Effect of Bacteriophage R17 Infection on Host-directed Synthesis of Ribosomal Ribonucleates

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Studies were performed on the synthesis of ribosomal ribonucleates in cells of *Escherichia coli* K-12 infected by the ribonucleic acid (RNA) bacteriophage R17. Host-specific RNA was measured in the presence of phage RNA by in vitro hybridization of the purified ribonucleates with *E. coli* deoxyribonucleic acid. The results showed that, although the overall rate of RNA synthesis was only slightly affected by phage infection, the level of host RNA synthesis was decreased by 70 to 80%. Fractionation of the purified ribonucleates by sucrose gradient sedimentation, followed by hybridization of fractions sedimenting in the 23S and 16S regions, revealed that the level of ribosomal RNA synthesis was also decreased by 70 to 80%, and that this inhibition occurred during the first 15 to 20 min after infection. These findings are discussed in light of what is known about the inhibition of host RNA synthesis by other virus systems.

Infection of *Escherichia coli* with a virulent phage such as T2 or T4 causes a rapid suppression of ribonucleic acid (RNA) synthesis in the host cell (5). In bacteria infected with an RNA phage, on the other hand, no such inhibition is apparent, since no significant decrease in the overall rate of RNA synthesis occurs after infection (18, 27). However, Ellis and Paranchych (8) and Bishop (3) have shown that a rapid decline of ribosome biosynthesis takes place during the early stages of infection with an RNA phage. This observation was taken by Ellis and Paranchych (8) to be an indication that phage infection may cause a direct inhibition of ribosomal RNA synthesis. Support for this contention was provided recently by the autoradiographic studies of Granboulan and Franklin (13), who showed that R17 infection causes a shift of RNA synthesis from a nuclear site to the cytoplasm. This observation is presumed to reflect a replacement of host-directed RNA synthesis by a phage-directed RNA-synthesizing system. Furthermore, Watanabe and August (29) recently reported that the RNA phage R23 completely dominates the capacity of the host cell for RNA synthesis. It therefore appears likely that the decrease in ribosome biosynthesis in RNA phage-infected bacteria may indeed arise from an inhibition of host RNA synthesis, although a direct demonstration of this inhibition of host RNA synthesis has not yet been reported. Such a demonstration would require the complete resolution of host and phage ribonucleates. Unfortunately, this separation is

not easily accomplished by the usual RNA fractionation procedures, such as sucrose gradient sedimentation or methylated albumin chromatography (8, 16, 24). Recent studies, however, have shown that specific hybridization occurs between *E. coli* ribosomal RNA and the homologous deoxyribonucleic acid (DNA; 31, 32), but not between *E. coli* DNA and coliphage RNA (7). This has provided the possibility of using DNA-RNA hybridization as a means of measuring host-specific RNA synthesis against a background of phage RNA replication. Such a procedure was used in the present study, and it was found that phage R17 infection of *E. coli* K-12 leads to a 70 to 80% reduction in the synthesis of host 23S and 16S ribonucleates. This reduction appears to be complete by about 20 min after infection.

MATERIALS AND METHODS

Growth of bacteria and bacteriophage. Bacteriophage R17 and its host bacterium *E. coli* K-12, Hfr₁, have been previously described (27). The basic tris-(hydroxymethyl)aminomethane (Tris)-maleate synthetic growth medium (TMM) used was as described by Paranchych (26). Assay of phage infectivity was carried out as described by Paranchych and Graham (27), and viable counts of bacteria were determined by plating the appropriate bacterial dilutions on agar plates containing Trypticase Soy Broth (BBL).

Purification of phage R17 and its RNA. Preparation and purification of phage R17 was carried out as described by Paranchych and Graham (27), and phage RNA was prepared from purified phage by phenol extraction as described by Strauss and Sinsheimer (28).

Measurement of acid-insoluble and alkali-stable nucleates. The incorporation of radioactive uracil into acid-insoluble nucleates was measured by use of the filter-paper disc method of Mans and Novelli (22).

RNA samples used for hybridization studies were tested for alkali-stable content by incubation at 37 C for 16 to 20 hr in 0.3 N KOH. The samples were then neutralized with trichloroacetic acid, and, after the addition of carrier protein (50 μ g/ml), an equal volume of 10% trichloroacetic acid was added. The mixtures were allowed to stand at 4 C for 30 min, after which they were passed through membrane filters; (GA-6; Gelman Instrument Co., Ann Arbor, Mich.) to isolate acid-insoluble material. After washing with sufficient 5% trichloroacetic acid to remove acid-soluble nucleotides, the filters were placed in liquid scintillation fluid and counted for radioactivity in a liquid scintillation spectrometer. In all cases, the alkali-stable material comprised less than 0.5%, and usually less than 0.2%, of the total acid-insoluble radioactivity.

Labeling and extraction of intracellular ribonucleates. A log-phase culture of Hfr₁ containing about 5×10^7 bacteria per ml was grown for one generation (about 50 min) in the presence of 0.3 μ Ci/ml of ³H-labeled uracil. The cells were sedimented by centrifugation for 10 min at $10,000 \times g$, washed with TMM, and resuspended to a concentration of 10^8 per ml in fresh, prewarmed, nonradioactive medium. Growth was continued to a density of 3×10^8 to 5×10^8 bacteria per ml, at which time the culture was divided into two portions, one of which was infected with phage R17 at a multiplicity of about 20 plaque-forming units (PFU) per bacterium. At 20 min after infection, samples were removed from the cultures and rapidly chilled in an ethyl alcohol-dry ice bath (the RNA from these samples was used to determine the specific activity of ³H-labeled RNA in the ³²P-labeled RNA used for hybridization). The remainder of each culture was exposed to 10 μ Ci of ³²P per ml and incubated for an additional 25-min period, after which the cultures were quickly chilled in an ethyl alcohol-dry ice bath.

The chilled samples were sedimented by centrifugation for 10 min at $10,000 \times g$, washed with TMM, and finally resuspended in a small volume of 0.01 M Tris chloride (pH 7.3) containing 0.01 M MgCl₂, 50 μ g of deoxyribonuclease per ml, and 250 μ g of lysozyme per ml. After freezing in an ethyl alcohol-dry ice bath, the samples were quickly thawed at 37 C, and, after standing at 20 C for 5 min, they were shaken for 1 min with sodium dodecyl sulfate at a final concentration of 1% (w/v). The resulting mixtures were extracted three times with equal volumes of 80% phenol (all extractions were carried out at 0 to 2 C), and were precipitated twice with three volumes of ethyl alcohol at -20 C. Finally, the RNA was dissolved in 1.0 ml of 0.01 M Tris chloride (pH 7.3) containing 10^{-3} M ethylenediaminetetraacetate (EDTA), and was passed through a column (1 by 30 cm) of Sephadex G-75. The leading ultraviolet-absorbing peak, which contained both ribosomal and 4S ribonucleates, was then further fractionated by sucrose density gradient sedimentation (22). The resulting RNA samples were then used in hybridization experi-

ments. No further treatment of the fractionated samples was necessary, since control tests showed that the sucrose (up to 1% final concentration) had no effect on the capacity of the RNA to hybridize with *E. coli* DNA.

Preparation of E. coli DNA. DNA was isolated initially according to the method of Marmur (23) from Hfr₁ grown in Trypticase Soy Broth. Such DNA still contained traces of ribonuclease, however, which were removed by the procedure suggested by Gillespie and Spiegelman (12). This involved incubation of the DNA for 2 hr at 37 C with predigested pronase (50 μ g/ml), followed by repeated phenol extraction until no detectable ribonuclease activity remained. The final preparation was dialyzed against $0.01 \times \text{SSC}$ (standard saline citrate: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). Ribonuclease activity was detected by measuring the decrease in acid-insoluble radioactive RNA after incubation of the RNA for 20 to 24 hr at 37 C with the DNA.

DNA-RNA hybridization. Detection of DNA-RNA hybrids was performed according to the method of Gillespie and Spiegelman (12), except that the hybridization buffer contained the following: 0.01 M Tris chloride (pH 7.3), 0.5 M NaCl, and 10^{-3} M EDTA. Denaturation of the *E. coli* DNA was accomplished by making the DNA preparation (20 to 25 μ g/ml) 0.1 N with respect to NaOH (5 min), followed by neutralization with HCl and raising the concentration of NaCl to 0.5 M. Denatured DNA solutions containing 100 μ g of DNA were passed through membrane filters (B-6; Schleicher and Schuell Co., Keene, N. H.; presoaked in hybridization buffer for at least 10 min, and washed with 10 ml of the same buffer). The filters with adsorbed DNA were then washed with 50 ml of hybridization buffer. Under these conditions, at least 90% of the DNA adsorbed to the filters, as judged by the absorbance at 260 m μ of the filtrates. The DNA filters were subsequently dried at room temperature for at least 4 hr and at 80 C for an additional 2 hr in a vacuum. Hybrids were formed by immersing the DNA filters in scintillation vials containing 2.0 ml of radioactive RNA in hybridization buffer, and incubating the vials for 5 hr at 67 C. After this, the vials were chilled in an ice bath, and the filters were removed and washed with the hybridization buffer. The filters were then immersed in 2.0 ml of buffer (0.01 M Tris chloride, 0.3 M NaCl, pH 7.3) containing 20 μ g of ribonuclease per ml and were incubated at 30 C for 1 hr. Finally, each filter was individually washed with the same buffer (without ribonuclease), dried, and counted in a liquid scintillation spectrometer.

Treatment of hybridization data. The ³²P specific radioactivity of purified RNA was determined by measuring the ³²P content of a known weight of the RNA (based on its optical density at 260 m μ). To determine the amount of RNA synthesized during the ³²P-labeling period, samples were removed from the uninfected and phage-infected cultures immediately before, and at the end of, the ³²P-labeling period. The difference between the tritium specific radioactivities of these samples was then used as a measure of the net synthesis of RNA during the ³²P-labeling period.

Hybridization of purified RNA with *E. coli* DNA

was carried out as described in the previous section, and curves of counts per minute of ^{32}P hybridized versus the RNA concentration were plotted. Although all the graphs displayed a tendency towards a plateau, indicative of saturation of a limited number of DNA-binding sites, a plateau was never actually achieved. Instead, there was a small continuous linear slope of increasing hybridization up to the highest RNA concentration tested ($10\text{ }\mu\text{g/ml}$). This continuous increase of hybridization at higher RNA concentrations was attributed to RNA of nonribosomal origin, and was corrected for in the following manner. The slope evident at the higher RNA concentrations was extended to the ordinate axis, and a line parallel to this slope was then drawn from the origin. This was taken to represent the hybridization contributed by the contaminating RNA. At all RNA concentrations employed, the value read from this line was subtracted from the observed value, and the final figures were replotted as a function of RNA concentration. The curves thus obtained showed apparent saturation at RNA concentrations of 1.5 to $2.0\text{ }\mu\text{g/ml}$. The amount of ^{32}P radioactivity hybridized (in the plateau region) was then converted to microgram units by reference to the ^{32}P specific radioactivity of the RNA, and comparisons were made of the amounts of hybridization of ^{32}P -labeled RNA from uninfected and phage-infected cells.

Enzymes. The enzymes used in this study were purchased from the following companies: pancreatic ribonuclease and lysozyme, Sigma Chemical Co., St. Louis, Mo.; electrophoretically pure deoxyribonuclease, Worthington Biochemical Corp., Freehold, N.J.; pronase, Calbiochem, Los Angeles, Calif.

Radioactivity measurements. The liquid scintillation fluid used contained 6 g of 2,5-diphenyloxazole (PPO) and 0.5 g of *p*-bis[2-(5-phenyloxazolyl)]-benzene (POPOP) per liter of toluene. Radioactive counting was carried out in a Beckman model LS200 scintillation spectrometer.

RESULTS

Effect of R17 infection on the overall rate of ribonucleate synthesis. Previous reports have shown that synthesis of RNA continues after phage infection, even though newly made phage RNA does not appear in measurable quantities until 10 to 15 min after infection (6, 25, 27). It is evident, therefore, that host RNA synthesis is not completely inhibited immediately after infection. Cooper and Zinder (6), however, reported a slight lag in the synthesis of RNA immediately after infection with phage f2. Since this lag has never been observed with phage R17, the following experiment was carried out to re-examine the overall rate of ribonucleate synthesis in R17-infected cells.

A log-phase culture of Hfr₁ was made to contain $0.5\text{ }\mu\text{C/ml}$ of ^{14}C -labeled uracil (final uracil concentration $10\text{ }\mu\text{g/ml}$). The incubation was continued for 12 min, at which time the culture was divided into two portions, one of which was infected with phage R17 at a multi-

plicity of infection of 10 PFU per bacterium. Both portions were allowed to continue growth, and 0.1-ml samples were periodically withdrawn from each portion for measurement of acid-insoluble radioactivity, as described in Materials and Methods. Since it has been shown previously (4) that, under similar conditions, the amount of an RNA precursor being incorporated into DNA is only about 5% of that being incorporated into RNA, it has been assumed in the present experiment that the incorporation of ^{14}C -labeled uracil into an acid-insoluble product is a measure of RNA synthesis only. A typical result is illustrated in Fig. 1, where it can be seen that the rate of RNA synthesis remained unchanged during the early minutes of infection. At approximately 20 min after infection, however, the rate of nucleate synthesis in the infected culture slightly exceeded that in the control culture. This increased rate of synthesis continued until the onset of lysis at approximately 80 min after infection. The previous reports that synthesis of RNA continues after infection were thus confirmed, but a lag period immediately after infection, of the type reported for phage f2 by Cooper and Zinder (6), was not evident. Apparently, phage R17 differs from phage f2 in this respect.

Lack of hybridization between phage ribonucleates and *E. coli* DNA. Before attempting to measure the amount of host RNA made in infected cells, it was first necessary to demon-

FIG. 1. Incorporation of ^{14}C -labeled uracil by uninfected and phage R17-infected cells. The conditions of the experiment are described in the text. The position of the arrow indicates the time of addition of phage R17. Symbols: ●, uninfected culture; ○, R17-infected culture.

strate that phage R17 RNA does not possess any significant level of base complementarity with *E. coli* DNA. Doi and Spiegelman (7) showed that the RNA from the related bacteriophage MS2 fulfilled such a condition. Figure 2 shows that the same holds true for R17 RNA, under conditions allowing optimal binding of *E. coli* stable ribonucleates to *E. coli* DNA. At the usual saturating concentration of *E. coli* RNA (see later sections), 0.34% of the DNA was engaged in hybrid formation, whereas, at this same concentration of R17 RNA, less than 0.01% of the DNA hybridized. Prolonging the incubation period did not reveal any additional binding sites for the phage RNA. Figure 3 shows that non-radioactive *E. coli* ribosomal RNA can compete efficiently for DNA hybridization sites with ^3H -labeled *E. coli* ribonucleates. On the other hand, no detectable competition was observed between phage RNA and the ^3H -labeled RNA. It is evident, therefore, that R17 RNA does not exhibit any significant hybridization to *E. coli* DNA, nor does it interfere with the formation of true *E. coli* RNA-*E. coli* DNA hybrids.

Hybridization of unfractionated ribonucleates. Before examining the ability of fractionated RNA from infected and noninfected cells to hybridize with *E. coli* DNA, studies were first carried out to determine the extent of hybridization of *E. coli* DNA with unfractionated ribonucleates. A culture of Hfr₁ in TMM was grown to 6.5×10^8 bacteria per ml; the culture was then divided into two portions, one of which was infected with phage R17 at a multiplicity of infection of 15 PFU per bacterium. After 20 min, both cultures were pulse-labeled for 5 min with ^3H -labeled

FIG. 2. Hybridization of *Escherichia coli* RNA and R17 RNA to *E. coli* DNA. Hybridization was performed with filters containing 90 μg of *E. coli* DNA in 2.0 ml of hybridization buffer at 67 C for the indicated times. Symbols: $\bullet$, ^{32}P -labeled *E. coli* RNA, 3-hr incubation; $\circ$, ^{32}P -labeled R17 RNA, 3-hr incubation; $\blacktriangle$, ^{32}P -labeled R17 RNA, 8-hr incubation. Specific activity of *E. coli* RNA, 5,240 counts per min per μg ; of R17 RNA, 2,820 counts per min per μg .

FIG. 3. Competition for DNA hybridization sites. Filters containing 90 μg of *Escherichia coli* DNA were incubated for 5 hr at 67 C with 2.0 ml of hybridization buffer containing 5 μg of ^3H -labeled *E. coli* ribosomal RNA and various amounts of nonradioactive RNA. Symbols: $\circ$, plus R17 RNA; $\bullet$, plus *E. coli* ribosomal RNA. The 100% value represented the binding of ^3H -labeled RNA to 0.36% of the DNA.

uracil, after which they were chased for 10 min with a 100-fold excess of nonradioactive uracil. The cultures were then rapidly chilled in an ethyl alcohol-dry ice bath and extracted for RNA as described in Materials and Methods. Figure 4 illustrates the results obtained when various amounts of the RNA prepared in this manner were hybridized with *E. coli* DNA. It may be seen that the amount of hybridizable RNA synthesized during the ^3H -pulse in the infected culture was approximately 40% of that synthesized in the noninfected one. It is evident, therefore, that there is at least a 60% inhibition of host RNA synthesis in infected bacteria by 20 min after infection. It should be noted that, in this particular experiment, the bacterial density (6.5×10^8 cells per ml) was somewhat higher than that required for optimal efficiency of phage infection. In other experiments performed subsequently, phage infection was carried out at lower cell densities, and up to 80% inhibition of host RNA synthesis was obtained.

Hybridization of fractionated 23S and 16S ribonucleates. The experiment described in the previous section demonstrated a 60% decrease in the synthesis of the stable ribonucleates in the infected cell. Stable host ribonucleates are predominantly ribosomal, but also include soluble RNA (sRNA) and stable messengers that may exist. It was decided to examine the 23S and 16S

FIG. 4. Hybridization of *Escherichia coli* DNA with unfractionated RNA from uninfected and R17-infected cells. The conditions of the experiment are described in the text. Hybridization was performed in 2.0 ml of hybridization buffer with filters containing 90 µg of *E. coli* DNA. The incubations were at 67°C for 5 hr. Symbols: ●, RNA from uninfected bacteria; ○, RNA from phage R17-infected bacteria.

ribonucleate components of ribosomal particles to determine whether they were decreased to the same extent as total host RNA, and also to compare these two components with each other.

The experimental design is described in Materials and Methods. Figure 5 shows the sucrose gradient fractionation of one pair of purified RNA preparations, and the fractions taken for hybridization tests. In this instance, sedimentation was performed at high RNA concentration, and at low ionic strength in the absence of Mg^{++} . Under these conditions, 23S host RNA and 27S phage RNA were not resolved. The tritium radioactivity shows the positions of stable host 23S, 16S, and 4S ribonucleates. It is evident that the stability of these ribonucleates was not affected by R17 infection. The ^{32}P profiles of the two preparations, however, were different. In the uninfected preparation, the ^{32}P followed very closely the tritium label, showing that essentially all of the ^{32}P label was incorporated into stable 23S, 16S, and 4S ribonucleates. In the infected preparation, on the other hand, the leading peak of ^{32}P represented a mixture of host 23S RNA and single-stranded phage RNA. Moreover, a large amount of ^{32}P -labeled RNA sedimenting over the range 6S to 20S contained, in addition to the residual host 16S RNA, various intermediate or replicative forms of phage RNA. The sedimenta-

FIG. 5. Sucrose gradient sedimentation of RNA from control and R17-infected cells. The conditions of the experiment are described in the text. The purified RNA was submitted to sucrose gradient sedimentation in a linear 5 to 20% sucrose gradient containing 0.01 M Tris chloride (pH 7.3) and 10^{-3} M EDTA. Centrifugation was for 5.5 hr at 37,000 rev/min in the SW39 rotor. (A) RNA from uninfected cells, 0.65 mg. (B) RNA from infected cells, 0.80 mg. Solid line, ^{32}P counts per minute; dashed line, 3H counts per minute.

tion properties of these latter ribonucleates have been documented (9, 16).

Figure 6 shows the results of hybridization tests with the 23S and 16S ribonucleate fractions selected as shown in Fig. 5. After correcting for nonribosomal RNA as described in Materials and Methods, it was found that the amount of hybridizable RNA synthesized in the infected culture during the ^{32}P -labeling period was approximately 20% of that synthesized in the control culture during the same period. It should be noted that the RNA synthesized during the ^{32}P -labeling period represented only about one-fifth of the total RNA extracted from the cells (determined by measuring the specific activity of the 3H -labeled RNA in samples taken just prior to, and at the end of, the 25-min ^{32}P -labeling period). Thus, each microgram of 23S RNA from either uninfected or infected cells consisted of a mixture of about 0.2 µg of ^{32}P -labeled RNA, and 0.8 µg of RNA made prior to the addition of the ^{32}P , this 0.8 µg being represented by the tritium label. The 16S RNA fraction also contained a 4:1 ratio of 3H -labeled-to- ^{32}P -labeled RNA. It is

FIG. 6. Effect of RNA concentration on hybridization of *Escherichia coli* DNA with RNA from uninfected and R17-infected cells. The RNA fractions indicated in Fig. 5 (23S and 16S) were each dialyzed against 0.01 M Tris chloride (pH 7.3) containing 10^{-3} M EDTA. Varying amounts of each RNA, in 2.0 ml of hybridization buffer, were incubated at 67°C for 5 hr with filters containing 90 µg of *E. coli* DNA. Symbols: ●, RNA from uninfected cells; ○, RNA from phage R17-infected cells.

evident, therefore, that the hybridization experiments were in reality a measure of the competition between a relatively small amount of ^{32}P -labeled host RNA and a larger amount of the same type of RNA formed prior to the application of the ^{32}P label. The degree of this competition reflects the relative proportions of ^{32}P -labeled host RNA in each microgram of RNA tested. It follows, therefore, on the basis of the results shown in Fig. 6, that each microgram of 23S or 16S RNA extracted from infected cells was comprised approximately of 0.8 µg of ^3H -labeled host RNA, approximately 0.05 µg of ^{32}P -labeled host RNA, and about 0.15 µg of ^{32}P -labeled phage RNA.

An interesting feature which was common to all the hybridization curves obtained in these studies was the apparent lack of true saturation with increasing RNA concentration. As can readily be seen from inspection of Fig. 4 and 6, the curves tended to saturate around RNA concentrations of 2 µg/ml, but a persistent slope of increased hybridization occurred at higher RNA concentrations. A similar phenomenon was observed by McConkey and Dubin (21) for 16S

and 23S ribonucleates under certain conditions, and was attributed by them to the presence of residual messenger ribonucleates. The method used to correct for this persistent hybridization at high RNA concentrations is described in Materials and Methods. After having made corrections in this manner, the percentage of DNA engaged in binding at the saturation level was calculated for each species of RNA extracted from uninfected cells. The mean values obtained from four determinations for each RNA species were as follows: total unfractionated RNA, 0.37%; 23S RNA, 0.21%; 16S RNA, 0.25%. These values are generally in good agreement with saturation values for bacterial ribosomal ribonucleates obtained in other laboratories (1, 12, 21, 31). It is to be noted, however, that the sum of the values for 23S and 16S ribonucleates was greater than the value for the corresponding unfractionated RNA. This was in agreement with Attardi et al. (1), who concluded that *E. coli* 23S and 16S ribonucleates possess some common binding sites on *E. coli* DNA.

Kinetics of the decline of ribosomal RNA synthesis in phage-infected cells. To determine whether R17 infection of Hfr₁ causes a gradual decrease in host RNA synthesis, or whether the pattern of inhibition resembles more closely that found with ribosomes (8), the following additional experiment was carried out. A series of cultures were prelabeled with ^3H -labeled uracil as described in Materials and Methods; they

TABLE 1. Relative synthesis of ribosomal ribonucleates at various times after infection^a

Sample	Time of labeling with ^{32}P (min after infection)	Amt of hybridizable RNA synthesized relative to control	
		23S RNA	16S RNA
		%	%
Control.....		100	100
Infected.....	10-35	48.2	50.0
Infected.....	20-45	21.7 ^b	18.0 ^b
Infected.....	30-55	24.4	28.9

^a The cell density in each culture was approximately 3×10^8 bacteria per ml at the time of infection. In all cases, the fraction of surviving bacteria at 10 min after infection was less than 4%. Radioactive labeling prior to infection was with ^3H -labeled uracil, and labeling after infection was with ^{32}P . Hybridizable RNA was assayed as described in the text. Each value was obtained from the appropriate pair of saturation curves (control and infected) after correcting for non-ribosomal RNA as described in Materials and Methods.

^b Average of two separate experiments.

were then divided into two portions, and one portion was infected with phage R17. At various times after infection, each pair of cultures (control and infected) was exposed to ^{32}P for 25 min, and then each of the cultures was assayed for hybridizable ^{32}P -labeled RNA, as described in the previous section.

From the results summarized in Table 1, it is evident that the decrease of host ribosomal RNA synthesis was largely manifested during the first 15 to 20 min. After this time, there was apparently no further decrease in the level of host RNA synthesis. Reference to Ellis and Paranchych (8) shows this pattern of inhibition of ribosomal RNA synthesis in infected cells to be almost identical to that of the formation of intact ribosomes.

DISCUSSION

The results described in this communication have shown that, although RNA synthesis proceeds at a similar overall rate in uninfected and R17-infected cells, the nature of the ribonucleates produced differs. Reference to Table 1 shows that there was a marked decrease of ribosomal RNA synthesis during the first 20 min after infection, following which no further decrease of RNA synthesis occurred. This observation provides strong support for the contention (8) that the inhibition of ribosome biosynthesis in R17-infected cells is a direct result of a decrease in the synthesis of ribosomal RNA. This aspect of RNA phage infection thus resembles the effects of some RNA-containing animal viruses, notably poliovirus and mengovirus (11, 15), which also cause an inhibition of ribosomal RNA synthesis during the early stages of infection.

It is worthwhile considering the possible mechanisms by which phage R17 might cause the inhibition of ribosomal RNA synthesis. In this respect, there is one important difference between RNA phages and the small RNA-containing animal viruses such as poliovirus and mengovirus. The studies of August et al. (2) and of Weissmann et al. (30), with use of crude extracts of *E. coli* before and after infection by the RNA phages f2 and MS2, respectively, revealed that the in vitro assayable level of the host DNA-dependent RNA polymerase was not significantly decreased as a result of infection. Infection of host cells by poliovirus or mengovirus, on the other hand, caused a considerable decrease in the in vitro activity of the nuclear "aggregate enzyme" (11, 15).

Further investigations in this laboratory, of the isolated *E. coli* DNA-RNA polymerase complex (analogous to the "aggregate enzyme" of

animal cells) from uninfected and R17 phage-infected cells, have shown that the integrity of this complex and its in vitro activity (based on endogenous template only) are fully retained after infection. Furthermore, the purified enzyme obtained from uninfected and R17 phage-infected *E. coli* possessed the same sedimentation properties (Hudson and Paranchych, *unpublished data*). In addition, it was shown that R17 RNA was unable to depress the rate of RNA synthesis in vitro by the isolated *E. coli* DNA-RNA polymerase complex. This was in agreement with the studies of Fox et al. (10), who showed that ribonucleates were generally incapable of affecting DNA-primed RNA synthesis in vitro once their enzyme (*Micrococcus lysodeikticus* RNA polymerase) became associated with a DNA template. The above results, together with the observed lack of homology between R17 RNA and *E. coli* DNA, render it unlikely that the phage could directly interact with the host transcription complex, or that it otherwise causes a blockage of host transcription in vivo. It is possible, however, that an inhibition of host transcription in vivo could result from the attachment of the phage protein coat to the complex, or from the action of an inhibitory protein coded for by the phage genome. This inhibition, however, would have to be reversible, since transcription by the host DNA is restored upon disruption of infected cells.

It is of interest to compare the ability of phage R17 to inhibit ribosomal RNA synthesis with that of other RNA phage systems. For example, an inhibition of ribosomal RNA synthesis has been reported to occur in cells infected with both the ZIK/1 (3a) and β (28) phages. Moreover, Watanabe and August (29) have determined the amount of phage RNA (i.e., that RNA recovered in phage particles) synthesized during various time intervals after infection. In the case of phage R23, this value was 60% for RNA made later than 30 min after infection. Furthermore, a large fraction of the remaining RNA was found to be ribonuclease-resistant phage RNA, indicating an almost complete domination of RNA synthesis in the cell by the phage. On the other hand, when a similar experiment was done on cells infected with either phage f2 or phage Q_{β} , only 5% of the RNA synthesized later than 30 min after infection was recovered in phage particles. It thus appears that the RNA phages f2 and Q_{β} give rise to a less marked inhibition of ribosomal RNA synthesis than do phages R17 and R23. This observation must, therefore, be taken into account by any hypothesis attempting to explain the phage-induced inhibition of host RNA synthesis.

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