications imposing specificity in addition to this one (Symonds et al., 1963). However, it is known that DNA synthesis is not a prerequisite for host-controlled modification and that both strands of the DNA helix must be labeled to be recognized as "foreign" DNA (Arber and Dussoix, 1962). This rationale predicts that there are two cell products involved in these phenomena, namely, an enzyme capable of

TABLE 6. *Linkage analysis**

Unselected marker	Selected marker							
	leu⁺		*pro*⁺		*lac*⁺		*gal*⁺	
	K-12 F⁻	K-12-(B/r) F⁻	K-12 F⁻	K-12-(B/r) F⁻	K-12 F⁻	K-12-(B/r) F⁻	K-12 F⁻	K-12-(B/r) F⁻
leu⁺ (%)	—	—	91	35	91	32	86	35
pro⁺ (%)	66	23	—	—	93	60	81	37
lac⁺ (%)	50	21	81	45	—	—	64	21
gal⁺ (%)	26	9	34	10	59	38	—	—

* The male donor was Hfr Hayes (AB259); the K-12 F^- was AB266; the K-12-(B/r)F^- was AB2601.

recognizing certain DNA elements, and an enzyme(s) capable of modifying all DNA elements within the cell.

The anomalies of the sexual crosses between K-12 and B/r strains of *E. coli* can be explained as follows. The introduction of K-12 chromosome fragments smaller than approximately one-third of the total genome into the B/r cell results in its degradation before integration can occur. This degradation results from a specific nuclease in the B/r F^- cell, which recognizes the K-12 chromosome as "foreign" DNA. However, the introduction of larger fragments saturates the nuclease so that integration occurs before complete hydrolysis. Recombination of the K-12 exogenote and the B/r endogenote would "genetically rescue" the K-12 DNA from further degradation (Arber and Dussoix, 1962). [Since Arber and Dussoix (1962) have shown that modification occurs without DNA synthesis, one could argue that the exogenote could escape destruction if it is modified before being completely destroyed by the specific nuclease. Thus, the appearance of recombinants in restricted crosses can also be pictured as a race between hydrolysis of the exogenote and its modification by the recipient cell. Both mechanisms may contribute to the rescue of the exogenote.] This would account for the delayed appearance of recombinants until about one-third of the chromosome is deposited in the female cell, and consequently the reduced frequencies of recombinants. The appearance of recombinants in restricted crosses can be pictured as a race between hydrolysis of the exogenote and its integration into the recipient's chromosome. This explanation also provides an excellent account of the reduced linkage of unselected markers to selected markers; i.e., once a large piece of chromosome is deposited in the recipient cell, integration can occur; however, the probability that the nuclease has fragmented the exogenote is high, and the frequency with which markers are cotransferred would be reduced. Pittard (1964) has demonstrated that, in crosses between K-12 Hfr derivatives and F^- K-12 strains lysogenic for the bacteriophage P1, linkage of unselected markers to selected markers is greatly reduced. Both observations are compatible with the above explanation, since it is known that DNA elements originating in *E. coli* K-12 are restricted by *E. coli* strain B or *E. coli* strain K-12 lysogenic for phage P1 (Arber and Dussoix, 1962).

If indeed the mating responses of the K-12 and B/r F^- strains are explained on the basis of host-controlled modification and restriction, then these two mechanisms must be under the genetic control of a region between the *thr* and *pil* loci which determines the mating response of these two strains.

The two hybrid strains, the B/r–(K-12) hybrid which mated as a K-12 F^- and the K-12–(B/r) hybrid which mated as a B/r F^-, presented a way of testing this explanation. A lysate of lambda prepared from K-12 was assayed for plaque-forming units on the K-12 host strain and on the K-12–(B/r) hybrid strain. The results (Table 8) show that the efficiency of plating (EOP) was reduced to 5×10^{-5} on the K-12–(B/r) hybrid strain. A lysate of lambda prepared on the K-12–(B/r) strain was assayed for plaque-forming units on the same two bacterial strains, and, as can be seen from Table 8, the EOP of the lysate was reduced to 10^{-4} on the K-12 strain. There was no apparent difference in phage adsorption as measured by the loss of free phage from the supernatant fluid, and it would appear that these two K-12 strains, which differ by a small genetic region near the *thr* locus, restrict and modify the bacteriophage lambda in a manner identical to the K-12 and B strains of *E. coli*.

It was not possible to test the restriction and modification of the bacteriophage lambda by the B/r and B/r–(K-12) strains. Although lambda particles are adsorbed by these strains, as measured by the disappearance of free phage from the supernatant fluid, no reproductive cycle can be detected. However, Glover et al. (1963) showed that the F-merogenotes F-*lac* and F-*gal* are also restricted and modified and can be assayed for modification and restriction in a manner similar to the bacteriophage lambda. The B/r and B/r–(K-12) strains were tested for their restricting and modifying properties by measuring the efficiency of transfer of these episomes.

The B/r F-*lac*$^+$/*lac*$^-$ *sm*^{s} strain was used as a male donor for four different F^- strains, the B/r F^-, K-12–(B/r) F^-, K-12 F^-, and the B/r–(K-12) F^-, and selection was made for *lac*$^+$ colonies. The transfer of F-*lac* was reduced to 10^{-2} from B/r to K-12 and B/r–(K-12) strains (Table 8). All the *lac*$^+$ colonies that did appear were stable haploids. These colonies were probably the result of recombination between a large fragment introduced by the F-*lac* element and

the F^- chromosome. This is supported by the finding that *pro*$^+$ colonies in these crosses appeared at a frequency a little less than that for *lac*$^+$ colonies, and that the *lac*$^+$ recombinants also received, in some cases, the unselected *leu*$^+$ and *pro*$^+$ markers. However, in the nonrestricting crosses, the *lac*$^+$ colonies examined were all F′ diploids and all those tested showed nonlinkage of *leu*$^+$ and *pro*$^+$; the frequency of *lac*$^+$ colonies was at least 100 times that for *pro*$^+$ colonies. Similar results were obtained when the donor was a K-12 F-*gal*$^+$/*gal*$^-$ strain and the above F^- strains were again used as recipients in bacterial crosses. The restrictive effect occurred in the predicted strains (Table 8). Finally, the wild-type sex-factor, F_1, was also tested and found to be restricted and modified, as predicted by these strains (Table 8).

On the basis of these experiments with F-*lac*, F-*gal*, F_1, and the bacteriophage lambda, it was concluded that the K-12 and B/r–(K-12) strains restrict and modify DNA elements in an identical manner, and the B/r and K-12–(B/r) strains restrict and modify DNA elements in an identical manner.

TABLE 8. *Restriction and modification of DNA elements**

Origin of DNA element	Relative efficiency of plating or transfer			
	K-12	K-12–(B/r)	B/r	B/r–(K-12)
Lambda·K-12	1.0	5×10^{-5}	—	—
Lambda·K-12–(B/r)	10^{-4}	1.0	—	—
F-*lac*·B/r	10^{-2}	1.0	1.0	2×10^{-2}
F-*gal*·K-12	1.0	5×10^{-2}	5×10^{-2}	1.0
F^+·K-12	1.0	5×10^{-2}	—	—

* The following strains were used: AB266: *E. coli* K-12; K-12 F^-, AB2601: *E. coli* K-12–(B/r) hybrid; B/r F^-, AC2514: *E. coli* B/r–(K-12) hybrid; K-12 F^-, AC2513: *E. coli* B/r; B/r F^-, AC2516: *E. coli* B/r; F-*lac*$^+$/*lac*$^-$, AB2604: *E. coli* K-12; F-*gal*$^+$/*gal*$^-$, AB264: *E. coli* K-12; F^+.

DISCUSSION

Dussoix and Arber (1962) generalized that all DNA elements would be susceptible to host-controlled modification and restriction, not just the DNA of the phage lambda. These mechanisms require two enzymes: (i) a specific nuclease capable of recognizing "foreign" DNA, and (ii) an enzyme(s) capable of altering the host DNA so that it is not recognized as "foreign" DNA by itself. These enzymes must be under the control of at least two structural genes. The restricting and modifying properties of *E. coli* strains K-12, B, and C, and the bacteriophage P1 are distinguishable from one another (Arber and Dussoix, 1962). The restricting and modifying properties of some strains of *Salmonella* and their phages are also different (Uetake, Toyama, and Hagiwara, 1964).

As discussed above, the anomalous results obtained between strains K-12 and B are explained on the basis of host-controlled modification and restriction. This is supported by the fact that the genetic region governing the mating response of the K-12 and B female recipients also controls the modifying and restricting properties of these two strains.

The restricting and modifying loci are concluded to be linked to one another on the basis of the following argument. If the two loci were separated by genetic recombination so that the K-12 restriction and B/r modification were present (or vice versa), the result would be lethal. If they were not linked to each other, then it would be quite difficult to introduce the two loci together with the *thr* locus. In fact, they are transferred with the *thr* locus about 60% of the time. The actual linkage would be greater than 60%, since the linkage is reduced in these crosses.

It is unlikely that the K-12 and B/r restricting and modifying loci are not allelic. If they were not allelic, one would expect to find recombinants from these crosses with both the K-12 and B/r restricting and modifying properties superimposed upon one another. This would be analogous to the K-12 strain lysogenic for phage P1, in which both the P1 and K-12 modifying and restricting mechanisms operate simultaneously (Arber and Dussoix, 1962). However, the hybrid strains from male K-12 × female B/r crosses and male B/r × female K-12 crosses had either K-12 or B restricting and modifying properties.

Thus, the genetic loci responsible for restriction and modification of DNA in strains K-12 and B of *E. coli* are located between the *thr* and *pil* loci. The restricting and modifying genes are linked to each other, and these two sets of genes are allelic in the K-12 and B strains of *E. coli*.

The anomalous kinetics of recombinant formation, reduced frequency of recombinant forma-

tion, and reduced linkage of unselected markers to selected markers in bacterial crosses between K-12 and B strains of *E. coli* can be interpreted as the manifestation of host-controlled restriction and modification on the *E. coli* chromosome.

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