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Identification of Related Genes in Phages φ80 and P22 Whose Products Are Inhibitory for Phage Growth in *Escherichia coli* IHF Mutants

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Bacteriophage λ grows in both IHF$^+$ and IHF$^-$ host strains, but the lambdoid phage φ80 and hybrid phage λ(QSR*rha*$^+$)$_{80}$ fail to grow in IHF$^-$ host strains. We have identified a gene, *rha*, in the φ80 region of the λ(QSR*rha*$^+$)$_{80}$ genome whose product, Rha, inhibits phage growth in an IHF$^-$ host. A search of the GenBank database identified a homolog of *rha*, ORF201, a previously identified gene in phage P22, which similarly inhibits phage growth in IHF$^-$ hosts. Both *rha* and ORF201 contain two possible translation start sites and two IHF binding site consensus sequences flanking the translation start sites. Mutations allowing λ(QSR*rha*$^+$)$_{80}$ and P22 to grow in IHF$^-$ hosts map in *rha* and ORF201, respectively. We present evidence suggesting that, in an IHF$^+$ host, λ(QSR*rha*$^+$)$_{80}$ expresses Rha only late in infection but in an IHF$^-$ host the phage expresses Rha at low levels early in infection and at levels higher than those in an IHF$^+$ host late in infection. We suspect that the deregulation of *rha* expression and, by analogy, ORF201 expression, is responsible for the failure of φ80, λ(QSR*rha*$^+$)$_{80}$, and P22 to grow in IHF mutants.

Bacteriophages λ, φ80, and P22 are members of a family of temperate bacteriophages that are similar in genetic organization, with analogous functions being located in the same relative positions on each genome (4). This common genetic structure permits the formation of viable hybrids by homologous recombination between phages. Hybrid phages maintain the genomic organization characteristic of lambdoid phages and, so, contain copies of genes from one or the other parent phage. Thus, hybrid lambdoid phages may have characteristics unique to one or the other parent. For example, λ, but not φ80, grows in hosts defective for the DNA-binding protein, integration host factor (IHF) (19a, 25). Studies from this laboratory indicate that a previously unidentified gene, *rha*, located downstream of p_R', in the QSR$_{80}$ region, is responsible for this failure of growth in IHF$^-$ bacteria. For the purposes of this study, we will refer to the hybrid phage as λ(QSR*rha*$^+$)$_{80}$.

IHF is a member of a group of bacterial DNA-binding proteins which wrap DNA into higher-order structures (8, 27). The *Escherichia coli himA* and *himD* genes encode the two subunits of the IHF heterodimer (10, 23). IHF is a sequence-specific DNA-binding protein which interacts primarily with the minor groove and bends DNA (6, 28, 31). Although first identified because of its requirement for the site-specific recombination reaction that integrates phage λ into the bacterial genome, IHF participates in a diverse array of both phage and host activities. Considering only lambdoid phages, IHF is required for (i) λ site-specific recombination, which integrates and excises λ from the bacterial chromosome, (ii) efficient cleavage at *cos* sites, which generates unit size genomes for packaging, (iii) activation of *c*II gene expression (cII protein is required for the production of cI repressor protein, integrase, and an antisense RNA, all of which direct the phage toward lysogeny), (iv) modulation of gene expression from the p_L promoter (14, 15), and (v) modulation of φ80 Rha protein interference with phage development (for reviews, see references 1, 9, 11, 12, and 26).

The majority of the λ(QSR*rha*$^+$)$_{80}$ genome is derived from λ, with only the rightmost 3% of the genome, including the QSR region, being derived from φ80 (24). Two λ(QSR*rha*$^+$)$_{80}$ derivatives were used to locate the *rha* gene. One derivative, λ(QSR*rhaΔ01*)$_{80}$, contains a deletion of approximately 1.7 kb in the QSR region which removes the *rha* gene. The other, λ(QSR*rhaR126P*)$_{80}$, has a point mutation in the *rha* gene (note that the name of this mutation is based on data reported in this article). Mixed infections of λ(QSR*rha*$^+$)$_{80}$ and λ(QSR-*rhaΔ01*)$_{80}$ or λ(QSR*rha*$^+$)$_{80}$ and λ(QSR*rhaR126P*)$_{80}$ led to the conclusion that λ(QSR*rha*$^+$)$_{80}$ produces a diffusible product, Rha, which mediates the growth inhibition in IHF$^-$ hosts. These experiments also indicated that the mutation in λ(QSR*rhaR126P*)$_{80}$ results in the production of an altered protein which competes with Rha at an unknown site of action. The antitermination protein for the late operon in phage φ80, Q$_{80}$, can activate *rha* expression from a λ(QSR*rha*$^+$)$_{80}$ lysogen, confirming the location and indicating that *rha* is transcribed in the same direction as the QSR genes (24, 25).

Mozola et al. (24) compared the growth rates of λ(QSR-*rha*$^+$)$_{80}$ and λ and showed that λ(QSR*rha*$^+$)$_{80}$ infection of IHF$^-$ bacteria at 32°C results in very limited cell survival, no cell lysis, pronounced inhibition of both bacterial and phage DNA syntheses, and no appreciable inhibition of transcription or translation. In contrast, λ infection results in limited cell survival, cell lysis, and no inhibition of bacterial or phage DNA synthesis. Infection with the revertant λ(QSR*rhaΔ01*)$_{80}$ does not inhibit phage DNA synthesis in the IHF$^-$ host at 32°C. On the basis of these data, those authors proposed that Rha interferes with DNA synthesis (24).

In the present study, we identify the *rha* gene and show that regulation of its expression is altered in an IHF$^-$ host. We also

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TABLE 1. Bacterial strains, relevant genotypes, and sources

Strain	Description	Source[a]
K37	Su^0 F^- *himA*$^+$ *himD*$^+$	
K2691	K37 Δ*himA sma-sma*	
DH5α	*endA1 hsdR17* ($r_K^-m_K^+$) *supE44 thi-1 recA1 gyrA relA1* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*) U169	M. Imperiale
K6113	DH5α + pRha	
K6114	DH5α + pRhaΔAB	
K6115	DH5α + pRhaΔAS	
K6116	DH5α + pRhaΔSB	
K6117	DH5α + pRhaSB	
K6118	DH5α + pRhaΔ01	
K6119	DH5α + pRevSB	
K6320	DH5α + pRevKS	
K6337	DH5α + pRevAS	
K7535	*S. typhimurium* LT7 *proAB47* Str^r	L. Baron
CGSC4288	*E. coli* Δ*pro-lac* Spc^r	L. Baron
WR4028	*E. coli-S. typhimurium* LT7 hybrid, λ^s $P22^s$ *xyl*$^+$	L. Baron
WR3035	CGSC4288 F′ *lac*$^+$ *pro*$^+$ (P22)	L. Baron
K7577	CGSC4288 F′ *lac*$^+$ *pro*$^+$ (P22ΔKE101)	

[a] Strains for which no source is listed were constructed in this laboratory.

show that the product of the P22 ORF201 gene functions similarly to the *rha* gene product.

MATERIALS AND METHODS

Bacterial strains and phages. Bacterial strains and relevant genotypes are shown in Table 1. Phages λ(QSR*rha*$^+$)$_{80}$ [previously called λ(QSR)$_{80}$], λ(QSR-*rha*Δ*01*)$_{80}$, and λ(QSR*rhaR126P*)$_{80}$ [previously called λ(QSR*rha02*)$_{80}$] are described in references 24 and 25. See below for details on P22 phages.

Media. Media were prepared as previously described (13).

Cloning. The methods described in Sambrook et al. (29) were employed. pRha and pRhaΔ01 were constructed by isolating the *Kpn*I-cos fragments from λ(QSR *rha*$^+$)$_{80}$ and λ(QSR*rha*Δ*01*)$_{80}$, respectively, ligating the fragments into the *Kpn*I site of pUC19, blunting the ends, and ligating a second time (Fig. 1). Restriction enzyme analysis was employed to identify the orientation of the fragments. pRhaAS was constructed by digesting pRha with *Ava*I and *Sst*I, ligating the 0.4-kb fragment into the *Sst*I site of pUC19, blunting the ends, and ligating a second time. Likewise, pRhaSB was constructed by digesting pRha with *Sst*I and *Bam*HI and cloning the 0.3-kb fragment into the *Sst*I and *Bam*HI sites of pUC19. The pRha.M, pRhaAS.M, and pRhaSB.M derivatives of these plasmids contain the same fragments cloned into the same sites described above but in the M13mp19 vector. The Rha deletion plasmids were constructed by digesting the pRha plasmid with appropriate restriction enzymes, isolating the appropriate fragments, and then ligating these fragments. For example, pRhaΔAB was constructed by digesting pRha with *Ava*I and *Bam*HI, isolating the 5.3-kb fragment containing vector sequences and the *Kpn*I-*Ava*I and *Bam*HI-cos portions of the (QSR)$_{80}$ region, and ligating its ends together. pRhaΔAS and pRhaΔSB were constructed in an analogous manner with appropriate restriction enzymes. pRevKS was constructed by digesting λ(QSR*rhaR126P*)$_{80}$ first with *Kpn*I, isolating the 3.5-kb *Kpn*I-cos fragment, redigesting with *Sst*I, and cloning the 2.0-kb *Kpn*I-*Sst*I fragment into the *Sst*I site of pUC19. pRevAS was constructed by digesting pRevKS with *Ava*I and *Sst*I and cloning the 0.4-kb fragment into the *Ava*I and *Hin*cII sites of pUC19. The M13 derivative of this plasmid, pRevAS.M, contains the same fragment cloned into the same sites in M13mp19. pRevSB was constructed by digesting λ(QSR*rhaR126P*)$_{80}$ first with *Kpn*I, isolating the 3.5-kb *Kpn*I-cos fragment, redigesting with *Sst*I and *Bam*HI, and cloning the 0.3-kb fragment into the *Sst*I and *Bam*HI sites of pUC19. pGTGRha contains a PCR product created with primers described below, digested with *Eco*RI and *Hin*dIII, and cloned into the *Eco*RI and *Hin*dIII sites of pQE30. This construct is deleted for the DNA encoding the six histidine residues which is normally just upstream of the polylinker in pQE30. pRhapep was constructed by cloning a PCR product created with primers described below, digested with *Bam*HI and *Hin*dIII, and cloned into the *Bam*HI and *Hin*dIII sites of pQE30. This plasmid retains the code for six histidine residues just upstream of the plasmid insert. pUC19, M13mp19, and pQE30 plasmid DNAs were purchased from either Boehringer Mannheim Biochemicals or Qiagen. Restriction enzymes and other plasmids were obtained from Qiagen, Bethesda Research Laboratories, Boehringer Mannheim Biochemicals, and New England Biolabs and used according to the manufacturer's instructions.

Marker rescue. Marker rescue experiments were performed by incubating DH5α bacteria (containing a relevant plasmid) and phage λ(QSR*rha*$^+$)$_{80}$ in Luria-Bertani broth which was 10 mM in $CaCl_2$ with vigorous shaking at 37°C for 4 h. Cultures were treated with chloroform, and the phage titer was determined on lawns formed from IHF^+ (K37) and IHF^- (K2691) bacterial strains at 32°C.

PCR. PCRs were carried out as described in reference 29. The insert for plasmid pGTGRha was generated with λ(QSR*rha*$^+$)$_{80}$ DNA as a template and the following oligonucleotide primers obtained from the University of Michigan DNA facility: 5′GGAATTCGTGAGACAGGCTCACTTTCTA3′ and 5′CCCA AGCTTAGTGAATGCCTGCTGTAATG3′. The insert for plasmid pRhapep was generated with λ(QSR*rha*$^+$)$_{80}$ DNA as a template and the following oligonucleotide primers: 5′CGGGATCCAATAATCCGTCAGTTAT3′ and 5′CCC AAGCTTGCTTTGAGCGCGTTTCT3′.

DNA sequencing. PCR sequencing was performed with [γ-^{32}P]dATP according to the manufacturer's instructions included in the Promega fmol kit. Priming oligonucleotides were obtained from the University of Michigan DNA facility. The oligonucleotide primer 5′TTCTGGCTGCGTTACCTGCAT3′ and a pRha DNA template were used in the sequencing PCRs. Dideoxynucleotide sequencing was carried out with [α-^{35}S]dATP with the United States Biochemical Sequenase version 2.0 kit. M13 forward and reverse universal primers obtained from Promega were used with M13 templates (pRha.M, pRhaAS.M, pRhaSB.M, and pRevAS.M) for this type of sequencing. The oligonucleotide primer 5′GT TGTCGAGGGTCGTGA3′ was also used to sequence from the M13 templates.

Construction of the P22ΔKE101 lysogen. Phage P22ΔKE101 was supplied by S. Casjens (5). *Salmonella typhimurium* LT7 AB47$^-$ F′ *lac*$^+$ *pro*$^+$ contains a chromosomal deletion that includes the P22 *attB* site. The F′, however, contains a P22 *attB* site, so P22 is integrated into the F′ in LT7 *proAB47* P22 lysogens. Lysogens were obtained by spotting P22 phage onto a bacterial lawn, isolating bacteria from the turbid area, and selecting bacteria which were resistant to P22 infection. F′ elements with P22 prophage were then transferred to *E. coli* CGSC4288.

Zygotic induction. Strains WR3035, K7577, K37, and K2691 were subcultured to approximately 10^8 cells per ml at 32°C. Donor and recipient strains in Luria-Bertani broth were mixed at a 1:5 ratio and agitated very slowly at 32°C for 1.5 h to allow transfer of the F′ and associated prophage. The mated strains were diluted 1:10 in Luria-Bertani broth and agitated vigorously for 4 h at 32°C. The titer of progeny P22 phage was determined by using as the lawn an *E. coli-S. typhimurium* hybrid strain, WR4028, which allows growth of P22 grown in *E. coli.*

Protein purification. The QiaExpress system from Qiagen was used to overexpress and purify a Rha peptide. pRhapep contains a PCR product encoding Rha amino acids 14 to 146, cloned into the *Bam*HI and *Hin*dIII sites in the polylinker of Qiagen vector pQE30. IPTG (isopropyl-β-D-thiogalactopyranoside)-induced expression from pRhapep produced an N-terminal $(His)_6$-tagged Rha peptide. The peptide was purified according to the manufacturer's instructions.

Production of anti-Rha polyclonal antisera. Female New Zealand White rabbits were inoculated with the Rha peptide in Freund's complete adjuvant. Three boosts of the Rha peptide in Freund's incomplete adjuvant were given at 3-week intervals. Rabbit serum was prepared as described in reference 17.

Western blotting (immunoblotting). Protein extracts were prepared by resus-

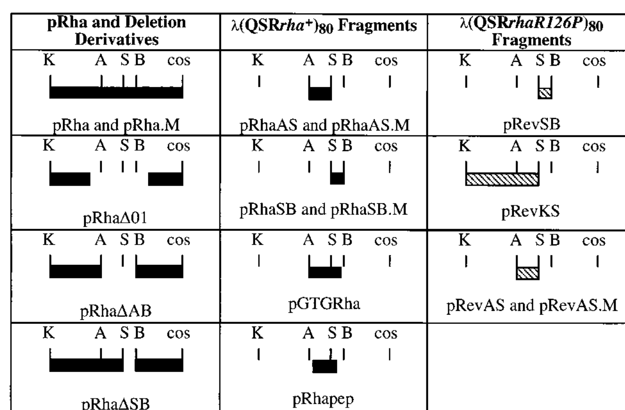

FIG. 1. Rha plasmid series (see Materials and Methods for details of construction). The first column shows the plasmid inserts in pRha and the derivative deletion constructs. The second and third columns show the fragments of λ(QSR *rha*$^+$)$_{80}$ and λ(QSR*rhaR126P*)$_{80}$ which were cloned into plasmids. Solid and hatched bars, DNAs derived from λ(QSR*rha*$^+$)$_{80}$ and λ(QSR*rhaR126P*)$_{80}$, respectively. Restriction enzymes: A, *Ava*I; B, *Bam*HI; K, *Kpn*I; S, *Sst*I. cos, right cohesive end of φ80, which is identical to that of λ.

TABLE 2. Marker rescue of plasmid-borne *rha* mutations by λ(QSR*rha*$^+$)$_{80}$

Strain (plasmid)[a]	Frequency of phage which grow in IHF$^-$ bacteria[b]
K6113 (pRha)	1×10^{-5}
K6118 (pRhaΔ01)	4×10^{-3}
K6114 (pRhaΔAB)	1×10^{-3}
K6116 (pRhaΔSB)	2×10^{-3}
K6119 (pRevSB)	2×10^{-6}
K6320 (pRevKS)	2×10^{-4}
K6337 (pRevAS)	4×10^{-4}

[a] The portion of λ(QSR*rha*$^+$)$_{80}$ or λ(QSR*rhaR126P*)$_{80}$ included in each plasmid is diagrammed in Fig. 1.

[b] Titer on K2691 (IHF$^-$)/titer on K37 (IHF$^+$).

pending bacterial pellets in loading buffer and boiling for 5 min. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15% 29:1 polyacrylamide/bisacrylamide) and electroblotted onto nitrocellulose. Rha protein was measured according to the manufacturer's instructions by using the Amersham ECL kit.

Nucleotide sequence accession number. The nucleotide sequence reported here has been deposited in GenBank under accession number L40418.

RESULTS

Marker rescue to localize the *rha* gene. Deletion mapping was used to locate the position of the *rha* gene within the (QSR)$_{80}$ region. Previous studies had shown that mutations which allow λ(QSR*rha*$^+$)$_{80}$ to grow in *E. coli* IHF mutants map to the (QSR)$_{80}$ region. Therefore, we assumed that the 1.7-kb deletion in the (QSR)$_{80}$ region of the λ(QSR*rhaΔ01*)$_{80}$ revertant includes at least part of the *rha* gene. To determine the location of the *rha* gene, we constructed a set of deletions which further dissected the region covered by the *rhaΔ01* deletion. These deletions were created by cleaving with appropriate restriction enzymes in the (QSR)$_{80}$ insert of plasmid pRha (Fig. 1). Constructs that had lost the *rha* gene were identified by using λ(QSR*rha*$^+$)$_{80}$ in a marker rescue experiment. If the deletion removed essential parts of the *rha* gene, then recombinants formed between an infecting λ(QSR*rha*$^+$)$_{80}$ and the plasmid should grow in an IHF mutant because they are deleted for *rha*. Table 2 shows the results of these marker rescue experiments. Recombinants that grow in IHF mutants were obtained in crosses with two deletion plasmids, pRhaΔAB and pRhaΔSB. The frequency of obtaining phage which grow in IHF$^-$ bacteria was 100-fold higher in a lysate of λ(QSR*rha*$^+$)$_{80}$ grown on bacteria containing pRhaΔAB or pRhaΔSB than in a lysate grown on bacteria containing pRha. The higher yield indicates that recombination between λ(QSR*rha*$^+$)$_{80}$ and each of these fragments generated Rha$^-$ phage. Further, these results indicate that the *Ava*I-*Bam*HI and *Sst*I-*Bam*HI restriction fragments each contain a portion of the *rha* gene.

The *rha* open reading frame was more precisely located by using plasmids containing fragments of the (QSR)$_{80}$ region from λ(QSR*rhaR126P*)$_{80}$ (Fig. 1). λ(QSR*rhaR126P*)$_{80}$ is a mutant of λ(QSR*rha*$^+$)$_{80}$ which grows in IHF mutants and does not contain any large deletions. Since the responsible mutation is located in the QSR region, this phage likely contains a point mutation in the *rha* gene which alters the function of the Rha protein (25). We have named the *rhaR126P* mutation on the basis of sequence data presented below. Lysates of λ(QSR*rha*$^+$)$_{80}$ grown on bacteria containing either of two plasmids, pRevKS or pRevAS, yielded phage that grow in IHF mutants at a 100-fold higher frequency than did lysates grown on bacteria containing pRha. The higher yield presumably indicates the presence of recombinants and locates the point mutation in λ(QSR*rhaR126P*)$_{80}$ within the *Ava*I-*Sst*I restriction fragment. There was no increase in the number of phage plated on IHF$^-$ strains in lysates of λ(QSR*rha*$^+$)$_{80}$ grown on a strain containing pRevSB, indicating that the *rhaR126P* mutation does not lie within the *Sst*I-*Bam*HI restriction fragment.

Sequence of the *rha* gene and the *rhaR126P* allele. The sequence of the region that apparently contains the *rha* gene was determined. Sequencing the inserts in plasmids pRhaAS.M and pRhaSB.M revealed several open reading frames. Mozola and Friedman determined that Q_{80} can activate expression of *rha*, indicating that *rha* is transcribed as part of the late operon which initiates at $p_R{}'$ (25). Only one of the possible *rha* open reading frames reads in the direction of $p_R{}'$ transcription and overlaps the location of the *rhaR126P* mutation. There are two possible translation start sites for this open reading frame, a GUG and an AUG, and each has a ribosome binding site consensus sequence (30) upstream (Fig. 2).

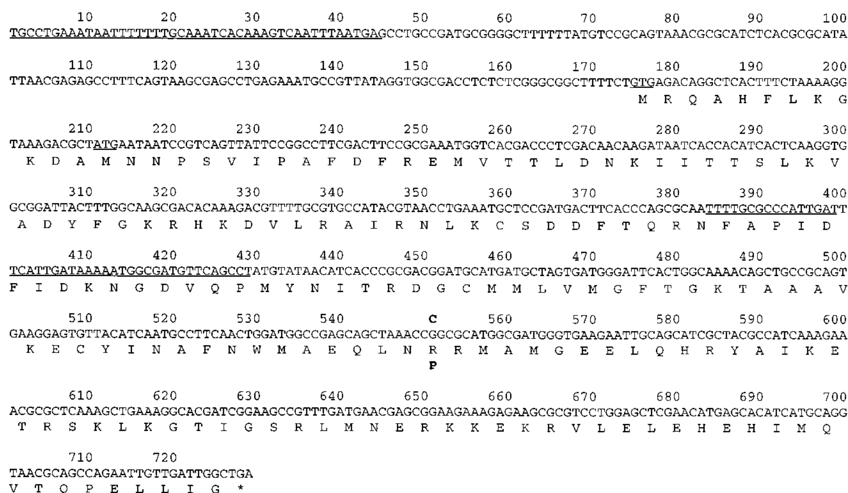

FIG. 2. Sequence of the φ80 *rha* gene and upstream sequences. The two possible translation start sites and the two putative IHF binding sites are underlined. The *rhaR126P* mutation and the resulting amino acid change are boldfaced.

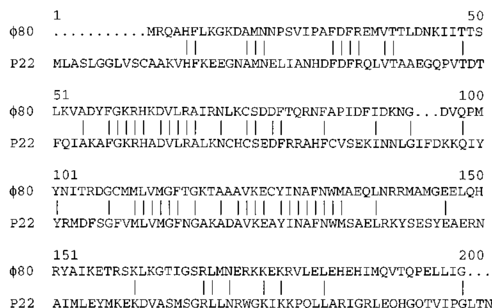

FIG. 3. Alignment of φ80 Rha and P22 ORF201 deduced amino acid sequences. The predicted amino acid sequences, beginning at the GTG translation start site, for both the φ80 and the P22 Rha proteins are shown. Identical amino acids are indicated by vertical bars.

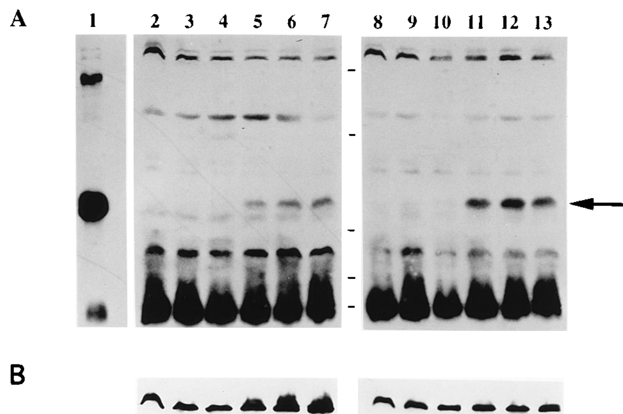

FIG. 4. Expression of φ80 Rha and *E. coli* NusA over the course of infection with $\lambda(\text{QSR}rha^+)_{80}$. Western blot analysis was performed as described in Materials and Methods. Lane 1, extract isolated from K37 containing pGTGRha; lanes 2 and 8, extracts from uninfected strains K37 (IHF^+) and K2691 (IHF^-), respectively; lanes 3 to 7, extracts isolated from strain K37 infected by $\lambda(\text{QSR}rha^+)_{80}$ at 5, 10, 20, 30, and 40 min postinfection, respectively; lanes 9 to 13, extracts isolated from strain K2691 infected by $\lambda(\text{QSR}rha^+)_{80}$ at 5, 10, 20, 30, and 40 min postinfection. The location of the Rha protein is indicated (arrow). (A) Proteins identified by anti-Rha antibody; (B) *E. coli* NusA protein identified by anti-NusA antibody.

The *rha* sequence was analyzed to locate possible IHF binding sites (6) by using the MacTargsearch program of Goodrich et al. (16). This program compares DNA sequence information with the sequences of known IHF binding sites and calculates a similarity score. These authors calculated similarity scores, ranging from 46.2 to 76.6, for 27 sites known to bind IHF in vitro. There are two IHF binding site consensus sequences in or near the *rha* open reading frame, centered at −150 and +233 relative to the GUG translation start site (the IHF binding site consensus sequences are underlined in Fig. 2). These predicted IHF sites have similarity scores of 51.3 and 42.3, respectively.

The predicted Rha protein contains 184 or 172 amino acids, depending on which translation start site is used. Sequencing the pRevAS plasmid containing the *rhaR126P* point mutation revealed a single base change of G to C at position 377 (numbered from the GUG start site). This mutation changes amino acid 126 from arginine to proline (Fig. 2).

Identification of a functional homolog of *rha* in phage P22. By using the Genetics Computer Group DNA sequence analysis program, a TFASTA search was performed to identify proteins homologous to Rha (7). Only one protein, translated from P22 ORF201, showed a high degree of homology to Rha. There is 34.8% amino acid identity between the predicted amino acid sequences of the two open reading frames, including regions which are more than 90% identical (Fig. 3). The two open reading frames are located in analogous positions on the P22 and φ80 genomes. The first three genes in the late operon of lambdoid phages are the λ *S*, *R*, and *Rz* homologs (32). φ80 *rha* and P22 ORF201 are located immediately downstream of the λ *Rz* homologs (5; also, this study). The two genes each have two possible translation start sites, one GUG and one AUG. Like the φ80 *rha* gene, P22 ORF201 also has two IHF consensus binding sequences. One IHF site is located upstream of the translation start sites and one is located within the open reading frame (shown for *rha* in Fig. 2).

To determine if P22 ORF201 also interferes with phage growth in an IHF mutant, P22 was tested for its ability to grow in an *E. coli* IHF^- host. P22, an *S. typhimurium*-specific phage, cannot infect *E. coli*. Therefore, the P22 genome was introduced into *E. coli* by a method which circumvents the attachment step, transfer on an F′. When the F′ with the integrated phage genome is transferred to the recipient, provided there is no P22 repressor, the prophage is induced in a process called zygotic induction (19). Strain WR3035, which carries an F′ *pro-lac* containing a P22 prophage, was mated with either the IHF^+ strain K37 or the isogenic IHF^- strain K2691. The mating at 32°C with the IHF^+ strain resulted in a 1,000-fold greater yield of phage than did the mating with the IHF^- strain (titers, 5×10^9 and 4×10^6, respectively).

To determine if the P22 ORF201 gene product might be responsible for this failure of P22 growth in the IHF mutant, we compared the growth of P22ΔKE101, a P22 derivative carrying a partial deletion of ORF201 (5), in isogenic IHF^+ and IHF^- hosts. As in the experiments outlined above, we used an F′ *pro-lac* derivative with an integrated prophage (in strain K7577) to introduce the P22ΔKE101 genome into the IHF^+ and IHF^- bacteria. There was not a significant difference in phage yield from matings to the IHF^+ and IHF^- recipients at 32°C (titers, 1×10^9 and 5×10^9, respectively), indicating that P22ΔKE101 grows well in both strains.

Production of Rha during $\lambda(\text{QSR}rha^+)_{80}$ infection. We employed Western analysis to determine if the timing and level of *rha* expression differs during $\lambda(\text{QSR}rha^+)_{80}$ infection of IHF^+ and IHF^- strains. As shown in Fig. 4A, Rha is expressed late in infection of an IHF^+ host, as measured at 20, 30, and 40 min postinfection. Rha protein is not detectable at earlier times. In contrast, small amounts of Rha protein are expressed early in infection of an IHF^- host, as measured at 5 and 10 min postinfection. Greater quantities of Rha appear to be produced in an IHF^- host than in an IHF^+ host late in infection, as measured at 20, 30, and 40 min postinfection. Although these data suggest that both the timing and the amount of Rha protein production may be affected by the presence of IHF, they are not definitive.

Reprobing the same Western blot revealed that levels of the bacterial protein NusA were relatively constant in protein samples derived from IHF^- cells, both early and late in infection, and in samples derived from IHF^+ cells early in infection. These results confirm that protein extracts from equal numbers of cells had been loaded in each of these lanes. However, protein samples taken from infected IHF^+ cells at 20, 30, and 40 min postinfection show higher levels of NusA (Fig. 4B). Equivalent numbers of bacterial cells were infected, and the multiplicity of phage infection, 5, was sufficient to ensure that over 95% of the bacteria were infected. Similar volumes of each culture were used to prepare the protein extracts. It is

unclear whether the difference in the level of NusA is due to an increase in the cell mass of the IHF^+ bacterium or a decrease in the cell mass of the IHF^- bacterium, perhaps due to cell lysis. It is important to note, however, that, even though the amount of NusA detected late in infection in the IHF^- cell extracts is less than that in IHF^+ extracts, the level of Rha protein is higher in the IHF^- cell extracts.

DISCUSSION

We have identified the *rha* gene in the QSR region of phage φ80. Its product, an approximately 20-kDa protein, can cause a failure in phage growth. Surprisingly, even though the genes immediately upstream of *rha* have homologs in λ (the Q, S, R, and Rz genes), there is no homolog of *rha* in λ. However, we have identified a structural and functional homolog of *rha* in another lambdoid phage, P22.

Using specific antiserum to identify the Rha protein, we have shown that the timing and levels of *rha* expression differ in isogenic IHF^+ and IHF^- bacteria following infection by $\lambda(\mathrm{QSR}rha^+)_{80}$. The timing of expression is delayed and the levels of Rha protein late in infection are significantly lower in the IHF^+ bacterium. For comparison, Western blots were reprobed with an antiserum raised against a host protein, NusA. The levels of NusA are similar in extracts isolated at early and late times following infection of the IHF^- host, while they appear to be elevated in lanes loaded with extracts isolated at later times following infection of the IHF^+ host. This indicates that, compared with the IHF^+ infection, higher levels of Rha are present in extracts derived from the IHF^- infection, even though there may be less total protein loaded in these lanes.

Although we are unable to explain why the presence of Rha aborts the phage infection, previous studies suggest some possibilities. It was shown that infection with $\lambda(\mathrm{QSR}rha^+)_{80}$, but not λ or $\lambda(\mathrm{QSR}rha\Delta 01)_{80}$, inhibits host DNA synthesis. Since *rha* is deleted in the $\lambda(\mathrm{QSR}rha\Delta 01)_{80}$ phage and there is no *rha* homolog in λ, we suggest that *rha* may be responsible for inhibiting host DNA synthesis. Although infection with λ does not inhibit host DNA synthesis, infection with P22 does. Hilliker, employing a series of hybrid phages, concluded that the P22 gene(s) responsible for shutoff of host DNA synthesis is linked to the replication genes 18 and 12. It is, however, unlikely that ORF201 encodes the host DNA synthesis function. Examination of the genetic structure of the phage hybrids used in those studies reveals that the hybrids do not carry P22 ORF201 (18).

Clearly, the deregulated expression of *rha* can seriously impede phage infection, and our studies indicate that IHF may influence the regulation of *rha* expression. How might IHF function in this role? A number of studies have shown that IHF can act at many levels to regulate gene expression as well as gene product action. In λ, IHF has been shown to influence transcription, translation, and protein action (see references 1, 9, 11, 12, and 26 for reviews).

Transcriptional regulation of *rha* would seem to be a likely mechanism of IHF action. Since the *rha* gene is part of the late operon, expression of the operon, rather than of the *rha* gene itself, would be regulated by IHF. Although we have no information about transcription of the late operon in φ80, we do have information about late operon transcription in λ. Kur et al. (20, 21) reported that there are IHF binding sites in and around the λ p_R' promoter, the promoter controlling late gene expression, including a binding site which overlaps the −35 region of p_R'. Transcription from p_R' on a plasmid construct is 50% higher in an IHF^- bacterium than in an IHF^+ bacterium, indicating that IHF can reduce expression from the p_R' promoter (20, 21). Although the Q antitermination protein clearly plays the central role in regulation of late gene expression, IHF apparently plays an auxiliary role.

Control of Rha production at the level of translation is suggested in two ways: (i) there are two translation start signals near the 5′ end of the *rha* coding region, and (ii) there are two IHF binding site consensus sequences flanking these start signals. If either or both of the possible IHF binding sequences contribute to the regulation of *rha* expression, control must be exerted posttranscriptionally because these sites are far removed from the promoter. Studies by Mahajna et al. (22) serve as a precedent for proposing translational regulation by IHF. Those workers proposed that perhaps IHF binding to RNA controls translation of the λ *c*II mRNA. Similarly, it is possible that IHF binding to the *rha* mRNA might modulate expression of *rha* by regulating the level of Rha produced or by determining which translation start site is used. A regulatory scheme based on alternative translation signals has been described for the λ S gene (2, 3). Our Western analysis, however, did not show two species of Rha protein. Since the sizes of the proteins produced by initiating translation at each of the two start sites are predicted to be 19.1 and 20.4 kDa, it is possible that the similar electrophoretic mobilities of such proteins would prevent resolution of the two forms.

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REFERENCES

1. **Becker, A., and H. Murialdo.** 1990. Bacteriophage lambda DNA: the beginning of the end. J. Bacteriol. **172:**2819–2824.
2. **Blasi, U., C.-Y. Chang, M. T. Zagotta, K. Nam, and R. Young.** 1990. The lethal lambda S gene encodes its own inhibitor. EMBO J. **9:**9481–9489.
3. **Blasi, U., K. Nam, D. Hartz, L. Gold, and R. Young.** 1989. Dual translational initiation site control function of the lambda S gene. EMBO J. **8:**3501–3510.
4. **Campbell, A., and D. Botstein.** 1983. Evolution of the lambdoid bacteriophages, p. 365–380. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Evolution of the lambdoid bacteriophages. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Casjens, S., K. Eppler, R. Parr, and A. R. Poteete.** 1989. Nucleotide sequence of the bacteriophage P22 gene 19 to 3 region: identification of a new gene required for lysis. Virology **171:**588–598.
6. **Craig, N. L., and H. A. Nash.** 1984. E. coli integration host factor binds to specific sites in DNA. Cell **39:**707–716.
7. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
8. **Drlica, K., and Y. J. Rouviere.** 1987. Histonelike proteins of bacteria. Microbiol. Rev. **51:**301–319.
9. **Feiss, M.** 1986. Terminase and the recognition, cutting, and packaging of lambda chromosomes. Trends Genet. **2:**100–104.
10. **Flamm, E. L., and R. A. Weisberg.** 1985. Primary structure of the hip gene of Escherichia coli and of its product, the beta subunit of integration host factor. J. Mol. Biol. **183:**117–128.
11. **Freundlich, M., N. Ramani, E. Mathew, A. Sirko, and P. Tsui.** 1992. The role of integration host factor in gene expression in Escherichia coli. Mol. Microbiol. **6:**2557–2563.
12. **Friedman, D. I.** 1988. Integration host factor: a protein for all reasons. Cell **55:**545–554.
13. **Friedman, D. I., C. T. Jolly, and R. J. Mural.** 1973. Interference with the expression of the N gene function of phage lambda in a mutant of Escherichia coli. Virology **51:**216–226.
14. **Giladi, H., M. Gottesman, and A. B. Oppenheim.** 1990. Integration host factor stimulates the phage lambda pL promoter. J. Mol. Biol. **213:**109–121.
15. **Giladi, H., S. Koby, M. E. Gottesman, and A. B. Oppenheim.** 1992. Supercoiling, integration host factor, and a dual promoter system participate in the control of the bacteriophage lambda pL promoter. J. Mol. Biol. **224:**937–948.
16. **Goodrich, J. A., M. L. Schwartz, and W. R. McClure.** 1990. Searching for and

predicting the activity of sites for DNA binding proteins: compilation and analysis of the binding sites for Escherichia coli integration host factor (IHF). Nucleic Acids Res. **18:**4993–5000.
17. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
18. **Hilliker, S.** 1974. Specificity of regulatory elements in temperate bacteriophages. Doctoral thesis. Massachusetts Institute of Technology, Cambridge.
19. **Jacob, F., and E. L. Wollman.** 1961. Sexuality and the genetics of bacteria. Academic Press, New York.
19a. **Kikuchi, A., and R. Weisberg.** Unpublished data.
20. **Kur, J., N. Hasan, and W. Szybalski.** 1989. Physical and biological consequences of interactions between integration host factor (IHF) and coliphage lambda late p′R promoter and its mutants. Gene **81:**1–15.
21. **Kur, J., N. Hasan, and W. Szybalski.** 1990. Alterations in the p′R promoter of coliphage lambda modify both its activity and interaction with the integration host factor (IHF). Mol. Gen. Genet. **221:**411–420.
22. **Mahajna, J., A. B. Oppenheim, A. Rattray, and M. Gottesman.** 1986. Translation initiation of bacteriophage lambda gene *c*II requires integration host factor. J. Bacteriol. **165:**167–174.
23. **Miller, H. I., and D. I. Friedman.** 1980. An E. coli gene product required for lambda site-specific recombination. Cell **20:**711–719.
24. **Mozola, M. A., D. L. Carver, and D. I. Friedman.** 1985. A phi 80 function inhibitory for growth of lambdoid phage in him mutants of Escherichia coli deficient in integration host factor. II. Physiological analysis of the abortive infection. Virology **140:**328–341.
25. **Mozola, M. A., and D. I. Friedman.** 1985. A phi 80 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. Virology **140:**313–327.
26. **Nash, H. A.** 1981. Integration and excision of bacteriophage lambda: the mechanism of conservation site specific recombination. Annu. Rev. Genet. **15:**143–167.
27. **Pollock, T. J., and H. A. Nash.** 1983. Knotting of DNA caused by a genetic rearrangement. Evidence for a nucleosome-like structure in site-specific recombination of bacteriophage lambda. J. Mol. Biol. **170:**1–18.
28. **Robertson, C. A., and H. A. Nash.** 1988. Bending of the bacteriophage lambda attachment site by Escherichia coli integration host factor. J. Biol. Chem. **263:**3554–3557.
29. **Sambrook, J., E. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
30. **Shine, J., and L. Dalgarno.** 1974. The 3′ terminal sequence of Escherichia coli 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71:**1342–1346.
31. **Yang, C. C., and H. A. Nash.** 1989. The interaction of E. coli IHF protein with its specific binding sites. Cell **57:**869–880.
32. **Young, R.** 1992. Bacteriophage lysis: mechanism and regulation. Microbiol. Rev. **56:**430–481.