The Isolation and Properties of Non-restricting Mutants of Two Different Host Specificities Associated with Drug Resistance Factors

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Non-restricting (r^-) mutants of two different host specificities carried by resistance transfer factors have been isolated. As previously found with other host specificities, the non-restricting mutants were of two phenotypes: those that retained the ability to modify DNA, and those which had lost the ability to modify DNA. These mutants were tested for complementation with wild-type host specificities carried either on the *Escherichia coli* chromosome, on resistance transfer factors, or on the phage P1. No complementation was observed and possible explanations for this finding are considered.

INTRODUCTION

The host range of some bacteriophages can be altered as a result of a single cycle of growth in a new host strain, and this alteration is termed 'host controlled modification' (Luria, 1953; Arber & Dussoix, 1962). In *Escherichia coli* strains K12 and B, afterwards called K and B, two distinct host specificity functions, restriction and modification, have been recognized which operate with the same specificity. Restriction involves cleavage of at least one strand of the non-modified phage DNA (Meselson & Yuan, 1968). Modification is specifically associated with the phage DNA also, and protects the phage from this restriction. In the case of the male specific phage fd, modification involves methylation of adenine residues in the DNA (Arber, 1968).

The chromosomal genes determining these two phenotypic characters have been mapped close to *thr* and *serB* in *Escherichia coli*, both by bacterial conjugation and by co-transduction with these markers by phage P1, and the strain specificities K, B and 15 were shown to be allelic (Boyer, 1964; Colson, Glover, Symonds & Stacey, 1965; Hoekstra & De Haan, 1965; Lederberg, 1966; Wood, 1966; Glover & Colson, 1969). However, the elucidation of the genetic fine structure of the host specificity genes has not been feasible, mainly due to the lack of quantitative techniques for selecting clones of different restriction or modification phenotypes. Information concerning the number and function of these genes has come from study of the phenotypes of non-restricting mutants. The non-restricting (r^-) mutants found have been of two phenotypes, those retaining modification ability (m^+), and those which were also defective in modification (m^-) (Colson *et al.* 1965; Wood, 1965, 1966; Lederberg,

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1966). The existence of two phenotypes for non-restricting mutants suggested that the genetic determinants for host specificity involved a minimum of two genes. Confirmation of this hypothesis has been obtained as a result of complementation studies in partial diploids constructed with F prime factors. These studies have revealed that at least two genes are involved in restriction; that one of these genes, together with a third, is required for modification; and a fourth gene has been postulated (Boyer & Roulland-Dussoix, 1969; Glover, unpublished results).

Two host specificities, *hsI* and *hsII* (Bannister & Glover, 1968), are associated with the drug-resistance factors (R factors) responsible for the transmissible resistance to antibiotics in Enterobacteriaceae (Watanabe, 1963; Meynell, Meynell & Datta, 1968). In this paper we present a genetic analysis of the determinants involved in the control of these host specificities, based upon exploration of the types of restrictionless mutants which can be obtained, and the behaviour of these mutants in complementation tests.

METHODS

Media. Oxoid no. 2 broth: Oxoid no. 2 broth powder 25 g.; distilled water 1.0 l. Oxoid agar: Oxoid no. 2 broth solidified with 12.5 g./l. Davis New Zealand agar. VB agar: VB salts (Vogel & Bonner, 1956) solidified with 15 g./l. Davis New Zealand agar. Water top agar: Difco Bacto agar 6 g.; distilled water 1.0 l. Difco agar: Oxoid tryptone 10 g.; NaCl 8 g.; glucose 1 g.; Difco Bacto agar 10 g.; distilled water 1.0 l. Sugars used at a final concentration of 2 % (w/v). Tetracycline used at a final concentration of 50 µg./ml. Streptomycin used at 20 µg./ml. Amino acids used at 20 µg./ml.

Bacteriophages: phage λ (Lederberg & Lederberg, 1953) and its virulent mutant, λ_{vir} (Jacob & Wollman, 1954).

Bacterial strains. The strains of *Escherichia coli* used are listed in Table 1.

Table 1. *Bacterial strains*

Strain	Characteristics	Reference
c600	<i>thr leu thi lac hsK</i>	Appleyard, 1954
c600 (P1)	P1 lysogen of c600	Glover, Schell, Symonds & Stacey, 1963
c600 (λ c1857)	λ c1857 lysogen of c600	Sussman & Jacob, 1962
J5-3	<i>pro met hsK</i> (λ)	Clowes & Rowley, 1954
4K	<i>thr leu thi serB lac r⁻m⁻K</i>	Colson <i>et al.</i> 1965
7K	<i>thr leu thi r⁻m⁺K</i>	Colson <i>et al.</i> 1965
15	<i>thi thy hs15</i>	Stacey, 1965
c	<i>thr</i>	Glover & Colson, 1969
B	<i>trp hsB</i>	Glover & Colson, 1969
B6	<i>thr leu ilvA r⁻m⁺B</i>	Glover & Colson, 1969
B8	<i>thr leu ilvA r⁻m⁻B</i>	Glover & Colson, 1969

R factors. R124 *fi*⁺ and carrying resistance to tetracycline; R132 *fi*⁻ and carrying resistance to streptomycin and sulphonamide; R199 *fi*⁻ and carrying resistance to tetracycline; R313-T-1 *fi*⁻ and carrying resistance to tetracycline. R124 carries *hsI*, the other three R factors carry *hsII* (*hsI* and *hsII* define the host specificities determined by these R factors (Bannister, 1969)). The R factors were the gift of Dr Elinor Meynell and Dr Naomi Datta.

Techniques

Overnight cultures of bacteria were obtained by inoculating 5 ml. of Oxoid no. 2 broth from a single colony and incubating at 37°. Efficiency of plating (e.o.p.) is the ratio of the plaque count on the test strain to the plaque count on a permissive strain. A quick test for restriction was employed in the isolation of restrictionless mutants. Colonies were streaked on Difco agar plates and spotted with approximately 0.01 ml. of dilutions of phage λ vir.

Transfer of R factors

An overnight culture of the donor strain was diluted 1/10 into fresh broth, and incubated on an inclined rotor at 37° for 1½ hr. This culture was diluted 1/10 into an overnight culture of recipient and incubated at 37° for 30 min., then streaked out on VB agar selective for R⁺ clones of the recipient, and incubated at 37° for 24 to 36 hr. Colonies were purified by two serial single-colony isolations, and tested for all the characters of the recipient and the R factor.

RESULTS

Spontaneous restrictionless mutants of hsII

Routinely, R⁺ strains were grown overnight from inocula taken from single colonies only. On two separate occasions, the R⁺ colony used as an inoculum was found to be a non-restricting mutant, retaining modification ability (i.e. r⁻m⁺). These mutants were obtained from two different R factors, R199 and R313-T-1, and the two mutant R factors were termed R199-1 and R313-1 respectively.

Restrictionless mutants produced by ethylmethane sulphonate mutagenesis

Although two spontaneous mutants of the hsII host specificity had arisen, screening several hundred single colonies of strain J5-3 carrying R313-T-1 hsII, and of J5-3 R124 hsI, failed to reveal any other restrictionless mutants. To obtain additional mutants of both hsI and hsII, mutagenesis with ethylmethane sulphonate was used, and the mutagenesis was followed by an enrichment procedure developed by Hubaček & Glover (in preparation). This procedure relies on the fact that λ .K can lysogenize restrictionless mutants of hsI or hsII efficiently and cannot readily lysogenize wild-type restricting bacteria. The mutagenesis was performed in R⁺ strains lysogenic for the heat-inducible phage λ c1857, and after infection with wild-type λ , the phage was induced by exposure to 42°. Restrictionless mutants which had become lysogenic for wild-type λ were protected against induction, while the restricting bacteria were lysed. The results of five separate experiments, showing the proportion of non-restricting mutants found after enrichment and the phenotypes of the mutants, are presented in Table 2. The e.o.p. of λ .K on hsI⁺ strains is about 10⁻⁴, whereas the e.o.p. of λ .K on hsII⁺ strains is only 10⁻², and this difference in the restriction of λ .K by the two host specificities may account for the greater yield of restrictionless mutants from the strain carrying R124. Two phenotypes, r⁻m⁻ and r⁻m⁺, were observed, and for hsI, where the total number of mutants scored is higher, these two phenotypes occur in roughly equal proportions.

The uniqueness of hsI and $hsII$

It is clear from the results presented in Tables 3 to 6 that the host specificities hsI and $hsII$ are not identical with any of the host specificities previously described. A strain carrying hsI (or $hsII$) restricts phage λ provided the phage λ was not grown on a strain carrying hsI (or $hsII$). Likewise, phage λ grown on a strain of *E. coli* C or an r^-m^- mutant of *E. coli* K or B carrying hsI (or $hsII$) is restricted by *E. coli* K, B, 15 and K (P1).

Table 2. *The production of restrictionless mutants of R factor-carried host specificities*

Overnight cultures of R^+ bacteria were diluted 1/10 into fresh Oxoid no. 2 broth, incubated for 1½ hr, harvested by centrifugation and resuspended in 1/10 the original volume of M9 buffer containing 0.02 M-ethylmethane sulphonate. After 60 min. the cultures were diluted 1/50 with M9 buffer and centrifuged. The pellet was resuspended in half the centrifugation volume, in Oxoid no. 2 broth, and incubated overnight. The cultures were now diluted 1/10 in fresh broth, and aerated for c. 2 hr, followed by a further 1/2 dilution with Oxoid no. 2 broth containing M/100 Mg^{2+} . Wild-type phage λ .K was added, and after 30 min. the culture was transferred to 42° and aerated for 60 min. Appropriate dilutions were made and poured in soft agar overlays on VB supplemented plates, and incubated for 48 hr before picking and testing colonies. Unless otherwise stated, incubation was at 30°.

R factor	No. of colonies tested	No. of r^-m^+	No. of r^-m^-
R 124	40	13	14
R 124	50	7	14
R 132-SS-I	58	3	5
R 313-T-I	40	1	0
R 313-T-I	30	1	0

Table 3. *Efficiency of plating of phage λvir grown on Escherichia coli K, B, C, BRI and CRI*

Host strain*	Phage†					
	λ . K	λ . KRI	λ . B	λ . BRI	λ . C	λ . CRI
K	1.0	1.0	5×10^{-4}	1×10^{-3}	4×10^{-4}	1×10^{-4}
KRI	4×10^{-4}	1.0	7×10^{-5}	5×10^{-3}	4×10^{-4}	6×10^{-4}
KRII	2×10^{-2}	2×10^{-2}	7×10^{-5}	1×10^{-4}	2×10^{-4}	1×10^{-4}
B	2×10^{-4}	5×10^{-5}	1.0	1.0	1×10^{-4}	5×10^{-4}
BRI	2×10^{-4}	1×10^{-3}	7×10^{-5}	1.0	1×10^{-5}	2×10^{-4}
BRII	7×10^{-4}	6×10^{-3}	2×10^{-3}	6×10^{-3}	1×10^{-2}	7×10^{-4}
C	1.0	1.0	1.0	1.0	1.0	1.0
CRI	5×10^{-6}	1.0	7×10^{-6}	1.0	2×10^{-5}	1.0
CRII	4×10^{-3}	1×10^{-2}	1×10^{-4}	1×10^{-4}	1×10^{-2}	1×10^{-2}

* K is C600, B is Btrp, C is Cthr, RI is R 124, RII is R 313-T-1.

† λ . K is phage grown on strain K; λ . KRI is phage grown on strain KRI, etc.

Complementation studies

Complementation was tested by transferring the R factor, or an *hs* mutant R factor, to strains carrying the other host specificities, and examining the restriction and modification properties of the resulting strains, with λvir . Controls showing the e.o.p. of variously modified λvir on R^+ and R^- strains are contained in Tables 3 to 6,

the phenotypes of R⁺ strains constructed with *hsI* and *hsII* r⁻ mutants are recorded in Tables 7 to 9. Although the wild-type host specificities are always expressed, no complementation of the restrictionless mutants by the wild-type host specificity genes was observed. Restriction was not restored to any *hsI* or *hsII* r⁻ mutant in the

Table 4. *Efficiency of plating of phage λvir grown on Escherichia coli K, B, C, KR II, BR II and CR II*

Host strain	Phage					
	λ. K	λ. KR II	λ. B	λ. BR II	λ. C	λ. CR II
K	1.0	1.0	5 × 10 ⁻⁴	5 × 10 ⁻⁴	4 × 10 ⁻⁴	1 × 10 ⁻⁴
KRI	4 × 10 ⁻⁴	1 × 10 ⁻³	7 × 10 ⁻⁵	5 × 10 ⁻⁵	4 × 10 ⁻⁴	2 × 10 ⁻⁵
KRII	2 × 10 ⁻²	1.0	7 × 10 ⁻⁵	7 × 10 ⁻³	2 × 10 ⁻⁴	1 × 10 ⁻³
B	2 × 10 ⁻⁴	5 × 10 ⁻⁵	1.0	1.0	1 × 10 ⁻⁴	2 × 10 ⁻⁴
BRI	2 × 10 ⁻⁴	7 × 10 ⁻⁵	7 × 10 ⁻⁵	7 × 10 ⁻⁴	1 × 10 ⁻⁵	1 × 10 ⁻⁴
BRII	7 × 10 ⁻⁴	1 × 10 ⁻²	2 × 10 ⁻³	1.0	1 × 10 ⁻²	7 × 10 ⁻²
C	1.0	1.0	1.0	1.0	1.0	1.0
CRI	5 × 10 ⁻⁶	1 × 10 ⁻⁴	7 × 10 ⁻⁶	2 × 10 ⁻⁵	2 × 10 ⁻⁵	5 × 10 ⁻⁵
CRII	4 × 10 ⁻³	1.0	1 × 10 ⁻⁴	1.0	1 × 10 ⁻²	1.0

K is C600, B is *btrp*, C is *cthr*, RI is R 124, RII is R 313-T-1.

Table 5. *Efficiency of plating of phage λvir grown on Escherichia coli K, KRI, KRII and K(P I)*

Host strain	Phage			
	λ. K	λ. KRI	λ. KRII	λ. K(P I)
K	1.0	1.0	1.0	1.0
KRI	4 × 10 ⁻⁴	1.0	1 × 10 ⁻³	3 × 10 ⁻⁴
KRII	2 × 10 ⁻²	2 × 10 ⁻²	1.0	5 × 10 ⁻²
K(P I)	1 × 10 ⁻⁴	1 × 10 ⁻⁴	1 × 10 ⁻⁴	1.0

K is C600, RI is R 124, and RII is R 313-T-1.

Table 6. *Efficiency of plating of phage λvir grown on Escherichia coli 15, 15RI, 15RII, C, CRI and CRII*

Host strain	Phage					
	λ. 15	λ. 15RI	λ. 15RII	λ. C	λ. CRI	λ. CRII
15	1.0	1.0	1.0	3 × 10 ⁻²	5 × 10 ⁻²	1 × 10 ⁻²
15RI	1 × 10 ⁻²	1.0	1 × 10 ⁻²	1 × 10 ⁻³	1 × 10 ⁻²	1 × 10 ⁻⁴
15RII	1 × 10 ⁻²	1 × 10 ⁻²	1.0	1 × 10 ⁻²	1 × 10 ⁻²	1 × 10 ⁻²
C	1.0	1.0	1.0	1.0	1.0	1.0
CRI	1 × 10 ⁻³	1.0	1 × 10 ⁻⁵	5 × 10 ⁻⁴	1.0	1 × 10 ⁻⁴
CRII	1 × 10 ⁻²	1 × 10 ⁻²	1.0	1 × 10 ⁻²	1 × 10 ⁻²	1.0

RI is R 124, RII is R 313-T-1.

presence of intact K, B or P I host specificities (Table 7), or to *hsI* mutants in the presence of intact *hsII* (Table 9), or to r⁻ mutants of K and B by intact *hsI* and *hsII* (Table 8). Nor is modification restored to any r⁻m⁻ mutants, although the m⁺ phenotype of r⁻m⁺ mutants is expressed (Tables 7 to 9).

Table 7. Phenotypes displayed by R^+ strains carrying restrictionless mutants of $R124$, $R313$ -T-I, and $R132$

R factor	Host-specificity phenotype of R factor	Host specificity phenotype of the R^+ strain when the host strain is		
		K^*	B	$K(P1)$
R 124	$r_I^+ m_I^+$	$r_I^+ m_I^+ r_K^+ m_K^+$	$r_I^+ m_I^+ r_B^+ m_B^+$	$r_I^+ m_I^+ r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 124-1	$r_I^- m_I^+$	$r_I^- m_I^+ r_K^+ m_K^+$	$r_I^- m_I^+ r_B^+ m_B^+$	$r_I^- m_I^+ r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 124-2	$r_I^- m_I^-$	$r_I^- m_I^- r_K^+ m_K^+$	$r_I^- m_I^- r_B^+ m_B^+$	$r_I^- m_I^- r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 124-3	$r_I^- m_I^+$	$r_I^- m_I^+ r_K^+ m_K^+$	$r_I^- m_I^+ r_B^+ m_B^+$	$r_I^- m_I^+ r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 313-T-1	$r_{II}^+ m_{II}^+$	$r_{II}^+ m_{II}^+ r_K^+ m_K^+$	$r_{II}^+ m_{II}^+ r_B^+ m_B^+$	$r_{II}^+ m_{II}^+ r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 313-1	$r_{II}^- m_{II}^+$	$r_{II}^- m_{II}^+ r_K^+ m_K^+$	$r_{II}^- m_{II}^+ r_B^+ m_B^+$	$r_{II}^- m_{II}^+ r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 313-2	$r_{II}^- m_{II}^-$	$r_{II}^- m_{II}^- r_K^+ m_K^+$	$r_{II}^- m_{II}^- r_B^+ m_B^+$	$r_{II}^- m_{II}^- r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 313-3	$r_{II}^- m_{II}^+$	$r_{II}^- m_{II}^+ r_K^+ m_K^+$	$r_{II}^- m_{II}^+ r_B^+ m_B^+$	$r_{II}^- m_{II}^+ r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 132-1	$r_{II}^- m_{II}^+$	$r_{II}^- m_{II}^+ r_K^+ m_K^+$	$r_{II}^- m_{II}^+ r_B^+ m_B^+$	$r_{II}^- m_{II}^+ r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 132-2	$r_{II}^- m_{II}^-$	$r_{II}^- m_{II}^- r_K^+ m_K^+$	$r_{II}^- m_{II}^- r_B^+ m_B^+$	$r_{II}^- m_{II}^- r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$
R 199-1	$r_{II}^- m_{II}^+$	$r_{II}^- m_{II}^+ r_K^+ m_K^+$	$r_{II}^- m_{II}^+ r_B^+ m_B^+$	$r_{II}^- m_{II}^+ r_K^+ m_K^+ r_{P1}^+ m_{P1}^+$

* K strains used were C600 and J5-3.

The restriction phenotype was determined by measuring the efficiency of plating of variously modified phage λvir on the test strain, and the modification phenotype was determined by measuring the efficiency of plating on standard strains of the phage λvir grown on the test strain.

Table 8. Phenotypes displayed by restrictionless mutants of *Escherichia coli* K and B carrying $R124$ or $R313$ -T-I

Strain	Phenotype of R^- strain	Phenotype of R^+ carrying	
		$R124 (r_I^+ m_I^+)$	$R313$ -T-I ($r_{II}^+ m_{II}^+$)
C600	$r_K^+ m_K^+$	$r_K^+ m_K^+ r_I^+ m_I^+$	$r_K^+ m_K^+ r_{II}^+ m_{II}^+$
7K	$r_K^- m_K^+$	$r_K^- m_K^+ r_I^+ m_I^+$	$r_K^- m_K^+ r_{II}^+ m_{II}^+$
4K	$r_K^- m_K^-$	$r_K^- m_K^- r_I^+ m_I^+$	$r_K^- m_K^- r_{II}^+ m_{II}^+$
B	$r_B^+ m_B^+$	$r_B^+ m_B^+ r_I^+ m_I^+$	$r_B^+ m_B^+ r_{II}^+ m_{II}^+$
B6	$r_B^- m_B^+$	$r_B^- m_B^+ r_I^+ m_I^+$	$r_B^- m_B^+ r_{II}^+ m_{II}^+$
B8	$r_B^- m_B^-$	$r_B^- m_B^- r_I^+ m_I^+$	$r_B^- m_B^- r_{II}^+ m_{II}^+$

Table 9. Phenotypes of C600 $R132$ strains also carrying $R124$ or a restrictionless mutant or $R124$

Strain	Host-specificity phenotype		
	$r_K^+ m_K^+$	$r_{II}^+ m_{II}^+$	$r_I^+ m_I^+$
C600 $R132$ $R124$	$r_K^+ m_K^+$	$r_{II}^+ m_{II}^+$	$r_I^+ m_I^+$
C600 $R132$ $R124$ -1	$r_K^+ m_K^+$	$r_{II}^+ m_{II}^+$	$r_I^- m_I^+$
C600 $R132$ $R124$ -2	$r_K^+ m_K^+$	$r_{II}^+ m_{II}^+$	$r_I^- m_I^-$
C600 $R132$ $R124$ -3	$r_K^+ m_K^+$	$r_{II}^+ m_{II}^+$	$r_I^- m_I^+$

DISCUSSION

The absence of efficient quantitative techniques for selecting different restriction or modification phenotypes seriously limits genetic fine structure analysis of host specificities. Although crude mapping and establishment of the allelic relationships

of the determinants for the host-specificity types K, B and 15 has been established by bacterial conjugation and cotransduction with phage P1, study of the restrictionless mutants gave the most information concerning the number and function of the genes involved. Complementation studies in partial diploids proved that a minimum of three genes was necessary for the expression of restriction and modification. Provisionally, the following nomenclature for these genes is used: *hsm* is a gene specifying a product essential for modification, *hsr* is a gene specifying a product essential for restriction, and *hss* is a gene specifying a product essential for both restriction and modification (Arber & Linn, 1969).

The host-specificity types *hsI* and *hsII* are carried on R factors, whose genomes are relatively small compared with the chromosome of *Escherichia coli*. Mapping of R factors by time of entry studies is not possible since all R factor-carried determinants appear to enter the recipient at the same time (Meynell *et al.* 1968). Mapping by phage P1 transduction is also excluded, since phage P1 normally transduces the entire R factor (Watanabe & Fukasawa, 1961; Kondo, Harada & Mitsushashi, 1962). Within these limitations, the present analysis of *hsI* and *hsII* shows that the phenotypes r^{-m-} and r^{-m+} were found for restrictionless mutants of *hsI* and *hsII*, the r^{-m-} class being observed approximately as frequently as the r^{-m+} class, and we conclude that both arose as a result of single mutations. By analogy with the K and B host specificities, we anticipate that the *hsI* and *hsII* host specificities are controlled by at least three genes, *hss*, *hsr* and *hsm* and that the two phenotypes found, r^{-m-} and r^{-m+} are caused by mutations in *hss* and *hsr* respectively.

Further investigation of these host specificities is dependent on complementation studies. Unfortunately, there is incompatibility between R factors of the same *fi* type, such that two *fi*⁻, or two *fi*⁺, R factors will not co-exist stably in the same bacterium (Harada *et al.* 1961; Mitsushashi *et al.* 1962; Watanabe *et al.* 1964). Only one R factor (R124) is known to carry the *hsI* host specificity, and all R factors carrying *hsII* are *fi*⁻ (Bannister, 1969). Thus it is not possible to test complementation between mutants of *hsI*, or between mutants of *hsII*. The only complementation tests possible were between two different host-specificity systems. Complementation does occur between mutants of K and B (Boyer & Roulland-Dussoix, 1969; Arber & Linn 1969; Glover, unpublished results) and it was therefore reasonable to test for complementation between *hsI* and *hsII*, and between either *hsI* or *hsII* and the host specificities K, B and that carried by phage P1. However, in no instance was complementation observed.

The assumption was made that the most suitable test for complementation was the restoration of restriction to an r^{-m+} (*hsr*⁻) mutant in the presence of the wild-type host specificity of a different type. In addition, r^{-m-} (presumed *hss*⁻) mutants were included, and modification was also scored. This assumption requires that the genetic control of host specificities *hsI* and *hsII* is analogous to the genetic control of K and B host specificities. On the basis of the three-gene model, complementation would involve the association of dissimilar gene products from two host-specificity systems to form a functional enzyme. If the analogous gene products from different host-specificity types were slightly different, association of the gene products might not occur, or the product of association might not function correctly, and complementation would not be observed. A second explanation of the absence of complementation is possible. The apparent similarity of the *hsI* and *hsII* host specificities to the K and B host

specificities may be only superficial, and at the molecular level there may be fundamental differences.

It has been argued, in relation to host specificity mutants of phage P1, that the absence of complementation with the intact κ and ν host specificities is no indication that complementation could not occur, because such restrictionless mutants of phage P1 are normally selected in κ strains. Hence, the phage P1 mutants are preselected for the inability to complement with the κ host specificity, and, it may be assumed, with the ν host specificity also. This criticism is also true of the mutants of hsI and $hsII$, which have been selected in a κ strain. However, mutants of κ and ν host specificities, known to complement other mutants (7 κ and $\nu 6$ in Table 8), failed to complement with the intact hsI and $hsII$ host specificities. Hence it may be assumed that the absence of complementation is indicative of an inherent difference between the host specificity systems, rather than to the nature of the mutants.

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