A φ80 Function Inhibitory for Growth of Lambdoid Phage in Him Mutants of *Escherichia coli* Deficient in Integration Host Factor

I. Genetic Analysis of the Rha Phenotype

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Bacteriophage φ80 and λ-φ80 hybrid phage of the type λ(QSR)$_{80}$, in which the rightmost 10% of the λ genome is replaced by corresponding φ80 material, are unable to grow lytically in *himA* and *hip/himD* mutants of *Escherichia coli* K12 at 32°. The genetic element responsible for the growth defect, *rha*, has been mapped to the (QSR)$_{80}$ region and was located more precisely by restriction enzyme and DNA heteroduplex analysis of mutations that result in loss of the Rha phenotype. Such an Rha mutant carrying a 1.5-kb deletion beginning 0.58 kb from the right end of the chromosome and extending leftward locates the *rha* locus at least in part within this region of (QSR)$_{80}$. In addition, a substitution derivative of λ(QSR)$_{80}$ was isolated which does not exhibit the Rha phenotype. In this phage, λ-80*hy*95, the right half of the (QSR)$_{80}$ region is replaced by DNA homologous to the 95–100% segment of λ. In mixed infections in the *himA*42 host at 32°, λ$^+$ does not complement λ(QSR)$_{80}$ for growth and the burst size of the coinfecting λ$^+$ is reduced in comparison to that in a single infection. Deletion mutants of λ(QSR)$_{80}$ that grow normally in *himA*42 at 32° in single infections are inhibited for growth in mixed infections with λ(QSR)$_{80}$. These results suggest the existence of a *trans*-acting function which inhibits phage growth in the absence of HimA or Hip/HimD function. It is likely that the *rha* gene either encodes that function or indirectly controls its action. © 1985 Academic Press, Inc.

INTRODUCTION

Temperate coliphages λ and φ80 are members of the lambdoid family of phages. Members of this relatively large group of phages are distinguished from one another primarily on the basis of immunity; each phage produces a specific repressor protein which in the prophage state renders the lysogenic cell immune to superinfecting phage of the same type (Jacob and Wollman, 1961; Hershey and Dove, 1971). The genomes of the lambdoid phages are arranged in functional units, with genes involved in similar functions clustered together on the genetic map.

The genomes of λ and φ80 bear a close resemblance to one another with respect to the relative location of genes specifying analogous functions (Sato *et al.*, 1968; see Fig. 1 for details).

The discovery that viable hybrids between λ and φ80 could be formed upon mixed infection (Signer, 1964; Franklin *et al.*, 1965; Yamagishi *et al.*, 1965) showed that there is functional interchangeability among at least some λ and φ80 gene products and regulatory sites. In addition, it predicts that nucleotide sequence homology exists between the two phages; this allows for the formation of hybrids by homologous recombination. A number of λ-φ80 hybrids have been described (compiled in Szybalski and Szybalski, 1979; Aizawa and Matsushiro, 1975; Furth *et al.*, 1978; see Fig. 1).

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Regulation of gene expression in the lambdoid phages has been characterized in detail for phage λ (reviewed by Friedman and Gottesman, 1983). These studies reveal a pattern of regulation based primarily on a series of transcription termination signals and the antitermination functions N and Q. Detailed transcriptional mapping studies have shown that the patterns of gene expression in ϕ80 infection are analogous to those of λ (Lozeron and Szybalski, 1969).

Him and Hip mutants of *Escherichia coli* were originally selected as host mutants unable to support λ integrative recombination (Miller and Friedman, 1977; Williams *et al.*, 1977; Miller *et al.*, 1979). Subsequently, the *himA* and *hip/himD*[3] genes were shown to encode the two subunits of a host factor, IHF (integration host factor), required for *in vitro* λ site-specific recombination (Kikuchi and Nash, 1978; Nash and Robertson, 1981; Miller and Nash, 1981; Miller *et al.*, 1981; Nash, 1981). The *himA* locus maps at minute 38 and the *hip/himD* locus maps at minute 19 on the *E. coli* K12 chromosome (Miller *et al.*, 1979). Studies with *himA* and *hip/himD* mutants show that, *in vivo*, IHF participates in λ lysogeny at two levels: it is required as a cofactor in the integrative recombination reaction catalyzed by λ Int protein which leads to insertion of the λ genome into the *E. coli* chromosome (Miller and Friedman, 1980; Miller and Nash, 1981), and it is essential for the expression of the λ repressor (cI) and integrase (Int) functions (Miller, 1981; Hoyt *et al.*, 1982; Oppenheim *et al.*, 1982).

In addition to causing defects in λ integration, *himA* and *hip/himD* mutations are pleiotropic, affecting other site-specific recombination reactions and influencing the expression of certain genes and/or the activity of various gene products. These additional properties of HimA-Hip/HimD mutants include: reduction in the integration frequency of phage with integrase and attachment site specificities different from those of λ, such as ϕ80 and P2 (Miller *et al.*, 1979); reduction in the frequency of precise excision of the transposable genetic elements Tn5 and Tn10 (Miller and Friedman, 1980); inability to support the lytic growth of phage Mu (Miller and Friedman, 1980; Yoshida *et al.*, 1982) and phage 21 (M. Feiss, personal communication); non-motility due to a defect in expression of the *hag* gene (N. Craig, H. Nash, H. Miller, and M. Simon, personal communication), and reduced expression of *ilvB* (Friedman *et al.*, 1984; Friden *et al.*, 1984).

In addition to these characteristics, HimA and Hip/HimD mutants also fail to support the lytic growth of λ-ϕ80 hybrid phage carrying the QSR region of ϕ80. This phenotype, which we have named Rha (reduced on *HimA*), was first observed for the *hip157* mutant by A. Kikuchi and R. Weisberg (personal communication). We describe here a genetic analysis of the Rha phenotype. In the accompanying paper we report studies on the physiology of the abortive infection of λ(QSR)₈₀ phage in *himA* mutants.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. E. coli and bacteriophage strains used are described in Tables 1 and 2, respectively. λ(QSR)₈₀.580, the λ(QSR)₈₀ phage used in this study, was isolated and characterized in this laboratory (Mozola, 1982). Restriction enzyme analysis and DNA–DNA heteroduplex analysis showed the substitution in the 90–100 map unit region of the chromosome found in λ(QSR)₈₀.580 to be essentially indistinguishable from that found in λ-80*hy5*, a well-characterized λ-ϕ80 hybrid with a crossover point at 90.6 λ map units (Fiandt *et al.*, 1971; see Fig. 1).

Media. TB and LB media have been described (Miller and Friedman, 1980). LBMM is LB + 10 mM MgSO₄ and 0.2% maltose. TMG buffer for dilution of bacteria and phage is 10 mM Tris (pH 7.4), 10 mM MgSO₄, and 0.01% gelatin.

Phage growth and complementation tests.

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[3] Although *himD* and *hip* mutations appear to map in the same gene, there is some question over appropriate nomenclature. To avoid confusion, we will refer to markers in that region as *hip/himD*.

TABLE 1

BACTERIAL STRAINS

Strain	Relevant genotype	Source
K37	Su^0 $himA^+$ $himD^+$	M. Yarmolinsky
K100	K37 $recA^-$	M. Gottesman
K124	K37 (λ^+)	
K159	K37 $(\lambda imm434)$	
K484	$uvrA$ $himA^+$	M. Pearson
K648	$himAam79$	
K770	$hip157$	A. Kikuchi
K807	$himB114$	
K870	$himA42hip157$	
K936	K37 $himA42$	
K1215	K37 $[\lambda imm434 \, (QSR_{80})]$	
K1237	K936 $[\lambda imm434 \, (QSR_{80})]$	
K1299	K37 $himA\Delta82 - $ Tn10	H. Miller
K1382	K37 $himD63$	H. Miller
K1483	Su^0 $(\lambda cI_{857}cII^+\Delta(O\text{-}uvrB))$	S. Adhya
K1555	Su^0 $(\lambda cI_{857}P^+\Delta(Q\text{-}uvrB))$	S. Adhya
K1556	Su^0 $(\lambda cI_{857}R^+\Delta(A\text{-}uvrB))$	S. Adhya
K1557	Su^0 $(\lambda cI_{857}G^+\Delta(H\text{-}uvrB))$	S. Adhya
K1675	K484 $himA42$	
K1923	$himD::mud$	H. Miller
K1926	K37 $himD74 - $ Tn10	H. Miller

Note. All bacterial strains are derivatives of *Escherichia coli* K12. Strain numbers are those of this laboratory. Only genotypes relevant to this work are shown. Strains with no source listing were constructed in this laboratory.

Phage growth was tested by streaking lysates onto lawns of starved bacterial cultures in TB top agar that had been poured onto TB plates. For titering phage as plaque-forming units, dilutions of phage were mixed with 4 drops of a fresh overnight bacterial culture grown in LBMM and adsorbed at room temperature for 10 min. TB top agar (2.5 ml) was then added and the mixture poured onto a TB plate and incubated appropriately.

Measurement of phage burst sizes and single-step growth experiments were performed essentially as outlined in Friedman *et al.* (1973). Phage complementation tests were performed in a manner similar to burst experiments except that bacteria were concentrated by pelleting and resuspension in 10 mM MgSO$_4$ to a final concentration of 2×10^9/ml before infection. Phage were added to a multiplicity of infection (m.o.i.) of 5 in single infections

and 5 of each phage type in mixed infections.

Phage crosses. A culture of bacteria (0.5 ml) grown to 2×10^8/ml in LBMM was infected with phage at an m.o.i. of 5 of each phage (one phage in crosses against prophage in lysogenic bacteria) and adsorption was carried out for 15 min at room temperature. TMG (5 ml) was then added, the infected bacteria were pelleted, resuspended in 5 ml LBMM, and incubated in a shaking water bath until lysis (2–3 hr). The resulting lysate was then treated with chloroform and plated on appropriate selective bacterial lawns.

Isolation of Rha mutants. Rha$^-$ mutants were independently isolated as follows. $\lambda(QSR)_{80}$ or $\lambda cI_{857}red3(QSR)_{80}$ lysates were grown in K37 ($himA^+recA^+$) or K100 ($himA^+recA^-$) at 35° from individual plaques picked from K37 lawns. The lysates were then plated on K936 ($himA42$) lawns at 32°, and one plaque from each K936 lawn was picked and used to make a lysate in K37. The lysates were then titered on K37 and K936 at 32° to verify the Rha$^-$ phenotype.

Preparation of phage DNA. Phage particles were concentrated and purified by pelleting followed by banding in a CsCl

TABLE 2

BACTERIOPHAGE STRAINS

Relevant genotype	Source
λ^+	M. Yarmolinsky
λcI_{60}	M. Gottesman
$\lambda(QSR)_{80}$	
$\lambda cI_{60}(QSR)_{80}$	
$\lambda cI_{60}Pam3$	
$\lambda cI_{857}80hy5$	R. Weisberg
$\lambda cI_{857}red3(QSR)_{80}$	
$\lambda imm434cI_2$	M. Yarmolinsky
$\lambda imm434nin5$	
$\lambda imm434(QSR)_{80}$	
$\lambda imm434nin5(QSR)_{80}$	
$\phi80c$	M. Gottesman

Note. Only the relevant genotypes are shown. Strains with no source listing were isolated or constructed in this laboratory. Additional mutant phage isolated in this work are discussed in the text.
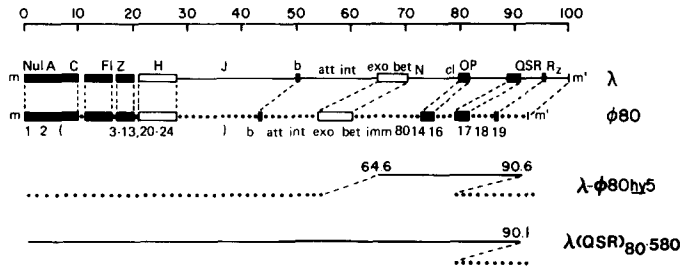
FIG. 1. Genetic and homology map of λ and ϕ80. The linear phage chromosomes are depicted and are drawn approximately to scale. Not all genes are shown. The scale is in λ map units with 1 unit equaling approximately 485 nucleotide base pairs (Daniels *et al.*, 1983a, b). The λ genetic map is adapted from Szybalski and Szybalski (1979). The ϕ80 map is according to Youderian (1977) and Youderian and King (1981). DNA sequence homology data are from Fiandt *et al.* (1971). Regions of strong homology are indicated by solid boxes, regions of partial homology by open boxes, and regions of nonhomology by solid and dotted lines for λ and ϕ80, respectively. The structure of λ-80*hy*5 was determined from heteroduplex analysis by Fiandt *et al.* (1971) as compiled in Szybalski and Szybalski (1979). λ(QSR)$_{80}$.580 is discussed under Materials and Methods.

equilibrium density gradient. Phage DNA was prepared by pronase treatment and phenol extraction. When only small amounts of phage DNA were needed for restriction enzyme analysis, DNA was prepared according to the rapid λ DNA isolation procedure of Davis *et al.* (1980) except that ribonuclease A was not used in the resuspension buffer.

Restriction enzyme digestion and agarose gel electrophoresis. Phage DNAs were digested with restriction enzymes according to the directions of the supplier. Cohesive end fragments were denatured by incubating the samples at 70° for 3 min, then placing the samples on ice. DNA fragments were separated by electrophoresis through horizontal 0.8% agarose (Sea Kem) gels and stained with ethidium bromide.

DNA-DNA heteroduplex analysis. DNA-DNA heteroduplexing, sample spreading, preparation of grids for electron microscopy, and uranyl acetate staining and platinum–palladium shadowing of grids were performed according to the procedures of Davis *et al.* (1971). In the heteroduplexing process, reanneling was for 45 min at room temperature at a formamide concentration of 32%. DNA spreads were made with a 70% formamide hyperphase and a 40% formamide hypophase. M13 and SV40 DNAs were used as single- and double-stranded DNA size markers, respectively.

RESULTS

Growth Characteristics of λ(QSR)$_{80}$

The Rha phenotype is demonstrated in Table 3. At 32°, λ(QSR)$_{80}$ is unable to form plaques on all *himA* mutants tested, which include missense (*himA*42), nonsense (*himAam*79), and deletion (*himA*-

TABLE 3

PLATING CHARACTERISTICS OF λ(QSR)$_{80}$

Bacterial strain	Relevant genotype	λ$^+$		λ(QSR)$_{80}$	
		32°	42°	32°	42°
K37	*him*$^+$	++	++	++	++
K936	*himA*42	++	++	−	+
K648	*himAam*79	++	++	−	+
K1299	*himA*Δ82	++	++	−	+
K1382	*himD*63	++	++	++	++
K1923	*himD*::mud	++	++	−	+
K1926	*himD*74	++	++	−	+
K770	*hip*157	++	++	−	+
K870	*himA*42*hip*157	++	++	−	+
K807	*himB*114	++	++	++	++

Note. Streak tests for plaque formation were performed as described under Materials and Methods. ++, Large plaques, +, small plaques, −, no plaques.

Δ82) mutants. Of the four *hip/himD* mutants tested, all are unable to support λ(QSR)₈₀ growth at 32° except for the *himD63* mutant. We note, however, that the *himD63* mutant is leaky for Mu growth and thus may retain considerable IHF activity. The *himB114* (*gyrB*) mutation (Miller *et al.*, 1979) has no effect on λ(QSR)₈₀ growth.

Nonhybrid phage φ80 also exhibits the Rha phenotype, as evidenced by its inability to plate on *himA42* at 32°. The finding that both φ80 and λ(QSR)₈₀ fail to plate on *himA* mutants at 32° suggests that the (QSR)₈₀ region is specifically responsible for the Rha phenotype, and that the defect does not result from an incompatibility between λ and φ80 functions peculiar to the hybrid. We have tested a λ-φ80 hybrid with the head and tail, attachment site, and QSR regions from φ80 and the remainder of the genome from λ (λ-80*hy5*; see Fig. 1), and find that it does not plate on *himA42* at 32°.

The growth restriction of λ(QSR)₈₀ in *himA* and *hip/himD* mutants is partially relieved at 42°. λ(QSR)₈₀ is able to form plaques at 42° on all *himA* and *hip/himD* mutants tested (Table 3). However, plaque size is significantly reduced (except on the leaky *himD63* mutant) when compared to the size of those formed by λ(QSR)₈₀ plating on a Him⁺ host or λ⁺ plating on the various Him⁻ mutants. The temperature dependence of the Rha phenotype is confirmed by the results of burst experiments shown in Table 4. In these experiments,

TABLE 4

QUANTITATIVE BURST ASSAY OF λ(QSR)₈₀

Bacterial strain	Relevant genotype	Burst size of λimm434(QSR)₈₀		
		32°	37°	42°
K37	*himA⁺*	360	560	430
K936	*himA42*	<0.01	77	160

Note. Burst experiments were performed as described under Materials and Methods. Burst sizes are given as phage per infected cell assayed 90 min after phage adsorption.

λimm434(QSR)₈₀ was used; this phage exhibits the Rha phenotype and differs from λ(QSR)₈₀ only in that it contains the immunity region (*cI, cro*) of phage 434 substituted for that of λ (Kaiser and Jacob, 1957). The data indicate that temperature has no effect on the burst size of λimm434(QSR)₈₀ in the *himA⁺* host. In the *himA42* host, however, growth is extremely defective at 32°, intermediate at 37°, and approaching normal levels at 42°, although still lower than bursts in the *himA⁺* host. Thus, the Rha phenotype becomes progressively less pronounced as the temperature is raised.

Genetic Mapping of Rha

Genetic mapping provided an additional means of localizing the element responsible for the growth defect of λ(QSR)₈₀ in Him⁻ strains. λimm434(QSR)₈₀ was crossed with a series of defective λ prophages which have deletions beginning within bacterial genes flanking the right prophage attachment site (*attR*) and extending leftward to varying extents into the prophages, removing genetic information essential for phage growth. The structures of the defective prophages harbored by the lysogens and the strategy of the mapping are shown in Fig. 2. The only phages with λ immunity produced by infection of these lysogens with λimm 434(QSR)₈₀ should be double recombinants as shown in Fig. 2. Such recombinants can be scored for the Rha phenotype by plating the progeny of the crosses on λimm434 lysogens of *himA⁺* and *himA⁻* strains at 32°.

The results of the crosses are shown in Table 5. The Rha phenotype can be crossed out by recombination with the prophages in K1556 and K1557 as evidenced by the large number of immλ phage produced that are able to plate on the HimA⁻ lysogen. The higher fraction of recombinants losing the Rha phenotype by recombination with K1557 is consistent with the deletion of less prophage material to the right of the QSR region in this phage. The Rha phenotype cannot be crossed out by recombination with the prophages in

K1483 ▬▬▬▬▬▬▬▬▬▬▬▬▬▬▬▬
K1555 ▬▬▬▬▬▬▬▬▬▬▬▬▬▬
K1556 ▬▬▬▬▬▬▬▬▬▬▬
K1557 ▬▬▬▬▬▬▬

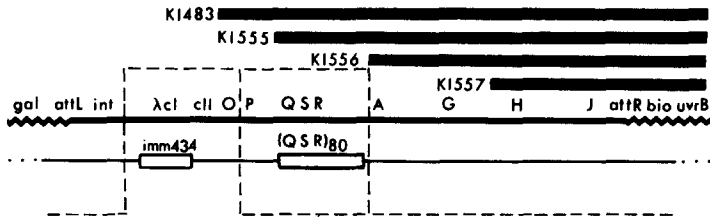| gal | attL | int | λcI | cII | O | P | Q S R | A | G | H | J | attR | bio | uvrB |

imm434　　　　　　(Q S R)$_{80}$

FIG. 2. Strategy for mapping Rha employing deletion prophage. The procedure for the mapping crosses and the relevant genotypes of strains used are presented under Materials and Methods. The mapping strategy is discussed in the text. The prophage map is not drawn to scale and not all genes are shown. Bacterial genetic material adjacent to the defective λ prophage is indicated by wavy lines. Solid bars indicate the extent of material deleted in the deletion prophage lysogens. Only the immunity and QSR regions of the genome of the superinfecting λimm434(QSR)$_{80}$ are shown. Dashed lines indicate two possible double recombinations to generate immλ progeny phages.

either K1555 or K1483, both of which are deleted for (QSR)$_\lambda$. In this case, the immλ phages that do form plaques on K1237 are mutants (see below). The results of these recombination experiments confirm that the QSR substitution found in λ(QSR)$_{80}$ contains the element responsible for the Rha phenotype.

Analysis of Rha Mutants

Mutants of λ(QSR)$_{80}$ that are able to grow in himA$^-$ and hip/himD$^-$ hosts at 32° (Rha$^-$) are found in lysates of λ(QSR)$_{80}$ and nonhybrid φ80 at a frequency of 10^{-4} to 10^{-5}. Regardless of the host used in the selection (i.e., himA$^-$ or hip/himD$^-$), all

TABLE 5

RESULTS OF Rha MAPPING EMPLOYING DELETION PROPHAGE

Cross λimm434(QSR)$_{80}$ ×	Titer at 32° on		Fraction plating on K1237
	K1215 (himA$^+$)	K1237 (himA$^-$)	
K1483	6.8×10^6	5.6×10^2	8.2×10^{-5}
K1555	1.3×10^7	3.4×10^2	2.6×10^{-5}
K1556	7.0×10^6	1.1×10^6	0.16
K1557	2.2×10^7	1.3×10^7	0.59

Note. K1215 and K1237 are λimm434(QSR)$_{80}$ lysogens of K37 (himA$^+$) and K936 (himA42), respectively. Lysogens carrying (QSR)$_{80}$ were used for selection of immλ recombinants to prevent replacement of (QSR)$_{80}$ with (QSR)$_\lambda$ by recombination during growth on selective lawns. See Fig. 2 for a map of the prophage deletions.

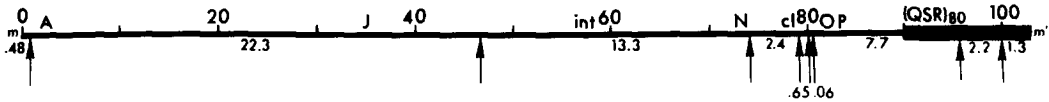Rha$^-$ mutants isolated grow with equal efficiency on the different Him$^-$ strains at 32°.

Two mutants derived from λ(QSR)$_{80}$, Rha-01 and Rha-02, were tested for plating on a variety of Him$^-$ mutants. Both mutants grow well at both 32° and 42° on all him/hip mutants tested. Thus the mutants do not exhibit allele specificity with regard to growth in himA and hip/himD mutants. The fact that the Rha$^-$ mutants grow in the himAΔ82 deletion mutant, the himD::mud insertion mutant, and the himA42hip157 double mutant strongly suggests that growth of these phages is totally independent of any need for HimA–Hip/HimD activity.

Restriction Enzyme Analysis of Rha

Restriction enzyme analysis of the DNA from Rha$^-$ mutants was used to determine if any of these mutants have deletions, substitutions, or insertions within their genomes. In addition to mutants Rha-01 and Rha-02, 10 more Rha$^-$ mutants were selected for analysis. (Rha-21 through Rha-30, all generated in a Red$^+$RecA$^+$ background; see Materials and Methods.) Phage DNA was isolated, digested with the restriction endonuclease BglII, and the resultant DNA fragments separated by agarose gel electrophoresis. A BglII restriction map of λ(QSR)$_{80}$ is shown in Fig. 3. Results of BglII digestion analysis of Rha$^-$ mutant DNAs are shown in Fig. 4.

FIG. 3. *Bgl*II restriction map of λ(QSR)$_{80}$. The linear map of λ(QSR)$_{80}$ with the approximate placement of some genes is shown. The scale is in λ map units. *Bgl*II sites are indicated by vertical arrows. Fragment sizes are shown in kilobase pairs (kb) between the arrows. Restriction sites and fragment sizes are according to Daniels *et al.* (1983a) and deWet *et al.* (1980). The total length of λ is 48,502 base pairs (Daniels *et al.*, 1983b). The (QSR)$_{80}$ substitution increases the length of the phage genome by approximately 1.3 kb. Placement of the λ-φ80 junction at approximately 90 λ map units is according to Fiandt *et al.* (1971).

Lane 3 shows the *Bgl*II digestion pattern of λ(QSR)$_{80}$ DNA. The third largest fragment is the λ-φ80 junction fragment of approximately 7.7 kilobase pairs (kb). The fifth and sixth bands from the top of the
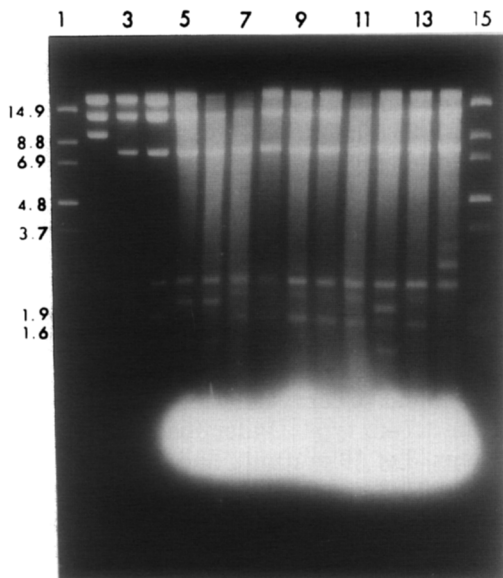

FIG. 4. Electrophoretic analysis of *Bgl*II-digested λ(QSR)$_{80}$ mutant DNAs. Restriction analysis with *Bgl*II was carried out as described under Materials and Methods. Lanes 1 and 15—*Ava*I-digested λcI$_{857}$ DNA used as fragment size markers. Fragment sizes are indicated in kb (Daniels *et al.*, 1983a). All other DNAs were treated with *Bgl*II. Lane 2—λcI$_{857}$; lane 3—λ(QSR)$_{80}$; lane 4—Rha-01; Lanes 5 through 14—Rha-21 through Rha-30. The DNA digests in lanes 5 through 14 are from phage DNA prepared by the rapid λ DNA isolation method and therefore show a background smear of digested *E. coli* DNA and a heavily stained area of RNA at the bottom of the gel. For λ(QSR)$_{80}$ derivatives, the 0.06- and 0.65 kb fragments (see Fig. 3) are not visible on this gel.

gel represent the 2.2- and 1.3-kb fragments internal to the (QSR)$_{80}$ substitution. Mutants Rha-21, Rha-22, and Rha-28 (lanes 5, 6, and 12) produce restriction fragment patterns like that of λ(QSR)$_{80}$. The faint band present between the 1.3- and 2.2-kb bands is a fragment composed of the 1.3-kb right-end piece and the 0.48-kb left-end fragment resulting from incomplete denaturation of the cohesive ends. We have found this to be a problem inherent in digests of DNA prepared by the rapid DNA isolation procedure employed.

Although not shown in Fig. 4, mutant Rha-02 also produces a *Bgl*II pattern like that of λ(QSR)$_{80}$. In addition, Rha-02 and the λ(QSR)$_{80}$ parent produce identical restriction fragment patterns after digestion with *Eco*RI, *Hind*III, *Bam*HI, *Ava*I, *Sst*I, and *Kpn*I (data not shown). Because Rha-02, Rha-21, Rha-22, and Rha-28 have restriction fragment patterns indistinguishable from that of λ(QSR)$_{80}$, they must contain either point mutations or small deletions.

Mutants Rha-01, Rha-23, Rha-24, Rha-25, Rha-26, Rha-27, and Rha-29 (lanes 4, 7, 8, 9, 10, 11, and 13) produce an apparently common restriction fragment pattern in which the 2.2- and 1.3-kb pieces internal to the (QSR)$_{80}$ substitution are lost and are replaced by a new fragment of approximately 1.75 kb. [Size estimates were made from plots of log DNA fragment size vs electrophoretic mobility (Helling *et al.*, 1974) and band position relative to the λ*Ava*I markers.] The net result is a loss of about 1.7 kb compared to λ(QSR)$_{80}$. Since the 1.75-kb piece is the only new fragment appearing in this digest, the pattern is consistent with these

mutants containing a deletion that removes the rightmost BglII site in λ(QSR)$_{80}$ (see Fig. 3). This interpretation is supported by the fact that the 7.7-kb $\lambda-\phi80$ junction fragment is apparently unaffected. The faint band seen in lanes 7 through 11 and 13 (rapid DNA preps) above the new 1.75-kb fragment again represents undenatured right and left terminal fragments (the new 1.75-kb fragment is now the right terminal fragment). Note that this faint band is not seen in the Rha-01 digest in lane 4, in which DNA purified from λ phage particles was used. Estimates of the size of the putative deletion in Rha-01 by digestion with the restriction enzymes $Hind$III, BamHI, AvaI, and SstI range from 1.4 to 1.8 kb (data not shown; see deWet et $al.$, 1980 for restriction maps).

Mutant Rha-30 (Fig. 4, lane 14) produces a unique BglII pattern. The 7.7-kb $\lambda-\phi80$ junction fragment is unaltered. Both internal (QSR)$_{80}$ fragments of 2.2 and 1.3 kb are missing and are replaced by a single fragment of higher molecular weight (the fainter band of still higher molecular weight again represents undenatured end fragments). Like the mutants with a net loss of 1.7 kb, Rha-30 appears to have lost the rightmost BglII site in λ(QSR)$_{80}$. The size of the new fragment is estimated to be about 2.9 kb. This results in a net loss of approximately 0.60 kb as compared to (QSR)$_{80}$, and can be explained by a deletion in the region around the rightmost BglII site in λ(QSR)$_{80}$. Although the restriction fragment of both Rha-30 and the common class of mutants losing 1.7 kb suggest that these mutants have simple deletions, patterns of this type could also arise from substitution events resulting in a net loss of DNA. Rha-30 carries such a substitution and will be discussed below.

We have also analyzed the $Hind$III restriction fragment patterns of DNAs from two Rha$^-$ mutants generated from nonhybrid $\phi80$. One mutant has a $Hind$III pattern identical to $\phi80$ and thus is presumed to be a point mutant or small deletion. The other mutant has a pattern indicating that it contains a deletion indistinguishable in size and position from the common 1.7-kb deletion (data not shown).

Although the above restriction analysis cannot position the exact boundaries of each deletion, the fact that 7 of the 12 mutants analyzed produced identical BglII digestion patterns consistent with a similar or identical deletion suggests that they may have been generated by a common recombinational event. Since these seven mutants were independently isolated, they obviously cannot be repeated isolations of progeny of one original mutant. One explanation for the repeated isolation of the common class of 1.7-kb deletions is that their formation results from recombination between short regions of DNA sequence homology (Albertini et $al.$, 1982). Such recombination can be catalyzed either by the $E.$ $coli$ RecA (Howard-Flanders and Theriot, 1966) or λ Red (Echols and Gingery, 1968; Signer and Weil, 1968; Schulman et $al.$, 1970) systems. The following results support this explanation.

Rha$^-$ mutants generated under various recombination-deficient conditions (see $Materials$ and $Methods$) were analyzed by BglII digestion of their DNAs and were found to be of the following types. In a Red$^+$RecA$^-$ background, 5 of 10 mutants were found to carry deletions and all were of the 1.7-kb type. Under Red$^-$RecA$^+$ conditions, 3 of 10 mutants had deletions and one of these was not of the 1.7-kb type (Rha-9, a 1.5-kb deletion). Under Red$^-$RecA$^-$ conditions, only 1 of 20 mutants was a deletion mutant and it was not of the 1.7-kb type (Rha-18, a 1.9-kb deletion). The two extraordinary deletions are located in the same general region of the (QSR)$_{80}$ substitution as the common class 1.7-kb deletions (Mozola, 1982). The deletion mutations, and the as yet unmapped putative point mutations, will be used to define the locus or loci (rha) responsible for the Rha phenotype.

DNA-DNA Heteroduplex Analysis

The conclusion that mutants Rha-01, Rha-9, and Rha-18 carry deletions within

the $(QSR)_{80}$ region was verified by DNA-DNA heteroduplex analysis.

Figure 5 shows an electron micrograph of a representative heteroduplex molecule formed between the DNAs of $\lambda(QSRrha\Delta01)_{80}$ and $\lambda imm434nin5(QSR-rha^+)_{80}$. The $imm434$ substitution and the $nin5$ deletion serve as markers to orient

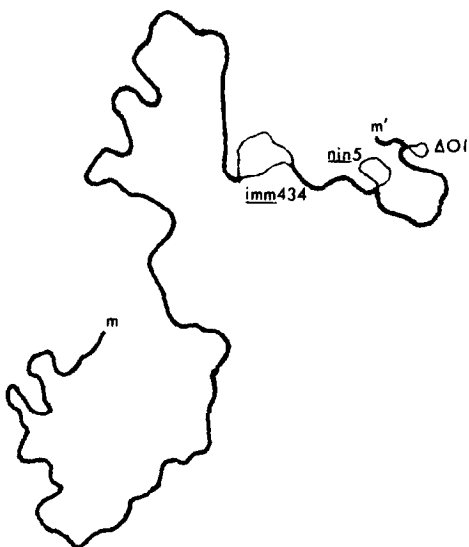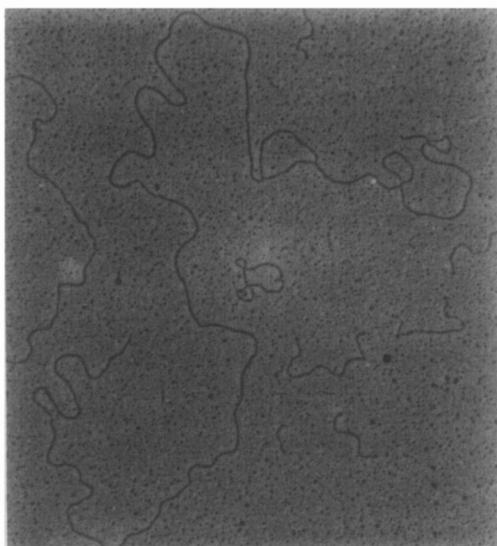the left and right ends of the molecule; $nin5$ is a deletion of approximately 2.86 kb between 83.7 and 89.5 units on the λ map (deWet et al., 1980). Measurements given are averages of measurements of individual molecules and errors represent 1 standard deviation. A deletion loop of 1.71 (±0.22) kb is located 0.73 (±0.07) kb from the right end of the molecule (m'). Both the size and position of the deletion are consistent with the restriction analysis data. The rightmost BglII site in $\lambda(QSRrha^+)_{80}$ is located 1.3 kb from the right end, m'. The deletion visualized by heteroduplex analysis would remove this BglII site, as indicated by the digestion experiments. The $nin5$ deletion loop was measured at 2.88 (±0.35) kb, in agreement with previous estimates.

The mutant with a deletion estimated to be 1.5 kb from BglII digestion analysis ($rha\Delta9$) was compared with $\lambda imm434-nin5(QSRrha^+)_{80}$ by DNA heteroduplex analysis. This heteroduplex revealed a deletion loop measured to be 1.46 (±0.13) kb located 0.58 (±0.09) kb from the right end. The mutant with an estimated 1.9-kb deletion($rha\Delta18$) was also heteroduplexed as above and showed a deletion loop measured to be 1.91 (±0.27) kb located 0.68 (±0.06) kb from the right end.

Mutant Rha-30, as discussed previously (see Fig. 4), produces a unique restriction pattern consistent with a deletion in the $(QSR)_{80}$ region resulting in a net loss of about 0.60 kb as compared to $\lambda(QSR-rha^+)_{80}$. Heteroduplex analysis revealed, however, that this mutant has a substitution of the right terminal 2.87 (±0.22) kb of $(QSR)_{80}$ (including the rha locus as defined by the deletions) by a nonhomologous segment of 2.41 (±0.18) kb. This difference of approximately 0.46 kb roughly agrees with the net loss of 0.60 kb indicated by restriction enzyme analysis. Rha-30 has been renamed $\lambda-80hy95$. A heteroduplex of $\lambda-80hy95$ vs $\lambda imm-434nin5(QSRrha^+)_{80}$ is shown in Fig. 6. The substituted DNA in this mutant was shown to be homologous to λ DNA based upon the analysis of the heteroduplex formed between $\lambda-80hy95$ and $\lambda imm-434nin5$ (Fig. 7). This analysis shows the
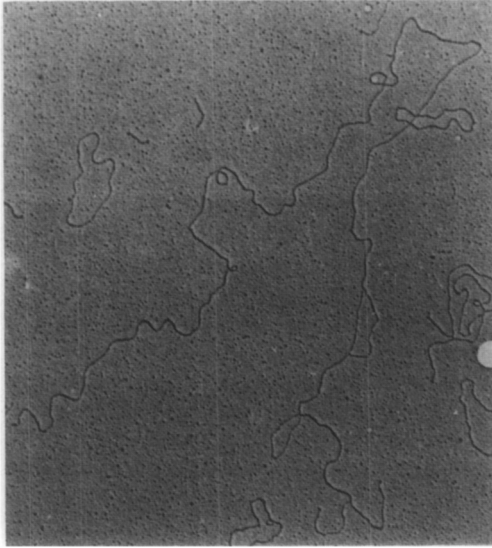
tween $(QSR)_{80}$ and $(QSR)_\lambda$ at 90.1% λ (0.3 kb = 0.6 λ map units), consistent with a crossover region predicted by the λ-$\phi80$ homology map (Fiandt *et al.*, 1971) and similar in position to the crossover in λ-$80hy5$ placed at 90.6% λ (Fiandt *et al.*, 1971; see Fig. 1). The length of the homologous right half of the QSR region in this heteroduplex, 2.47 kb, translates to 5.04 λ map units. Measuring from the right end, this would place the crossover at the right branch of the nonhomology bubble at 95.0% λ. A region of homology between λ and $\phi80$ exists at this position on the λ map (Fiandt *et al.*, 1971; see Fig. 1). No recombinants in this region have been previously reported.

The results of the physical analysis of Rha$^-$ mutants discussed above are summarized in Fig. 8 in the form of a map of
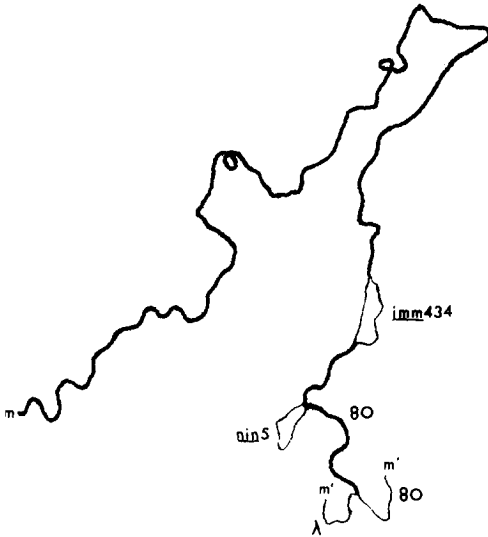

FIG. 6. Representative heteroduplex DNA molecule formed between λ-$80hy95$ and $\lambda imm434nin5$-$(QSRrha^+)_{80}$. The lengths discussed in the text are averages of measurements of nine individual molecules.

right terminal 2.47 (±0.09) kb to be homologous between the two DNAs. A nonhomology bubble representing the left half of the QSR region is seen adjacent to the homologous right end. The position of the left branch of the nonhomologous region with respect to the *nin5* loop places the left end of the nonhomology region be-
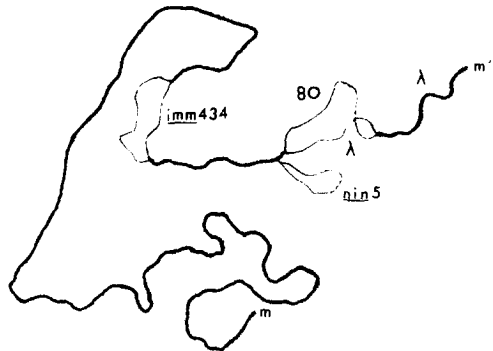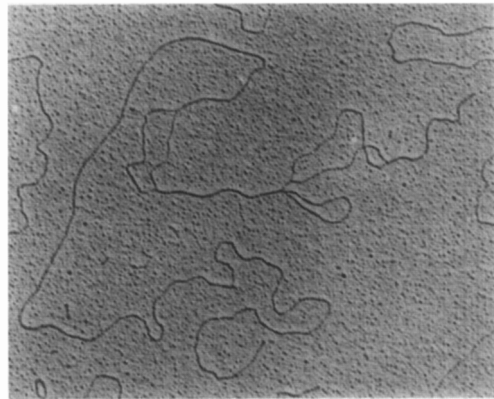

FIG. 7. Representative heteroduplex DNA molecule formed between λ-$80hy95$ and $\lambda imm434nin5$. The lengths discussed in the text are averages of measurements of 11 individual molecules.
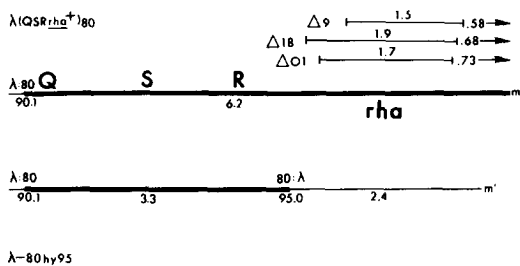
FIG. 8. Physical map of the *rha* locus. The positions of three deletions defining the *rha* locus in $\lambda(QSR)_{80}$ are shown. Measurements from DNA heteroduplex analysis are given in kb. Shown below the deletion map is the structure of the substitution mutant λ-$80hy95$. $\phi80$ DNA is indicated by heavy lines and DNA homologous to λ by light lines. Crossover points between λ-homologous and $\phi80$ DNA as determined by heteroduplex analysis are indicated in λ map units. The approximate placement of the $\phi80$ genes 17, 18, and 19 are indicated here by "Q," "S," and "R."

the *rha* locus. Interestingly, Fiandt *et al.* (1971) observed a deletion similar to the *rha* deletions described here in a stock of $\phi80pt_1$, a $\phi80$ tryptophan-transducing phage (Yamagishi *et al.*, 1966; Deeb *et al.*, 1967). This unselected deletion was of about 1.5 kb beginning approximately 0.75 kb from the right $\phi80$ terminus. We have not tested $\phi80pt_1$ for the Rha phenotype.

The λ-$80hy95$ mutant apparently acquired the substituted λ-homologous material during propagation in a nonlysogenic host (K37) in the absence of any coinfecting phage. The 95–100% λ-homologous segment must have been rescued from a cryptic lambdoid prophage present in the K37 chromosome. Such a cryptic prophage, *qsr'*, known to consist partly of DNA with homology to the QSR-m' region of λ, has been identified in *E. coli* K12 strains (Anilionis *et al.*, 1980) and maps near the *gal* operon. Using Southern hybridization analysis, we have determined that the *qsr'* cryptic prophage is present in our K37 strain, explaining the origin of the substitution found in λ-$80hy95$ (unpublished results). Other substitution mutants of λ arising by recombination with the *qsr'* cryptic prophage have been described previously (Kaiser, 1980).

Complementation Behavior of $\lambda(QSR)_{80}$

Representative results of complementation tests performed in K37 (*himA$^+$*) and K936 (*himA42*) at 32° are shown in Table 6 and details of these experiments are discussed below.

In K936, $\lambda cI^-(QSRrha^+)_{80}$ gives no burst

TABLE 6

COMPLEMENTATION BEHAVIOR OF $\lambda(QSR)_{80}$

	Burst size	
Infection	K936 (*himA$^-$*)	K37 (*himA$^+$*)
1. λcI^-	234	282
2. $\lambda imm434cI^-$	130	518
3. $\lambda cI^-(QSRrha^+)_{80}$	0.15	588
4. $\lambda imm434cI^-(QSRrha\Delta01)_{80}$	179	682
5. $\lambda imm434cI^-(QSRrha02)_{80}$	152	565
6. $\lambda(QSRrha02)_{80}$	193	181
7. $\lambda cI^-(QSRrha^+)_{80}$ + $\lambda imm434cI^-$	1.0 7.0	226 96
8. $\lambda cI^-(QSRrha^+)_{80}$ + $\lambda imm434cI^-(QSRrha\Delta01)_{80}$	0.6 13	228 129
9. $\lambda cI^-(QSRrha^+)_{80}$ + $\lambda imm434cI^-(QSRrha02)_{80}$	29 88	127 82
10. λcI^- + $\lambda imm434cI^-(QSRrha\Delta01)_{80}$	198 169	224 184
11. λcI^- + $\lambda imm434cI^-(QSRrha02)_{80}$	154 165	219 181
12. $\lambda(QSRrha02)_{80}$ + $\lambda imm434cI^-(QSRrha\Delta01)_{80}$	141 145	108 259
13. λcI^- + $\lambda imm434cI^-$	144 113	125 89

Note. Complementation tests were performed at 32° as described under Materials and Methods. Burst sizes are given as phage per infected cell assayed 120 min after phage adsorption. Bursts of *imm*λ phage were scored from duplicate platings on K159, a $\lambda imm434$ lysogen of K37, at 32°. Bursts of *imm434* phage were scored from duplicate platings on K124, a λ lysogen of K37, at 32°.

in a single infection (0.15, line 3). In a mixed infection with $\lambda imm434cI^-$ (line 7), the burst of $\lambda cI^-(QSRrha^+)_{80}$ is still very low (1.0). In addition, the presence of $\lambda cI^-(QSRrha^+)_{80}$ reduced the burst of $\lambda imm434cI^-$ almost 20-fold (from 130 in single infection, line 2, to 7.0). This result indicates that a coinfecting heteroimmune phage with the QSR region of λ is unable to complement $\lambda(QSR)_{80}$ phage for growth in $himA42$ at 32° and that the $(QSR)_\lambda$ phage is negatively complemented by $\lambda(QSR)_{80}$. Complementation experiments have been performed with various combinations of $(QSR)_{80}$ and $(QSR)_\lambda$ phage (e.g., $\lambda imm434(QSRrha^+)_{80}$ + λ, $\lambda(QSR-rha^+)_{80}$ + $\lambda imm21$); the general pattern of lack of complementation of the $(QSR)_{80}$ phage and negative complementation of the coinfecting phage was always observed. The extent of negative complementation was variable, however, ranging from 2- to 90-fold.

Line 8 in Table 6 shows the results of a mixed infection between $\lambda cI^-(QSRrha^+)_{80}$ and the deletion mutant $\lambda imm434cI^-$-$(QSRrha\Delta01)_{80}$. The burst of the rha^+ phage is not significantly increased (from 0.15 to 0.6), and the burst of the deletion mutant is reduced from 179 (line 4) to 13. The $rha\Delta01$ allele is therefore recessive to rha^+. The rha^+ allele and the putative point mutation $rha02$ exhibit a different complementation pattern (line 9). In this mixed infection, both phage produce significant bursts, although the burst of the rha^+ phage is still somewhat low (29). Finally, we point out the following. Lines 10 and 11 in Table 6 show that neither the deletion nor point mutant negatively complement a coinfecting heteroimmune λ phage. The result in line 12 shows that the deletion and point mutants do not interfere with each other's growth. In K37, all single and mixed infections produce normal bursts of each phage, demonstrating the specificity for IHF of the observed phenotypes.

The following experiment was performed to determine if the rha^+ phage must be replicating in order to cause the negative complementation of the coinfect-ing phage. A repressed λ prophage can be transactivated for late gene expression from the late promotor P_R' if Q is supplied by a superinfecting helper phage, because P_R' is not subject to cI repression (Thomas, 1966). Recall that in burst experiments, both λ and the deletion mutant $\lambda imm434cI^-(QSRrha\Delta01)_{80}$ are negatively complemented by $\lambda(QSRrha^+)_{80}$ in $himA42$ at 32°. If Rha expression is under Q control (as expected from its map position), it should be transactivated by infection with a heteroimmune phage containing the Q gene of $\phi80$, but not by one with the Q gene of λ, because the Q gene products of λ and $\phi80$ are not complimentary (Schlief, 1972). Conforming with this prediction, we find that on a lawn of a $\lambda imm434(QSRrha^+)_{80}$ lysogen of $himA42$ (K1237) at 32°, λ^+ forms large plaques while $\lambda(QSRrha\Delta01)_{80}$ does not form plaques. In addition to showing that replication of the rha^+ phage is not necessary in order to cause the inhibition, this result suggests that expression of a diffusible gene product encoded by the rha locus is responsible for the $trans$-acting growth inhibition. Further, this result shows that rha^+ is under Q control and can be transactivated by Q_{80}, but not by Q_λ. Therefore, we conclude that phage carrying the $rha\Delta01$ mutation do not produce the rha gene product but are still sensitive to its effects.

DISCUSSION

The element responsible for the inability of $\lambda(QSR)_{80}$ to grow lytically in HimA$^-$ and Hip/HimD$^-$ mutants of $E.$ $coli$ at 32° has been mapped to the $(QSR)_{80}$ region of the phage genome, and placed more precisely within the right half of the $(QSR)_{80}$ substitution by restriction enzyme and heteroduplex analysis of substitution and deletion mutations that result in the Rha$^-$ phenotype.

The complementation studies lead us to conclude that rha encodes a diffusable gene product capable not only of preventing growth of $\lambda(QSR)_{80}$, but also of acting in $trans$ to inhibit growth of coinfecting

phage with the $(QSR)_{80}$ or $(QSR)_\lambda$ regions. The data effectively rule out an alternative model, i.e., that *rha* defines a site that in some manner, in the absence of IHF, consumes a product required for the growth of lambdoid phages at low temperature. We summarize the observations leading to this conclusion. First, rha^+ is dominant to the $rha\Delta01$ deletion mutant or rha^0 $[(QSR)_\lambda]$. These observations are consistent with either model. Second, Rha activity can be transactivated from a repressed prophage by $\lambda(QSR)_{80}$, but not by $\lambda(QSR)_\lambda$. Since in neither case does the prophage replicate autonomously, the *rha* DNA cannot simply provide a site that titrates some essential activity. However, it is possible that the site could operate if it was in some way activated. For instance, the transactivation results are consistent with transcriptionally activated *rha* DNA titrating an essential function. Third, one Rha$^-$ mutant, Rha-02, is dominant, permitting growth of both the mutant and a Rha$^+$ phage under restrictive conditions. The most likely explanation for the phenotype of this mutant is the production of an altered Rha product that can act in *trans* to interfere with the activity of the wild-type Rha product. An explanation of the Rha-02 mutant on the basis of an altered site would require postulating a change to a more active site in attracting the putative function, but less active in the Rha interaction inhibiting phage growth. A change with such contradictory characteristics seems highly unlikely. Collectively, the results of the complementation studies are most consistent with the *rha* locus encoding a diffusible gene product.

One obviously interesting question raised by these experiments is the basis for the temperature sensitivity of the Rha phenotype. Although we have no results to explain this observation, we point out that $\phi80$ is normally temperature sensitive for growth. Processes previously shown to be temperature sensitive in $\phi80$ include a step in the morphogenesis process late in infection and excision of an integrated prophage (Aizawa and Matshurio, 1975).

Thus, the *rha*-encoded protein may simply be inactive at high temperature.

How does IHF circumvent the growth inhibition of lambdoid phages by Rha? Again we can only offer informed speculation. IHF has been shown to be a DNA-binding protein; the sequence at which it binds has been identified (Nash, 1981). It is possible that IHF interferes with Rha function by binding to the phage genome, e.g., the bound IHF might compete with Rha for a site. Alternatively, IHF may exert its effect by inactivating Rha through protein/protein interaction or by preventing translation of the *rha* gene. IHF is thought to regulate λ cII synthesis at the translational level (Hoyt et al., 1982). It is unlikely that IHF represses transcription of the *rha* gene. Transactivation experiments suggest that *rha* is transcribed from the late promoter P_R' and not from a unique promoter. Since $\lambda(QSRrha^+)_{80}$ grows normally under HimA$^+$-Hip/HimD$^+$ conditions, P_R' must be active.

The identification of Rha adds another to the small list of functions found in lambdoid phages that are apparently unique to only one of the members of this group, having no analogous functions in other lambdoid phages.

ACKNOWLEDGMENTS

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