

# 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

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Derivatives of phage  $\lambda$  with the rightmost 3% of the genome (the QSR region) from the related phage  $\phi 80$  fail to grow at low temperatures (e.g., 32°) in *Escherichia coli* hosts deficient in either protein component of IHF (integration host factor), the products of the *himA* and *hip/himD* genes. The abortive infection of  $\lambda(QSR)_{80}$  in mutants defective for IHF was studied in detail. This infection is characterized by a lack of cell lysis and an inhibition of phage DNA replication after an initial period of normal synthesis. An inhibition of host DNA replication also occurs after a similar period of apparently normal synthesis, and the abortive  $\lambda(QSR)_{80}$  infection is lethal to the host. An assay of  $\beta$ -galactosidase activity in  $\lambda(QSR)_{80}$ -infected cells provided indirect evidence that RNA and protein synthesis continue late into the abortive infection. The defective growth is imposed by the product of the *rha* gene located in the  $(QSR)_{80}$  genetic material. Two-dimensional electrophoretic analysis of phage proteins produced in ultraviolet (uv)-irradiated phage-infected host cells has demonstrated the existence of a protein that is encoded or whose synthesis is regulated by the *rha* locus. Based on these findings, possible roles for a HimA-Hip/HimD-controlled *rha* gene product in a late stage of  $\phi 80$  development are discussed. © 1985 Academic Press, Inc.

### INTRODUCTION

In the accompanying paper (Mozola and Friedman, 1984), we described the genetic analysis of *rha*, the element responsible for the inability of lambdoid phage  $\phi 80$  and  $\lambda(QSR)_{80}$  hybrid phage to grow lytically in HimA and Hip/HimD mutants of *E. coli* K12 at 32°. The *himA* and *hip/himD* genes encode, respectively, the  $\alpha$  and  $\beta$  subunits of IHF (integration host factor; Nash and Robertson, 1981; Miller *et al.*, 1981). Complementation analysis suggests that this growth restriction of  $\lambda(QSR)_{80}$  in *himA*<sup>-</sup> and *hip/himD*<sup>-</sup> cells is mediated by a *trans*-acting diffusible gene product encoded by the *rha* locus. The locus encoding this function (or con-

trolling its expression) has been found only in the QSR region of phage  $\phi 80$  and not in other lambdoid phages that have been studied. Most of our experiments have employed *himA*<sup>-</sup> bacteria as the IHF defective host; this paper will focus on work with the *himA42* and *himA $\Delta$ 82* mutants.

In addition to the growth restriction of  $\phi 80$  and  $\lambda(QSR)_{80}$  phage, four other examples of IHF involvement in the lytic growth of bacteriophage are known. Phage Mu is unable to grow lytically in HimA<sup>-</sup> and Hip/HimD<sup>-</sup> mutants (Miller and Friedman, 1980; Yoshida *et al.*, 1982). The block to Mu growth apparently occurs at an early step, as Mu DNA synthesis (B. Waggoner and M. Pato as cited in Yoshida *et al.*, 1982) and late RNA synthesis (Yoshida and Howe as cited in Yoshida *et al.*, 1982) are defective in a HimA<sup>-</sup> host. HimA<sup>-</sup> cells survive the abortive Mu in-

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fection (Miller and Friedman, 1980). Mutants of Mu capable of growing in HimA mutants (MumuA) have been isolated (Miller and Friedman, 1980; Yoshida *et al.*, 1982) and map within or near the Mu A gene (Yoshida *et al.*, 1982), a Mu function expressed early in infection (Wijffelman and van de Putte, 1974).

Lambdoid phage 21 and a  $\lambda$ -21 hybrid (hybrid 33) carrying the leftmost 5% of the chromosome from 21 are also incapable of lytic growth in *himA*<sup>-</sup> and *hip/himD*<sup>-</sup> mutants (M. Feiss, personal communication). Mutants of  $\lambda$ -21 hybrid 33 able to grow in HimA<sup>-</sup> strains have been isolated; these variants carry mutations (*her*) that map at the left end of the chromosome in the 21 analog of the  $\lambda$  *Nul* gene (M. Feiss, personal communication), a gene encoding a protein directly involved in DNA packaging (Hohn, 1975; Becker *et al.*, 1977). Therefore, in the case of 21 and  $\lambda$ -21 hybrid 33, the block to lytic growth in HimA<sup>-</sup> and Hip/HimD<sup>-</sup> mutants is manifested at a late stage in the phage development process.

Two mutants of  $\lambda$  also exhibit growth defects in *himA*<sup>-</sup> or *hip/himD*<sup>-</sup> hosts. The mutant  $\lambda$ cin, which has an alteration in the t<sub>R1</sub> terminator (Wulff, 1976; Rosenberg *et al.*, 1978), does not grow in *himA* or *hip/himD* mutants (Williams *et al.*, 1977; Miller *et al.*, 1979). This phenotype has not been studied in detail and is not understood. A recently isolated  $\lambda$  mutant,  $\lambda$ cos154, grows poorly in *himA*<sup>-</sup> or *hip/himD*<sup>-</sup> hosts. This phage has a single base pair change in a site thought to be required for the recognition of a host factor that participates in the cleavage reaction at the *cos* site that generates unit-size  $\lambda$ DNA molecules for packaging into phage particles (Bear *et al.*, in press). A reason why  $\lambda$ cos154 grows in *him*<sup>+</sup> bacteria is suggested by *in vitro* studies which show that either of two host factors are required for cleavage at *cos*, IHF, or a previously undescribed protein (Gold and Becker, 1983; M. Gold, personal communication). Presumably, it is this second protein that acts at the site eliminated by the *cos154* mutation.

In addition to its role in  $\lambda$  lysogeny, then, IHF is potentially involved in a variety of processes associated with the lytic growth of *E. coli* bacteriophage. In this paper, we report results of a physiological analysis of the abortive infection of  $\lambda$ (QSR)<sub>80</sub> in *himA*<sup>-</sup> hosts at 32°, undertaken in an attempt to determine at what stage of the lytic cycle  $\lambda$ (QSR)<sub>80</sub> development is blocked in hosts defective in IHF. Toward this end, we have measured five parameters of the infection process: host cell killing, host cell lysis, phage DNA synthesis, effects on host DNA synthesis, and, indirectly, through an assay of  $\beta$ -galactosidase activity, RNA and protein synthesis. In addition, using two-dimensional polyacrylamide gel electrophoresis, we have analyzed specific patterns of phage protein synthesis in  $\lambda$ (QSR)<sub>80</sub>-infected bacteria in an attempt to identify a protein or proteins encoded or whose synthesis is controlled by the *rha* locus.

#### MATERIALS AND METHODS

##### *Bacterial and Bacteriophage Strains*

Most of the *E. coli* and bacteriophage strains used in this study are described in the accompanying paper (Mozola and Friedman, 1984). The following additional strains listed with relevant markers were used. Bacteria: K2524 ( $\Delta$ lacu168), obtained from M. Smith; K3057 (a derivative of K2524 carrying a BMG 67 prophage), constructed in this laboratory; K3058 (a derivative of K3057 carrying the *himA* $\Delta$ 82 mutation), constructed in this laboratory. Phage: BMG 67; a  $\lambda$ imm21lac phage, relevant details of its structure are shown in Fig. 7.

##### *Media*

Media for propagation of bacteria and phage have been described (Miller and Friedman, 1980; Mozola and Friedman, 1984). 3M-L medium used in thymidine-incorporation assays contains (per liter) 100 ml 10 $\times$  M9 salts (per 100 ml: 6 g Na<sub>2</sub>HPO<sub>4</sub>, 3 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g NaCl, and 1 g NH<sub>4</sub>Cl), 2.5 ml 1 M MgSO<sub>4</sub>, 20 ml 20%

maltose, 50 ml 20% Difco casamino acids, 100 ml 10× nucleotide base mix (2 mM each adenine, guanine, cytosine, and uracil in 0.015 M KOH), and 10 ml 2 mg/ml tryptophan. The medium is mixed from separate sterile solutions. RM medium used for protein-labeling experiments contains (per liter) 40 ml 25× RM salts (per 100 ml: 2.5 g NH<sub>4</sub>Cl, 3.75 g KCl, 6.2 g KH<sub>2</sub>PO<sub>4</sub>, and 14.2 g Na<sub>2</sub>HPO<sub>4</sub>), 0.4 ml 0.25 M CaCl<sub>2</sub>, 1 ml 1 M MgSO<sub>4</sub>, 0.3 ml 10 mM FeCl<sub>3</sub>, 1.6 ml glycerol, and 20 ml 20% maltose. The medium is mixed from separate sterile solutions. RM-Mg is RM + 10 mM MgSO<sub>4</sub>. TS buffer contains (per liter) 3.7 g Na<sub>2</sub>HPO<sub>4</sub>, 3 g KH<sub>2</sub>PO<sub>4</sub>, and 4 g NaCl adjusted to pH 6.0. For preparation of protein extracts, SDS lysing solution contains (per 462 μl) 50 μl 0.5 M Tris (pH 6.8), 80 μl 15% SDS, 10 μl glycerol, 40 μl 2-mercaptoethanol, and 272 μl distilled water. Lysis buffer contains (per 10 ml) 5.7 g urea, 0.2 ml Nonidet P-40, 0.5 ml 2-mercaptoethanol, 0.4 ml pH 5-7 ampholines (LKB), 0.1 ml pH 3-10 ampholines (LKB), and 4.2 ml distilled water.

#### *Cell Survival Assay*

To test the degree of survival of phage-infected bacteria, cultures infected as in complementation experiments (Mozola and Friedman, 1984) were titered for surviving bacteria after the initial adsorption period. Titering was done in duplicate on TB plates and the plates were incubated at 32°.

#### *Cell Lysis Assay*

Lysis of phage-infected bacteria was assayed by following changes in turbidity of the cultures at intervals postinfection. Cells were grown in LBMM at 32° to a concentration of approximately  $2 \times 10^8$ /ml and subdivided 10 ml each into side-arm culture flasks. The cells were infected with phage at a multiplicity of infection (m.o.i.) of 5 and incubated at either 32° or 42° in a shaking water bath. Turbidity measurements were taken at the time of infection and at 30-min intervals there-

after using a Klett-Summerson colorimeter equipped with a red filter.

#### *<sup>3</sup>H[Thymidine] Incorporation Assays*

For assay of phage-specific DNA synthesis by [<sup>3</sup>H]thymidine incorporation, bacteria were grown in 3M-L medium at 32° to a concentration of  $2 \times 10^9$ /ml. To inhibit host DNA synthesis, mitomycin C (Sigma) was added to a concentration of 80 μg per ml of  $2 \times 10^8$ /ml cells. An aliquot of cells was reserved for the untreated control and stored on ice. Bacteria with mitomycin C were incubated in the dark at 37° for 10 min. Treated and untreated bacteria were then pelleted by low-speed centrifugation and resuspended to a concentration of  $1 \times 10^9$ /ml in 10 mM MgSO<sub>4</sub>. For each sample, 2 ml of concentrated cells were infected with phage at an m.o.i. of 5 and incubated on ice in the dark for 20 min to facilitate adsorption. The infected cells were diluted into 18 ml prewarmed 3M-L in culture flasks in a shaking water bath at 32°. An aliquot was removed, treated with chloroform, and assayed for unadsorbed phage. At intervals between 1 and 90 min after dilution, 1-ml aliquots were removed and added to tubes containing 1 ml of 3M-L plus 10 μCi of [*methyl*-<sup>3</sup>H]thymidine (ICN) at 32°. After a 1-min pulse, incorporation was stopped by placing the tubes on ice and immediately adding 2.5 ml of an ice-cold mixture composed of 1 part of a 1 mg/ml solution of nonradioactive thymidine and 1.5 parts of 15% trichloroacetic acid (TCA). After at least 30 min on ice, the TCA-insoluble material was collected by suction filtration on 2.4-cm diameter Whatman GF-C glass fiber filters. The filters were washed with 40 ml of ice-cold 5% TCA, followed by 10 ml of room-temperature 95% ethanol. The filters were dried under a lamp for 5 min, and radioactivity was counted in vials containing 4 ml of scintillation cocktail. Experiments measuring total host plus phage DNA synthesis were performed exactly as described above except that the mitomycin C treatment was eliminated. Background levels of radio-

activity were established by adding TCA and 3M-L + [<sup>3</sup>H]thymidine simultaneously to uninfected samples 5 min after dilution. For mitomycin C-treated cultures, the background was on the order of 100–200 cpm. For untreated cultures, the background was 1000–2000 cpm.

#### *Production and Measurement of $\beta$ -Galactosidase*

Bacteria were grown in LBMM to approximately  $2 \times 10^8$ /ml, pelleted, and resuspended in 0.1 vol 10 mM MgSO<sub>4</sub>. Phage were added to an m.o.i. of 5 and allowed to adsorb for 20 min on ice. Infected cells were diluted 1:10 into LB medium. The bacteria were incubated at 32°, aliquots removed at the indicated times, placed on ice, and then assayed for  $\beta$ -galactosidase using essentially the method outlined by J. Miller (1972). Aliquots were removed at  $t = 0$  to assay phage adsorption.

#### *Analysis of Phage Protein Synthesis*

*Pulse labeling of infected cells.* Phage-infected bacteria were pulse labeled with [<sup>35</sup>S]methionine by a procedure modified from that described by Murialdo (1979). Bacteria were grown in RM medium at 32° to a concentration of  $5 \times 10^8$ /ml, pelleted, and resuspended in RM to a concentration of  $4 \times 10^9$ /ml. The concentrated cells were spread in a thin layer and irradiated with uv light (General Electric lamp No. G8T5) at a dose of  $6.0 \times 10^3$  ergs/mm<sup>2</sup> while being agitated on a rotary shaker. MgSO<sub>4</sub> was added to the cells after irradiation to a final concentration of 10 mM. Cells ( $4 \times 10^8$ , 0.1 ml) in 5-ml glass centrifuge tubes were infected at an m.o.i. of 5 and adsorption was carried out on ice in the dark for 30 min. Phage added were from lysates dialyzed against 10 mM Tris (pH 7.4) plus 10 mM MgCl<sub>2</sub>. After adsorption, RM-Mg medium prewarmed to 32° was added to a final volume of 0.5 ml and the samples were incubated in a 32° shaking water bath. At 30 min post-adsorption, 40  $\mu$ Ci of [L-<sup>35</sup>S]methionine (Amersham) was added and incubation

continued for 15 min at which time incorporation was stopped by chilling the tubes in a 0° ice-water bath. The tubes were stored on ice until all samples had been collected.

*Preparation of protein extracts.* To each tube prepared as described above, 3 ml RM at 4° was added and the cells were pelleted by centrifugation at 4° in a Sorvall SS34 rotor at 12,000 rpm for 10 min. Protein extracts were prepared according to a procedure obtained from P. Bloch. The pellets with a residual amount of medium (total volume about 5  $\mu$ l) were vortexed until a slurry was formed and transferred to 1.5-ml microfuge tubes. SDS lysing solution (13  $\mu$ l) was added and the samples were incubated in a boiling water bath for 4 min to lyse the cells. Lysis buffer (100  $\mu$ l) was added and the samples were stored at -70° until used.

*Two-dimensional gel electrophoresis.* Two-dimensional electrophoresis of proteins was performed according to the procedures of O'Farrell (1975). A 30- $\mu$ l sample was loaded onto the first-dimension isoelectric focusing gel, which was run to equilibrium and establishes an effective pH gradient of about 4.5 to 7.0. Polyacrylamide gels (15%) were used in the second dimension. The gels were run in a 4° cold room at 4.5 W per gel (constant power) until the bromophenol blue dye front was about 1 cm from the bottom of the gel (4 to 5 hr). The proteins were fixed and stained with Coomassie blue and destained as described by O'Farrell (1975). The gels were placed onto Whatman chromatography paper and dried under vacuum over a boiling water bath for 2 hr. Kodak XAR-5 X-ray film was used for autoradiography, which was for 5 to 11 days depending upon the age of the sample used for electrophoresis.

## RESULTS

### *Cell Survival*

The fraction of HimA<sup>+</sup> or HimA<sup>-</sup> cells in a culture surviving infection with  $\lambda$  or  $\lambda$ (QSR)<sub>80</sub> phage was measured. Results are shown in Table 1. Although the infec-

TABLE 1  
CELL SURVIVAL FOLLOWING  $\lambda(QSR)_{80}$  INFECTION

| Phage                          | Percentage of cells surviving infection |                                  |
|--------------------------------|-----------------------------------------|----------------------------------|
|                                | K936 ( <i>himA42</i> )                  | K37 ( <i>himA</i> <sup>+</sup> ) |
| $\lambda cI^- (QSRrha^+)_{80}$ | 17                                      | 1.6                              |
| $\lambda cI^-$                 | 7                                       | 0.7                              |
| $\lambda Pam3$                 | 100                                     | 100                              |

Note. Cell survival following phage infection at 32° was assayed as described under Materials and Methods.

tion of a *himA42* (K936) host at 32° with  $\lambda cI^- (QSRrha^+)_{80}$  is abortive and does not result in a burst of phage (Mozola and Friedman, 1984), only 17% of the infected bacteria survive. This indicates that, at least in the majority of infected cells, there is sufficient expression of one or more phage functions to irreversibly inhibit bacterial growth. In comparison, 100% of the *himA42* bacteria survived infection with a replication-defective ( $\lambda Pam3$ ) phage. Therefore, in the *himA*<sup>-</sup> host,  $\lambda cI^- (QSRrha^+)_{80}$  may express higher levels of a function lethal to the host than does the  $\lambda P^-$  phage or, alternatively,  $\lambda cI^- (QSRrha^+)_{80}$  may express a function that leads to cell killing for which there is no analog in  $\lambda$ . The productive infection of  $\lambda cI^-$  in *himA42* showed 7% cell survival.

#### Cell Lysis

Cell lysis following  $\lambda$  or  $\lambda(QSR)_{80}$  infection was assayed turbidimetrically. The results of infections of *himA42* (K936) are shown in Fig. 1 and those of *himA*<sup>+</sup> (K37) in Fig. 2. At 32°, lysis of the  $\lambda cI^-$ -infected *himA42* culture is evident by 90 min postinfection and complete by 180 min. In contrast, the  $\lambda cI^- (QSRrha^+)_{80}$ -infected *himA42* culture shows no lysis; the turbidity of the culture increases continually until it reaches a plateau at 180 min. At 42°, both the  $\lambda cI^-$  and  $\lambda cI^- (QSRrha^+)_{80}$ -infected *himA42* cultures show complete lysis. However, in the  $\lambda cI^- (QSRrha^+)_{80}$

infection there is a delay of about 30 min as compared to the  $\lambda cI^-$  infection.

In the case of the *himA*<sup>+</sup> host, all cultures show complete lysis by 120 min (Fig. 2). By comparison of Figs. 1 and 2, it can be seen that for the productive infections, lysis is somewhat more rapid in a *himA*<sup>+</sup> than in a *himA42* host. In both *himA*<sup>+</sup> and *himA*<sup>-</sup> hosts at 42°, the  $\lambda cI^-$  infection leads to lysis earlier than does the  $\lambda cI^- (QSRrha^+)_{80}$  infection. There is no difference at 32° in the lysis patterns of  $\lambda cI^-$  and  $\lambda cI^- (QSRrha^+)_{80}$ -infected *himA*<sup>+</sup> cultures.

The results of these experiments show that there is no cell lysis resulting from the abortive infection of the *himA42* host at 32° by  $\lambda cI^- (QSRrha^+)_{80}$ . However, as

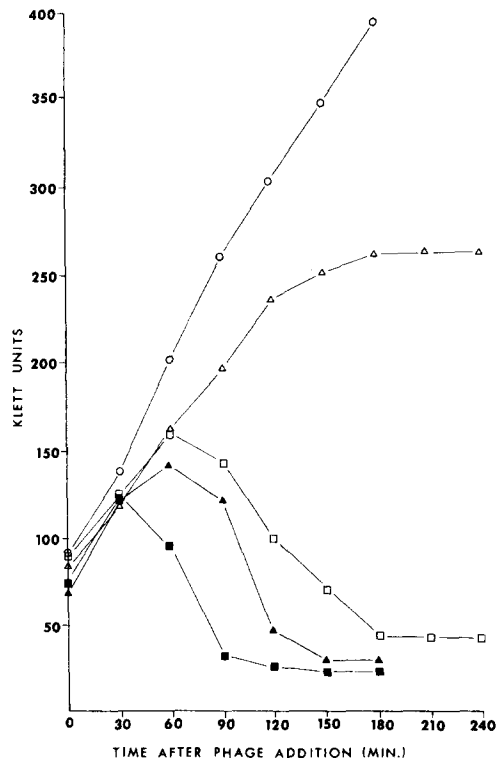


FIG. 1. Lysis of phage-infected *himA42* cultures. Cell lysis was assayed turbidimetrically as described under Materials and Methods. Late exponential phase K936 (*himA42*) cells were infected with phage at an m.o.i. of 5 at 0 min. O, Uninfected cells at 32°; □,  $\lambda cI^-$ , 32°; ■,  $\lambda cI^-$ , 42°; △,  $\lambda cI^- (QSRrha^+)_{80}$ , 32°; ▲,  $\lambda cI^- (QSRrha^+)_{80}$ , 42°.

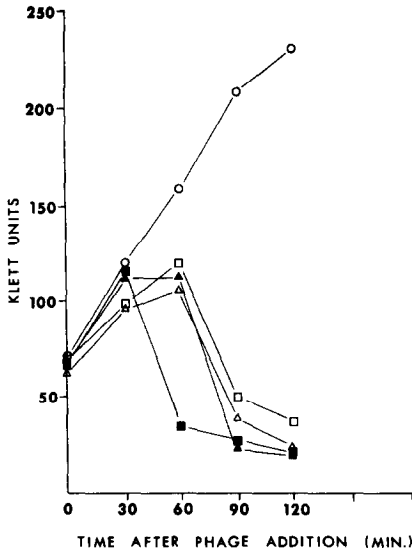


FIG. 2. Lysis of phage-infected *himA*<sup>+</sup> cultures. See legend to Fig. 1. ○, Uninfected cells at 32°; □,  $\lambda cI^-$ , 32°; ■,  $\lambda cI^-$ , 42°; △,  $\lambda cI^-(QSRrha^+)_{80}$ , 32°; ▲,  $\lambda cI^-(QSRrha^+)_{80}$ , 42°.

shown in Fig. 1, the  $\lambda cI^-(QSRrha^+)_{80}$ -infected culture reaches a plateau in turbidity at 180 min, rather than continuing to grow. In comparison, the uninfected culture shows 50% higher turbidity at this time. This property of the *himA*<sup>-</sup>-infected culture is consistent with the high degree of cell killing observed in the abortive infection of the *himA42* host by  $\lambda cI^-(QSRrha^+)_{80}$  at 32°.

*$\lambda(QSR)_{80}$  DNA Replication*

Rates of phage-specific DNA synthesis during  $\lambda(QSR)_{80}$  infection of *himA*<sup>+</sup> and *himA42* cells were measured by assaying incorporation of [<sup>3</sup>H]thymidine ([<sup>3</sup>H]TdR) into trichloroacetic acid (TCA)-precipitable material. *E. coli* DNA synthesis was inhibited by pretreatment of *uvrA*<sup>-</sup>*himA*<sup>+</sup> (K484) or *uvrA*<sup>-</sup>*himA42* (K1675) bacteria with mitomycin C (MC) using a procedure adapted from that of Young and Sinsheimer (1967). Results of an assay of phage DNA synthesis in *himA42* at 32° are shown in Fig. 3.

Untreated, uninfected cells show an approximately fourfold increase in the rate

of [<sup>3</sup>H]TdR incorporation during an 80-min time course. This is consistent with the measured doubling time for K484 and K1675 in 3M-L medium at 32° of approximately 45 min. A 10-min pretreatment of the cells with 80  $\mu$ g MC/ml of  $2 \times 10^8$ /ml cells results in an approximately 20-fold reduction in the rate of [<sup>3</sup>H]TdR incorporation as measured at 1 min post-infection (time after dilution of the phage-infected or uninfected control cultures into prewarmed medium). By 10 min post-infection, the rate of [<sup>3</sup>H]TdR incorporation in the MC-treated culture is almost 100-fold lower than that of the untreated culture and decreases further with time.

An analysis of the phage-infected MC-treated *himA42* cultures reveals the fol-

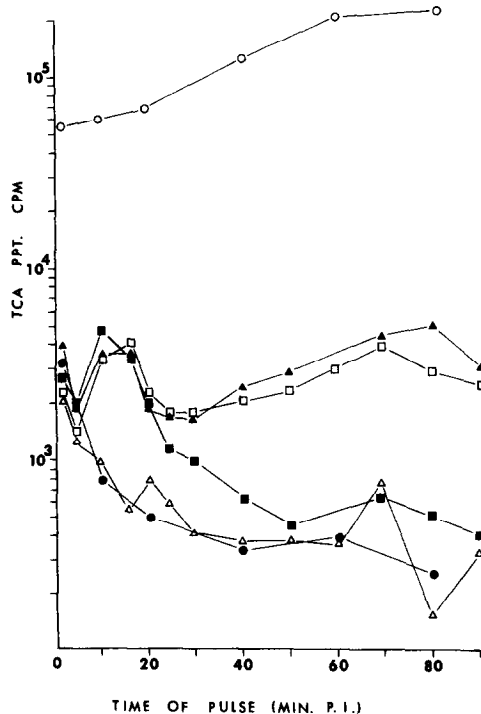


FIG. 3. Phage-specific DNA synthesis in  $\lambda(QSR)_{80}$ -infected *uvrA*<sup>-</sup>*himA42* cells at 32°. Phage-specific incorporation of [<sup>3</sup>H]TdR into TCA-insoluble material in mitomycin C-treated K1675 was measured as described under Materials and Methods. ○, Uninfected cells - MC; ●, uninfected cells + MC. Infections of MC-treated cells: □,  $\lambda cI^-$ ; ■,  $\lambda cI^-(QSRrha^+)_{80}$ ; △,  $\lambda cI^-Pam3$ ; ▲,  $\lambda(QSRrha\Delta01)_{80}$ . P. I. = postinfection.

lowing. By 10 min postinfection, phage-specific DNA synthesis is evident in all cultures with one exception. The replication-defective  $\lambda$ CI<sup>-</sup>Pam3 infection parallels that of the MC-treated uninfected culture and thus serves as an infection-positive and replication-negative control. We look next at the productive infections by  $\lambda$ CI<sup>-</sup> and the Rha<sup>-</sup> mutant  $\lambda$ (QSRrha $\Delta$ 01)<sub>80</sub> (see accompanying paper, Mozola and Friedman, 1984). At 20 to 25 min postinfection, a transient depression in the rate of [<sup>3</sup>H]TdR incorporation is observed, and after 25 min postinfection, the rate of [<sup>3</sup>H]TdR incorporation increases until a maximum is reached at 70 to 80 min postinfection. At this time, the rate of [<sup>3</sup>H]TdR incorporation in these infections is 20-fold above background levels. By 80 or 90 min postinfection, some cultures show decreasing rates of [<sup>3</sup>H]TdR incorporation, indicating that the phage infection cycle is nearing an end.

Looking next at infection by  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub> in the *himA*<sup>-</sup> host, there is an obvious defect in DNA replication. At early times, up to 20 min postinfection, [<sup>3</sup>H]TdR incorporation follows the pattern of the *himA*<sup>+</sup> infection. After 20 min, however, the rate of DNA synthesis drops rapidly and reaches a basal level not appreciably above background by 50 min postinfection. The turnoff of phage DNA synthesis at late times seen in  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub> in K1675 is a consequence of the host being *himA*<sup>-</sup>, because under *himA*<sup>+</sup> conditions at 32°  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub> shows a pattern of [<sup>3</sup>H]TdR incorporation indistinguishable from those produced by  $\lambda$ CI<sup>-</sup> and  $\lambda$ (QSRrha $\Delta$ 01)<sub>80</sub> (Fig. 4).

#### Effect of $\lambda$ (QSR)<sub>80</sub> Infection on Host DNA Synthesis

The effect of  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub> infection on DNA synthesis by *himA*<sup>+</sup> and *himA42* bacteria was measured by an assay similar to that used for investigating patterns of phage-specific DNA synthesis, except that the bacteria were not treated with mitomycin C prior to infection and the isogenic *uvrA*<sup>+</sup> strains K37 (*himA*<sup>+</sup>) and K936

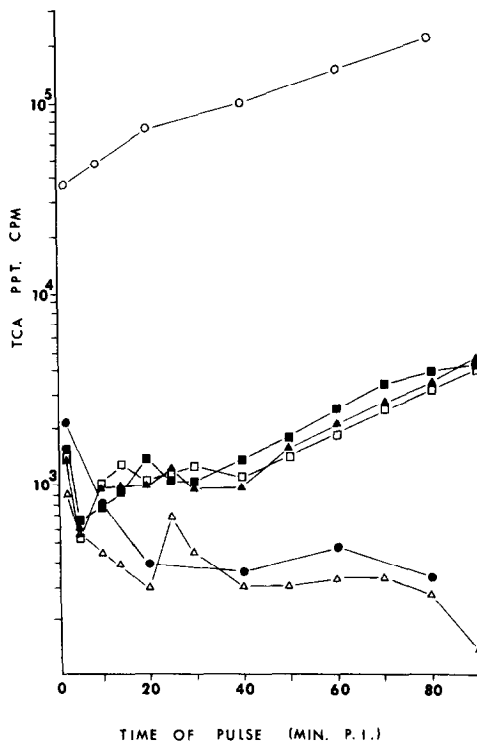


FIG. 4. Phage-specific DNA synthesis in  $\lambda$ (QSR)<sub>80</sub>-infected *uvrA*<sup>-</sup>*himA*<sup>+</sup> cells at 32°. See legend to Fig. 3. ○, Uninfected cells - MC; ●, uninfected cells + MC. Infections of MC-treated cells: □,  $\lambda$ CI<sup>-</sup>; ■,  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub>; △,  $\lambda$ CI<sup>-</sup>Pam3; ▲,  $\lambda$ (QSRrha $\Delta$ 01)<sub>80</sub>.

(*himA42*) were used. In this assay, total DNA synthesis, both phage and host, is measured. Figure 5 shows the patterns of total DNA synthesis in the *himA42* host at 32°. The infection with  $\lambda$ CI<sup>-</sup>Pam3, defective for phage DNA synthesis, shows an approximately twofold reduction in the rate of total DNA synthesis in comparison to an uninfected culture. Infection with  $\lambda$ CI<sup>-</sup> produces, except at early times, rates slightly higher than those for the uninfected culture, suggesting that somewhat more than 50% of the total DNA synthesis observed in this infection is  $\lambda$  specific. This, of course, is based on the assumption that  $\lambda$ CI<sup>-</sup> has the same effect on *E. coli* DNA synthesis as does  $\lambda$ CI<sup>-</sup>Pam3. Similar findings have been reported by Cohen and Chang (1970) in a study of the effects of  $\lambda$  infection on *E.*

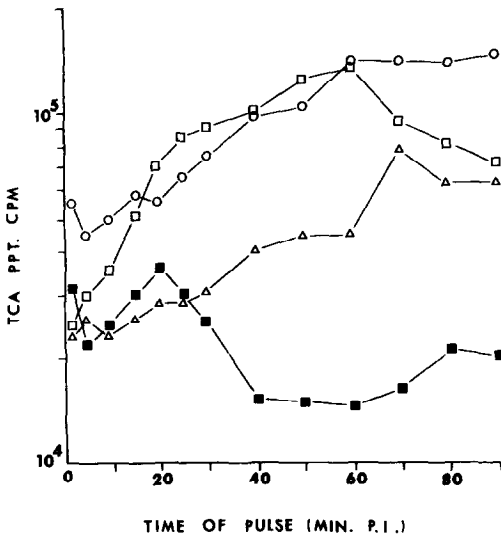


FIG. 5. Effect of  $\lambda$ (QSR)<sub>80</sub> infection on host DNA synthesis in *himA42* at 32°. Total DNA synthesis in phage-infected K936 was assayed as [<sup>3</sup>H]TdR incorporation into TCA-insoluble material as described under Materials and Methods. ○, Uninfected cells. Infections: □,  $\lambda$ CI<sup>-</sup>; ■,  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub>; △,  $\lambda$ CI<sup>-</sup>Pam3.

*coli* metabolism, although they observed somewhat higher rates of DNA synthesis attributable to  $\lambda$ .

Not only is  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub> defective for DNA synthesis at late times in the *himA42* host at 32°, it also exhibits a profound effect on host DNA synthesis. From 15 to 20 min postinfection, total DNA synthesis is higher in the  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub>-infected culture than in the  $\lambda$ CI<sup>-</sup>Pam3 infection. This correlates with the early phage-specific DNA synthesis exhibited by  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub> under HimA<sup>-</sup> conditions (see Fig. 3). However, by 30 min postinfection, a marked decrease in total DNA synthesis is observed. The rate remains low throughout the remainder of the experiment. At 70 min postinfection, total DNA synthesis in the  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub>-infected culture is reduced approximately eightfold in comparison to the uninfected culture. The  $\lambda$ CI<sup>-</sup>Pam3-infected culture shows only a twofold reduction in total DNA synthesis compared to the uninfected control. Since the  $\lambda$ CI<sup>-</sup>Pam3 phage is defective for  $\lambda$ -

specific DNA synthesis, the additional reduction in total DNA synthesis seen with  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub> can only be explained by a further inhibition of *E. coli* DNA synthesis.

Figure 6 shows results of an assay of total DNA synthesis in the *himA*<sup>+</sup> host (K37) at 32°. The incorporation pattern seen in the  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub> infection parallels that produced by  $\lambda$ CI<sup>-</sup> infection, showing that the *himA*<sup>-</sup> allele is responsible for the enhanced inhibition of DNA synthesis observed in the  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub> infection of K936.

#### Expression of *lacZ* following Infection with $\lambda$ (QSR)<sub>80</sub>

Expression of the *lacZ* gene, whose product is  $\beta$ -galactosidase, was used to assay the effect of infection with (QSR)<sub>80</sub> phages on transcription and translation. In the system we employed (see Fig. 7), a cloned *lacZ* gene is located on a  $\lambda$ imm21 prophage vector and is under the control

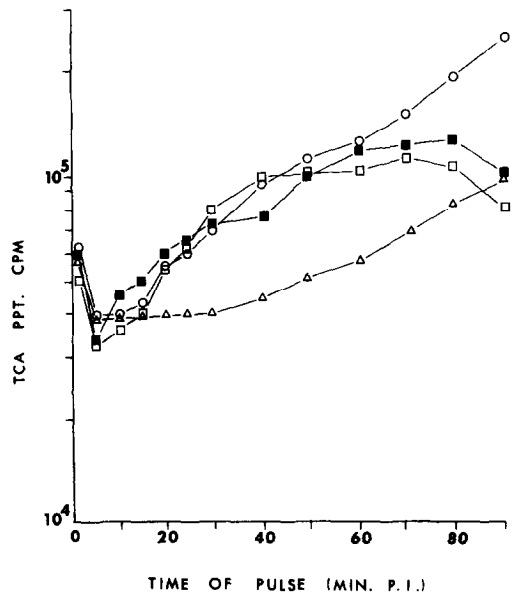


FIG. 6. Effect of  $\lambda$ (QSR)<sub>80</sub> infection on host DNA synthesis in *himA*<sup>+</sup> at 32°. See legend to Fig. 5. ○, Uninfected cells. Infections: □,  $\lambda$ CI<sup>-</sup>; ■,  $\lambda$ CI<sup>-</sup>(QSRrha<sup>+</sup>)<sub>80</sub>; △,  $\lambda$ CI<sup>-</sup>Pam3.



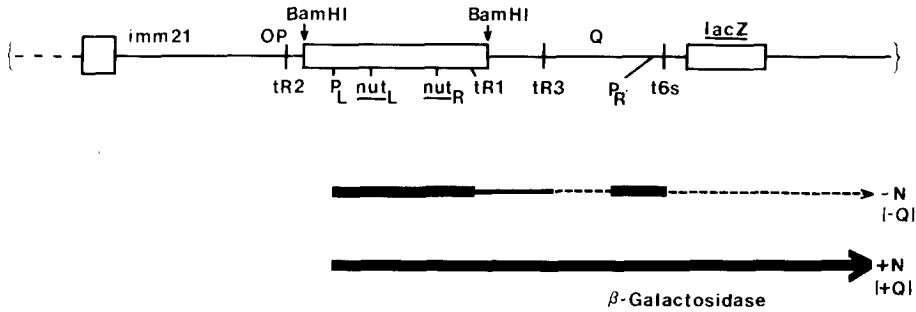


FIG. 7. Relevant features of the  $\lambda$ imm21lacZ transducing phage BMG 67. BMG 67 was constructed from a  $\lambda$  cloning vector designed to test promoter activity (M. Gottesman, D. Court, R. Zagursky, and S. Sullivan, personal communication). *Top*: A map of the relevant regions of the phage is shown. Portions of  $\lambda$ , including  $p_L$ ,  $nut_R$ , and tR1, have been cloned into a BamHI site downstream of the tR2 terminator. The *lacZ* gene has been cloned into an EcoRI site downstream of Q. The map is not drawn to scale. *Middle*: The transcription pattern in the absence of the  $\lambda$  N antitermination function is shown. Since the phage is carried as a prophage, the 21 promoters are repressed. Transcription of *lacZ* initiates only at the cloned  $\lambda$  promoters,  $p_L$  and  $p_R'$ . However, in the absence of the N protein, most transcription is terminated before Q and *lacZ*. Therefore, only small amounts of  $\beta$ -galactosidase are synthesized. *Bottom*: The transcription pattern in the presence of the  $\lambda$  N function is shown. Following infection with  $\lambda$ (QSR)<sub>80</sub>, N protein is synthesized and acts at *nut* sites to render subsequent transcription resistant to the tR1 and tR3 terminators and partially resistant to the t6s terminator. This transcription results in the synthesis of Q protein and therefore high levels of  $\beta$ -galactosidase. The Q protein is an antitermination function acting at a site downstream of the  $p_R'$  promoter. The transcription of *lacZ* in the presence of N represents the combined antitermination activity exerted by both the N and Q proteins. Note that the infecting phages used in these experiments have the Q gene from  $\phi$ 80 which does not influence transcription initiating at the  $\lambda$   $p_R'$  promoter. An indication of the level of transcription is shown by the nature of the line; the thicker the line, the greater the transcription, with the least amount of transcription represented by a dashed line.

of a cloned phage promoter (M. Gottesman, D. Court, R. Zagursky, and S. Sullivan, personal communication). This phage is called BMG 67. Full expression of *lacZ* requires that the  $\lambda$  N transcription antitermination function be supplied in *trans*. Because  $\lambda$ (QSR)<sub>80</sub> phage expresses N, this system can provide a measurement of transcription and translation following infection with that phage. Moreover, the requirement for N means that genes located both on the phage and bacterial (prophage) genomes must be transcribed and translated in order for high levels of  $\beta$ -galactosidase to be expressed.

Results of a representative experiment are shown in Table 2. Two bacterial hosts carrying BMG 67 as a prophage were used in this experiment; K3057 (*himA*<sup>+</sup>) and K3058 (*himA* $\Delta$ 82). There is little interference with the expression of *lacZ* in the

$\lambda$ cI<sup>-</sup>(QSR*rha*<sup>+</sup>)<sub>80</sub> infection of the *himA*<sup>-</sup> host K3058 at 32°. The level of  $\beta$ -galactosidase activity is fourfold above background at 20 min postinfection and eightfold above background at 40 min postinfection. The significance of these increases is apparent when compared to the increases observed following infection with  $\lambda$ cI<sup>-</sup>(QSR*rha* $\Delta$ 01)<sub>80</sub>, a derivative that grows normally in *himA*<sup>-</sup> hosts. In this case the levels of  $\beta$ -galactosidase expressed are 9- and 13-fold above background, respectively, at 20 and 40 min. Thus, there apparently is not a general shutdown of RNA and protein synthesis in the abortive  $\lambda$ (QSR)<sub>80</sub> infection, even at times in the infection considerably later than the point at which inhibition of DNA synthesis is observed. An answer to the question of whether or not the twofold difference in stimulation of  $\beta$ -galactosidase activity

TABLE 2  
ASSAY OF  $\beta$ -GALACTOSIDASE ACTIVITY IN  
 $\lambda$ (QSR)<sub>80</sub>-INFECTED CELLS

| Lysogen                               | Superinfecting phage                                                   | Time postinfection (min) |      |      |
|---------------------------------------|------------------------------------------------------------------------|--------------------------|------|------|
|                                       |                                                                        | 0                        | 20   | 40   |
| K3057<br>( <i>himA</i> <sup>+</sup> ) | None                                                                   | 440                      | 420  | 630  |
|                                       | $\lambda$ cI <sup>-</sup> (QSR <i>rha</i> <sup>+</sup> ) <sub>80</sub> | 460                      | 2020 | 3810 |
|                                       | $\lambda$ cI <sup>-</sup> (QSR <i>rha</i> $\Delta$ 01) <sub>80</sub>   | 480                      | 1570 | 3530 |
| K3058<br>( <i>himA</i> $\Delta$ 82)   | None                                                                   | 115                      | 120  | 120  |
|                                       | $\lambda$ cI <sup>-</sup> (QSR <i>rha</i> <sup>+</sup> ) <sub>80</sub> | 80                       | 490  | 1070 |
|                                       | $\lambda$ cI <sup>-</sup> (QSR <i>rha</i> $\Delta$ 01) <sub>80</sub>   | 155                      | 990  | 1640 |

Note.  $\beta$ -Galactosidase activity in phage-infected cells at 32° was assayed as described under Materials and Methods. The units of activity were calculated using the method described by J. Miller (1972).

seen in the *himA*<sup>-</sup> infections with  $\lambda$ cI<sup>-</sup>(QSR*rha*<sup>+</sup>)<sub>80</sub> and  $\lambda$ cI<sup>-</sup>(QSR*rha* $\Delta$ 01)<sub>80</sub> reflects a small reduction in transcription or translation imposed by the *rha*<sup>+</sup> allele awaits further study.

For purposes of comparison, we have included data on *lacZ* expression in K3057 (*himA*<sup>+</sup>) following infection with the two phages. Infection with both phages results in higher  $\beta$ -galactosidase activities in the *himA*<sup>+</sup> bacterium than in the *himA*<sup>-</sup> host. In this case, the level of stimulation is the same for both phages.

#### Proteins Encoded or Controlled by the *rha* Locus

Results of the physiological experiments described above, together with the genetic analysis presented in the accompanying paper (Mozola and Friedman, 1984), suggest that a *trans*-acting protein is responsible for the growth defect, negative-complementation phenomena, and inhibitory effects on DNA replication associated with the *Rha* phenotype. In light of this, we looked for proteins encoded by the *rha* locus which are expressed in the abortive infection of  $\lambda$ (QSR*rha*<sup>+</sup>)<sub>80</sub> in a *HimA*<sup>-</sup> background. In these experiments, we used electrophoretic analysis of proteins synthesized following phage infection of uv-

irradiated *himA42* cells at 32° to look for a *rha*-encoded protein. Proteins were pulse labeled with [<sup>35</sup>S]methionine between 30 and 45 min after infection, which represent a period approximately midway through a productive  $\lambda$  infection cycle under the growth conditions used here. Two-dimensional electrophoretic separation of proteins was carried out by the method of O'Farrell (1975). In this procedure, proteins are separated by charge (isoelectric focusing) in the first dimension and by molecular weight through an SDS-polyacrylamide gel in the second dimension.

Figure 8 shows the two-dimensional pattern of background host proteins produced in uninfected uv-irradiated K1675 (*uvrA*<sup>-</sup>*himA42*) cells at 32° labeled with [<sup>35</sup>S]methionine between 30 and 45 min postinfection. Figures 9 through 11 show

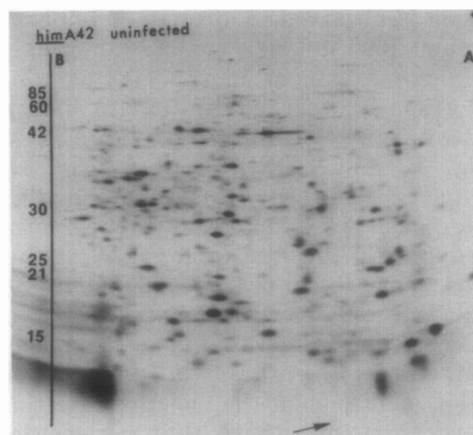


FIG. 8. Proteins produced in uninfected *himA42* cells. An autoradiograph of a two-dimensional electrophoretic gel showing proteins labeled with [<sup>35</sup>S]methionine in uv-irradiated uninfected K1675 (*uvrA*<sup>-</sup>*himA42*) cells at 32° is shown. The time of labeling corresponds to the 30- to 45-min period postphage infection for those studies in which phage were added (see Figs. 9, 10, and 11). Autoradiography was for 11 days. The basic and acidic sides of the first-dimension isoelectric focusing gel are indicated by "B" and "A", respectively. An approximate molecular weight scale for the second-dimension SDS-polyacrylamide gel is shown (molecular weight  $\times 10^{-3}$ ). The position of the 7K-8K protein discussed in the text is marked by an arrow.

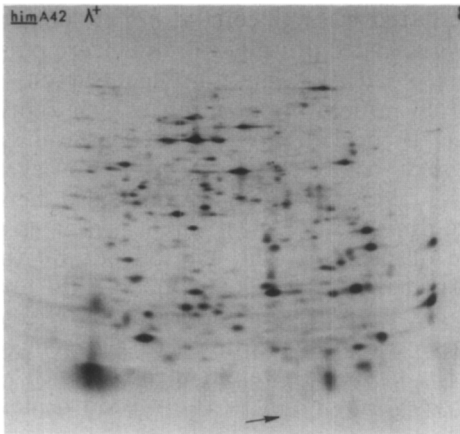


FIG. 9. Proteins produced in  $\lambda^+$ -infected *himA42* cells. For details see legend to Fig. 8.

the patterns of proteins labeled following infection with  $\lambda^+$ ,  $\lambda(\text{QSR}rha^+)_{80}$ , and  $\lambda(\text{QSR}rha\Delta01)_{80}$ . One candidate for a protein encoded or controlled by the *rha* region is observed. A protein of approximately 7000 to 8000 molecular weight (7K-8K) is present in the  $\lambda(\text{QSR}rha^+)_{80}$  gel (Fig. 10; marked by arrow), but is absent from the uninfected (Fig. 8),  $\lambda^+$  (Fig. 9), and  $\lambda(\text{QSR}rha\Delta01)_{80}$  (Fig. 11) samples. Besides the 7K-8K spot, no other differences were noted in the patterns produced by the *rha*<sup>+</sup> and *rha* $\Delta01$  infections. Thus, the appearance of the 7K-8K protein correlates with the presence of an intact *rha* region on the phage. The demonstration of synthesis of this protein under conditions of the abortive infection supports our hypothesis that *rha* encodes a diffusible gene product responsible for the inhibition of phage growth observed in *HimA*<sup>-</sup> and *Hip/HimD*<sup>-</sup> hosts at 32°. Other differences between the protein patterns of the  $\lambda^+$  and  $\lambda(\text{QSR}rha^+)_{80}$  infections are evident; these are not thought to involve *rha*, but presumably arise from other differences between the QSR regions of  $\lambda$  and  $\phi80$  and will not be considered here.

We have also analyzed the patterns of phage protein synthesis under conditions permissive for growth of  $\lambda(\text{QSR}rha^+)_{80}$ , i.e., in a *uvrA*<sup>-</sup>*himA*<sup>+</sup> strain at 32°. A

spot corresponding to the 7K-8K protein discussed above is seen in the  $\lambda(\text{QSR}rha^+)_{80}$  infection, but not in the uninfected,  $\lambda^+$ ,  $\lambda(\text{QSR}rha\Delta01)_{80}$ , or  $\lambda$ -80hy95 samples, and thus again correlates the synthesis of the 7K-8K protein with an intact *rha* locus (data not shown). Furthermore, this result indicates that *rha* is expressed in the *himA*<sup>+</sup> host, but that this expression is not deleterious to phage growth under *himA*<sup>+</sup> conditions.

#### DISCUSSION

The abortive infection of  $\lambda(\text{QSR})_{80}$  in *himA*<sup>-</sup> cells at 32° is characterized by: (1) cell killing, (2) absence of cell lysis, (3) an inhibition of phage DNA synthesis after an early period of apparently normal synthesis, (4) a marked inhibition of host DNA synthesis, and (5) continued RNA and protein synthesis. These results, as well as those of the genetic analysis presented in the accompanying paper, are best explained by postulating the existence of a diffusible protein product encoded or controlled by the *rha* locus that is responsible for the observed inhibitory effects on phage and host physiological processes. A protein encoded or controlled by the

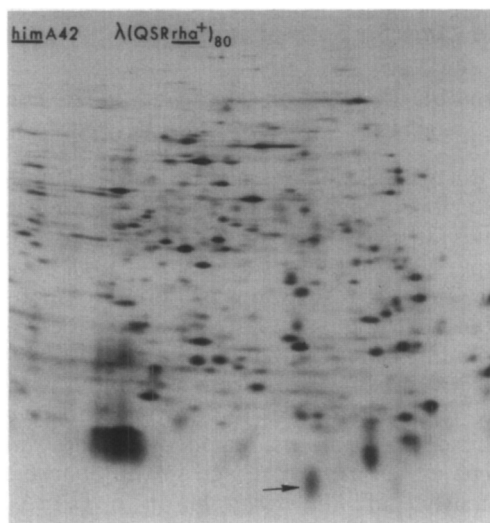


FIG. 10. Proteins produced in  $\lambda(\text{QSR}rha^+)_{80}$ -infected *himA42* cells. For details see legend to Fig. 8.

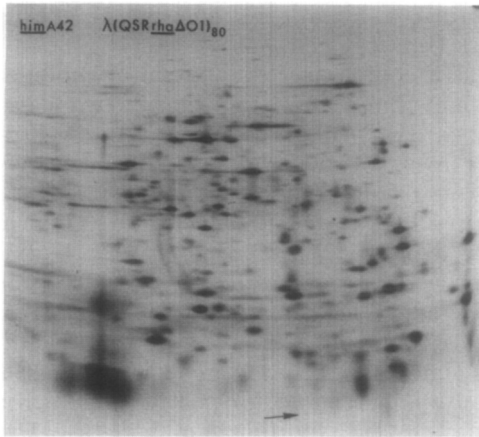


FIG. 11. Proteins produced in  $\lambda(QSRrha\Delta O1)_{80}$ -infected *himA42* cells. For details see legend to Fig. 8.

*rha* locus has been identified on 2-D gels; this protein is synthesized during the abortive  $\lambda(QSR)_{80}$  infection in a *himA42* host. To simplify the discussion, the consequences of the abortive infection of  $\lambda(QSR)_{80}$  phage will be referred to as the Rha phenotype with no intention of distinguishing whether the phenotype is caused directly by the *rha* gene product or by another product under control of the *rha* gene product.

The defect in DNA synthesis is the most obvious deficiency observed when a *rha*<sup>+</sup> phage infects a *him*<sup>-</sup> bacterium at 32°. Whether or not this defect is the direct cause of the abortive infection has not been established. We also do not know if the  $(QSR)_{80}$ -associated inhibitions of phage and host DNA synthesis are caused by a common function. The fact that both phage and host DNA synthesis are inhibited at similar times postinfection is consistent with a common function operating on both types of replication. The finding that the Rha inhibitions of both phage and host DNA synthesis are not seen until 25 min after initiation of the infection is consistent with location of *rha* within the late operon transcribed from *p<sub>R</sub>'* (see accompanying paper).

Although these studies do not lead to a definitive model explaining the Rha phe-

notype, they do provide a basis for informed speculation about Rha. It is likely that *rha* is not present to confound Him workers and therefore we suggest that the *rha* gene product or its agent has a role, albeit dispensible, in normal lytic growth of  $\phi 80$ . The defect in the *him*<sup>-</sup> infection is likely, then, to be a failure in the normal control or modulation of Rha expression in the absence of significant IHF activity.

How might uncontrolled Rha expression be manifested? The defect in DNA synthesis might be the cause of the various Rha phenotypes. For instance, inappropriate Rha activity might produce abnormal or "dead-end" forms of phage DNA that are unsuitable for DNA packaging. This would explain both the lack of phage production in the abortive infection (see accompanying paper) and the lack of cell lysis. The inhibition of host DNA synthesis could also result directly from *rha* gene product activity, or indirectly from a general host cell response to the abortive phage infection. A similar sequence of events has been proposed by Toothman and Herskowitz (1980a, b, c) to explain the phenomenon of Rex-dependent exclusion of superinfecting lambdoid phage by certain  $\lambda$  lysogens, in which an abortive phage infection resembling that of  $\lambda(QSR)_{80}$  in a *himA*<sup>-</sup> host takes place. A role for an IHF-regulated  $\phi 80$  function in phage DNA replication or packaging is not unreasonable in light of the observed defect in growth of Mu in HimA mutants and the demonstrated role for IHF activity in lambdoid phage DNA packaging (see Introduction).

Considering the apparently normal DNA synthesis pattern exhibited by  $\lambda(QSR)_{80}$  during the first 20 to 25 min of the abortive infection of *himA42*, it is tempting to speculate that the primary defect in the abortive phage infection may be a failure to switch from early Theta form to late rolling-circle DNA replication (reviewed by Furth and Wickner, 1983). Such a situation exists in a  $\lambda gam$ <sup>-</sup> infection; normally Gam protects an intermediate DNA structure in the Theta form

to rolling-circle transition from attack by the *E. coli* RecBC nuclease (Enquist and Skalka, 1973). A  $\lambda$ gam<sup>-</sup> infection, although defective for rolling-circle replication, still produces a phage burst (unlike the abortive  $\lambda$ (QSR)<sub>80</sub> infection) since monomeric DNA can be recombined by homologous recombination functions ( $\lambda$ Red or *E. coli* RecA) to produce dimers suitable for packaging (Enquist and Skalka, 1973; Dawson *et al.*, 1975, 1976). A *himA* mutation does not interfere with this type of recombination as evidenced by the fact that a  $\lambda$ bio11 phage (which is *red*<sup>-</sup> and *gam*<sup>-</sup>) grows in *HimA*<sup>-</sup> hosts at both 32 and 42° (Miller and Friedman, 1977). Thus, although it is possible that the *rha* gene product plays a role in late DNA replication, it is likely that the early phage DNA synthesis seen in the abortive *rha*<sup>+</sup> infection does not lead to intact progeny monomers, but instead produces incomplete or aberrant DNA structures unsuitable for packaging. Preliminary results of experiments analyzing the fate of  $\lambda$ (QSR*rha*<sup>+</sup>)<sub>80</sub> DNA during the abortive infection are consistent with this idea.  $\lambda$ (QSR*rha*<sup>+</sup>)<sub>80</sub> DNA is not degraded in any gross manner in *himA42* at 32°, but it appears that *cos* cleavage to generate unit-size DNA molecules for packaging does not occur (A. Granston, personal communication).

A second explanation for the Rha phenotype is that *rha* encodes a function involved in cell lysis. The location of *rha* in the late operon near known lysis functions is certainly consistent with this hypothesis. In the abortive  $\lambda$ (QSR)<sub>80</sub> infection of *himA42* at 32°, the *rha* gene product, not under normal *HimA*-*Hip*/*HimD* control, may act prematurely in the phage infection cycle and damage the cell membrane. The importance of the membrane in energy generation and macromolecular synthesis could explain why such damage might lead to loss of energy from the cell and subsequently a cessation in both phage and host DNA synthesis, death of the host cell, and abortion of the phage lytic cycle. However, the finding that RNA and protein synthesis continue at late times in the abortive infection argues

against a hypothesis of general energy loss from the cell.

IHF could exert its control or modulation of Rha activity in either of two ways. First, IHF could regulate the level of synthesis of the *rha* gene product. According to this idea, in the absence of active IHF in the *himA*<sup>-</sup> host, control of *rha* expression is altered leading to improper expression of Rha which results in the abortive infection. The abnormal expression could be either in the amount or time at which the gene is expressed. There is precedent for postulating such a role for IHF. Synthesis of both the  $\lambda$ *int* and *cII* gene products is normally controlled by the level of IHF (Miller, 1981). Second, IHF might modulate the activity of Rha either by interacting directly with the Rha function or with a site on the nucleic acid where that function normally binds. Consistent with this latter idea is the fact that IHF is a DNA-binding protein (Nash, 1981). A definitive elucidation of the mechanism of Rha-IHF interaction should prove useful in further defining the role of IHF in cellular metabolism.

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