Genet. Res., Camb. (1970), **15**, *pp.* 237–250
Printed in Great Britain

Functional analysis of host-specificity mutants in *Escherichia coli*

By S. W. GLOVER

M.R.C. Molecular Genetics Unit, Department of Molecular Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR

(*Received* 21 *November* 1969)

SUMMARY

Evidence from a functional analysis of host-specificity mutants in merodiploids is presented which supports the suggestion that three genes, *hss*, *hsr* and *hsm*, are necessary for the expression of host-controlled restriction and modification. The host-specificity phenotype expressed by the merodiploids provides evidence that at least two genes, *hss* and *hsr*, are concerned in the expression of host-specific restriction of DNA and one of these genes, *hss*, is responsible for the strain specificity of the restriction enzyme. A class of modification-deficient mutants isolated from restriction-deficient, modification-proficient mutants, was also tested for complementation in merodiploids and the phenotype of these merodiploids provides evidence that at least two genes, *hss* and *hsm*, are concerned in the expression of host-specific modification of DNA and one of these genes, *hss*, is responsible for the strain specificity of the modification enzyme. How these three genes function at the molecular level is discussed in terms of models based on the interaction of subunits to form oligomeric enzymes.

1. INTRODUCTION

Many strains of Enterobacteriaceae are able to recognize DNA from other so-called foreign strains. As a result of this recognition the invading DNA molecule from the foreign strain may be degraded by a strain-specific endonuclease which produces a small number of double strand breaks at defined sites along the DNA molecule (Messelson & Yuan, 1968). Thus the DNA of an infecting phage may be degraded and will be unable to replicate, and the phage is then said to be restricted. However, a small fraction of bacteria infected with such a phage do not restrict phage growth, and these bacteria produce bursts of progeny phages which are host-modified so that they are no longer restricted in subsequent rounds of infection in the same host strain. This host modification is a process which acts directly on DNA and in one particular case takes the form of specifically altering the base adenine by methylation (Arber & Dussoix, 1962; Arber & Smith, 1966). Modification of T-even phages involves glucosylation of DNA (Fukasawa & Saito, 1963; Hattman & Fukasawa, 1963; Shedlovsky & Brenner, 1963; Symonds *et al.* 1963).

Genetic analysis of host-controlled restriction and modification of phage λ carried out in several different laboratories reveals that mutants deficient in restriction

but not in modification, and mutants deficient in both processes, can be readily isolated (Glover *et al.* 1963; Wood, 1966; Lederberg, 1966). Mutants deficient in both restriction and modification have also been isolated from the former class as a result of a second mutation (Glover & Colson, 1969). These mutations all map close together on the *Escherichia coli* chromosome. No fine structure genetic analysis has been reported, but Glover & Colson (1969) observed a small number of recombinants in crosses between mutants and suggested that these could be accounted for by postulating three genes which control host-specific restriction and modification.

This paper* describes the isolation of F′ factors which carry the genes controlling host-restriction and modification and their use in an analysis of the functions controlled by each gene. The nomenclature of Arber & Linn (1969) will be used; they have defined the symbols *hss* for a gene which determines the synthesis of a polypeptide responsible for site recognition on DNA; *hsr* for a gene responsible for the synthesis of a polypeptide involved in endonuclease activity and *hsm* for a gene responsible for the synthesis of a polypeptide involved in modification.

2. METHODS

Bacteria. The bacterial strains used are listed in Table 1.

Bacteriophages. Phage λ and a virulent mutant *λvir* (Jacob & Wollman, 1954); P1 (Lennox, 1955); male specific phages MS-2 (Davis, Strauss & Sinsheimer, 1961) and fd (Marvin & Hoffman-Berling, 1963).

Media. Buffer for bacteria (g/l.) KH_2PO_4 3·0; Na_2HPO_4 7·0; NaCl 4·0; $MgSO_4.7H_2O$ 0·2. Phage buffer (g/l.) KH_2PO_4 3·0; Na_2HPO_4 7·0; NaCl 5·0; $MgSO_4$ (0·1 M solution) 10·0 ml; $CaCl_2$ (0·001 M solution) 10·0 ml; gelatin (1 % solution) 1·0 ml. M9 medium (g/l.) KH_2PO_4 3·0; Na_2HPO_4 7·0; NaCl 0·5; NH_4Cl 1·0; $MgSO_4$ (0·1 M solution) 10·0 ml; glucose 0·02. M9 medium was solidified with 1·5 % Davis New Zealand agar. Difco agar (g/l.) Oxoid tryptone 10·0; NaCl 8·0; glucose 1·0; Difco Bacto agar 10·0. L-broth (g/l.) Difco tryptone 10·0; yeast extract 5·0; NaCl 10·0. L-agar was L-broth solidified with 1·5 % Difco Bacto agar. Soft agar was either L-broth solidified with 0·6 % Difco Bacto agar or water soft agar containing 0·6 % Difco Bacto agar. L-amino acid supplements were added to minimal medium at 20 μg/ml; thiamin at 10 μg/ml and streptomycin at 200 μg/ml.

Phage techniques. The general techniques were as described by Adams (1950) and special techniques relating to λ were those described by Arber (1958, 1960).

Spot tests for restriction and modification. Restriction was scored with *λvir*.K, *λvir*.B and *λvir*.C by the method described by Colson *et al.* (1965). Modification was scored using standard indicator strains of *E. coli* K, B and C by the method of Colson *et al.* (1965).

* While this paper was in preparation the results of an essentially similar study have been published (Boyer & Roulland-Dussoix, 1969). Preliminary experiments were reported by Glover (1968) and a summary of these results was included in a review (Arber & Linn, 1969).

Test for restriction on merodiploids. Merodiploids grown in supplemented M9 were suspended in 3 ml of water soft agar with 0·01 M-$MgSO_4$ and mixed with standard dilutions of *λvir*.K, *λvir*.B, *λvir*.C and *λvir*.KB and the contents of the tubes poured on supplemented M9 agar plates. The efficiency of plating (e.o.p.) of the phages was scored after 24–36 h incubation at 37 °C.

Tests for modification on merodiploids. Phage from isolated *λvir* plaques on merodiploids was resuspended in phage buffer, diluted, and the e.o.p. of this phage, on standard indicator strains K, B, C and F'K/B, was scored on Difco agar plates after incubation for 24 h at 37 °C.

Table 1. *Bacterial strains*

Strain	Host-specificity	Reference/origin
C600	$r_K^+m_K^+$	Appleyard (1954)
HfrH	$r_K^+m_K^+$	Hayes (1953)
AB2463	$r_K^+m_K^+$	Howard-Flanders & Theriot (1966)
KLF1	$r_K^+m_K^+/r_K^+m_K^+$	Low (1968)
B251	$r_B^+m_B^+$	Arber & Dussoix (1962)
CB0156	$r_B^-m_B^-(2)/r_B^-m_B^-(2)$	By Dr Claire Berg from B7.1
C	r^-m^-	Bertani & Weigle (1953)
4K	$r_K^-m_K^-$	From C600
7K	$r_K^-m_K^+$	From C600
7K-2	$r_K^-m_K^-(2)$	From 7K
B2	$r_B^+m_B^+$	From B251
B8	$r_B^-m_B^-$	From B2
B1	$r_B^-m_B^-$	From B2
B6	$r_B^-m_B^+$	From B2
B11	$r_B^-m_B^+$	From B2
B1.1	$r_B^-m_B^-(2)$	From B11
B7.1	$r_B^-m_B^-(2)$	From B11
B15.1	$r_B^-m_B^-(2)$	From B11
B15.2	$r_B^-m_B^-(2)$	From B11

Single cycle experiments with merodiploids. Merodiploids were grown in supplemented M9 to about 5×10^8 bacteria/ml, centrifuged and resuspended in 0·01 M-$MgSO_4$ and starved for 30 min at 37 °C. Phage *λvir* was added at a multiplicity of about 0·2 and adsorbed for 10 min at 37 °C. An equal volume of L-broth (2× conc.) was added and the mixture was aerated for 10 min at 37 °C. The infected bacteria were filtered and unadsorbed phage in the filtrate assayed. The filter was washed with 20 ml L-broth and the bacteria resuspended in a further 20 ml L-broth and refiltered. This procedure was repeated. A sample of the infected bacteria was diluted 100-fold in L-broth and aerated for 60 min then treated with chloroform and the progeny phage were assayed on standard indicator strains. A second sample of the infected bacteria was immediately diluted and plated on standard indicator strains to measure the number of infectious centres. A third sample was immediately treated with chloroform to kill the bacteria and then assayed to measure the amount of unadsorbed phage still remaining after repeated filtration (residual phage). In no experiment did this exceed 10 % of the number of infectious centres.

Modified single burst experiments with merodiploids. Infected bacteria prepared as for the single cycle experiment after the removal of unadsorbed phage were

diluted and distributed in 1 ml amounts to 110 tubes. The tubes were incubated at 37 °C for 90 min and then 0·2 ml samples were added to water soft agar suspensions of *E. coli* C, and the contents of the tubes poured over Difco agar plates. The remainder of the suspension was kept overnight at 4 °C. Phage from those tubes which yielded plaques on *E. coli* C was then plated on standard indicator strains.

F′ factors carrying host-specificity mutations. An F′ factor KLF1 (Low, 1968) carrying wild-type host-specificity from *E. coli* K was obtained from Dr Brooks Low. Other F′ factors carrying host-specificity genes were obtained by the following method. A phage P1 lysate was prepared on the required strain and host-specificity genes transduced to HfrH *thi serB* by selecting *serB*$^+$ transductants (Glover & Colson, 1969). The HfrH strain was then mated with AB2463 *recA* and *thr*$^+$ *leu*$^+$ colonies selected. After purification colonies were tested for restriction and modification with *λvir* and for fertility with male-specific phages MS-2 and fd and by mating with a suitable recipient. Homozygous merodiploids were obtained either directly by transferring the F′ KLF1 to an appropriately marked F$^-$ recipient or as a result of spontaneous segregation from *rec*$^+$ recipients infected with the F′ KLF1.

Construction of merodiploids. Both the F′ donor and F$^-$ recipient strains were grown overnight in supplemented M9. The donor culture was diluted 1 in 10 into supplemented M9 and grown to about 2×10^8 bacteria/ml. Equal volumes of donor and recipient cultures were then mixed and incubated at 37 °C for 30 min. Mating was stopped by blending and suitable dilutions were plated on M9 supplemented to select colonies of the recipient strain which were now carrying the F′ from the donor strain and to contraselect the donor. After 48 h incubation at 37 °C 20 colonies were picked and purified, tested for restriction with *λvir* on supplemented M9 which did not permit the growth of spontaneous F$^-$ segregants, and tested for fertility with male-specific phages MS-2 and fd. When the recipient strain was *recA* it was frequently necessary to add 10 % L-broth to M9 media in order to stimulate sufficient growth of the culture to support the growth of phage λ.

3. RESULTS

(i) *The isolation of modification-deficient mutants from* E. coli $r_B^- m_B^+$

Glover & Colson (1969) pointed out that on any model which supposes that three genes are concerned in the control of restriction and modification we can expect to obtain at least three classes of mutants with the phenotype r$^-$m$^-$. One class would arise as a result of a single *hss* mutation from wild type and as a consequence of this mutation the mutant would be unable to recognize host-specific sites on DNA. The other two classes could be obtained as a result of second mutations induced in an r$^-$m$^+$ mutant. The first of these would be r$^-$m$^-$ simply as a result of a mutation in *hss* as described above, the other would be r$^-$m$^-$ as a result of a mutation in *hsm*. These authors isolated second-step r$^-$m$^-$ mutants from $r_K^- m_K^+$ but were unable to distinguish two classes in complementation tests. The tests were designed to measure the modification of phage λ produced as a result of zygotic induction in matings between two independent second-step r$^-$m$^-$ mutants.

The discovery by Low (1968) that F′ factors carrying any desired region of the *E. coli* chromosome could be readily isolated rendered possible complementation tests between host-specificity mutants in merodiploids. For this purpose second-step r^-m^- mutants of *E. coli* B were isolated from an $r_B^-m_B^+$ mutant. This mutant was made lysogenic for phage λ and grown to about 2×10^8 bacteria/ml in L-broth, centrifuged and resuspended in 5 ml of L-broth containing 60 μg/ml *N*-methyl-*N*-nitro-nitrosoguanidine and kept at 37 °C for 15 min. The bacteria were then centrifuged and the mutagen removed by repeated washing and centrifugation. The final suspension was diluted 1 in 10 into L-broth and incubated at 37 °C until the titre reached about 2×10^8 bacteria/ml. L-agar plates were then seeded with dilutions of the treated culture and incubated. Plates, containing about 50 colonies per plate, were replica-plated on to Difco agar plates seeded with soft agar overlays containing, respectively, *E. coli* $r_B^+m_B^+$ and $r_B^-m_B^-$. After incubation, colonies from the master plates which produced phage able to lyse the $r_B^-m_B^-$ indicator but not the $r_B^+m_B^+$ indicator were picked and tested for restriction and modification. Four colonies were obtained which were deficient in both restriction and modification and designated $r_B^-m_B^-(2)$ to indicate that the r^-m^- phenotype was obtained as a result of two mutational steps.

(ii) *Functional analysis of host-specificity mutants*

Dominance. Merodiploids were constructed as described in Methods and the restriction and modification phenotype of the diploids was scored with *λvir*. Experiments 3, 4 and 5 (Table 2) show that the wild-type alleles are dominant to all of the mutations tested, since the phenotype of merodiploids between $r_K^+m_K^+$ and the mutants was similar to that of homozygous $r_K^+m_K^+/r_K^+m_K^+$ diploids (Expt 1) and in fact not markedly different from that of the haploid $r_K^+m_K^+$. The reduction in efficiency of plating of λ.C exercised by the diploid $r_K^+m_K^+/r_K^+m_K^+$ *recA* (Expt 2) was always significantly greater than when *rec*$^+$ strains were used. The reason for this is not clear, but it may simply be related to the fact that the growth of *recA* mutants is always poor and this may simply result in less efficient growth of phage λ so that many infected cells do not yield progeny. Consistent with this is our observation that the plaques of phage *λvir* on *recA* strains, as well as being fewer than on *rec*$^+$ strains, are frequently less well defined and much smaller.

The merodiploid $r_K^+m_K^+/r_B^+m_B^+$ (Expt 6) and the reciprocal (Expt 14) restricted λ.K, λ.B and λ.C, indicating that the two restrictions, K-specific and B-specific, can be expressed together in the same cell. It is interesting that λ.C was not restricted in this diploid to an extent greater than in either haploid strain, although it is restricted in *E. coli* K (P1) to a degree approaching that of the calculated restrictions imposed by $r_K^+m_K^+$ and $r_{P1}^+m_{P1}^+$ combined. It could be that this reflects something in common between sites which are K-specific and those sites on the DNA which are B-specific, while K and P1-specific sites are clearly different. However, it is more likely that it merely reflects that fraction of cells which are phenotypically non-restricting at the time of challenge. Homozygous diploids $r_B^-m_B^-(2)/r_B^-m_B^-(2)$

Table 2. *Restriction and modification phenotypes of merodiploids*

Expt. no.	Haploid phenotype: F′	Haploid phenotype: Recipient strain	Merodiploid: Restriction* λ.KB‡	λ.K	λ.B	λ.C	Merodiploid: Modification† KB	K	B	C	Merodiploid phenotypes
1	$r_K^+m_K^+$	$r_K^+m_K^+$ C600	1·0	1·0	0·0005	0·0004	—	1·0	0·0003	1·0	$r_K^+m_K^+$
2	$r_K^+m_K^+$	$r_K^+m_K^+$ AB2463	1·0	1·0	0·00001	0·000003	—	1·0	0·0005	1·0	$r_K^+m_K^+$
3	$r_K^+m_K^+$	$r_K^-m_K^+$ 7K	—	1·0	0·0009	0·0008	—	1·0	0·0006	1·0	$r_K^+m_K^+$
4	$r_K^+m_K^+$	$r_K^-m_K^-$ 4K	—	1·0	0·0002	0·0009	—	1·0	0·0004	1·0	$r_K^+m_K^+$
5	$r_K^+m_K^+$	$r_K^-m_K^-$ 7K-2	—	1·0	0·001	0·0015	—	0·8	0·0003	1·0	$r_K^+m_K^+$
6	$r_K^+m_K^+$	$r_B^+m_B^+$ B2	1·0	0·004	0·0004	0·0005	0·5	1·0	0·5	1·0	$r_{KB}^+m_{KB}^+$
7	$r_K^+m_K^+$	$r_B^-m_B^+$ B6	1·0	0·0003	0·0002	0·0004	0·5	0·9	0·6	1·0	$r_{KB}^+m_{KB}^+$
8	$r_K^+m_K^+$	$r_B^-m_B^+$ B11	1·0	0·002	0·0003	0·0004	0·5	1·0	0·5	1·0	$r_{KB}^+m_{KB}^+$
9	$r_K^+m_K^+$	$r_B^-m_B^-$ B8	1·0	1·0	0·003	0·003	—	0·8	0·0001	1·0	$r_K^+m_K^+$
10	$r_K^+m_K^+$	$r_B^-m_B^-$(2) B7.1	1·0	0·0004	0·0007	0·0001	1·0	1·0	1·0	1·0	$r_{KB}^+m_{KB}^+$
11	$r_K^+m_K^+$	$r_B^-m_B^-$(2) B1.1	1·0	0·0003	0·0003	0·0001	1·0	1·0	1·0	1·0	$r_{KB}^+m_{KB}^+$
12	$r_K^+m_K^+$	$r_B^-m_B^-$(2) B15.1	1·0	1·0	0·01	0·03	0·001	1·0	0·001	1·0	$r_K^+m_K^+$
13	$r_K^+m_K^+$	$r_B^-m_B^-$(2) B15.2	1·0	1·0	0·01	0·02	0·001	1·0	0·001	1·0	$r_K^+m_K^+$
14	$r_B^+m_B^+$	$r_K^+m_K^+$ AB2463	1·0	0·0001	0·0001	0·0001	0·5	1·0	1·0	1·0	$r_{KB}^+m_{KB}^+$
15	$r_B^-m_B^+$B6	$r_K^+m_K^+$ AB2463	1·0	0·0001	0·0001	0·0001	0·5	1·0	1·0	1·0	$r_{KB}^+m_{KB}^+$
16	$r_B^-m_B^+$B6	$r_B^-m_B^-$ B8	1·0	0·001	1·0	0·0002	0·0001	0·0001	1·0	1·0	$r_B^+m_B^+$
17	$r_K^+m_K^+$	r^-m^- C	1·0	1·0	0·001	0·001	—	1·0	0·0002	1·0	$r_K^+m_K^+$
18	$r_B^-m_B^-$(2) 7.1	$r_B^-m_B^-$(2) B7.1	1·0	1·0	1·0	1·0	—	0·0001	0·01	1·0	$r_B^-m_B^-$
19	$r_K^-m_K^-$ 4K	r^-m^- C	1·0	1·0	1·0	1·0	—	0·0003	0·0001	1·0	r^-m^-
20	$r_K^-m_K^+$ 7K	$r_K^-m_K^+$ 7K	1·0	1·0	1·0	1·0	—	1·0	0·0003	1·0	$r_K^-m_K^+$
21	$r_K^-m_K^+$ 7K	r^-m^- C	1·0	1·0	1·0	1·0	—	1·0	0·0001	1·0	$r_K^-m_K^-$
22	r^-m^- C	r^-m^- C	1·0	1·0	1·0	1·0	—	0·0005	0·0001	1·0	r^-m^-
23	$r_B^-m_B^-$ B8	$r_B^-m_B^-$ B8	1·0	1·0	1·0	1·0	—	0·0005	0·0003	1·0	r^-m^-
24	$r_B^-m_B^+$	r^-m^- C	1·0	1·0	1·0	1·0	—	0·0001	1·0	1·0	$r_B^-m_B^+$
25	$r_B^+m_B^+$	r^-m^- C	—	0·001	1·0	0·0001	—	0·0001	0·8	1·0	$r_B^+m_B^+$
26	$r_B^+m_B^+$	$r_B^-m_B^-$ B1	—	0·0001	1·0	0·0001	—	0·0001	1·0	1·0	$r_B^+m_B^+$

* Restriction indicates the e.o.p. of *λvir*.KB, *λvir*.K, *λvir*.B and *λvir*.C on the merodiploids.

† Modification indicates the e.o.p. of phage produced by the merodiploids on standard indicators *E. coli* K, B, C and on the merodiploid F′ $r_K^+m_K^+/r_B^+m_B^+$.

‡ λ.KB indicates phage which is able to plate with an efficiency of 1·0 on the standard indicators *E. coli* K, B and C.

— = Not tested.

(Expt 18), $r_K^- m_K^+/r_K^- m_K^+$ (Expt 20) and $r_B^- m_B^-/r_B^- m_B^-$ (Expt 23) displayed phenotypes virtually identical to those of the haploid strains from which they were derived.

(iii) *Complementation of restriction-deficient mutants*

The merodiploid $r_B^- m_B^+/r_B^- m_B^-$ (Expt 16) restricted λ.K and λ.C, a property not possessed by either component of the diploid when tested separately. Clearly the B-specific restriction expressed by this diploid is due to complementation between these two non-restricting mutants, which must therefore be restriction-deficient because of mutations in different genes. Of the two genes postulated to be involved in restriction, the $r_B^- m_B^+$ mutant must carry an intact *hssB* gene since it confers normal B-specific modification to phage λ. We conclude, then, that this mutant is *hsr*$^-$ while the $r_B^- m_B^-$ mutant is *hssB*$^-$ and as a consequence both restriction- and modification-deficient. Recombination between these two genes was reported by Glover & Colson (1969). They obtained $r_B^+ m_B^+$ recombinants from crosses between $r_B^- m_B^+$ and $r_B^- m_B^-$ and $r_K^+ m_K^+$ recombinants from crosses between $r_K^- m_K^+$ and $r_K^- m_K^-$. From this evidence we conclude that in *E. coli* B and in *E. coli* K host-specific restriction requires the function of two genes, *hss* and *hsr*.

The merodiploids $r_K^+ m_K^+/r_B^- m_B^+$ (Expts 7 and 9) restricted λ.K, which neither component of the diploid does when tested separately. Clearly the B-specific restriction deficiency in $r_B^- m_B^+$ has been complemented by a function provided by wild-type $r_K^+ m_K^+$. Similar results were obtained with the reciprocal diploid $r_B^- m_B^+/r_K^+ m_K^+$ (Expt 15). From these results we conclude that the genotype of $r_B^- m_B^+$ is *hssB*$^+$ *hsr*$^-$ *hsm*$^+$ and that complementation between *hssB*$^+$ and *hsr*$^+$ from $r_K^+ m_K^+$ results in B-specific restriction in the diploid. As expected, these merodiploids, the merodiploid $r_K^+ m_K^+/r_B^+ m_B^+$ and its reciprocal (Expts 6 and 14) all produce phage which is able to plate on *E. coli* K, B and C with an e.o.p. of 1·0. In addition, it plates on the merodiploid $r_K^+ m_K^+/r_B^+ m_B^+$ and its reciprocal at an efficiency of 1·0. This phage is therefore designated λ.KB and carries both K-specific and B-specific modifications. Phage with these properties has been previously obtained as a small fraction of the progeny produced by $r_B^- m_B^+$ cells after infection with λ.K (Kellenberger, Symonds & Arber, 1966).

(iv) *Complementation of modification-deficient mutants*

No complementation was observed between $r_K^+ m_K^+$ and $r_B^- m_B^-$ single-step mutants, from which we conclude that the $r_B^- m_B^-$ single-step mutants carry mutations in *hssB* and that wild-type K cannot provide the function normally associated with *hssB*. This result agrees with that obtained in Expt 16, in which the same $r_B^- m_B^-$ mutant was complemented by $r_B^- m_B^+$.

However, when second-step $r_B^- m_B^-(2)$ mutants obtained after mutagenesis of $r_B^- m_B^+$ were tested in merodiploids with $r_K^+ m_K^+$ then a quite different result was obtained. Two of these mutants (Expts 10 and 11) were complemented by $r_K^+ m_K^+$ and the diploid expressed B-specific restriction. This result confirms that obtained with $r_K^+ m_K^+/r_B^- m_B^+$ merodiploids (Expts 7 and 9) but in addition to complementation for B-specific restriction, complementation for B-specific modification was also

observed, indicating that in these $r_B^- m_B^-(2)$ second-step mutants the modification deficiency can be complemented by a function provided by the $r_K^+ m_K^+$ component. We conclude therefore that two genes are necessary for the expression of modification. One of these is *hssB* and is defective in $r_B^- m_B^-$ single-step mutants, but not in $r_B^- m_B^-(2)$ second-step mutants B7.1 and B1.1. The other is *hsm*, which is defective in $r_B^- m_B^-(2)$ second-step mutants B7.1 and B1.1, and it is this function which is provided by $r_K^+ m_K^+$ in the merodiploids constructed between wild-type K and these mutants. The other two $r_B^- m_B^-(2)$ second-step mutants, B15.1, and B15.2, derived from $r_B^- m_B^+$ could not be complemented by $r_K^+ m_K^+$ in merodiploids (Expts 12 and 13) from which we conclude that they carry *hssB* mutations.

Merodiploids constructed between host specificity mutants and *E. coli* C did not reveal any host-specificity functions which *E. coli* C could exercise to restore the restriction and modification deficiencies in the mutants tested (Expts 17, 19, 21 and 22, 24 and 25).

(v) *Single cycle growth experiments with phage* λvir *in partial diploids*

The phenotypes of the merodiploids described in the previous section were scored by measuring the efficiency of plating of *λvir* and scoring the efficiency of plating, on standard indicator strains, of phage obtained from plaques on these merodiploids. To test whether the low efficiency of plating of λ observed as a result of complementation in the diploids reflected accurately the restriction of growth of λ in these bacteria, merodiploids were infected with *λvir* and the number of infectious centres produced on standard indicators was scored. The infected cultures were then allowed to lyse and the modification of the progeny phage produced after this cycle of growth was determined by plating on standard indicators. The results of these experiments in Table 3 show that only a small fraction of infected merodiploids produce progeny phage, and the λ they produce plate equally well on all of the indicator strains. Thus the results obtained by measuring the efficiency of plating of *λvir* on lawns of the merodiploids truly reflect the restriction of growth in infected cells. One of these merodiploids, $r_K^+ m_K^+ / r_B^- m_B^+$, was infected with *λvir*.C and the infected bacteria diluted into a large number of tubes so that single bursts could be examined. This experiment, summarized in Table 4, shows that, although the bursts were small and subject to considerable variation, some contained phages able to grow on the three indicator strains *E. coli* K, B and C.

Stability of the merodiploids. A merodiploid of the constitution r^+/r^- that frequently segregated r^- bacteria would not restrict λ very efficiently. To determine therefore the stability of merodiploids constructed with the F′ KLF1, merodiploids KLF1 *ara*$^+$/*ara*$^-$ were made with *rec*$^+$ and *recA* strains and the frequency of segregation of *ara*$^-$ colonies was measured. *ara*$^-$ colonies were segregated at frequencies of 0·1 % in *rec*$^+$ and 0·3 % in *recA* merodiploids. All of the *recA ara*$^-$ colonies tested were F^- while out of ten *rec*$^+$ *ara*$^-$ colonies tested, seven were male and three were not. It is clear then that segregation of r^+/r^- merodiploids at this rate would not significantly influence the efficiency of plating of phage λ. Nevertheless, a segregating merodiploid of the constitution $r_K^+ m_K^+ / r_B^+ m_B^+$ might produce

Table 3. *Single cycle growth experiments with* λvir *in merodiploids*

Phage	Host	No. of infected bacteria/ml	No. of infectious centres/ml			No. of progeny phage/ml			
			On K	On B	On F′K/B	On K	On B	On C	On F′K/B
λvir.C	$r_K^+m_K^+$	$2{\cdot}5 \times 10^8$	$1{\cdot}6 \times 10^4$	—	—	5×10^6	—	5×10^6	—
λvir.C	$r_K^+m_K^+/r_B^+m_B^+$	$1{\cdot}5 \times 10^8$	$8{\cdot}0 \times 10^4$	$5{\cdot}0 \times 10^4$	—	$2{\cdot}0 \times 10^6$	$2{\cdot}1 \times 10^6$	$3{\cdot}0 \times 10^6$	—
λvir.K	$r_K^+m_K^+/r_B^+m_B^+$	$2{\cdot}0 \times 10^8$	—	$2{\cdot}7 \times 10^5$	—	$8{\cdot}0 \times 10^8$	3×10^8	9×10^8	—
λvir.B	$r_K^+m_K^+/r_B^+m_B^+$	$3{\cdot}0 \times 10^8$	$8{\cdot}4 \times 10^5$	$4{\cdot}0 \times 10^5$	—	$2{\cdot}0 \times 10^7$	$1{\cdot}0 \times 10^7$	$1{\cdot}4 \times 10^7$	—
λvir.C	$r_K^+m_K^+/r_B^-m_B^+$	$7{\cdot}6 \times 10^7$	$3{\cdot}5 \times 10^3$	$4{\cdot}5 \times 10^3$	$5{\cdot}9 \times 10^3$	$5{\cdot}5 \times 10^5$	$3{\cdot}1 \times 10^5$	$4{\cdot}0 \times 10^5$	$3{\cdot}5 \times 10^6$
λvir.K	$r_K^+m_K^+/r_B^-m_B^+$	$7{\cdot}5 \times 10^7$	6×10^4	2×10^4	2×10^4	2×10^5	$1{\cdot}4 \times 10^5$	2×10^5	$1{\cdot}5 \times 10^5$
λvir.B	$r_K^+m_K^+/r_B^-m_B^+$	$1{\cdot}8 \times 10^8$	$1{\cdot}2 \times 10^4$	$1{\cdot}3 \times 10^5$	—	3×10^6	$1{\cdot}0 \times 10^6$	3×10^6	3×10^6
λvir.C*	$r_K^+m_K^+/r_B^-m_B^+$	$8{\cdot}7 \times 10^7$	$1{\cdot}25 \times 10^4$	$3{\cdot}8 \times 10^3$	—	$6{\cdot}5 \times 10^5$	$3{\cdot}6 \times 10^5$	$9{\cdot}5 \times 10^5$	—

* Samples from this experiment were used for the experiment presented in Table 4.

results which mimicked a stable merodiploid with respect to the restriction and modification of phage λ. To test this possibility the efficiency of plating of *λvir* was measured on mixtures of *E. coli* K and *E. coli* B varying from 99·5 % of K and 0·5 % of B to 99·5 % of B and 0·5 % of K. The efficiencies of plating of *λvir* on these mixtures are presented in Table 5, from which it is quite clear that no mixture of *E. coli* K and B mimics the behaviour of K/B merodiploids.

Table 4. *Modified single burst experiment with* λvir. *C on the merodiploid* $F' r_K^{\pm} m_K^{\pm} / r_B^{-} m_B^{\pm}$

Tube* no.	Number of plaques		
	On C	On K	On B
21	3	4	0
43	4	2	0
47	1	0	0
60	24	12	6
67	10	2	6
86	1	2	0
92	1	4	0
104	4	5	5
109	4	7	0

* Total number of tubes was 110, of which those listed (nine) produced phage and the remainder contained no phage.

Table 5. *The plating efficiency of phage λ on mixtures of* E. coli *K and B*

Mixed indicator		Efficiency of plating		Fraction of progeny phage forming plaques on		
K (%)	B (%)	*λvir*.K	*λvir*.B	K	B	C
0	100	3×10^{-4}	1·0	0·0005	1·0	1·0
0·5	99·5	3×10^{-2}	1·0	0·05	1·0	1·0
5·0	95	2×10^{-1}	1·0	0·1	0·6	1·0
50	50	3×10^{-1}	3×10^{-1}	0·3	0·3	1·0
90	10	1·0	1×10^{-1}	0·9	0·1	1·0
95	5	1·0	3×10^{-2}	1·0	0·5	1·0
99·5	0·5	1·0	1×10^{-3}	1·0	0·05	1·0
100	0	1·0	5×10^{-4}	1·0	0·0004	1·0

(vi) *Independence of P1 host-specificity and bacterial host specificities*

All combinations of *E. coli* K and *E. coli* B host-specificity mutations were tested against P1 host-specificity mutations for complementation in P1 lysogenic bacteria. The restriction and modification of phage λ in these lysogens was measured and no complementation was detected in any combination. This result indicates that P1 host-specificity is quite distinct from that of *E. coli* K and *E. coli* B. It should be pointed out that all of the P1 mutants were isolated in $r_K^{\pm} m_K^{\pm}$(P1) lysogens and were thus preselected for the fact that they could not be complemented by *E. coli* K. However, no host-specificity mutants of *E. coli* K or B could be complemented by

wild-type $r^+_{P1}m^+_{P1}$ and in this case there was no preselection for non-complementable mutants.

(vii) *Further analysis of* $r^-_B m^-_B(2)$ *second-step mutants*

The simplest interpretation of the $r^-_B m^-_B(2)$ second-step mutants B7.1 and B1.1, which are complemented by $r^+_K m^+_K$ for both restriction and modification deficiency, is that they have the genotype *hssB*$^+$ *hsr*$^-$ *hsm*$^-$, and that, in merodiploids with wild-type *hssK*$^+$ *hsr*$^+$ *hsm*$^+$, B specificity is expressed by complementation between *hssB*$^+$ and *hsr*$^+$ and *hsm*$^+$. However, recent results (Hubacek & Glover, 1970) indicate that this may not be the case. A merodiploid was constructed between B2.1 *hssB*$^+$ *hsr*$^-$ *hsm*$^+$ and B7.1 and scored for restriction and modification of phage λ. To our surprise this merodiploid expressed B-specific restriction. The second-step mutant B7.1 was then tested in a merodiploid with *hssK*$^+$ *hsr*$^-$ *hsm*$^+$, and this diploid restricted λ.K, λ.B and λ.C and the phage produced was λ.KB. From these experiments we conclude that B7.1 has an intact *hssB* gene and that *hsr* must also be wild type since it appears to function to complement the restriction deficiency in $r^-_K m^+_K$ and $r^-_B m^+_B$ mutants, both mutations in B7.1 must therefore have been in *hsm*.

What remains then is to explain why the phenotype of the mutant B7.1 is $r^-_B m^-_B$ and why the phenotype of B11.10, the parent from which B7.1 was derived, is $r^-_B m^+_B$. Preliminary experiments indicate that in merodiploids with 7K *hssK*$^+$ *hsr*$^-$ *hsm*$^+$ the mutant B11.10 can complement the restriction deficiency of 7K and as a result λ.B is restricted. Furthermore, in these same merodiploids the restriction deficiency of B11.10 is complemented by *hssK*$^+$ *hsr*$^-$ *hsm*$^+$ and as a result λ.K is also restricted. We conclude therefore that B11.10 carries a single mutation in *hsm* and is *hssB*$^+$ *hsr*$^+$. What kind of mutation then in *hsm* could produce the $r^-_B m^+_B$ phenotype of B11.10? Hubacek & Glover (1970) have recently shown that among mutants selected for temperature-sensitive restriction and modification many carried mutations in *hsm* only, and they were able to conclude from a functional analysis of these mutants that *hsm* is necessary for the expression of restriction. The mutation in B11.10 would therefore seem to be a mutation in *hsm* which impaired its ability to function in restriction but did not impair its ability to function in modification. The second-step mutation in B7.1 would also be in *hsm* and as a result of this mutation the modification function of *hsm* is impaired.

4. DISCUSSION

These results are in general agreement with those of Boyer & Roulland-Dussoix (1969). At the genetic level the results of the complementation analysis presented indicate that at least two and probably three genes are involved in the expression of host-specific restriction. In the nomenclature of Arber & Linn (1969) two of these genes are *hss* and *hsr* and the merodiploid *hssB*$^+$ *hsr*$^-$ *hsm*$^+$/*hssB*$^-$ *hsr*$^+$ *hsm*$^+$ expresses B-specific restriction as a result of complementation. These results also show that the strain-specificity of restriction is determined by *hss*, since in mero-

diploids the specificity of restriction which is expressed depends upon the component which brings in an intact *hss* gene. For example, the merodiploid *hssK*$^+$ *hsr*$^+$ *hsm*$^+$/*hssB*$^+$ *hsr*$^-$ *hsm*$^+$ can express B-specific restriction because the *hssB* gene is strain specific while *hsr*$^+$ is not. This result confirms the same conclusions drawn from an analysis of the phenotype of recombinants obtained as a result of genetic crosses between host-specificity mutants of *E. coli* K and B (Glover & Colson, 1969).

In addition, since the single-step mutant B8 which carries a mutation in *hssB* is restriction *and* modification deficient we can say that *hss* is also necessary for the expression of modification.

The results of the complementation analysis also indicate that at least two genes are involved in the expression of modification. One of them as shown above is *hss*. Complementation between *hssK*$^+$ *hsr*$^+$ *hsm*$^+$ and second-step $r_B^-m_B^-(2)$ mutants which almost certainly have two mutations in *hsm* indicate that the second gene necessary for modification is *hsm*, and similarly the specificity of the modification expressed is determined by *hss* and not by *hsm*.

Two types of restriction-deficient mutant can therefore be isolated—*hss*$^-$ and *hsr*$^-$—and likewise two types of modification-deficient mutant can be isolated—*hss*$^-$ and *hsm*$^-$. This simple picture is complicated by the fact that it appears that a class of r^-m^+ mutants actually carry *hsm* mutations, indicating that *hsm* is required for the expression of restriction and that some second-step r^-m^- mutants carry two mutations in *hsm*. We can draw no conclusions about the role of *hsr* in the expression of modification except that it certainly does not determine the strain specificity of modification.

The second-step mutants B15.1 and B15.2, which could not be complemented by *hssK*$^+$ *hsr*$^+$ *hsm*$^+$ in merodiploids, appear to be weakly transdominant since λ.B and λ.C were not as efficiently restricted by the merodiploids as they are by the haploid strain K; however, the phage produced was λ.K. B15.1 and B15.2 may therefore represent mutants in the fourth gene which has been postulated by Boyer & Roulland-Dussoix (1969) to account for transdominant mutants. However, alternative explanations seem equally plausible on present evidence. Either these particular merodiploids are less stable than others tested, resulting in the segregation of a significant fraction of r^- bacteria sufficient to raise the efficiency of plating of phages λ.B and λ.C to about 10^{-2} but sufficiently stable to produce phage which is at least 50 % λ.K; or these mutants carry mutations in *hssB* or *hsm* which render them phenotypically $r_B^-m_B^-$ but nevertheless produce altered polypeptides which effectively compete with the wild-type polypeptides produced by *hssK*$^+$ *hsr*$^+$ *hsm*$^+$, thus slightly impairing K-specific restriction and perhaps also modification.

At the molecular level we suppose that these genes act by determining the synthesis of three different polypeptides which interact to form oligomeric enzyme(s). From the results presented above it is sufficient to postulate that the *hss*-directed polypeptide and the *hsm*-directed polypeptide interact to produce a strain-specific modifying enzyme. For the strain-specific restriction enzyme we postulate that *hss* and *hsr*-directed polypeptides are necessary and almost certainly the

hsm-directed polypeptide is required as well. More conclusive evidence in support of this last notion, based upon a functional analysis of temperature-sensitive restriction and modification mutants, has recently been presented by Hubacek & Glover (1970).

I would like to acknowledge the collaboration of Mr Alexander Lukin in the early experiments reported here and the excellent technical assistance of Miss Anne Welsh. I would like to thank Werner Arber for making his review (Arber & Linn, 1969) available prior to publication.

REFERENCES

Adams, M. H. (1950). Methods for the study of bacterial viruses. *Methods in Medical Research* **2**, 1–73.

Appleyard, R. K. (1954). Segregation of new lysogenic types during growth of doubly lysogenic strain derived from *Escherichia coli* K-12. *Genetics, Princeton* **39**, 440–452.

Arber, W. (1958). Transduction des caractères *gal* par le bactériophage λ. *Archs. Sci. Genève* **11**, 259–330.

Arber, W. (1960). Polylysogeny for bacteriophage lambda. *Virology* **11**, 250–272.

Arber, W. & Dussoix, D. (1962). Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage λ. *J. molec. Biol.* **5**, 18–36.

Arber, W. & Linn, S. (1969). DNA modification and restriction. *Ann. Rev. Biochem.* **38**, 467–500.

Arber, W. & Smith, J. D. (1966). Host controlled modification of phage and its correlation with specific methylation of desoxyribunucleotides. IXth Int. Congr. Microbiol. p. 5.

Bertani, G. & Weigle, J. J. (1953). Host controlled variation in bacterial viruses. *J. Bact.* **65**, 113–121.

Boyer, H. W. & Roulland-Dussoix, D. (1969). A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. molec. Biol.* **41**, 459–472.

Colson, C., Glover, S. W., Symonds, N. & Stacey, K. A. (1965). The location of the genes for host-controlled modification and restriction in *Escherichia coli*. *Genetics, Princeton* **52**, 1043–1050.

Davis, J. E., Strauss, J. H. & Sinsheimer, R. L. (1961). Bacteriophage MS2: another RNA phage. *Science, N.Y.* **134**, 1427.

Fukasawa, T. & Saito, S. (1963). Abnormal growth of T-even phages in bacterial mutants defective in UPDG pyrophosphorylase. *Fedn Proc.* **22**, 406.

Glover, S. W. (1968). Host specificity in F′ heterogenotes of *Escherichia coli*. *J. gen. Microbiol.* **53**, i–ii.

Glover, S. W. & Colson, C. (1969). Genetics of host-controlled restriction and modification in *Escherichia coli*. *Genet. Res.* **13**, 227–240.

Glover, S. W., Schell, J., Symonds, N. & Stacey, K. A. (1963). The control of host induced modification by phage P1. *Genet. Res.* **4**, 480–482.

Hattman, S. & Fukasawa, T. (1963). Host-induced modification of T-even phages due to defective glucosylation of their DNA. *Proc. natn. Acad. Sci. U.S.A.* **50**, 297–300.

Hayes, W. (1953). The mechanism of genetic recombination in *Escherichia coli*. *Cold Spring Harb. Symp. quant. Biol.* **18**, 75–93.

Howard-Flanders, P. & Theriot, L. (1966). Mutations of *Escherichia coli* K12 defective in DNA repair and genetic recombination. *Genetics, Princeton* **53**, 1137–1150.

Hubacek, J. & Glover, S. W. (1970). Complementation analysis of temperature sensitive host specificity mutations in *Escherichia coli*. (In the Press.)

Jacob, F. & Wollman, E. L. (1954). Etude génétique d'un bactériophage tempéré d'Escherichia coli. I. Le système génétique du bactèriophage λ. *Annals. Inst. Pasteur, Paris* **87**, 654–673.

Kellenberger, G., Symonds, N. & Arber, W. (1966). Host specificity of DNA produced by *Escherichia coli*. 8. Its acquisition by phage λ and its persistence through consecutive growth cycles. *Z. Vererbungsl.* **98**, 247–256.

LEDERBERG, S. (1966). Genetics of host-controlled restriction and modification of deoxyribonucleic acid in *Escherichia coli*. *J. Bact.* **91**, 1029–1036.

LENNOX, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**, 190–206.

LOW, B. (1968). Formation of merodiploids in matings with a class of *rec*⁻ recipient strains of *Escherichia coli* K12. *Proc. natn. Acad. Sci., U.S.A.* **60**, 160–167.

MARVIN, D. A. & HOFFMAN-BERLING, H. (1963). Physical and chemical properties of two new small bacteriophages. *Nature, Lond.* **197**, 517.

MESSELSON, M. & YUAN, R. (1968). DNA restriction enzyme from *E. coli*. *Nature, Lond.* **217**, 1110–1114.

SHEDLOVSKY, A. & BRENNER, S. (1963). A chemical basis for the host-induced modification of T-even bacteriophages. *Proc. natn. Acad. Sci., U.S.A.* **50**, 300–306.

SYMONDS, M., STACEY, K. A., GLOVER, S. W., SCHELL, J. & SILVER, S. (1963). The chemical basis for host induced modification in phage T2. *Biochem Biophys. Res. Commun.* **12**, 220–222.

WOOD, W. B. (1966). Host specificity of DNA produced by *Escherichia coli*. Bacterial mutations affecting the restriction and modification of DNA. *J. molec. Biol.* **16**, 118–133.