

The *hsd* (Host Specificity) Genes of *E. coli* K12

Bela Sain* and Noreen E. Murray**

Department of Molecular Biology, University of Edinburgh, Edinburgh, Scotland

Summary. The *hsd* genes of *E. coli* K12 have been cloned in phage λ by a combination of in vitro and in vivo techniques. Three genes, whose products are required for K-specific restriction and modification, have been identified by complementation tests as *hsdR*, *M*, and *S*. The order of these closely linked genes was established as *R*, *M*, *S* by analysis of the DNA of genetically characterised deletion derivatives of λ *hsd* phages. The three genes are transcribed in the same direction but not necessarily as a single operon. Genetic evidence identifies two promoters, one from which transcription of *hsdM* and *S* is initiated and a second for the *hsdR* gene.

The *hsdR* gene codes for a polypeptide of molecular weight ~130000; *hsdM* for one of 62-65000 and the *hsdS* gene was associated with two polypeptides of approximately 50000. Circumstantial evidence suggest that one of these two polypeptides may be a degradation, or processed, derivative of the other. The *hsdS* polypeptide of *E. coli* B has a slightly higher mobility in an SDS-polyacrylamide gel than does that of *E. coli* K12.

A probe comprising most of the *hsdR* gene and all of the *hsdM* and *S* genes of *E. coli* K12 shares extensive homology with the DNA of *E. coli* B but none with that of *E. coli* C.

1. Introduction

Some strains of *E. coli* distinguish the DNA even of other *E. coli* strains from their own. Foreign DNA is degraded. This biological phenomenon of restriction resides in endonucleases produced by strains

which protect their own DNA by methylation, i.e. modification, (Arber 1971). The restriction endonucleases of the *E. coli* host specificity systems determined by chromosomal genes, in contrast to many other restriction enzymes, do not break DNA at specific targets (Horiuchi and Zinder 1972; Murray et al. 1973a). Nevertheless, these systems retain sequence specificity in that modification is the methylation of a specific DNA sequence (Ravetch et al. 1978; Lautenberger et al. 1978; Sommer and Schaller 1979; Kan et al. 1979; K. Ineichen and T.A. Bickle, personal communication). Only when this site is present, but unmodified, is the DNA susceptible to restriction (Kühnlein and Arber 1972; Smith et al. 1972; Murray et al. 1973b).

Elegant genetic experiments demonstrated three genes whose products were all required for restriction (Hubacek and Glover 1970). Both the *E. coli* B and K restriction endonucleases comprise three different polypeptides (Eskin and Linn 1972; Meselson et al. 1972). The B restriction endonuclease was also shown to methylate B recognition sequences, more particularly when one of the two DNA strands was already methylated (Voivis et al. 1974). However, an enzyme purified as a B-specific methylase lacked the largest of the three polypeptides (Lautenberger and Linn 1972). Furthermore, only two of the three *hsd* genes are essential for an active methylase in vivo since a λ *hsd* phage carrying only the *M* and *S* genes provides K-specific modification in a strain lacking all components of the *hsd* system (Borck et al. 1976).

Information relevant to DNA sequence-specificity resides within the subunit determined by the *hsdS* gene (Boyer and Roulland-Dussoix 1969; Arber and Linn 1969; Hubacek and Glover 1970). Thus a hybrid protein having polypeptides determined by the *hsdM* and *R* genes of *E. coli* B and the *hsdS* gene of *E. coli* K has K-specificity.

We have isolated λ *hsd* phages with the aim of

* Present Address: Institute of Biochemistry, Hungarian Academy of Sciences, P.O.B. 521, H-6701 Szeged, Hungary

** Present Address and offprint requests to: EMBL, Postfach 10.2209, D-69 Heidelberg, Federal Republic of Germany

facilitating detailed genetic and biochemical analyses of the *hsd* genes and their products. Previously, in the absence of selective systems or close flanking markers, even genetic mapping of the host specificity region was difficult. The λ *hsd* phages, and their deletion derivatives described in this paper readily permitted ordering of the *hsd* genes. Genetic evidence for two promoters was obtained even though the three closely-linked genes were shown to be transcribed in the same direction. Polypeptides, whose molecular weights are similar to those reported for the three subunits of the K-restriction endonuclease (Meselson et al. 1972), were identified as products of the *hsd* genes.

2. Materials and Methods

a) Phages and Phage Vectors. The vectors, λ *hsd* phages and all derivatives made for the experiments described in this paper are listed in Table 1. λ^+ , or *limm*²¹, were used as heteroimmune helper phages to integrate *hsd* phages into the *E. coli* chromosome; *h*⁸⁰*red*⁻*gam*210*cIKH54nin5shn*6° as a donor of *shn*6°, *h*⁸⁰*att*⁺*imm*⁺ to make a *cI*⁺ *nin*⁺ derivative of the λ *hsd* phage 651; λ *trpcI857srI* λ 4°*nin5srI* λ 5° to make the *cI857* derivative of 651; 570BR (Klein and Murray 1979) as a donor of *sbam*1° and *h*⁸⁰*trp46Nam7am53P*_L⁺*cI*P_R⁴³⁴*nin5Sam7* (NM 770) as a donor of hybrid immunity *Sam7*(Wilson and Murray 1979).

b) Bacterial Strains and Plasmids. Bacterial strains are listed in Table 2. The plasmid vector was pBR322 (Bolivar et al. 1977).

c) Media and Microbial Methods. Media and general methods are described elsewhere (Murray et al. 1977). Deletion mutants of *hsd* phages were selected by their increased resistance to chelat-

ing agents (Parkinson and Huskey 1971). Sodium pyrophosphate (pH 7.0) was added to BBL trypticase agar to a concentration of 5 mM. Dilysogens were isolated following mixed infection with a *hsd* phage and a heteroimmune helper. The restriction phenotypes of the dilysogens were determined from the relative efficiencies with which *lvir. C*, *lvir. K* and *lvir. B* plated on them, and the modification phenotypes by taking *lvir* from the area of lysis resulting from *lvir. C* and testing the efficiencies of plating on C, K and B strains. Dilysogens were cured of the *hsd* prophage and restoration of the original phenotype was confirmed. Complementation in lytic infection was tested following multiple rounds of infection of *hsd* mutants with *hsd* phages. Modification was assessed from the relative efficiencies of plating of the *hsd* phages on C, K, and B strains.

d) Preparation of DNA. Phages were prepared and purified as described by Wilson et al. (1977). Purified phage were dialysed against 10 mM Tris.HCl (pH 7.8), 1 mM EDTA, the DNA extracted by phenol (Kaiser and Hogness 1960) and dialysed against the same buffer. Plasmid DNA was purified from cleared bacterial lysates by banding in CsCl/ethidium bromide gradients (Clewell and Helinski 1969). Bacterial DNA was purified by a modified version of the Marmur procedure (Marmur 1961) essentially as described by Kaiser and Murray (1979).

e) Biochemicals and Reagents. These were as in Wilson and Murray (1979).

f) Restriction and Ligation. Restriction endonucleases were either purified by standard methods or purchased from New England Biolabs. T4 DNA ligase was purified by Sandra Bruce (Murray et al. 1979). All restriction digests were made in 10 mM Tris.HCl (pH 7.5), 10 mM MgCl₂, 10 mM 2-mercaptoethanol 50 mM NaCl with the exception of *EcoRI* and *BamHI* where 100 mM NaCl was used. The conditions for ligase reactions were as in Wilson et al. (1977).

g) Gel Electrophoresis of DNA Fragments. DNA fragments were separated in 1% (w/v) agarose gels in 0.04 M-Tris.acetate, pH 8.2 (Sharp et al. 1973). Small DNA fragments were separated in a

Table 1. Phage and Phage Vectors

Stock number	Genotype	Origin and use
631	λ lacZ <i>cIVKH54 nin5 Sam7</i>	Vector (Murray et al. 1977)
642	λ hsdMS <i>cI857 nin5</i>	Made from <i>hsdR</i> DNA in vector 569 ^a (Borck et al. 1976)
651	λ hsdMS <i>cIVKH54 nin5 Sam7</i>	Made from CR63 DNA in vector 631 (Borck et al. 1976)
655	λ hsdMS <i>cI857 nin5</i>	Derivative of 651 used to make dilysogens
781	λ supE <i>cI857 nin5</i>	Vector (Murray et al. 1977)
954	λ hsdMS <i>cIVKH54 shn6</i> °	Derivative of 651
1011	λ hsdM <i>cIVKH54 shn6</i> °	Deletion derivative of 954 generated by <i>HindIII</i>
1013	λ hsdMS _B <i>cIVKH54 shn6</i> °	S _B derivative of 1011
1017	λ hsdMS <i>cI</i> ⁺ <i>nin</i> ⁺	Derivative of 651 used for the isolation of deletions
1019	λ hsdMS <i>cI857 nin5</i>	Spi ⁻ extension of 655
1048	λ hsdMS <i>cI857 nin5</i>	Made from CR63 DNA in vector 781
1049	λ hsdM <i>cI857 nin5</i>	<i>hsd</i> fragment from 1011 in vector 781 in reverse orientation
1050	λ hsdRMS <i>cI857 nin5</i>	Spi ⁻ extension of 642
1051	λ hsdMS <i>imm</i> ²¹ <i>nin5</i>	Derivative of 1048 used to make dilysogens of Hfr90
1052	λ Eam <i>hsdMS imm</i> ²¹ <i>nin5</i>	Derivative of 1051
1064	λ sbam1° <i>hsdMS imm</i> ²¹ <i>nin5</i>	Derivative of 1052
1065	λ sbam1° <i>hsdS imm</i> ²¹ <i>nin5</i>	Deletion derivative of 1064 generated by <i>BamHI</i>
1072	λ hsdM <i>imm</i> ²¹	Derivative of 1049 used to make dilysogens of Hfr90
1073	λ hsdRMS <i>imm</i> ⁴³⁴ <i>nin5</i>	Derivative of 1050 used to make dilysogens of Hfr90
1074	λ hsdM <i>Nam P</i> _L ⁺ <i>cI P</i> _R ⁴³⁴ <i>nin5 Sam7</i>	Derivative of 1072; used in infection experiments
1075	λ hsdS <i>Nam P</i> _L ⁺ <i>cI P</i> _R ⁴³⁴ <i>nin5 Sam7</i>	Derivative of 1065; used in infection experiments
1076	λ hsdMS <i>Nam P</i> _L ⁺ <i>cI P</i> _R ⁴³⁴ <i>nin5 Sam7</i>	Derivative of 1051; used in infection experiments

^a The λ DNA of this vector is indistinguishable from 781

Table 2. Bacterial Strains

Strain	Relevant features	Source or reference	Use
C-la	<i>E. coli</i> C strain	Bertani and Weigle (1953)	Source of DNA; <i>hsdR</i> ⁻ <i>M</i> ⁻ <i>S</i> ⁻ strain
B	<i>E. coli</i> B strain	F.W. Studier	Source of <i>hsdS</i> _B DNA
C R63		Appleyard et al. (1956)	Source of <i>hsdS</i> _K DNA
C'600	<i>supE tonA</i>	Appleyard (1954)	Host for λ
QR47	<i>supE</i>	Weil and Signer (1968)	λ crosses
594	<i>sup</i> ^o	Weigle (1966)	Nonpermissive λ host
ED8654	<i>hsdR supE supF</i>	Murray et al. (1977)	Complementation tests; transfection host
<i>groN</i> 785	<i>sup</i> ^o <i>groN</i>	Georgopoulos (1971)	Permissive for hybrid immunity phages
<i>groN</i> 785 <i>supF</i>	<i>supF groN</i>	D. Piolli	Permissive for hybrid immunity <i>Sam7</i>
S159	<i>sup</i> ^o <i>uvrA</i>	Jaskunas et al. (1975)	Infection of u.v. irradiated cells
S159 (λ <i>imm</i> ^{4,3,4} <i>supF</i>)	<i>supF imm</i> ^{4,3,4} <i>uvrA</i>	Murray and Wilson (1979)	Infection of u.v. irradiated cells
C'600 (P2)	P2 lysogen	R. Thomas	Selection of Spi ⁻ phages
S03	<i>supE hsdS</i>	Wood (1966)	Complementation tests
HB101	<i>hsdS recA</i>	Boyer and Roulland-Dussoix (1969)	Complementation tests; transformation host
WA960	<i>hsdS</i> _B	Wood (1966)	Source of B specificity
WA2710 ^a	<i>hsdR hsdM hsdS</i> _B	W. Arber	Complementation tests
SKRI	<i>Eco</i> RI restricting	Murray and Murray (1974)	Measuring in vivo restriction by <i>Eco</i> RI
Hfr90	<i>hsdM</i> mutation VIII	Hubacek and Glover (1970)	Complementation tests
NM148	<i>thr nal</i> ^r		Recipient to make competent <i>hsdM</i> strain
NM474	<i>nal</i> ^r <i>hsdM</i>	Hfr90 by NM148	Transformation competent complementation host

Unless stated otherwise, all strains are *E. coli* K derivatives

^a This strain carries the double mutation B7.1 (Glover 1970)

10% polyacrylamide gel (Maniatis et al. 1975b) using pBR322 DNA digested with *Hae*III for marker fragments.

h) Transfection and Transformation. ED8654 and HB101 were made competent in the uptake of DNA by the CaCl₂ shock method of Lederberg and Cohen (1974).

i) Labelling of DNA, Hybridization and Autoradiography. Labelled probes were made by nick translation as described by Maniatis et al. (1975a) except that the unlabelled deoxynucleotide triphosphate concentration was 10 μ M each and the DNase I concentration was 10–50 ng/ml, depending on the activity of the enzyme. DNA was transferred to nitrocellulose filters from agarose gels (Southern 1975) from plaques (Benton and Davis 1977) and from colonies (Grunstein and Hogness 1975). The filters were processed in the conventional way and were pretreated with Denhardt (1966) solution before hybridization. The hybridization was achieved by overnight incubation at 37° in 5 \times SSC, 40% formamide and Denhardt components. Positive hybridization was detected by autoradiography, at -70° C, using presensitized X-ray film and a phosphotungstate intensifying screen.

j) Analysis of Polypeptides. Protein synthesis was detected by pulse-labelling after phage infection of ultraviolet irradiated cells (Jaskunas et al. 1975) and samples were analysed on gradient SDS polyacrylamide gels (see Wilson and Murray 1979).

3. Results

a) Construction and Isolation of λ hsd Phages

Populations of λ transducing derivatives of *E. coli* K12 were made by inserting into vector chromosomes fragments of bacterial DNA generated by *Eco*RI. λ hsd phages were readily selected from these transducing phages since only they protected their DNA against

degradation by K-specific restriction after propagation in a non-modifying host (Borck et al. 1976). Phages 642 and 651 (see Table 1) have been described by Borck et al. (1976); since phage 642 was derived from the DNA of an *hsdR*⁻ strain, other λ hsd phages (e.g. 1048) were isolated with the aim of obtaining wild-type *hsd* genes inserted into λ vectors in each of the two alternative orientations.

E. coli strain C has no component of the K host specificity system (Glover 1970), but lysogens in which *hsd* phages were integrated via a helper phage into the attachment site of the *E. coli* C chromosome provided K-specific modification of λ vir phages replicated in them. This was true for λ hsd phages irrespective of the orientation of the bacterial DNA fragment. Expression of the modification (*hsdM*) and specificity (*hsdS*) genes from a prophage in *E. coli* C is consistent with the inclusion of a promoter from which these genes are transcribed (Borck et al. 1976) even when the major λ promoters are repressed. Neither these lysogens, nor lysogens made in an *hsdR*⁻ K strain were able to restrict unmodified phages. This suggests that fragmentation of *E. coli* K DNA with *Eco*RI has either interrupted the *hsdR* gene or separated it from the *hsdM* and *S* genes.

b) Preliminary Characterisation of λ hsdMS DNA

A fragment of approximately 11 kilobase pairs (kb) was identified when the DNA from each of three

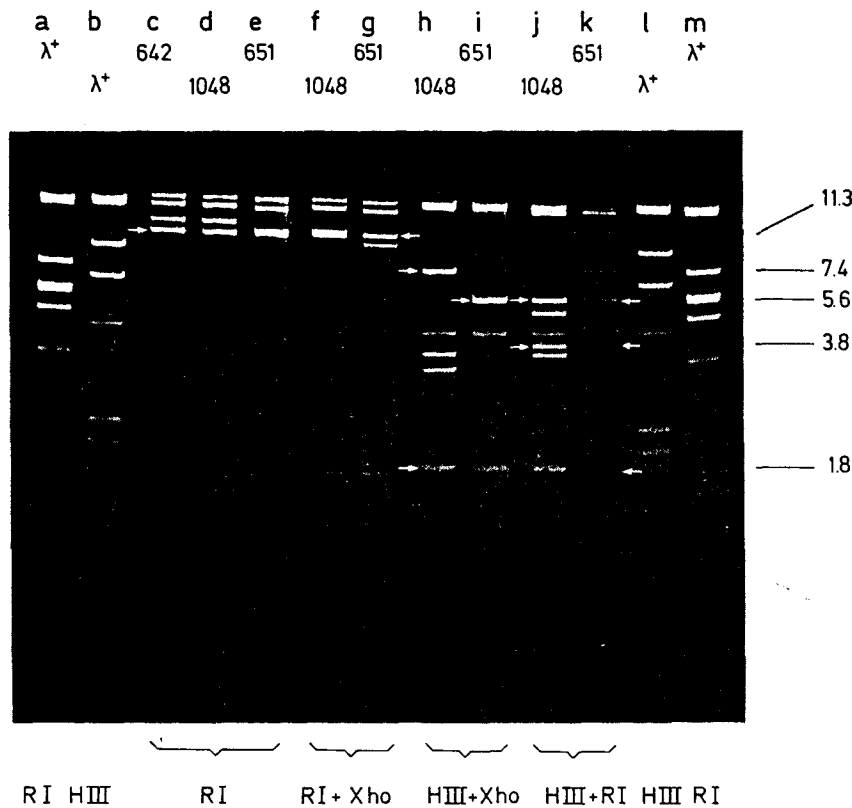


Fig. 1. Electrophoretic analysis of digests of DNA. The source of DNA is indicated at the head of each track and the enzyme used to digest the DNA is given at the foot of the track. The abbreviation RI is used for *EcoRI* and HIII for *HindIII*. The sizes of some important fragments are given in kilobase pairs (kb). The 7.4 kb fragment in track *h* and the 5.6 kb fragment in track *i* are diagnostic of the orientation of the bacterial DNA (see Fig. 2). The 1.8, 3.8, and 5.6 kb fragments common to tracks *j* and *k* are bacterial DNA. The vector DNA of phages 1048 and 651 are different (see Fig. 2). The largest fragment in each track represents cohered left and right terminal fragments of λ DNA.

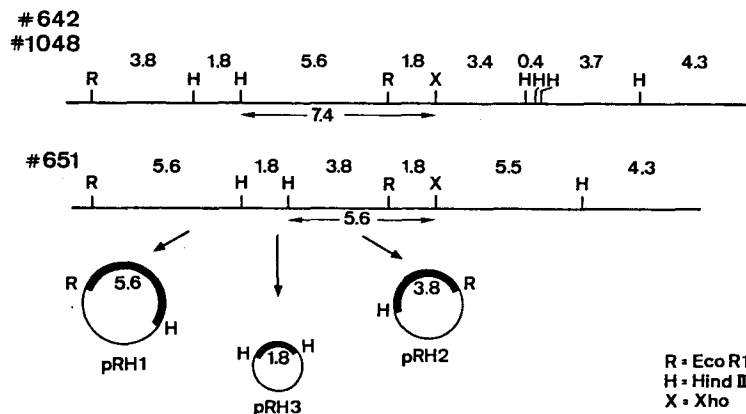


Fig. 2. Orientation of the bacterial DNA in *hsd* phages and the origin of pRH plasmids. The left arm of the λ vector (~ 20 kb) contains neither *EcoRI* nor *HindIII* targets and is not shown. The plasmid vector is pBR322 and fragments are inserted between the *EcoRI* and *HindIII* targets or, in the case of pRH3, at the *HindIII* site. Distances between adjacent restriction targets are expressed in kb. The distances of 7.4 and 5.6 kb between the *Xho* target and the nearer *HindIII* site within the insert identify the orientation.

independently isolated *hsd* phages No. 642, 1048, and 651, see Table 1) was digested with *EcoRI* (Fig. 1, tracks *c*, *d*, and *e*). For the DNA of phage 651 the bacterial insert was more readily separated from the right arm of the vector following digestion with *XhoI* and *EcoRI* (Fig. 1, track *g*). *XhoI* cuts only the vector DNA, and this but once; therefore its target provides

a convenient reference point close to the inserted bacterial DNA. Two targets for *HindIII* were located within the bacterial DNA (Figs. 1 and 2) and since these targets are positioned asymmetrically, double digests with *HindIII* and *XhoI* differentiated the orientation of the bacterial fragment. The bacterial DNA in the *hsdMS* phages 642 and 1048 is in the alterna-

tive orientation to that in phage 651 (Figs. 1 and 2, data for 642 not shown).

c) Further Cloning and Restriction Mapping of the *hsd* Region

A more detailed restriction analysis was undertaken with the aims of charting targets in the bacterial DNA prior to sequence studies and of cloning the entire *hsd* region on the assumption that *hsdR* is close to the *hsdM* and *S* genes.

The three fragments of bacterial DNA obtained after digestion of *hsdMS* with *EcoRI* and *HindIII* were transferred to plasmid pBR322. The resulting plasmids (see Fig. 2) were analysed directly by digestion with restriction enzymes and were also used to make radioactively labelled probes for the identification of overlapping DNA fragments in digests of *E. coli* DNA. The results of this type of analysis suggested digestion with *BglII* as an alternative means of isolating the *hsd* region.

A single *BglII* target was identified in pRH3 (see Fig. 2). This is the only *BglII* site in the bacterial DNA of phage 651 and is approximately 400 base pairs from the left *HindIII* site. Labelled probes made by nick-translation of pRH1 and pRH2 DNAs (see Fig. 2), when hybridized to *E. coli* K DNA fragmented with *BglII* and transferred to nitrocellulose filters after fractionation by electrophoresis through an agarose gel, identified two fragments of 8 and 10 kb respectively (data not shown). Even the smaller of these two fragments is large enough to code for three polypeptides of the sizes identified in the K-restriction endonuclease (Meselson et al. 1972) and each includes DNA extending beyond the *EcoRI* fragment originally isolated in *λhsdMS* (see Fig. 4). These two *BglII* fragments were therefore cloned. The cohesive ends of DNA fragments generated by either *BamHI* or *BglII* have the same nucleotide sequence. Fragments of *E. coli* K (CR63) DNA resulting from digestion with *BglII* were joined to pBR322 digested with *BamHI* and 800 of the resulting Ap^r Tc^s transformants of HB101 were screened for hybridization to *λ* and *λhsdMS* DNAs. One colony hybridized to both probes, identifying bacterial DNA that cross-hybridized to *λ* DNA (see Kaiser and Murray 1979). Among 5 colonies that hybridized to *λhsdMS* DNA, but not to *λ* DNA, were plasmids that included both of the predicted *BglII* fragments. This was verified indirectly since neither *BamHI* nor *BglII* recognizes the sequence generated by fusion of the heterologous cohesive ends. One plasmid, pBg3, included the DNA present in pRH1 and the others, e.g. pBg4 and pBg6, that present in pRH2. The structures of the plasmids

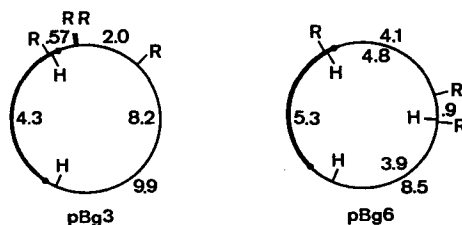


Fig. 3. The structures of the pBg plasmids. The heavy line represents vector DNA, solid circles the *BamHI*-*BglIII* joints. *R* indicates an *EcoRI* target, *H* a *HindIII* target. Numbers outside the circle indicate the lengths (in kb) between *EcoRI* targets and those within the lengths between *HindIII* targets. pBg4 is similar to pBg6 but the bacterial fragment is in the opposite orientation

<i>EcoRI</i>	~6	2.0	11.3 (<i>λhsdMS</i>)	1.9	4.3
<i>HindIII</i>		~12	1.8	3.9	5.5
<i>BglII</i>			8.3 (pBg3)	9.9 (pBg4 or 6)	
<i>BamHI</i>			3.1	7.6	11.1
<i>HpaI</i>				4.3	1.5
<i>SalI</i>			1.9	9.4	

Fig. 4. Restriction targets within the *hsd* region of *E. coli* K. The distances between targets are indicated in kb. The fragment of bacterial DNA in phage 651 (*λhsdMS*) is indicated and also those within the pBg plasmids. Fragments overlapping the cloned DNA were identified by hybridization to digests of *E. coli* DNA

deduced following digestion with *EcoRI* and *HindIII* are shown in Fig. 3. The small (0.2 kb) *EcoRI* fragment present in pBg3 was implied from partial digestion products of around 2.2 and 0.7 kb and was identified when complete digestion products were separated through a polyacrylamide gel (data not shown).

A more detailed restriction map of the *hsd* region of *E. coli* K (Fig. 4) was constructed on the basis of analysis of digests of plasmid and *λhsd* DNAs and of overlapping DNA fragments detected by cross-hybridization of labelled plasmid probes to various digests of *E. coli* K DNA (see also section f and Table 4).

d) Genetic Analysis of the *hsd* Region

The gene order within the *hsd* region was deduced by testing for functional genes in various plasmid derivatives and in *λhsd* phages and their deletion derivatives. *E. coli hsd* mutants were transformed with plasmid DNAs and the restriction and modification phenotypes of the transformants determined. The *hsd* genes within *λ* phages were detected either following lysogenization of *hsd*⁻ strains in the presence of a helper phage or following lytic infection. In the former case the properties of the lysogenic bacteria were determined. Absence of complementation could indicate the absence of the gene in question or merely the absence of the appropriate promoter sequence. In lytic infection by *λhsd* phages the incorporated

bacterial genes are transcribed from the λ promoters and modification of the resulting phage is a measure of the modification phenotype of the infected cells.

i) *Neither pBg Plasmid Includes a Functional hsdS Gene.* The pBg plasmids were recovered by transformation of the *hsdR*⁺*hsdS*⁻ strain HB101 and the transformants were checked for their ability to restrict unmodified λ vir. All were restriction deficient (r_K^-) and hence neither *Bgl*II fragment includes a functional *hsdS* gene.

ii) *Location of the hsdR Gene by Analysis of Plasmids.* The pBg plasmids (pBg3 and 4 or 6) do not contain the entire *hsd* region probably because the *hsdS* gene is cut by *Bgl*II; either *Bgl*II fragment could carry *hsdR* and/or *hsdM*. Transformants of the *hsdR*⁻*M*⁺*S*⁺ strain ED8654 were isolated and those derived from pBg3, but not pBg4 nor pBg6, were restriction proficient (r_K^+). Part of the *hsdR* gene is therefore within the 2.0 kb and not the 0.9 kb flanking *Eco*RI fragment (Fig. 4).

iii) *Location of hsdR Gene by in vivo extension of the λ hsdMS Phage.* λ hsdMS phages having the inserted bacterial DNA in each of the alternative orientation (642 and 655, a *cI857* derivative of 651, see Table 1 or Fig. 2) were integrated into the *E. coli* chromosome via rec-mediated crossing-over between the homologous bacterial DNA sequences. The lysogens were induced and those rare phages that had excised aberrantly and in so doing lost the λ gam gene were selected by their ability to form plaques on *E. coli* lysogenic for phage P2 (Zissler et al. 1971). Such phages (*Spi*⁻) were then tested for their efficiency of plaque formation on *E. coli* carrying the *Eco*RI restriction system. Phages that had acquired additional *Eco*RI targets, and presumably, therefore, adjacent chromosomal DNA sequences, were detected by their increased sensitivity to restriction (Murray and Murray 1974). DNA was made from these phages and a derivative of phage 655 (1019) was found to include *Eco*RI fragments of 0.9 and 4.3 kb while that derived from 642 (1050) included *Eco*RI fragments of 2.0 and 0.2 kb (see Figs. 3 and 4). Each of these phages was integrated by helper phage into the chromosome of an *E. coli* C strain and into that of an *hsdR*⁻K strain, ED8654. Lysogens of the former phage remained $r_K^- m_K^+$, but those of the latter phage, λ hsdRMS 1050, were $r_K^+ m_K^+$.

These results confirm the location of the *hsdR* gene deduced from analysis of the pBg plasmids and furthermore they require that all three genes are expressed from the repressed λ hsdRMS prophage. Lysogens i.e. λ hsdRMS (1050) of the *hsdR* strain ED8654 restricted unmodified λ vir less well than lysogens of

the *E. coli* C strain. This is consistent with interference in complementation by defective polypeptides in the *hsdR* strain.

iv) *Isolation and Characterization of Deletions.* Plasmids carrying parts of the *hsd* region have been described (See Figs. 2 and 3). Deletion mutants of λ hsd phages were made in vitro and in vivo. The former were generated by cutting out the DNA between either the two *Bam*HI or the two *Hind*III targets (see Fig. 4). For both experiments, targets in the vector were first removed by appropriate crosses (see Table 1) and DNA from the resulting phages digested to completion with the appropriate enzymes. λ hsd phages having deletions generated in vivo were isolated as phages with an enhanced resistance to chelating agents. The sensitivity of this selection was increased by first making a derivative of the λ hsdMS phage (651) which had a nearly normal DNA content (1017, see Table 1). The phenotypes of the deletion mutants were tested; also phage DNA was prepared and the extent of the deletions determined with respect to targets for restriction enzymes. The results of all the genetic analyses are summarised in Table 3 and Fig. 5a and unequivocally establish the order of genes within the *hsd* region as *hsdR*, *M*, and *S*.

Genetic evidence (section 3a) identifies a promoter for the *M* and *S* genes in λ hsdMS phages. All deletion phages that lack a functional *M* gene fail to express the *hsdS* gene in the prophage, but do so in the lytic state. Three of these deletions, those in phages 1065, Δ 7 and Δ 10, are confined to the bacterial DNA (Fig. 5a). It is concluded that these deletions remove a promoter from which transcription of the *M* and *S* genes can be initiated. Transcription of the *hsdR* gene therefore requires a different promoter and this could be accomplished either by divergent transcription from an overlapping control region, or by transcription in the same direction from a second, separate control region. The latter alternative predicts transcription of all three *hsd* genes in phage 1050 from the early λ promoter P_L whereas with the former only the *hsdM* and *S* genes would be transcribed from P_L , while *hsdR* would be transcribed late from P_R or P'_R . To distinguish these alternatives the direction of transcription of the *hsd* region with respect to λ promoters was determined. The proteins specified by the bacterial DNA in λ hsd phages were examined by labelling with ³⁵S-methionine following infection of UV-irradiated host cells.

e) *Analysis of Polypeptides Coded by λ hsd Phages*

Separation of the labelled polypeptides by electrophoresis through SDS polyacrylamide gels revealed

Table 3. Complementation for *hsd* functions

Plasmid or phage	Bacterial hosts			
	C-1 a <i>hsdR</i> ⁻ <i>M</i> ⁻ <i>S</i> ⁻ <i>r</i> _K ⁻ <i>m</i> _K ⁻	ED8654 <i>hsdR</i> ⁻ <i>r</i> _K ⁻ <i>m</i> _K ⁺	HB101 or 803 <i>hsdS</i> ⁻ <i>r</i> _K ⁻ <i>m</i> _K ⁻	Hfr90 <i>hsdM</i> ⁻ <i>r</i> _K ⁻ <i>m</i> _K ⁻
a) Plasmid				
pRH1			-	+ +
pRH3			-	
pBg3		+ +	-	+ +
pBg4 or 6		- +	-	-
b) Prophage				
642, 655, 1017	- +	- +	+ +	
1048	- +	- +	+ +	+ + ^a
1049	- -	- +	- -	+ + ^a
1065	- -	- +	- -	- -
1050	+ +	+ +	+ +	+ + ^a
ϕ4, 9	- +	- +	+ +	
ϕ5	- -	- +	- -	
ϕ6, 7, 10	- -	- +	- -	
c) Phage (lytic)				
1048		+		+
1049		-		+/-
1065		-		+/-
1017		+		+
ϕ4, 9		+		+
ϕ5		-		-
ϕ6, 7, 10		-		+/-

- and + indicate the restriction and modification phenotypes
+/- indicates partial complementation

Gaps in the Table indicate that tests were not made.

^a *cl*⁺ *imm*²¹ derivatives of 1048 and 1049 and a *cl*⁺ *imm*⁴³⁴ derivative of 1050, were used and λ⁺ as helper to make dilysogens. Curing of the dilysogens resulted in loss of the *r*_K⁺ *m*_K⁺ phenotype. Phages 1048 and 1049, but not 1065, complemented the modification lesion in the *hsdR*⁻ *hsdM*⁻¹⁺ *hsdS*_B strain, WA2710. Phage 1048 but not 1049 provides both K and B specific modification

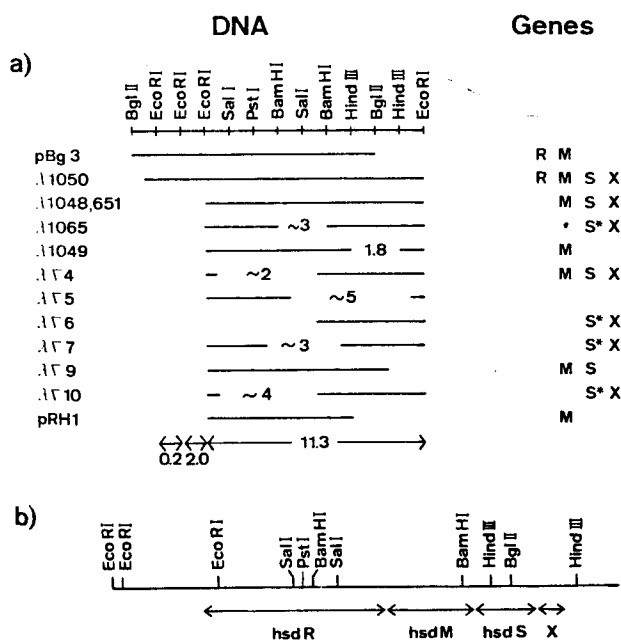


Fig. 5. **a** Analysis of deletion mutants. The bacterial DNA present in a phage, or plasmid, is indicated by a solid line. The ends of in vivo deletions are located between restriction targets; the distances are not to scale. The distances between *EcoRI* targets and the lengths of the deletions are indicated in kb. The functional *hsd* genes in phages or plasmids are listed to the right. X refers to a polypeptide identified in experiments described in Section 3e. This polypeptide has not been shown to affect the host specificity phenotype but is encoded by an adjacent gene. **b** A map drawn to scale indicates the probable locations of the *hsd* genes. This map uses the data presented in Section 3e. The lengths of the genes are calculated on the assumption that the molecular weights of the polypeptides are 130000 for *hsdR*, 63000 for *hsdM*, 50000 for *hsdS*, and 16000 for protein X. The change in molecular weight of the *hsdR* fusion polypeptide from 150000 to 80000 (see Fig. 6) when the DNA between the *BamHI* sites was deleted places at least 1.5 kb of the *hsdR* coding sequence between the *BamHI* sites. The map assumes that the second polypeptide of MW close to 50000 is a degradation product of the *hsdS* polypeptide. * Genetic evidence for *hsdS* is obtained only in the lytic phase when a promoter may be used

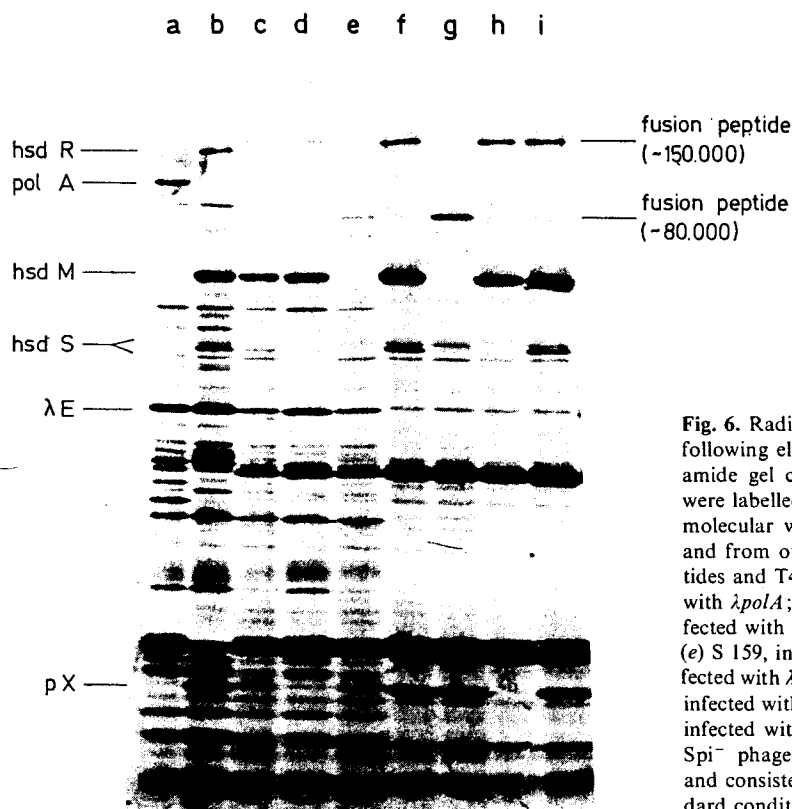


Fig. 6. Radioautographic analysis of the ^{35}S -labelled polypeptides following electrophoresis through a 7%–15% gradient polyacrylamide gel containing sodium dodecyl sulfate. The polypeptides were labelled early (3 to 10 min) after infection. The approximate molecular weights are estimated relative to known polypeptides and from other gels in which labelled bacteriophage T7 polypeptides and T4 DNA ligase were included: Track (a), S159 infected with λpolA ; (b) S159 infected with $\lambda\text{hsdRMS}=1050$; (c) S159 infected with $\lambda\text{hsdMS}=1048$; (d) S159 infected with $\lambda\text{hsdM}=1049$; (e) S159, infected with $\lambda\text{hsdS}=1065$; (f) S159 ($\lambda\text{imm}^{434}\text{supF}$), infected with $\lambda\text{hsdMS NamP}_L^c\text{cIP}_R^{434}=1076$; (g) S159 ($\lambda\text{imm}^{434}\text{supF}$), infected with $\lambda\text{hsdS NamP}_L^c\text{cIP}_R^{434}=1075$; (h) S159 ($\lambda\text{imm}^{434}\text{supF}$), infected with $\lambda\text{hsdM NamP}_L^c\text{cIP}_R^{434}=1074$; (i) as in (f). Note the Spi^- phage 1050 is deleted for phage functions present in 1048 and consistently shows some synthesis of late proteins under standard conditions for labelling early proteins

five polypeptides induced by the bacterial DNA in *hsd* phages. These polypeptides were detected by labelling early after infection with λhsdRMS 1050 (Fig. 6, track b) and four of the five were detected early after infection with phage 1048 (Fig. 6, track c), the fifth being replaced by a new polypeptide (data for phage 642 not shown but identical to that for 1048). In contrast, detection of polypeptides other than those encoded by the vector required late labelling following infection with λhsdMS phages having bacterial DNA in the alternative orientation (Fig. 7, e.g. tracks c and f).

Polypeptides transcribed from the early λ promoter, P_L are shown in Fig. 6. Infection with λhsdMS (1048, track c) induces five polypeptides not found in cells infected with λpolA (track a) and each of them can be associated with inserted bacterial DNA. The largest polypeptide MW 150000, (seen also in tracks d, f, h and i) has a mobility less than that of the λJ protein (see Fig. 7, tracks k and l vs c to j). This polypeptide behaves as expected if it is coded by most of the distal portion of *hsdR* fused to the proximal part of the vector gene, *red*. Hence, when the DNA between the *Bam*HI sites (see Figs. 4 or 5) is deleted, the fusion polypeptide is replaced by a smaller one (MW ~80000) (Fig. 6, 1065, track e and 1075 track g) and when the bacterial DNA

content is extended to include the entire *hsdR* gene (1050), the large fusion polypeptide is replaced by the product of the *hsdR* gene, a polypeptide slightly smaller than the *J* protein (Fig. 6 track b and see Fig. 7 track m). A molecular weight of ~130000 is in good agreement with the figure reported in the literature for the *hsdR* polypeptide of the B restriction endonuclease (Eskin and Linn 1972). As predicted, reversing the orientation of the *Eco*RI fragment in the λhsdMS phage, leads to loss of the large fusion polypeptide irrespective of the time of labelling (Fig. 7, tracks c to j show late labelling, data not shown for early labelling).

Deletion of the DNA between the two *Bam*HI targets not only changes the size of the large fusion polypeptide but is associated with the loss of a prominent polypeptide of MW 62–65000. The mobility of this fragment exceeds that of the product of T7 gene 8. MW, 65000 (Hausmann 1976), but is slower than that of T4 DNA ligase, MW ~59000 (Wilson and Murray 1979) (gels with these markers are not shown). The K restriction enzyme includes a polypeptide of MW ~62000 (Meselson et al. 1972) and our genetic analysis identifies a polypeptide of this size as that encoded by *hsdM* and shown to affect both restriction and modification (Hubacek and Glover 1970). This polypeptide appears to be unstable under some condi-

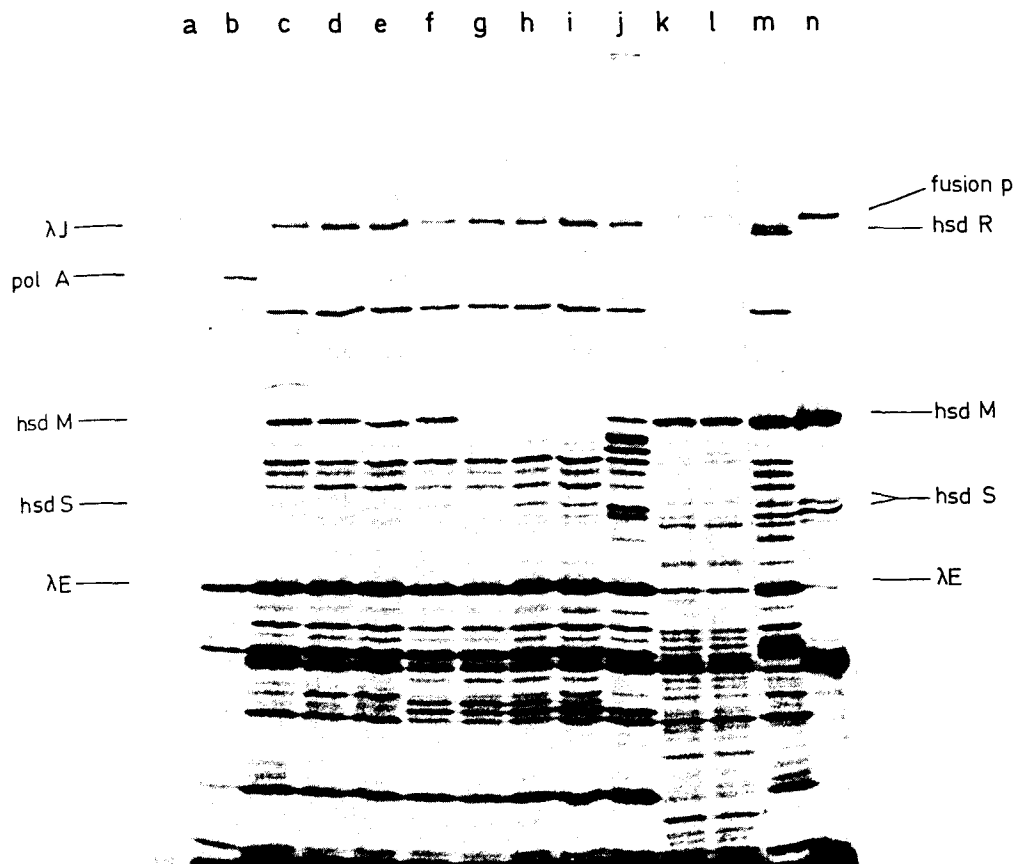


Fig. 7. Radioautographic analysis of the ^{35}S -labelled polypeptides following electrophoresis through a 7%–15% gradient polyacrylamide gel containing sodium dodecyl sulfate. The polypeptides were labelled late (15–25 min) after infection in tracks a–j and early (3–10 min) in tracks k to n. Track (a) S159, no phage (b) S159, *ipolA*; (c) S159, *λhsdMS*, 954; (d) S159, *λhsdM*, 1011; (e) S159, *λhsdMS_B*, 1013; (f) S159, *λhsdMS* V4 (indistinguishable from 1017); (g) S159, *λhsdV5* (h) S159, *λhsdV6*; (i) S159, *λhsdSV10*; (j) S159, *λhsdMSV3**; (k) S159, *λhsdMS* 1048; (l) S159, *λhsdM* 1049; (m) S159, *λhsdRMS* 1050; (n) S159 (*λimm⁴³⁴supF*), *λhsdMS NamP_L cIP_R⁴³⁴* 1074. *13 is not described in this paper; it retains the *hsdM* and *S* genes but has lost the phage genes through *red* to close to *N*. The presence of extra polypeptides and the apparent deficiency of the *hsdM* polypeptide following infection with this *ral*⁻ (Debrouwere et al. 1980) phage have not been investigated. The deletions 3, 4, 5, 6, and 10 were all derived from 1017 (see Table 1)

tions and on occasions has been detected as a doublet of bands.

Examination of a number of gels provided evidence for two polypeptides having mobilities consistent with molecular weights close to 50000. These polypeptides were not very prominent (Fig. 6, track b and c vs d) and they migrated close to other polypeptides; therefore alternative phages (see Wilson and Murray 1979) were used in which rightwards transcription from the λ promoters was blocked while unmoderated transcription from the leftwards λ promoter was maintained. Under these conditions the two bands are readily identified (Fig. 6, tracks f and i) and both are missing when the *λhsd* phage has the 1.8 kb *Hind*III fragment deleted (Fig. 6, track h). One, or both, of these polypeptides is the product of the *hsdS* gene.

The fifth polypeptide (MW less than 20000) iden-

tified as polypeptide X in Fig. 6, is also missing when the 1.8 kb *Hind*III fragment is deleted (tracks d & h). This polypeptide has been shown to be absent following infection with deletion derivatives 5 and 9 (data not shown but see Fig. 5 for characterization of deletions). There is no evidence that polypeptide X is relevant to host specificity since the modification phenotype of the deletion 9 derivative is unchanged (Table 3).

Effective expression of the bacterial genes in the *λhsdRMS* phage (1050) is from P_L and the direction of transcription is from *hsdR* through *M* to *S* (see Fig. 5b). Hence, in addition to the promoter between the *R* and *M* genes a second one upstream of *hsdR* is required.

Polypeptides characteristic of the K-restriction enzyme have been correlated with each of the three *hsd* genes. There remains, however, the complexity

that two polypeptides are apparently associated with the *hsdS* gene. It is difficult, though perhaps not impossible, to find the coding capacity for both polypeptides in the absence of overlapping genes (see Fig. 5b).

Labelling early after infection (see, for example, tracks *m* and *n* in Fig. 7) shows a marked preponderance of the smaller polypeptide while labelling late after infection (Fig. 7, tracks *c*, *f*, *i*, and *j*) an excess of the larger polypeptide. This could imply more efficient processing, or degradation, early after infection. If processing occurs, then in the absence of the *hsdM* polypeptide (Fig. 6, track *g*) the *hsdS* polypeptide is either not processed at all or, following processing, it is degraded. Alternatively, if there are two distinct species of polypeptides with similar molecular weights, the absence of one in track *g* requires that the *Bam*HI deletion (3.1 kb) which removes the *M* gene fuses the *hsdR* gene to the previously unidentified gene while leaving the *hsdS* gene intact. This deletion is probably not large enough since the mobility of the fusion product implies that at least 1.5 kb of the *hsdR* gene is deleted and the *hsdM* gene itself would require more than the remaining 1.6 kb.

f) Comparative Studies of *E. coli* B and C Strains

Preliminary evidence for homology between the *hsd* regions of *E. coli* K and B was obtained from a genetic experiment. A λ *hsd* phage having the S_K gene deleted (1011, Table 1) rescued the B-specificity gene from the *hsd* region of a strain conferring B specificity. λ *hsdMS_B* recombinants were selected after growth in a C strain on infection of an r_B^+ host. However, differences between the DNA of the *hsd* regions were detected since the bacterial DNA in the λ *hsdMS_B* phage included no *Hind*III targets but an extra target for *Eco*RI (data not shown).

The polypeptides labelled after infection with a

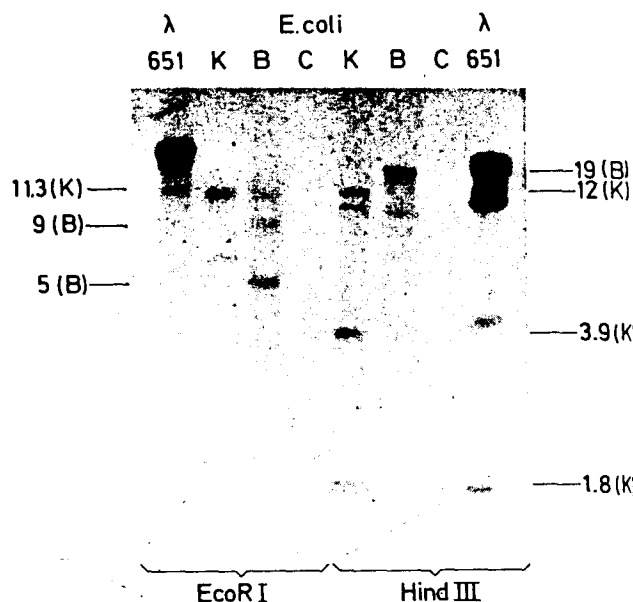


Fig. 8. Analysis of *E. coli* DNA fragments that cross-hybridize to a λ *hsdMS* probe. The DNA fragments were separated by electrophoresis through 1% agarose gel. The outer tracks are digests of the λ *hsdMS* phage used to make the labelled probe. The sizes of the DNA fragments that cross-hybridize to the *hsd* genes are given in kb - *Eco*RI fragments to the left, *Hind*III fragments to the right. K or B identifies the *E. coli* strain from which the DNA fragments were isolated. The sizes of other fragments that cross-hybridize to the λ vector DNA (Kaiser and Murray, 1979) are not given.

λ *hsdMS_B* phage (1013, see Table 1) are shown in Fig. 7 (track e). The mobilities of both the *hsdM* and *hsdS* polypeptides are slightly greater than the *M* and *S* polypeptides determined by the λ *hsd_K* phage.

Clones including the *hsd* genes were used as probes to detect homologous sequences in the DNA of *E. coli* B and C strains. An experiment using a λ *hsdMS* phage is shown in Fig. 8. In this experiment additional homologies between the vector and the *E. coli* chromosomes were detected (see Kaiser and Murray 1979)

Table 4. Homologous DNA sequences identified in *E. coli* strains

Probe	<i>E. coli</i> DNA					
	<i>Eco</i> RI			<i>Hind</i> III		
	K	B	C	K	B	C
λ <i>hsdMS</i>	11.3	9; 5	—	12; 1.8; 3.9	19	—
pRH3	11.3	9	—	1.8	19	—
pBg3	11.3; 2; 6 ^a	5; 2	5	12; 1.8	19	6
pBg4 (or 6)	11.3; 4.3; 0.9	9	5	1.8; 3.9; 5.5	19	7.5

The sizes of the DNA fragments that cross-hybridize to the *E. coli* K12 *hsd* genes and their flanking DNA sequences are given in kb; *Eco*RI fragments to the left, *Hind*III fragments to the right. K, B or C identifies the *E. coli* strain from which the DNA was isolated. Fragment sizes are approximate except those italicized which have been analysed in λ or plasmid derivatives

^a The small (0.2 kb) fragment identified in pBg3 DNA is too small to be detected in this experiment

but the absence of homology in the C strain is striking. Data from experiments using different plasmid probes are summarized in Table 4. Both of the *Hind*III sites in the region of the *hsdS* gene of *E. coli* K are missing in *E. coli* B and a new *Eco*RI site appears. This is in agreement with the analysis of the DNA of the λ *hsdMS_B* phage. While no evidence exists for even remnants of the *hsd* genes in *E. coli* C there is much cross hybridization between the K and B *hsd* genes.

4. Discussion

Polypeptides of sizes in keeping with those reported for purified K-restriction endonuclease have been correlated with each of the three *hsd* genes. The smallest of the three polypeptides is the product of the *hsdS* gene. The three *hsdK* genes in an *E. coli* C strain confer a K-specificity phenotype. While the identity of the three polypeptides encoded by the *hsd* genes with those in K-restriction endonuclease has not been established directly, we prefer this interpretation to the alternative that the smallest polypeptide might be the *rho* protein (Debrouwere et al. 1980). The latter suggestion was made to accommodate the anti-restriction role of the λ gene *ral* via its property of counteracting the host *rho* protein.

The apparent variability in the mobility of the *hsdS* polypeptide and hence the possibility of processing, or degradation perhaps by a phage product, remains to be investigated.

A fourth polypeptide, X, encoded by DNA close to, and downstream of *hsdS*, is not a prerequisite for functional expression of the *hsdM* and *S* genes in *E. coli* C. Currently, no information implicates this gene product in the phenomenon of host-specificity but a regulatory role is not ruled out.

λ *hsdMS* phages include a promoter for the *hsdM* and *S* genes. This promoter is removed by all those deletions so far shown to inactivate the *hsdM* gene. Transcription of the three *hsd* genes is from *hsdR* through *M* and *S*; hence a second promoter located upstream of *hsdR*, and present in the λ *hsdRMS* phage is identified. This organization could allow differential control in the production of modification and restriction activities. Only the *hsdM* and *S* polypeptides are essential for the methylase. The possibility of a sequential expression of modification and restriction complexes may now be investigated. However, on the assumption that restriction and modification result from the same polypeptide complex and that the preferred activity is dictated by the nature of the available DNA substrate (Vovis et al. 1974), control of this sort would only be advantageous when *hsd*

genes are transferred to a different bacterial strain. Modification activity determined by this phage is expressed before its associated restriction enzyme (Arber 1974). Experiments involving conjugational transfer of the genetic determinants for K-specific restriction and modification have suggested a similar sequential control (Glover and Colson 1969).

Preliminary cross-hybridization studies did not detect even the remnants of *hsd* genes in *E. coli* C but, as expected, demonstrated much homology between the allelic K and B regions. It seems probable that the *M* and *R* genes will share extensive homology and divergence in the *S* gene should reflect the different specificity of sequence recognition. The extent of the homology retained in the *S* genes will be revealed by sequence analysis. Other *E. coli* strains and even closely related species of bacteria may have similar host specificity systems. Indeed, on the basis of genetic tests, Bullas et al. (1980) have identified several allelic host specificity systems in *Salmonella* strains. The cloned *hsd* genes provide probes to screen for the homologous DNA predicted in allelic *hsd* genes.

Acknowledgements. We are indebted to W. Arber and J. Hubacek for providing bacterial strains and to our colleagues for enzymes and helpful interest, particularly A. Newman and R. Hayward for advice on polyacrylamide gels, K. Kaiser and K. Murray for critical reading of the manuscript, and G.R. Brown for technical assistance. The work was supported by the Medical Research Council and by a grant to B.S. from The Wellcome Foundation.

References

- Appleyard RK (1954) Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. *Genetics* 39:440-459
- Appleyard RK, McGregor JF, Baird KM (1956) Mutations to extended host range and the occurrence of phenotype mixing in temperate coliphage λ . *Virology* 2:565-574
- Arber W (1971) Host-controlled Variation. In: Hershey AD (ed) *The bacteriophage lambda*. Cold Spring Harbor Laboratories, New York, p 83
- Arber W, Linn S (1969) DNA modification and restriction. *Annu Rev Biochem* 38:467-500
- Arber W (1974) DNA modification and restriction. *Prog in Nucleic Acid Res* 14:1-37
- Benton WD, Davis RW (1977) Screening λ gt recombinant clones by hybridization to single plaques *in situ*. *Science* 196:180-182
- Bertani G, Weigle J (1953) Host controlled variation in bacterial viruses. *J Bacteriol* 65:113-121
- Bolivar F, Rodriguez RL, Greene PJ, Betlach MC, Boyer HW, Cross JH, Falkow S (1977) Construction and characterization of new cloning vehicles II. A multiple cloning system. *Gene* 22:75-93
- Borck K, Beggs JD, Brammar WJ, Hopkins AS, Murray NE (1976) The construction *in vitro* of transducing derivatives of phage lambda. *Mol Gen Genet* 146:199-207
- Boyer HW, Roulland-Dussoix D (1969) A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J Mol Biol* 41:459-472

- Bullas LR, Colson C, Neufeld R (1980) Deoxyribonucleic acid restriction and modification systems in *Salmonella*: Chromosomally located systems of different serotypes. *J Bacteriol* 141:275-292
- Clewell D, Helinski DR (1969) Supercoiled circular DNA-protein complex in *E. coli*. Purification and induced conversion to an open circular DNA form. *Proc Natl Acad Sci USA* 62:1159-1166
- Debrouwere L, Van Montagu M, Schell J (1980) The *ral* gene of phage λ III. Interference with *E. coli* ATP dependent functions. *Mol Gen Geñet* 179:81-88
- Denhardt DT (1966) A membrane-filter technique for the detection of complementary DNA. *Biochem Biophys Res Commun* 23:641-646
- Eskin B, Linn S (1972) The deoxyribonucleic acid modification and restriction enzymes of *Escherichia coli* B II. Purification, subunit structure, and catalytic properties of the restriction endonuclease. *J Biol Chem* 247:6183-6191
- Georgopoulos CP (1971) A bacterial mutation affecting N function. In: Hershey AD (ed) *The bacteriophage lambda*. Cold Spring Harbor Laboratories, New York, p 639
- Glover SW (1970) Functional analysis of host-specificity mutants in *Escherichia coli*. *Genet Res Camb* 15:237-250
- Glover SW, Colson C (1969) Genetics of host-controlled restriction and modification in *Escherichia coli*. *Genet Res* 13:227-240
- Grunstein M, Hogness DS (1975) Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. *Proc Natl Acad Sci USA* 72:3961-3965
- Hausmann R (1976) Bacteriophage T7 Genetics. *Curr Top Microbiol Immunol* 75:77-110
- Horiuchi K, Zinder ND (1972) Cleavage of bacteriophage ϕ 1 DNA by the restriction enzyme of *Escherichia coli* B. *Proc Natl Acad Sci USA* 69:3220-3224
- Hubacek J, Glover SW (1970) Complementation analysis of temperature sensitive host specificity mutations in *Escherichia coli*. *J Mol Biol* 50:111-127
- Jaskunas SR, Lindahl L, Nomura M, Burgess RR (1975) Identification of two copies of the genes for the elongation factor EF-Tu in *E. coli*. *Nature* 257:458-462
- Kaiser AD, Hogness DS (1960) The transformation of *Escherichia coli* with deoxyribonucleic Acid isolated from bacteriophage λ dg J *Mol Biol* 2:395-415
- Kaiser K, Murray NE (1979) Physical characterisation of the "Rac prophage" in *E. coli* K12. *Mol Gen Genet* 175:159-174
- Kan NC, Lautenberger JA, Edgell MH, Hutchison CA III. The nucleotide sequence recognized by the *Escherichia coli* K12 restriction and modification enzymes. *J Mol Biol* 130:191-210
- Klein B, Murray K (1979) Phage lambda receptor chromosomes for DNA fragments made with restriction endonuclease BamHI. *J Mol Biol* 133:289-294
- Kühnlein U, Arber W (1972) Host specificity of DNA produced by *Escherichia coli*. XV. The role of nucleotide methylation in *in vitro* B-specific modification. *J Mol Biol* 63:9-19
- Lautenberger JA, Linn S (1972) The deoxyribonucleic acid modification and restriction enzymes of *Escherichia coli* B. I. Purification, subunit structure, and catalytic properties of the modification methylase. *J Biol Chem* 247:6176-6182
- Lautenberger JA, Kan NC, Lackey D, Linn S, Edgell MH, Hutchinson III CA (1978) Recognition site of *Escherichia coli* B restriction enzyme on ϕ XsBI and Simian virus 40 DNAs: An interrupted sequence. *Proc Natl Acad Sci USA* 75:2271-2275
- Lederberg EM, Cohen SN (1974) Transformation of *S. typhimurium* by plasmid deoxyribonucleic acid. *J Bacteriol* 119:1072-1074
- Maniatis T, Jeffrey A, Kleid DG (1975a) Nucleotide sequence of the rightward operator of phage λ . *Proc Natl Acad Sci USA* 72:1184-1188
- Maniatis T, Jeffrey A, Van der Sande H (1975b) Chain length determination of small double strand and single strand DNA molecules by polyacrylamide gel electrophoresis. *Biochemistry* 14:3787-3794
- Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* 3:208-218
- Meselson M, Yuan R, Heywood J (1972) Restriction and modification of DNA. *Annu Rev Biochem* 41:447-466
- Murray NE, Batten PL, Murray K (1973a) Restriction of bacteriophage λ by *Escherichia coli* K. *J Mol Biol* 81:395-407
- Murray NE, Brammar WJ, Murray K (1977) Lambdoid phages that simplify the recovery of *in vitro* recombinants. *Mol Gen Genet* 150:53-61
- Murray NE, Bruce SA, Murray K (1979) Molecular cloning of the DNA ligase gene from bacteriophage T4. II. Amplification and preparation of the gene product. *J Mol Biol* 132:493-505
- Murray NE, Manduca de Ritis P, Foster LA (1973b) DNA targets for the *Escherichia coli* K restriction system analyzed genetically in recombinants between phages phi 80 and lambda. *Mol Gen Genet* 120:261-281
- Murray NE, Murray K (1974) Manipulation of restriction targets in phage λ to form receptor chromosomes for DNA fragments. *Nature* 251:476-481
- Parkinson JS, Huskey RJ (1971) Deletion mutants of bacteriophage lambda. I. Isolation and initial characterization. *J Mol Biol* 56:369-384
- Ravetch JV, Horiuchi K, Zinder ND (1978) Nucleotide sequence of the recognition site for the restriction-modification enzyme of *Escherichia coli* B. *Proc Natl Acad Sci USA* 75:2266-2270
- Sharp PA, Sugden B, Sambrook J (1973) Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using analytical agarose ethidium bromide electrophoresis. *Biochemistry* 12:3055-3063
- Smith J, Arber W, Kühnlein U (1972) Host specificity of DNA produced by *E. coli*. XIV. The role of nucleotide methylation in *in vivo* B-specific modification. *J Mol Biol* 63:1-8
- Sommer R, Schaller H (1979) Nucleotide sequence of the recognition site of the B-specific modification system in *E. coli*. *Mol Gen Genet* 168:331-335
- Southern E (1975) Detection of specific sequence among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503-518
- Voivis GF, Horiuchi K, Zinder N (1974) Kinetics of methylation of DNA by a restriction endonuclease from *Escherichia coli* B. *Proc Natl Acad Sci USA* 71:3810-3813
- Weigle J (1966) Assembly of phage lambda *in vitro*. *Proc Natl Acad Sci USA* 55:1462-1466
- Weil J, Signer ER (1968) Recombination in bacteriophage λ : II. Site-specific recombination promoted by the integration system. *J Mol Biol* 34:273-279
- Wilson GG, Murray NE (1979) Molecular cloning of the DNA ligase gene from bacteriophage T4 I. Characterization of the recombinants. *J Mol Biol* 132:471-491
- Wilson GG, Tanyashin VI, Murray NE (1977) Molecular cloning of fragments of bacteriophage T4 DNA. *Mol Gen Genet* 156:203-214
- Wood WB (1966) Host specificity of DNA produced by *E. coli*: Bacterial mutations affecting the restriction and modification of DNA. *J Mol Biol* 16:118-133
- Zissler J, Signer ER, Schaefer F (1971) The role of recombination in growth of bacteriophage lambda. In: Hershey AD (ed) *The bacteriophage lambda*. Cold Spring Harbor Laboratories, New York, p 455

Communicated by W. Arber

Received April 26 / May 21, 1980