THE LOCATION OF THE GENES FOR HOST-CONTROLLED MODIFICATION AND RESTRICTION IN *ESCHERICHIA COLI* K-12

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RECENT experiments have clarified certain aspects of the processes of modification and restriction by which certain systems of host-controlled modification (HCM) operate in *Escherichia coli* (ARBER 1962; ARBER and DUSSOIX 1962; DUSSOIX and ARBER 1962; GLOVER, SCHELL, SYMONDS and STACEY 1963). Modification is a process which acts directly on DNA and probably takes the form of specifically altering certain base sequences, perhaps by methylation (ARBER 1965; KLEIN and SAUERBIER 1965). Any DNA synthesized in a particular strain therefore bears a characteristic modification pattern. Restriction is a process which can occur when foreign DNA enters a cell, either by conjugation or through the intermediary of a phage particle. If this foreign DNA does not bear a modification pattern which is compatible with the recipient cell, it is rapidly degraded into small molecular weight components. The two aspects of HCM, therefore, have one thing in common: they both involve the recognition of a particular base sequence in DNA. However, basically they are very different, modification resulting in the addition of certain groups to DNA, while restriction leaves unmodified DNA in a condition in which it is open to attack by DNase.

This apparent dissimilarity in the processes of restriction and modification suggests that they are under independent genetic control. The first experimental evidence which seemed to support this idea came from experiments on the HCM system in which the modification and restriction are genetically controlled by the prophage P1. In this system (LEDERBERG 1957), DNA which has been synthesized in cells lysogenic for P1 carries a modification which enables it to infect either lysogenic or nonlysogenic cells of the same strain with equal efficiency. However, DNA synthesized in a nonlysogenic strain is rapidly degraded when transferred to P1-lysogenic cells. In a recent series of experiments two classes of P1 variants were isolated. In contrast to normal P1, which phenotypically can be represented P1r^+m^+, the first type of variant P1r^-m^+, causes no restriction but induces the normal type of modification, while the second type, P1r^-m^-, induces neither restriction nor modification (GLOVER *et al.* 1963).

Clearly one method of learning more about the genetic control of HCM in this system is to analyze it genetically. One phage cross between P1r^+m^+ and P1r^-m^- was performed to see if recombinants with the phenotype P1r^-m^+ could be identified, but without success (GLOVER and SCHELL, unpublished). Unfortunately

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nothing is known about the type of recombination which occurs with phage P1, and no other markers were available for carrying out crosses. Rather than develop the genetics of P1, it seemed preferable to investigate genetic control in another system of HCM in which genetic studies could be performed. For that reason, we began to study the system of HCM in *E. coli* K-12.

Some strains of *E. coli* impose a specific modification on their own DNA, and conversely restrict infecting DNA from other strains. This is exemplified by the three strains K-12, B and C, and can be demonstrated with phage λ (Table 1). It can be seen that, like wild-type P1, both K-12 and B can be phenotypically represented as r^+m^+ for restriction and modification, while C is akin to the r^-m^- mutant. Bacterial crosses between K, B and C could therefore be used to map the gene (or genes) responsible in K-12 and B for the r^+m^+ phenotype. However, it was decided to confine the mapping experiments to K-12 for two reasons. First, K-12 was the only strain for which a comprehensive genetic system was available. and second, any complications arising from interstrain crosses were ruled out. With this aim in view, K-12 mutants were isolated which, as in the P1 system, had the phenotypes r^-m^+ and r^-m^-. With the help of these mutants, it was then possible to show by conjugation experiments that the genes controlling both these mutant phenotypes were closely linked to the threonine locus in K-12.

MATERIALS AND METHODS

Media: See GLOVER (1962).

Bacteria: (a) *E. coli* K-12. The following Hfr strains were employed: Hfr, H, Hfr Cavalli, Hfr R1. Their points of origin and direction of chromosome transfer are illustrated in Figure 1. The F⁻ strain was C600. (b) *E. coli B.* This was the derivative B251 which is able to absorb phage λ (ARBER and DUSSOIX 1962). (c) *E. coli* C. See BERTANI and WEIGLE (1953).

Bacteriophage: A virulent mutant of λ (λv) (JACOB and WOLLMAN 1954).

Phage techniques: The general phage techniques are as described by ADAMS (1950). Special techniques relating to λ are those described by ARBER (1958, 1960).

Test for restriction and modification: (a) *Restriction:* An agar plate was overlaid with soft agar containing the bacterium to be tested. This was spotted with drops (0.01 ml) of three serial 100-fold dilutions of the challenge phages λ·B, λ·K and λ·C, allowing the efficiency of plating (e.o.p.) to be scored (Figure 2).

TABLE 1

Restrictive behavior of bacterial strains and host specificity of phage produced

	Approximate fraction of host bacteria which accept			Host specificity of phage produced	
Host bacteria	λ·B	λ·K	λ·C	λ·Kr^-m^+	λ·Kr^-m^-
B	1.0	10^{-4}	2×10^{-4}	10^{-4}	10^{-4} λ·B
K	4×10^{-4}	1.0	4×10^{-4}	1.0	10^{-4} λ·K
C	1.0	1.0	1.0	1.0	1.0 λ·C
Kr^-m^+	1.0	1.0	1.0	1.0	1.0 λ·K
Kr^-m^-	1.0	1.0	1.0	1.0	1.0 λ·C

Notation. Following the notation of ARBER and DUSSOIX (1962) the host specificity of a phage will be represented by the name of the phage followed by the name of the host strain in which it was last grown. For example, λ·B means phage lambda which has been grown in *E. coli* B while λ·C(P1) means phage lambda which has been grown in *E. coli* C(P1).

Abbreviations: HCM=host controlled modification; K=*E. coli* K-12; B=*E. coli* B; C=*E. coli* C.

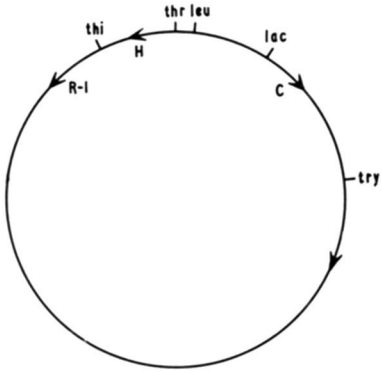

FIGURE 1.—The points of entry and directions of transfer of various Hfr strains are indicated by arrows on the circular chromosome map of *E. coli* K-12. Symbols on the outside of the circle represent bacterial markers, symbols on the inside indicate Hfr strains. *thi* = thiamine, *leu* = leucine, *lac* = lactose, *thr* = threonine, *try* = tryptophan. R-1 = Hfr Reeves 1 (REEVES 1959), C = Hfr Cavalli (CAVALLI-SFORZA 1950), Hfr H = Hfr Hayes (HAYES 1953).

(b) *Modification:* (i) *Usual procedure.* A plaque of λ grown on the strain to be tested was stabbed with a straight wire and the phage resuspended in phage buffer, to give approximately 10^5 phage particles per ml. Three serial tenfold dilutions were made, and drops (0.01 ml) of the phage suspension and the dilutions were spotted onto soft agar overlays of each of the three standard indicators B, K and C. The e.o.p. of the phage on the three indicators reveals its modification (Figure 3).

(ii) Quick method: To reduce the labour involved in testing a large number of phages, the following procedure was adopted. A plaque of λ grown on the strain to be tested is stabbed and the phage resuspended in 0.3 ml of phage buffer in a small tube. A straight wire is dipped vertically into the tube and used to stab repeatedly (about 12 times) along a radius of a thick Difco agar plate overlaid with soft agar containing the indicator bacteria. A phage that is not restricted by the indicator bacteria shows an area of lysis at each of the stabbing points, a phage which is restricted either lyses the indicator at the point of the first stab only, or not at all (Figure 4).

In the course of these experiments, we have repeatedly observed that some bacteria which have inherited HCM genes from the donor do not score as either donor-type or recipient-type

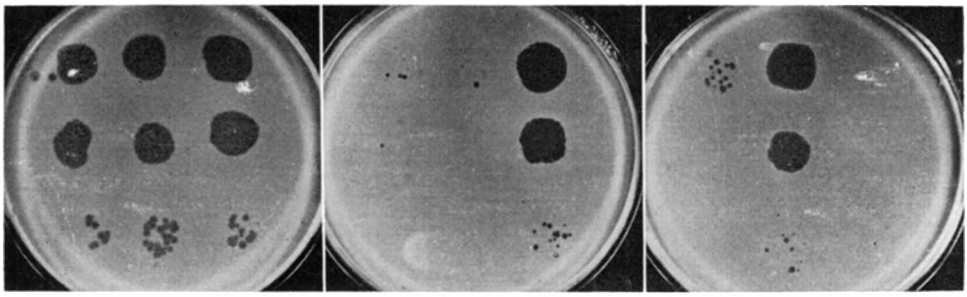

FIGURE 2.—*The test for restriction.* In the top row of each plate 10^5 challenge phages were added per droplet; in the middle row 10^3 phages; in the bottom row, 10 phages. In the left row on each plate the challenge phage was λ·C; in the middle row, λ·B; in the right row, λ·K. Plate at left: nonrestricting strain C; the e.o.p. of λ·C, λ·B and λ·K is approximately 1.0. Middle plate: restriction λ·C and λ·B on K. Plate at right: restriction of λ·C and λ·K on B.

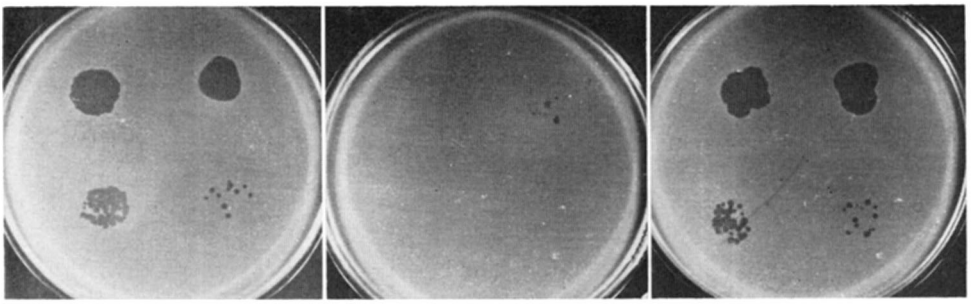

FIGURE 3.—*The test for modification.* Left plate: *E. coli* C as indicator. No restriction of test phage λ·B. Middle plate: *E. coli* K as indicator. Restriction of test phage λ·B. Right plate: *E. coli* B as indicator. No restriction of test phage λ·B.

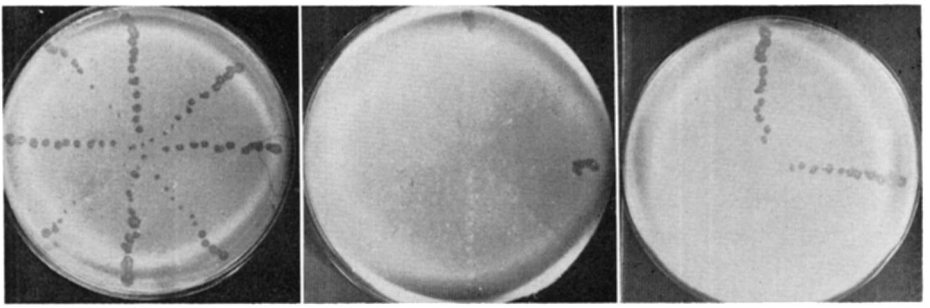

FIGURE 4.—*The test for modification*—quick method. Stabs at 12 o'clock and 3 o'clock are λ·B; remaining stabs are λ·C. Indicator bacteria are *E. coli* C (left plate); *E. coli* K (middle plate); *E. coli* B (right plate).

with respect to HCM on first isolation, but rather as intermediates. They have the capacity to segregate both donor and recipient types and can retain this capacity through many single-colony isolations. We have scored as donor-type all colonies which either on first isolation or after segragation produce the HCM phenotype of the donor. A detailed description of the properties of these unusual clones will be reported elsewhere (GLOVER and COLSON 1965).

RESULTS

(a) *Isolation of mutants:* A nitrosoguanidine treated culture of K-12 plates λ·C and λ·B at a higher efficiency than an untreated control. This increased e.o.p. for λ·C and λ·B can be accounted for by the presence of nonrestricting mutants in the culture. When the level of restriction was lowered 100-fold, approximately one out of 100 colonies tested was unable to restrict λ·C or λ·B. When the level of restriction was not so low, nonrestricting mutants were isolated by sib-selection for increased e.o.p. for λ·C and λ·B (COLSON and GLOVER 1965). Two types of nonrestricting mutant were isolated. One of these is able to confer K-12 specificity to λ grown in it while the other is not. Not only have these mutants lost the capacity to restrict phage λ, but they are also unable to restrict F-*lac* and chromosomal DNA. In conformity with the mutants of P1 previously mentioned, we can

represent normal phenotype of K-12 as Kr^+m^+ and the mutant phenotypes as Kr^-m^+ and Kr^-m^-.

(b) *Control crosses:* To locate the *r* and *m* genes in K-12 it was first necessary to examine the behavior of HCM mutants in conjugation experiments using conventional markers.

Two sets of crosses were performed to Kr^+m^+ and Kr^-m^- recipients, one (Table 2(1)) using a Kr^+m^+ donor and the other (Table 2(2)) a Kr^-m^- donor. The table lists the numbers of recombinants in certain classes arising from the crosses, and the actual linkage data for the region *thr-leu-lac*.

It can be seen from these results that when the donor is Kr^+m^+ the fertility and linkage values obtained from crosses involving the two recipients are similar. When the donor was Kr^-m^-, the fertility and linkage values obtained were lower using the restricting Kr^+m^+ recipient than using the Kr^-m^- recipient.

(c) *Interrupted matings:* Neither the inheritance of restriction nor modification can be scored quantitatively as a selected marker in crosses. To determine the time of entry of these loci we have carried out crosses of the type Hfr Kr^+m^+ $\times$ F$^-$$Kr^-m^-$. Mating was interrupted at various times during conjugation and recombinants for a selected early marker were isolated. The time of appearance of the r^+ and m^+ genes among these recombinants was compared to the appearance of other well known markers.

Two such crosses were performed with Hfr donors that had different points of origin and directions of chromosome transfer: (1) with Hfr Cavalli which transfers *lac*$^+$ early, and (2) with Hfr R-1 which transfers *thi*$^+$ early. The time of entry of the r^+ and m$^+$ markers is compared to that of the *thr*$^+$ and *leu*$^+$ genes for these crosses in Table 3. It can be seen that, with a precision of 5 minutes,

TABLE 2

Data on thr-lac-leu *linkage from control crosses with* Kr$^-$m$^-$ *and* Kr$^+$m$^+$ *donors and recipients*

		Recipients	
		Kr^+m^+	Kr^-m^-
(1) Kr^+m^+ donor: Hfr R-1 *met*$^-$$Kr^+m^+$ $\times$ C600 *thr*$^-$ *leu*$^-$ *thi*$^-$ *lac*$^-$ F$^-$ strains Kr^+m^+, Kr^-m^-			
Number of recombinants per 0.1 ml selected for:	*thr*$^+$ *leu*$^+$	290	278
	lac$^+$	109	131
Percent *thr*$^+$ *leu*$^+$ among:	*leu*$^+$	73	78
	lac$^+$	69	51
(2) Kr^-m^- donor: Hfr H *thi*$^-$ *r*$^-$*m*$^-$ *str-s* $\times$ C600 *thr*$^-$ *leu*$^-$ *thi*$^-$ *lac*$^-$ F$^-$ strains Kr^+m^+, Kr^-m^-			
Number of recombinants per 0.1 ml selected for:	*thr*$^+$ *leu*$^+$	167	1590
	lac$^+$	119	720
Percent *thr*$^+$ *leu*$^+$ among:	*leu*$^+$	72	92
	lac$^+$	27	82

In each cross 2.5×10^7 donor bacteria were mixed with 1×10^9 recipients in a total volume of 3 ml of broth. The mixture was rotated for 60 minutes, then 0.1 ml of a 10^{-3} dilution of the mixture was plated onto selective media to recover *thr*$^+$ *leu*$^+$ and *lac*$^+$ recombinants. In each cross, 200 selected recombinants were scored for the unselected marker.

TABLE 3

Data from crosses using interrupted mating with different Hfr donors

	Time of blending (min)			
	20	25	30	35
(1) *lac*+ transferred early:				
Hfr Cavalli *met*⁻ *T6-s* × *Kr*⁻*m*⁻ *thr*⁻ *leu*⁻ *thi*⁻ *lac*⁻ *T6-r* F⁻				
Number of *thr*+ *leu*+ among *lac*+ recombinants	0	0	0	8
Number of colonies with donor-type (*Kr*+*m*+)				
HCM among *lac*+ recombinants	0	0	0	7
Total number of *lac*+ recombinants scored	41	34	43	37
(2) *thi*+ transferred early:				
Hfr R-1 *met*⁻ *T6-s* × *Kr*⁻*m*⁻ *thr*⁻ *leu*⁻ *thi*⁻ *lac*⁻ *T6-r* F⁻				
Number of *thr*+ *leu*+ among *thi*+ recombinants	0	2	6	12
Number of colonies with donor-type (*Kr*+*m*+)				
HCM among *thi*+ recombinants	0	1	11	15
Total number of *thi*+ recombinants scored	50	50	50	50

In each cross equal volumes of donor and recipient bacteria at 1×10^8 cells per ml were mixed and rotated at 37°. At chosen times, 0.1 ml samples were diluted into 0.9 ml of buffer containing 4×10^{10} T6 particles per ml, and blended for 30 seconds. After diluting 100-fold, 0.1 ml samples were plated on selective media.

the time of entry of both these sets of genes is the same. Other similar crosses with other nonselective markers, on the other hand, showed no such correlation in the time of entry. In both crosses, the selected recombinants were either *r*+*m*+ or *r*⁻*m*⁻ showing that the restriction and modification genes are at least closely linked.

(d) *Analysis of linkage:* The percentages of donor type of HCM among *thr*+*leu*+, *thr*+*leu*⁻ and *thr*⁻*leu*+ recombinants obtained from crosses using HCM mutants are listed in Table 4. Contraselection against *thr*+ reduces the inheritance of donor-type HCM remarkably in each cross, while contraselection against *leu*+ has no significant effect.

This result was obtained using either wild-type or mutant donors and either wild-type or mutant recipients. It is evident therefore, that the mutations responsible for the r⁻m⁻ and r⁻m+ phenotypes have the same location, and this location is to the left of *thr*. Furthermore, the linkage of HCM to *thr*+ is approximately

TABLE 4

Linkage of donor-type HCM among recombinants from crosses

Donor	Recipient	Percent donor-type HCM among		
		thr+ *leu*+	*thr*+ *leu*⁻	*thr*⁻ *leu*+
Hfr R-1 *met*⁻ *r*+*m*+	C600 *thr*⁻*leu*⁻*thi*⁻*lac*⁻ F⁻ *r*⁻*m*⁻	84	77	4.5
Hfr H *thi*⁻ *str-s r*⁻*m*⁻	C600 *thr*⁻*leu*⁻*thi*⁻*lac*⁻*str-s* F⁻ *r*+*m*+	82	77	9.0
Hfr H *thi*⁻ *str-s r*⁻*m*⁻	C600 *thr*⁻*leu*⁻*thi*⁻*lac*⁻*str-r* F⁻ *r*+*m*+	84	80	8.0
Hfr H *thi*⁻ *str-s r*⁻*m*+	C600 *thr*⁻*leu*⁻*thi*⁻*lac*⁻*str-r* F⁻ *r*⁻*m*⁻	86	..	..

In each cross 100 of each class of selected recombinants were scored for restriction and modification.

the same as the linkage of *thr*$^+$ and *leu*$^+$ (see Table 3). Among over 1000 recombinants scored for restriction and modification, only donor or recipient type of HCM was observed and no recombinants were obtained.

DISCUSSION

Two types of mutant have been isolated from *E. coli* which have altered properties of HCM. The phenotype of one of them can be represented as r^-m^+, and that of the other as r^-m^-. These two types occur with about the same frequency. Similar mutants have been studied both in K-12 and in B by WOOD (1965). Out of more than 100 spontaneous and EMS-induced mutants that he isolated, approximately one half were r^-m^+ and the other half r^-m^-. This result is analogous to that obtained in experiments with P1 where again about one half of the 100 isolates were r^-m^+ and the others r^-m^-. This combined evidence strongly suggests that the r^-m^+ and the r^-m^- phenotypes are both due to single mutational events; in one case, the mutation affects only restriction while in the other it simultaneously affects both restriction and modification.

The conjugation experiments reported here have shown that the mutations responsible for both the r^-m^+ and the r^-m^- phenotypes map close to the threonine marker in K-12, and on the other side of it to the leucine marker.

Other experiments which confirm that the genes controlling HCM are located close to *thr* have recently been described by BOYER (1964), and by HOEKSTRA and DE HAAN (1965). These authors performed K × B crosses and showed that about 60% of the recipients acquiring the donor threonine locus had also acquired the restriction and modification properties of K. The reciprocal result was also demonstrated by BOYER in B × K crosses.

Although all these experimental results agree in that the gene or genes controlling the different functions involved in HCM map close to *thr*, yet the number of genes in this HCM region and the relationships between them still remain to be elucidated.

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SUMMARY

The genetic location of the control of host-controlled modification has been mapped close to *thr* and on the opposite side of it to *leu* by conjugation experiments between Hfr K-12 and recipients with altered properties of host-controlled modification.

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