Effect of R17 Bacteriophage-Specific Proteins on Ribosomal Ribonucleic Acid Synthesis of *Escherichia coli*

VIRGINIA L. SPANGLER AND WALLACE J. IGLEWSKI

Department of Microbiology, University of Oregon Medical School, Portland, Oregon 97201

Received for publication 7 December 1971

Bacteriophage R17 and a series of amber mutants of R17 were used to infect cultures of *Escherichia coli*. The ribonucleic acid (RNA) of these cultures was radioactively labeled, extracted, and subjected to polyacrylamide gel electrophoresis. Synthesis of ribosomal RNA was inhibited in cultures infected with the wild-type R17 and in cultures infected with phage mutants retaining the ability to produce viral polymerase. With the exception of one mutant tested, the degree of inhibition of ribosomal RNA synthesis in infected cells was correlated with the amount of viral polymerase synthesized. Maturation of 16S ribosomal RNA from its 17S RNA precursor appeared normal in infected cells, indicating that the inhibition of 16S ribosomal RNA synthesis occurred solely at the transcriptional level.

Infection of *Escherichia coli* with the bacteriophage R17 results in the inhibition of ribosomal ribonucleic acid (rRNA) synthesis (8). The R17 phage contains a small, single-stranded ribonucleic acid (RNA) genome which directs the synthesis of three proteins: coat protein, assembly protein, and viral RNA polymerase. The purpose of this investigation was to determine if one or more of these proteins is involved in the inhibition of rRNA synthesis. We approached the problem by infecting cultures of *E. coli* with amber mutants of R17 which lacked the ability to synthesize one or more of the viral proteins. RNA synthesis was measured by the amount of radioactive uridine incorporated into host and viral RNA species, which were separated according to their mobilities in polyacrylamide gels. We found that phage-induced inhibition of host rRNA synthesis occurred only when the infecting phage was able to produce the viral polymerase protein. The degree of inhibition was correlated with the amount of polymerase produced, except in the case of nonpolar coat mutant infections. The ability of infecting phage to synthesize either the coat or assembly proteins was not correlated with the inhibition of rRNA synthesis.

MATERIALS AND METHODS

Bacteria and bacteriophage. Amber mutants *amC8* and *amC13* of the bacteriophage R17 were provided by M. Capecchi of Harvard University. Amber mutants *amA31*, *amB24*, *amB22*, and *amC16*, along

with the nonpermissive and permissive indicator strains, *E. coli* S26 and *E. coli* S26RIE, respectively, were provided by R. Kamen of Harvard. Viral mutants were grown by the method of Tooze and Weber (19), and contained less than 1.0% revertants. *E. coli* C3000 (7) is a nonlysogenic, nonpermissive Hfr strain used in the RNA extraction experiments, and used to grow R17.

Media and solutions. In experiments in which cells incorporated radioactive precursor, cultures were grown in TCG1 synthetic medium (5). Virus stock and *E. coli* overnight cultures were grown in MS broth (3). RNA extractions were carried out in 0.1 M STE buffer, containing 0.1 M NaCl, 0.05 M tris-(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.4, and 1.0 mM ethylenediaminetetraacetic acid (EDTA). Electrophoresis buffers and solutions were prepared according to Pons and Hirst (15). Tritiated uridine (specific activity 20 Ci/mmol) was purchased from Schwartz/Mann, Boston, Mass. Omnifluor scintillation compound was purchased from New England Nuclear Corp., Boston, Mass.

Labeling and preparation of RNA. *E. coli* C3000 cultures were grown in 200 ml of TCG1 medium to 2×10^8 cells/ml and harvested by centrifugation at $11,700 \times g$ for 5 min. The resulting cell pellet was suspended in 3 ml of TCG1 medium containing phage at a multiplicity of 10 plaque-forming units (PFU)/cell. After a 3-min adsorption period, the cells were added to 100 ml of prewarmed medium and incubated in a shaking water bath at 37°C. Uninfected cells were treated identically; the adsorption period was simulated in medium containing no phage. At 45 min postinfection, each culture was pulse-labeled for 2 min with tritiated uridine at a final concentration of 2.5 μ Ci/ml, and chased for 10 min with a 300-fold excess

of unlabeled uridine. At the end of the chase period, 1 ml from each culture was pipetted into a chilled test tube containing 0.2 ml of 60% trichloroacetic acid plus 0.1 ml of chloroform. The rest of the culture was immediately poured over crushed TCG1 ice containing 0.01 M potassium cyanide, to stop further incorporation of label. The cells were then harvested by centrifugation at 5°C, and the cell pellets were suspended in cold STE buffer. RNA was extracted from the cells with phenol in the presence of 2.5% sodium dodecyl sulfate (SDS) by the method of Franklin (5). After alcohol precipitation, the RNA was suspended in 1 ml of sample buffer containing 20% sucrose for polyacrylamide electrophoresis (15).

RESULTS

It has been found that R17 infection of *E. coli* does not affect synthesis of transfer RNA (tRNA) (9). To examine the synthesis of 16S and 23S rRNA in infected and uninfected cultures, stable species of RNA were preferentially labeled with tritiated uridine by chasing with an excess of unlabeled uridine for 10 min. Labeled uridine was added to cultures prior to the onset of lysis in R17-infected cells and during the period of maximal inhibition of RNA synthesis (7). Cells were harvested, and total RNA was extracted and subjected to polyacrylamide gel electrophoresis. The resulting RNA profiles from R17-infected and uninfected cells are shown in Fig. 1A. Infection with the R17 phage results in the inhibition of 16S rRNA. The inhibition of 23S rRNA synthesis could not be determined from this profile because 23S rRNA had not been separated from 27S viral RNA. The identity of the heterogeneous RNA from infected cells migrating in fractions 42 to 72 is unknown.

To resolve 23S from 27S RNA, electrophoresis was carried out for 11.5 hr at 5 ma/gel in 2.4% polyacrylamide gels. Under these conditions tRNA migrated off the gel. The 23S rRNA band was resolved from 27S viral RNA and a new band of labeled RNA appeared between 16S and 23S RNA (Fig. 1B). The pulse-chase experiments shown in Fig. 2 indicated that this new component was probably the precursor to mature 16S rRNA, as described by Adesnik and Levinthal (1). The ratio of labeled 17S to 16S rRNA following a 2-, 4-, and 7-min chase was 1:0.9, 1:2.2, and 1:3.7, respectively. We included the radioactivity of this component, referred to as 17S rRNA, with that of 16S rRNA when determining the amount of label incorporated into rRNA during the 2-min pulse.

Data from electrophoretic profiles resolving viral RNA from rRNA were quantitated. The amount of radioactivity in the 16S and 23S RNA was corrected by dividing by the RNA recovery

FIG. 1. Polyacrylamide gel electrophoresis of ^3H -RNA extracted from R17-infected (---) and uninfected (—) cultures of *E. coli* C3000. (A) Electrophoresis was carried out for 8.5 hr in 2.2% polyacrylamide gels at 3 ma/gel. (B) Electrophoresis was carried out for 11.5 hr in 2.4% polyacrylamide gels at 5 ma/gel. A standard RNA preparation containing 4, 16, and 23S *E. coli* RNA and 27S viral RNA was used as a migration marker in the polyacrylamide gels. The RNA migrated from right to left and was fractionated by collecting 1.2-mm gel slices.

factor described in Table 1. The recovery factor consisted of the ratio of trichloroacetic acid-precipitable labeled RNA from chloroform-lysed cells to the amount of trichloroacetic acid-precipitable RNA recovered following phenol extraction of the culture. The factor is based on the assumption that the phenol extraction procedure does not preferentially extract any particular species of acid-precipitable RNA and that RNA is equally extractable from infected and control cultures. The recovery factor ranged from 0.67 to 0.85. The major loss of RNA during extraction was due to discarding from 5 to 10% of the aqueous phase at the phenol-buffer interphase during several phenol extractions. However, since the RNA is in solution, the concentration of individual species of RNA should not be affected by the loss of a portion of the solution. Additional extraction did not release additional labeled material. The amount of radioactivity in the 4S RNA served as an internal marker

FIG. 2. Polyacrylamide gel electrophoresis of ^3H -RNA extracted from an uninfected *E. coli* C3000 culture. Electrophoretic conditions were as in Fig. 1B. The 16S and 23S rRNA species are indicated. This 300-ml culture was pulse-labeled for 2 min with ^3H -uridine and chased by adding an excess of unlabeled uridine; 100-ml samples were harvested (A) 2 min, (B) 4 min, (C) 7 min later.

for the extraction procedure. When the amount of radioactivity in the extracted 4S RNA was divided by the recovery factor, incorporation of label into 4S RNA of infected and control cells was similar. This observation would be expected since 4S RNA synthesis is not altered in R17-infected cells (9). In addition, cultures infected with the wild-type R17 incorporated into 16S RNA 28% of the tritiated uridine incorporated by control cultures (Table 1). This value is consistent with the corresponding result of 29% obtained from hybridization experiments (8). For 23S rRNA, our results indicate that R17-infected cells in-

TABLE 1. Ribosomal ribonucleic acid synthesis in infected cultures of *Escherichia coli* C3000

Culture	Relative amounts of ^3H -uridine incorporated ^a	
	16S RNA (%)	23S RNA (%)
Uninfected control	100	100
R17-infected	28 $\pm$ 5	46 $\pm$ 7
<i>amA31</i> -infected	36 $\pm$ 3	44 $\pm$ 9
<i>amb24</i> -infected	80 $\pm$ 8	86 $\pm$ 10
<i>amb22</i> -infected	46 $\pm$ 7	36 $\pm$ 5
<i>amC8</i> -infected	100 $\pm$ 5	98 $\pm$ 7
<i>amC13</i> -infected	100 $\pm$ 9	98 $\pm$ 7
<i>amC16</i> -infected	97 $\pm$ 9	97 $\pm$ 9

^a The radioactivity of gel fractions containing 16S and 23S RNA was corrected for the amount of RNA lost during extraction procedures by dividing by a recovery factor (counts per minute of ^3H -RNA in final buffer solution per counts per minute of trichloroacetic acid-precipitable RNA in original culture). The corrected radioactivity was expressed as a per cent of that of the uninfected control, and each value in the table is the average per cent obtained from three or more experiments, with standard deviations indicated.

corporated 46% of the label incorporated by control cells. During electrophoresis, however, there may have been some slurring of the 27S viral RNA into the 23S rRNA.

The amber mutants of phage R17 used to infect cultures of *E. coli* are described in Table 2. In the nonpermissive host, the mutant *amA31* synthesizes coat protein and viral polymerase but does not synthesize assembly protein (19). The assembly protein enables the phage to adsorb to the cell (13). Phage *am31* grown in permissive *E. coli* S26 RIE was used to infect nonpermissive *E. coli* C3000 cells. The electrophoretic profiles of labeled RNA extracted from *amA31*-infected cells are shown in Fig. 3A. The amount of inhibition was similar to that observed in R17-infected cells (Table 1). The data indicate that the synthesis of assembly protein is not required for the inhibition of rRNA synthesis. As in experiments with the wild-type phage, viral RNA of *amA31* may have been incompletely separated from 23S rRNA, resulting in an apparent increase in the synthesis of 23S relative to 16S RNA.

The amber mutant *amb22* does not synthesize coat protein in the nonpermissive host (Table 2). Viral RNA produced during an infection with *amb22* is consequently not encapsulated. A second effect of the mutation in the coat protein cistron is the production of an abnormally large amount of viral polymerase (15). Among R17 and related phage, translation of the polymerase

TABLE 2. *Viral proteins synthesized during phage infections of nonpermissive Escherichia coli*^a

Phage	Proteins synthesized		
	Assembly	Coat	Polymerase per cent of wild type
R17	+	+	100
<i>amA31</i>	—	+	90–100
<i>amB24</i>	+	—	15–30
<i>amB22</i>	+	—	90–105 ^b
<i>amC8</i>	—	—	1
<i>amC13</i>	—	—	8
<i>amC16</i>	—	—	3

^a The proteins synthesized by mutants *amA31*, *amB24*, *amB22*, and *amC16* were determined by Tooze and Weber (19), and those by *amC8* and *amC13* by Gussin (6).

^b The percentage of polymerase synthesized in the nonpolar mutant *amB22*-infected cells has probably been underestimated. The in vivo polymerase activity was measured as a percentage of label incorporated into viral RNA of mutant-infected cells as compared to wild-type-infected cells (19). Polyacrylamide gel electrophoresis of labeled viral polymerase from *amB22*-infected cells indicate a large increase in the amount of polymerase synthesized (15). The latter result is in agreement with the 3- to 15-fold increase in viral polymerase synthesis observed in cells infected with the non-polar coat protein mutants of the f2 phage (14).

cistron is thought to be regulated by the binding of the coat protein to the viral mRNA (18). The lack of coat proteins in the *amB22*-infected cell precludes such regulation of translation, and viral polymerase accumulates in excess. RNA profiles (Fig. 3B) indicate that rRNA synthesis was inhibited in cells infected with *amB22*. The amounts of radioactive precursor incorporated into 16S and 23S RNA were 46 and 36%, respectively, of the control values (Table 1). Since inhibition of rRNA synthesis occurred in cultures infected with *amB22*, the synthesis of viral coat protein does not appear to be required for the inhibition.

The amber mutant *amB24* is a polar coat mutant (19). It lacks the ability to synthesize coat protein, and the polar effect results in a reduced synthesis of viral polymerase (15, 19) (Table 2). Electrophoretic profiles of RNA from cells infected with *amB24* and from uninfected cells are shown in Fig. 3C. The incorporation of tritiated uridine into rRNA by infected cells is inhibited, but not to the same extent as in cells infected with *amB22* (Table 1). The degree of inhibition of rRNA synthesis in cells infected with mutants *amB22* and *amB24* appears to be due to the amount of viral polymerase synthesized.

Little single-stranded viral RNA is observed in *amB22*- and *amB24*-infected cells under pulse-chase conditions (Fig. 3B, C). The limited viral RNA synthesis would be expected in *amB24*-infected cells because of the restricted viral polymerase synthesis (15, 19). However, the lack of single-stranded viral RNA in *amB22*-infected cells was unexpected since viral polymerase is produced during infection (15). A comparison of 2-min pulse-labeled viral RNA intermediates from cells infected with *amB22* and wild-type virus agrees with the expected increase in polymerase synthesis during infection with the mutant (Fig. 4A). The major difference between the mutant and R17-infected cells is the lack of single-stranded viral RNA in *amB22*-infected cells. The *amB22*-infected culture also contained a slightly larger amount of heterogeneous 4S to 23S RNA. An examination of the labeled RNA from *amB22* and R17-infected cells under pulse-chase conditions suggests that the viral RNA in *amB22*-infected cells may be degraded (Fig. 4B). Most of the labeled viral RNA from R17-infected cells migrates as 27S viral RNA, presumably extracted from mature progeny virus. No distinct peak of 27S viral RNA is observed in the *amB22*-infected cultures. In addition, a larger amount of the heterogeneous 5S to 23S RNA was consistently observed in the *amB22*-infected cells. We interpret these results as being due to the degradation of the viral RNA produced in *amB22*-infected cells. It seems likely that the viral RNA which is not encapsulated during infection with the coat protein mutant is probably being degraded. This elevated background of labeled 5S to 23S RNA undoubtedly affected the quantitation of rRNA synthesis in the *amB22*-infected cells.

Due to amber mutations in the polymerase cistron, phage *amC8*, *amC13*, and *amC16* do not synthesize viral polymerase in the nonpermissive host. Since these mutants cannot replicate the viral genome, no amplification of the viral messenger RNA occurs, and very little of the other viral proteins is synthesized (15). Infection of *E. coli* C3000 with any of these polymerase mutants had no effect on host synthesis of rRNA (Fig. 3D–F and Table 1).

Phages that inhibited host rRNA synthesis were the wild-type R17 and the mutants *amA31*, *amB22*, and *amB24*. Electrophoresis of RNA, shown in Fig. 2B and 3A–C, indicate that there was no accumulation of the precursor form of 16S rRNA in cultures infected with these phage. The amount of precursor synthesized was reduced, and this reduction probably accounts for the decreased amount of mature form observed. Our results therefore suggest that the inhibition

FIG. 3. Polyacrylamide gel electrophoresis of ^3H -RNA extracted from mutant phage-infected (---) and uninfected (—) cultures of *E. coli* C3000. Electrophoretic conditions were as in Fig. 1B. The 16S and 23S rRNA and 27S viral RNA species are indicated. (A) Assembly protein mutant *amA31*-infected and uninfected cultures. (B) Non-polar coat mutant *amB22*-infected and uninfected cultures. (C) Polar coat mutant *amB24*-infected and uninfected cultures. (D, E, and F) Uninfected and polymerase mutants *amC13*, *amC8*, and *amC16*-infected cultures, respectively.

of 16S rRNA synthesis occurred at the level of transcription rather than at the level of maturation.

DISCUSSION

The early events in the process of R17 infection, adsorption to the cell and penetration of the

viral nucleic acid, do not seem to be involved in the inhibition of host rRNA synthesis. This conclusion is based on results from infections with viral polymerase mutants *amC8*, *amC13*, and *amC16*. Grown in permissive *E. coli* cultures, these phages were adsorbed to nonpermissive *E. coli* cells, and events of infection proceeded

FIG. 4. Polyacrylamide gel electrophoresis of ^3H -RNA extracted from mutant *amB22* (---) and R17-infected (—) cultures. Electrophoresis was carried out for 3.5 hr in 2.2% polyacrylamide gels (8.5 by 0.6 cm) at 3 ma/gel. An RNA preparation containing 4, 16, and 23S *E. coli* RNA and purified 27S viral RNA, RF, and RI was used as a migrator marker in the gels. (A) Infected cultures pulse-labeled for 2 min with ^3H -uridine. (B) Infected cultures pulse-labeled for 2 min with ^3H -uridine and then chased for 10 min with an excess of unlabeled uridine at 45 min after infection.

normally until the amber mutation in the polymerase cistron was expressed. Without functional polymerase molecules, viral RNA, and thus viral proteins, were not synthesized in detectable amounts. No inhibition of host rRNA synthesis was observed in infections with our polymerase mutants.

The results of experiments with the other amber mutants indicate that synthesis of viral polymerase is required for the phage-associated inhibition of rRNA synthesis. Phages that were unable to synthesize assembly protein were able to cause the inhibition, as were phages unable to synthesize coat protein. In all experiments in which we observed inhibition of host rRNA synthesis, the

infecting phage was one which had retained its ability to synthesize viral polymerase and viral RNA. However, viral RNA synthesis does not appear to be associated with the inhibition of rRNA, since a temperature-sensitive polymerase mutant of R17 inhibits rRNA synthesis in the absence of viral RNA synthesis (10). Presumably this temperature-sensitive protein may possess two independent functions: (i) the synthesis of viral RNA and (ii) the inhibition of rRNA synthesis.

In *amB24*, *amA31*, and R17 infections, the degree of inhibition of rRNA synthesis correlates with the amount of viral polymerase synthesized in the infected cell. Restricted polymerase production by polar coat mutant *amB24* is accompanied by a slight inhibition of rRNA synthesis. Assembly protein mutant *amA31* and the wild-type R17 produce comparable quantities of viral polymerase, and induce correspondingly similar degrees of inhibition. The nonpolar coat mutant *amB22* produces a large excess of viral polymerase. However, the apparent inhibition of rRNA synthesis infected with *amB22* was not as great as that observed in cells infected with wild-type phage. The following may explain what we observed in *amB22* infections. We have previously noted that, in *amB22* infections, little 27S viral RNA is observed probably because it is unencapsulated and degraded. The initial nuclease degradation products of R17 RNA, as well as Q β RNA are 22S and 15S fragments (2, 17), which would be electrophoretically indistinguishable from rRNA. What we observed as rRNA synthesis in *amB22*-infected cells may have been the sum of the synthesis of rRNA and the initial cleavage products of viral RNA into 15S and 22S fragments. The actual synthesis of rRNA, therefore, may have been depressed in *amB22*-infected cells more than what we could observe. Viral RNA degradation may also have slightly affected observations of rRNA synthesis in *amB24*-infected cells. The effect would be much less pronounced in *amB24* experiments because of the restricted synthesis of viral RNA by *amB24* relative to that by *amB22* (19).

Cells infected with *amB22* were reported to synthesize 90 to 105% of the viral polymerase found in R17-infected cells (19). Viral polymerase was measured in vivo as the percentage of label incorporated into viral RNA of mutant-infected cells as compared to cells infected with wild-type virus. The degradation of unencapsulated viral RNA would have led to an underestimate of the viral polymerase in the *amB22*-infected cells and would explain the difference in polymerase syn-

thesis reported by Phillips et al. (15) and Tooze and Weber (19).

Kamen (11) and Kondo et al. (12) have shown that purified viral polymerase from RNA phage Q β consists of four polypeptide subunits: α , β , γ , δ . Three of these, α , γ , δ , were found to be derived from the host, the β subunit being the only one coded for by the viral genome. The ability of R17 and R17 mutants to synthesize viral polymerase therefore refers to the ability to synthesize the β subunit.

Our conclusion, that viral polymerase synthesis is required for the inhibition of rRNA synthesis, is inherent in the hypothesis of Travers et al. (20) on the mechanism of inhibition. They suggested that the viral polymerase of Q β and host deoxyribonucleic acid (DNA)-dependent RNA polymerase compete for a protein(s) which is necessary for both rRNA synthesis and phage RNA replication. The hypothesis was put forth to explain the relationship between a component of Q β polymerase to a transcription factor they found in *E. coli* extracts. Either the Q β component or the transcription factor, ψ r, could be used in an in vitro synthesizing system to specifically stimulate rRNA synthesis (20). The Q β component ψ r, consisted of the host-dependent subunits γ and δ . If the ψ r factors proves to be identical to one or both of these subunits, and exists in the *E. coli* cell in limiting quantity, competition for it between viral polymerase subunits and the host enzyme could reduce its availability for rRNA synthesis. The correlation we observed between the amount of viral polymerase and the degree of inhibition is consistent with, but does not prove, this mechanism of inhibition by competition for ψ r or another essential host factor(s). The following alternative mechanism could also correlate the amount of polymerase with the degree of inhibition. One of the host-dependent subunits, α , γ , or δ , may provide host DNA-dependent RNA polymerase or the viral polymerase with the ability to recognize and bind to rRNA cistrons on the *E. coli* chromosome. Unable to initiate transcription of the host DNA, the viral enzyme would block the binding of the host enzyme and thereby prevent transcription of rRNA genes. The correlation between viral polymerase and the degree of inhibition would be in this case contingent upon the relative association constants of viral and host polymerases with host DNA, which are presently undetermined. Experiments are in progress to investigate the possible binding of viral polymerase to *E. coli* DNA.

It is conceivable that a viral polymerase could bind ψ r and inhibit host rRNA synthesis, but be nonfunctional in terms of viral RNA-syn-

thesizing activity. Such an occurrence would explain the observation of Igarashi et al. that a temperature-sensitive polymerase mutant of R17 produced no viral RNA in *E. coli* at 43 C but caused a progressive decrease in rRNA synthesis (10). Why none of our polymerase amber mutants caused the inhibition may be due to the nature of the mutation. The amber mutations of *amC8*, *amC13*, and *amC16* cause premature terminations of polymerase translation. The resulting incomplete polypeptide may be unable to bind ψ r and thus have neither the ability to inhibit rRNA synthesis nor the ability to polymerize viral RNA. The temperature-sensitive mutation, however, allows translation of the entire polymerase molecule. At nonpermissive temperatures this polymerase may still be able to bind ψ r and thus inhibit rRNA synthesis although it loses its enzymatic activity for synthesis of viral RNA.

ACKNOWLEDGMENTS

This investigation was supported by Public Health research grant 5 R01 CA11465 from the National Cancer Institute to Wallace J. Iglewski and Public Health Service training grant AI 00139-11 from the National Institute of Allergy and Infectious Diseases to Arthur W. Frisch.

LITERATURE CITED

- Adesnik, M., and C. Levinthal. 1969. Synthesis and maturation of ribosomal RNA in *Escherichia coli*. *J. Mol. Biol.* 46:281-303.
- Bassel, B. A., and S. Spiegelman. 1967. Specific cleavage of Q β RNA and identification of the fragment carrying the 3'-OH terminus. *Proc. Nat. Acad. Sci. U.S.A.* 58:1155-1161.
- Davis, J. E., and R. L. Sinsheimer. 1963. The replication of bacteriophage MS2. Transfer of parental nucleic acid to progeny phage. *J. Mol. Biol.* 6:203-207.
- Ellis, D. B., and W. Paranchych. 1963. Synthesis of ribonucleic acid and protein in bacteria infected with an RNA bacteriophage. *J. Cell Comp. Physiol.* 62:207-213.
- Franklin, R. M. 1966. Purification and properties of the replicative intermediate of the RNA bacteriophage R17. *Proc. Nat. Acad. Sci. U.S.A.* 55:1504-1511.
- Gussin, G. N. 1966. Three complementation groups in bacteriophage R17. *J. Mol. Biol.* 21:435-453.
- Hotham-Iglewski, B., and R. M. Franklin. 1967. Replication of bacteriophage ribonucleic acid: alterations in polyribosome patterns and association of double-stranded RNA with polyribosomes in *Escherichia coli* infected with bacteriophage R17. *Proc. Nat. Acad. Sci. U.S.A.* 58:743-749.
- Hudson, J. B., and W. Paranchych. 1967. Effect of bacteriophage R17 infection on host-directed synthesis of ribosomal ribonucleates. *J. Virol.* 1:529-537.
- Hudson, J. B., and W. Paranchych. 1968. Synthesis of soluble ribonucleates in *Escherichia coli* infected with bacteriophage R17. *J. Virol.* 2:124-128.
- Igarashi, S. J., J. F. Elliot, and R. P. Bissonnette. 1970. Temperature sensitive mutation. V. Infection of *ts*-mutant of RNA phage and host cell metabolism. *Can. J. Microbiol.* 48:47-54.
- Kamen, R. 1970. Characterization of the subunits of Q β replicase. *Nature (London)* 228:527-533.
- Kondo, M., R. Gallerini, and C. Weissman. 1970. Subunit structures of Q β replicase. *Nature (London)* 228:525-527.
- Krahn, P. M., and W. Paranchych. 1971. Heterogenous distri-

- bution of A protein in R17 phage preparations. *Virology* 43:533-535.
14. Lodish, H. F., and N. D. Zinder. 1966. Mutants of the bacteriophage ϕ 2. VIII. Control mechanisms for phage-specific synthesis. *J. Mol. Biol.* 19:333-348.
 15. Phillips, L. A., J. L. Truden, W. J. Iglewski, B. Hotham-Iglewski, and R. M. Franklin. 1969. Replication of bacteriophage ribonucleic acid: alterations in polyribosome patterns in *Escherichia coli* infected with amber mutants of bacteriophage R17. *Virology* 39:781-790.
 16. Pons, M. W., and G. K. Hirst. 1968. Polyacrylamide gel electrophoresis of influenza virus RNA. *Virology* 34:385-388.
 17. Spahr, P. F., M. Farber, and R. F. Gesteland. 1969. Binding site on R17 for coat protein. *Nature (London)* 222:455-458.
 18. Sugiyama, T., and D. Nakada. 1968. Translational control of bacteriophage MS2 RNA cistrons by MS2 coat protein: Polyacrylamide gel electrophoretic analysis of proteins synthesized in vitro. *J. Mol. Biol.* 31:431-440.
 19. Tooze, J., and K. Weber. 1967. Isolation and characterization of amber mutants of bacteriophage R17. *J. Mol. Biol.* 28:311-330.
 20. Travers, A. A., R. Kamen, and R. Schleif. 1970. Factor necessary for ribosomal RNA synthesis. *Nature (London)* 228:748-751.