Development of Bacteriophage F1 in *Clostridium sporogenes*: Characterization of RNA Transcripts

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Received for publication 13 January 1975

RNA transcription was investigated during the development of F1 phage, which is specific for the strict anaerobe *Clostridium sporogenes*. RNA species transcribed during F1 phage infection were characterized with respect to time of appearance and molecular weight by polyacrylamide gel electrophoresis. Ten mRNA species were characterized, of which five were produced early in infection and five were synthesized late in infection. All the above 10 species were transcribed from one strand of F1 DNA, the heavy strand. Two additional mRNA species were transcribed from the light strand of F1 phage DNA later in infection. Throughout the F1 phage infective cycle, rRNA was continuously synthesized by cells of *C. sporogenes*.

Until recently, reports of bacteriophage specific for anaerobic bacteria have been limited to morphological studies of the phages and phage-infected host cells (2, 5, 12, 14). Several workers have also linked lysogeny with toxigenicity in *Clostridium perfringens* (16) and in *C. botulinum* (7, 8). However, almost no information is available concerning the molecular biology of phages that develop in anaerobic hosts.

We have previously investigated bacteriophage RNA transcription during the development of F1, a lytic bacteriophage that infects *Clostridium sporogenes*. This phage has an eclipse period of 30 min and a latent period of approximately 40 min, after which mature phage particles are liberated over a period of 20 min to give a maximum burst of 25 (20). During the infective cycle, RNA transcription occurs predominately from one strand of the phage genome, the heavy strand, from which three classes of mRNA are synthesized. One class, designated "immediate early," is synthesized in absence of phage-coded protein synthesis when cells are infected in presence of chloramphenicol (CAM). Delayed early mRNA is synthesized from 5 to 15 min after infection and has no species in common with immediate early (CAM) RNA. Between 15 and 20 min after infection, synthesis of the two early mRNA classes is replaced by synthesis of the late class, which continues until cell lysis (20).

In this report, the different classes of RNA produced in cells of *C. sporogenes* infected with F1 phage at various times after infection are characterized with respect to the number of

species, molecular weight, and ability to hybridize with F1 phage DNA.

MATERIALS AND METHODS

Bacteria and bacteriophage. *C. sporogenes* ATCC 8074 was used as the host in all experiments. Bacteriophage F1, which was obtained from J. F. M. Hoeniger, University of Toronto, was grown and purified as described previously (20).

Media and culture methods. Cultures of *C. sporogenes* were maintained in cooked meat medium (Difco). RNA was isolated from cells growing in M9 Trypticase medium, which contained, per liter: Na₂HPO₄ (6 g), KH₂PO₄ (3 g), NaCl (0.5 g), NH₄Cl (1 g), MgSO₄ (0.1 g), glucose (1 g), Trypticase (50 g), sodium thioglycolate (1 g), and vitamin B1 (0.1 g). All cultures were grown at 37 C under anaerobic conditions in flasks similar to those described by Bradley and Hoeniger (5).

Isolation of labeled RNA from phage-infected cells. Cells of *C. sporogenes* in the mid-log phase of growth in 10 ml of M9 Trypticase growth medium were infected with F1 phage at a multiplicity of infection of 2. After a period of 5 min in ice, during which 90% of the phage particles absorbed to the cells, anaerobic conditions were restored for varying periods of time before labeling. RNA used in hybridization experiments was labeled by a 2-min pulse of 25 μ Ci of [5-³H]uridine per ml (New England Nuclear Corp.; 25.6 Ci/mmol). RNA prepared for autoradiography was labeled for 5 min with 1 μ Ci each of [2-¹⁴C]uracil and [8-¹⁴C]adenine per ml (New England Nuclear Corp.; 56.5 and 60.5 mCi/mmol, respectively). The uptake of radioactive precursors was stopped by pouring the culture onto 10 ml of frozen buffer containing, per liter: Na₂HPO₄ (6 g), KH₂PO₄ (3 g), NaCl (0.5 g), and NH₄Cl (1 g), pH 7.2. The cells were centrifuged at 8,000 rpm at 5 C and resuspended in

0.4 ml of 25% sucrose in 0.01 M Tris, pH 6.8. Egg white lysozyme (Worthington Biochemical Corp., Freehold, N.J.) in 0.1 M EDTA was added to the cells to give a final concentration of 1 mg/ml. The cells were chilled for 15 min in ice, sodium dodecyl sulfate was added to give a final concentration of 2.5%, and the total volume was adjusted to 1 ml with 0.1 M EDTA in 0.1 M sodium acetate solution. The cell lysate was agitated gently at 62 C with 0.1 ml of 2% macaloid, a polyanionic clay obtained from Enelchem Products, Toronto, and 2 ml of water-saturated phenol. The phenol was removed by centrifugation, another 2 ml of phenol was added, and the extraction procedure was repeated three times. The RNA in the aqueous layer was precipitated by adding 0.1 ml of 3 M sodium acetate and 3 ml of ice-cold ethanol per ml of aqueous layer.

Isolation of RNA from 30S and 50S ribosomal subunits. *C. sporogenes* cells in the mid-log phase of growth in 40 ml of M9 Trypticase medium were labeled with a 5-min pulse of [^{14}C]uracil and [^{14}C]adenine or [^3H]uridine alone at different times after infection. The radioactive pulse was terminated by pouring 20-ml aliquots onto 10 ml of frozen M9 buffer. Polysome breakdown was enhanced by washing cells in ice-cold growth medium as described by Godson (10). The cells were centrifuged at 6,000 rpm for 10 min at 5 C and resuspended in 0.4 ml of 25% sucrose in 0.01 M Tris (pH 6.8), and the volume was adjusted to 2.0 ml by the addition of TMK buffer. TMK buffer contains 0.01 M Tris (pH 7.8), 0.014 M magnesium acetate, and 0.06 M KCl. Cell suspensions were sonicated for 3 min and then centrifuged at 15,000 rpm for 30 min ($30,000 \times g$). The pellet was discarded and the supernatant containing the ribosomal subunits was layered onto a 10-ml 15 to 30% linear sucrose gradient in TMK buffer. The sample was centrifuged for 16 h in a Spinco SW40 rotor at 23,000 rpm ($55,000 \times g$). After centrifugation, fractions of 150 μl were collected by piercing the bottom of the tube, and the OD_{260} of each fraction was determined. The fractions containing 50S and 30S subunits were collected with care to avoid cross-contamination and were examined with the electron microscope. To each 1 ml of the pooled fractions containing 30S or 50S subunits was added 0.2 ml of 0.1 M potassium acetate (pH 5.2) and 0.2 ml of 2% macaloid. RNA was extracted three times, using 2.0 ml of water-saturated phenol at 62 C as described earlier, and was precipitated by adding 2 volumes of ice-cold isopropanol. All RNA precipitates for electrophoresis were dissolved in buffer G (0.04 M Tris, pH 7.5, containing 0.2 M sodium acetate, 0.002 M EDTA, and 0.2% sodium dodecyl sulfate), which also contained 5% sucrose.

Isolation of ribosomal subunits from rifampin-treated cells. Rifampin has been used to eliminate endogenous mRNA associated with ribosomes in cells of *Escherichia coli* (15). Cells of *C. sporogenes* were infected with F1 phage at a multiplicity of infection of 2 in 40 ml of M9 Trypticase medium. At 20 min after infection, radioactive isotopes [^{14}C]uridine and [^{14}C]adenine or [^3H]uridine were added to the culture, which was shaken gently for 5 min at 37 C, after

which rifampin (5 $\mu\text{g/ml}$) was added. The flask was further incubated at 37 C for 30 min and the cells were poured into frozen M9 buffer. Ribosomal subunits were isolated as described above.

Polyacrylamide gel electrophoresis. RNA samples were analyzed by autoradiography and by examination of elution profiles after slicing the gels. For autoradiography, 2.5% polyacrylamide gels containing 0.6% agarose were prepared as described by Summers (18). Each gel was loaded with 50 μg of ^{14}C -labeled RNA (approximately 5×10^4 counts/min) and electrophoresed for 90 min at 5 mA per gel at room temperature, using buffer G. After electrophoresis the gels were processed for autoradiography as described by Fairbanks et al. (9). This method entails slicing the gels longitudinally and then drying them on filter paper under vacuum. Contact autoradiograms were prepared by using Kodak "no screen" X-ray film. The films were developed after 14 days and the autoradiograms were scanned with a Gilford spectrophotometer. For the elution of RNA species, gels were prepared by using a modification of the method described by Loening (13). Gels (5 cm long) were made containing 2.5% acrylamide to which 1% glycerol was added to improve gel slicing. Each gel was loaded with 50 μg of [^3H]RNA and electrophoresis was performed at room temperature (10 V/cm at 5 mA/gel). The gels were frozen in powdered dry ice and sliced into 1-mm sections, using a Joyce Loebl gel slicer (H. Mickle, Gomshall, Surrey, U.K.). RNA was eluted from the slices and 95% of the input counts per minute was recovered after incubation for 18 h at 60 C in 0.5 ml of $4 \times \text{SSC}$. (SSC is 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.6). The amount of radioactivity eluted from each of the slices was determined by adding 10 μl from each eluate to 10 ml of Aquasol (New England Nuclear Corp.); then each sample was counted in a Packard Tri-Carb liquid scintillation counter. The trichloroacetic acid-precipitable radioactivity of all eluates was determined and subsequently used in hybridization experiments.

Calculation of molecular weights of RNA species. Both the microdensitometer tracings and the elution profiles from the gel slices were used to find the number of labeled RNA species synthesized at each time period and their molecular weights. The method of Bishop et al. (3) was used to estimate the molecular weights. For markers, ^3H -labeled 23S and 16S RNAs isolated from *E. coli* were electrophoresed in parallel gels. The markers were assumed to have molecular weights of 1.1×10^6 and 5.6×10^5 , respectively. Slicing and autoradiography correlated well for both the number of species and their molecular weights.

DNA-RNA hybridizations. The [^3H]RNA eluted from the gels was hybridized to separated strands of F1 phage DNA by using the liquid hybridization technique as described earlier (20). Excess F1 DNA (6 to 10 $\mu\text{g/ml}$) was mixed in phenol-saturated $2 \times \text{SSC}$ with 4 μg of RNA per ml, which contained approximately 10^4 counts/min per ml. The hybridization mixtures were annealed at 60 C for 4 h, passed through nitrocellulose filters (25 mm, B6, Schleicher

and Schuell, Inc., Keene, N.H.) and washed with $2\times$ SSC. The filters were incubated with $20\text{ }\mu\text{g}$ of pancreatic RNase and 10 U of T_1 RNase (Worthington Biochemical Corp., Freehold, N.J.) per ml for 30 min at 37°C , rewashed with $2\times$ SSC, and dried. The radioactivity present in the filters was assayed in toluene-Omnifluor scintillation fluid. The procedures for the separation of the complementary strands of F1 phage DNA into light and heavy fractions using the ribopolymer poly(U,G) have been described previously (20).

RESULTS

Characterization of RNA by polyacrylamide gel electrophoresis. When RNA was isolated from uninfected cells of *C. sporogenes* labeled with ^{14}C , intense labeling of rRNA was observed in the positions of 23S, 16S, and approximately 17.5S; however, no distinct bands of host mRNA were visible (Fig. 1A). On 5-cm gels, tRNA (4S) and the 5S ribosomal component migrated out of the gels during the 90-min electrophoresis period and were not visible in any of the tracings. To identify which RNA species are transcribed from F1 DNA, ^3H -labeled RNA was isolated from cells at various times after infection and samples were electrophoresed on 2.5% polyacrylamide gels without agarose. The gels were sliced and [^3H]RNA was eluted and hybridized with separated strands of F1 DNA. Figures 2 to 5 show the elution profiles of [^3H]RNA from the gels

after slicing and the percentage of RNA eluted from the various fractions that hybridized with the heavy strand of F1 phage DNA.

Immediate early F1 mRNA. RNA that is synthesized early in F1 infection in cells treated with CAM (100 $\mu\text{g}/\text{ml}$) has been designated "immediate early" mRNA or "CAM" RNA (20). This class of mRNA is transcribed in absence of phage-coded protein synthesis. The microdensitometer tracing of the autoradiogram (Fig. 1C) and the elution profile (Fig. 2) show that after polyacrylamide gel electrophoresis, four distinct bands were obtained in addition to the 23S and 16S rRNA bands. Three species have been identified that have molecular weights greater than that of 23S RNA. The calculated molecular weights of these three species are 2.0×10^6 , 1.8×10^6 , and 1.65×10^6 . The fourth species occupies a position corresponding to the precursor of the host 16S rRNA. Each RNA species, after elution from the gels, was hybridized with separated strands of F1 phage DNA; however, none of the RNA species hybridized with the light strand, whereas all species hybridized with the heavy strand of the phage DNA (Fig. 2). In spite of the fact that all hybridizations were performed in DNA excess, the efficiency of hybridization of the F1-specific RNA separated by polyacrylamide gel electrophoresis was very low. This is in contrast to phage λ RNA, which after separation under the

FIG. 1. Microdensitometer traces of autoradiograms of ^{14}C -labeled RNA from F1-infected cells or from uninfected *C. sporogenes*. RNA (5×10^5 counts/min) labeled for 5 min at different times after infection was electrophoresed on 2.5% polyacrylamide gels for 90 min. The gels were sliced longitudinally and dried, and contact autoradiograms were prepared. The arrow indicates the position of 23S [^{14}C]RNA from *E. coli* W3350.

FIG. 2. Electrophoretic pattern of immediate early RNA from F1 phage. *C. sporogenes* cells were treated with 100 μ g of CAM per ml 10 min before infection. The CAM was either removed at the time of infection or left in the culture medium throughout the period of infection and labeling. RNA was labeled with [³H]uridine at 8 to 10 min after infection, 50 μ g of RNA was electrophoresed, and RNA was eluted from the gels, which had been sliced into 1-mm pieces. RNA was hybridized with the heavy strand of F1 DNA, using DNA in excess (RNA, 5 μ g/ml; DNA, 6 to 10 μ g/ml). Symbols: ●, Total counts per minute; □, percentage of input RNA hybridized to F1 heavy strand when RNA was made in cells treated with CAM before phage infection, but removed at time of infection; ▲, percentage of input RNA hybridized to F1 heavy strand when RNA was made in cells treated with CAM throughout infection and labeling period. The arrows indicate the position of 23S and 16S RNA from *E. coli* W3350, and the molecular weights of F1-specific RNAs are presented as multiples of 10⁶.

same conditions hybridizes with 90% efficiency to λ DNA (6). The F1 RNA-DNA hybridizations were performed in 50% dimethyl sulfoxide to eliminate secondary structure in the molecules, which was initially thought to be the cause of the low hybridization. The efficiency of hybridi-

zation was not, however, increased by this modification. At the present time, we have no explanation for the low efficiency of hybridization of the F1-specific RNA, after elution from polyacrylamide gels, with separated F1 DNA strands.

Cells of *C. sporogenes* that had been treated with CAM were washed with growth medium, infected with F1 phage, and labeled with [³H]uridine at 8 to 10 min after infection. The [³H]RNA isolated from these cells showed the same elution profile as that from cells in which CAM had been present throughout infection, except that RNA banding in the position of 23S and 16S hybridized with the heavy strand of F1 DNA whereas cells infected in presence of CAM

FIG. 3. Electrophoretic pattern of early mRNA from F1 phage. RNA labeled with [³H]uridine at 3 to 5 min after infection was electrophoresed on 2.5% polyacrylamide gel for 90 min. RNA eluted from 1-mm slices was hybridized with F1 heavy strand, using DNA in excess. Symbols: ●, Total counts per minute; □, percentage of input RNA hybridized to F1 heavy-strand DNA.

FIG. 4. Electrophoretic pattern of late RNA (20 to 22 min). RNA labeled with [³H]uridine at 20 to 25 min after infection was processed as described in the legend to Fig. 3. Symbols: ●, Total counts per minute; □, percentage of input RNA hybridized with F1 heavy-strand DNA.

contained 23S and 16S RNA that did not hybridize with F1 DNA (Fig. 2 and Table 1). The implication of this observation is discussed later.

Delayed early mRNA. F1 mRNA isolated from cells early in infection without CAM, 3 to 8 min (Fig. 1B) and 3 to 5 min (Fig. 3), contains the same three species present in CAM-treated cells plus two new species of low molecular weight (approximately 0.45×10^6 and 0.35×10^6). Previous hybridization experiments suggested that F1 RNA, synthesized in cells treated with CAM, can compete out 70% of the RNA made at 3 to 5 min under normal conditions in F1-infected cells (20). These two low-molecular-weight species appear to account for the [³H]RNA that cannot be competed by unlabeled

CAM RNA in DNA-RNA hybridization experiments. That these RNAs are transcribed from F1 phage DNA is shown by their ability to hybridize to the heavy strand of F1 DNA, which acts as the predominant template early in infection (Fig. 2 and 3). The light strand of F1 phage DNA, which was shown previously (20) not to be transcribed until later in infection, does not hybridize with any of the RNA species.

Late F1 mRNA. Late mRNA is the class of RNA synthesized from 15 min after infection until lysis of the cells. The [³H]RNA made after 20 min following phage infection cannot compete with any early unlabeled mRNA species in hybridization experiments and appears to be completely different from mRNA transcribed before 15 min after infection (20). Figures 4 and 5 show [³H]RNA elution profiles after poly-

FIG. 5. Electrophoretic pattern of late RNA (43 to 45 min). RNA labeled with [³H]uridine was processed as described in Fig. 3. Symbols: ●, Total counts per minute; □, percentage of input RNA hybridized with F1 heavy-strand DNA.

TABLE 1. Hybridization of F1 phage DNA with 23S and 16S RNA from F1-infected cells^a

Period (min) of labeling after F1 infection	Percent RNA hybridized with F1 phage	
	23S RNA ^b	16S RNA ^b
Uninfected <i>C. sporogenes</i>	0	0
3-5	0.7	0.4
20-22	0.95	1.12
43-55	2.9	2.1
8-10 (+CAM present before F1 infection) ^c	0.6	0.5
8-10 (+CAM present through- out F1 infection)	0	0

^a Heavy DNA strand of F1 was hybridized in DNA excess (DNA:RNA was 3:1) to 16S and 23S RNAs labeled with a 2-min pulse of [³H]uridine. The input radioactivity was approximately 10,000 counts/min.

^b RNA isolated from total cell RNA by polyacrylamide gel electrophoresis.

^c Chloramphenicol (CAM) was removed by centrifugation before F1 infection.

acrylamide gel electrophoresis; RNA was labeled at 20 to 22 min and 43 to 45 min, respectively. The gel pattern of RNA labeled between 20 and 22 min (Fig. 1D and 4) shows four mRNA species with molecular weights of approximately 2.3×10^6 , 1.7×10^6 , 1.5×10^6 , and 1.4×10^6 . At 43 to 45 min, five molecules were labeled with the loss of one of the larger species (1.5×10^6), which was replaced by two of smaller molecular weight (0.4×10^6 and 0.5×10^6). RNA species eluted from all the above bands hybridized with the heavy strand of F1 DNA, and the hybridization percentages are shown in Fig. 4 and 5. The light strand of F1 phage DNA hybridized only with the RNA peak of molecular weight 1.5×10^6 and at a very low efficiency (0.5%). Thus, the RNA synthesized at 20 min after infection consists of two different transcripts of the same molecular weight that both have a molecular weight of 1.5×10^6 . One RNA species is transcribed from the light strand, whereas the other is transcribed from the heavy strand of F1 phage DNA.

When RNA synthesized at 43 to 45 min after infection was subjected to polyacrylamide gel electrophoresis, the peak corresponding to 1.5×10^6 daltons was reduced considerably compared to 20-min RNA. The RNA synthesized at 43 to 45 min contained an RNA species of 0.5×10^6 daltons not present in late RNA synthesized at 20 min after infection. This species was also found to hybridize with the light strand of F1

phage DNA, giving a hybridization percentage of 1.2%.

23S and 16S RNAs. Table 1 shows the percentage of 23S and 16S RNAs that hybridized with the heavy strand of F1 phage DNA; a small percentage of the RNA in these bands always hybridized, except for the RNA made in uninfected cells and in cells treated with CAM throughout the period of infection and labeling (Fig. 2 and Table 1, line 5). The percentage of RNA in the 16S and 23S peaks hybridizing with F1 phage DNA appeared to increase during infection and reached a maximum at 45 min after infection (Table 1, lines 1-4). RNA from uninfected cells never hybridized with F1 DNA.

There are several possible explanations for the hybridization of 16S and 23S RNAs to F1 DNA. The F1 genome may code for mRNA that has the same molecular weight as *C. sporogenes* rRNA, i.e., 23S and 16S. Alternatively, these species of RNA might be rRNA that is transcribed from F1 DNA and is required for the translation of F1-specific delayed early and late proteins. A third possibility is that aggregation of the RNA molecules occurs in the gels, as a result of secondary structure, and the 23S and 16S fraction are composed of F1 messages of smaller molecular weight. Finally, RNA isolated after F1 infection which bands in the gels at positions of 23S and 16S could constitute degradation products of F1 mRNA species of high molecular weight.

The possibility of secondary structure in the F1 RNAs leading to their banding at 23S and 16S was investigated by heating early and late F1 RNAs with 50% dimethyl sulfoxide at 75°C for 5 min to remove all secondary structure (17). This treatment changed neither the elution profiles of the RNA after polyacrylamide gel electrophoresis nor the ability of the 23S and 16S species to hybridize with the heavy strand of F1 DNA.

To see whether RNA molecules in the 23S and 16S bands were associated with the ribosomes, 50S and 30S ribosomal subunits were isolated from uninfected cells and from cells infected with F1. RNA was labeled with [³H]uridine early in infection (5 to 10 min) or at late times (20 to 25 min and 40 to 45 min) and was isolated from the separated ribosomal subunits by the technique as described in Materials and Methods. The results of hybridization experiments between the 23S or 16S RNA species isolated from 50S or 30S ribosomal subunits and F1 heavy-strand DNA are shown in Table 2. A small percentage of the [³H]RNA hybridized with the heavy strand, and this percentage

TABLE 2. Hybridization of F1 phage DNA^a with [³H]RNA from ribosomal subunits

RNA isolated from	Percent RNA hybridized	
	23S RNA ^b	16S RNA ^c
Uninfected <i>C. sporogenes</i>	0	0
F1-infected cells		
5-10 min after infection	0.13	0.10
20-25 min after infection	0.23	0.28
40-45 min after infection	0.36	0.6

^a Heavy DNA strand of F1 was hybridized in DNA excess at a DNA:RNA ratio of 3:1 to RNA isolated from ribosomal subunits, which had been labeled for 5 min with [³H]uridine at the indicated times. The input radioactivity was approximately 30,000 counts/min.

^b Isolated from 50S ribosomal subunits.

^c Isolated from 30S ribosomal subunits.

increased with time after infection, reaching a maximum at 40 to 45 min, immediately before lysis. rRNA from uninfected *C. sporogenes* cells did not hybridize. The 23S and 16S RNAs from ribosomal subunits and from total RNA, after separations by electrophoresis, were annealed with the light strand of F1 phage DNA. None of these RNAs synthesized at any time during the F1 infective cycle were able to hybridize with the light strand.

The RNAs isolated from the 50S and 30S ribosomal subunits were electrophoresed on 2.5% polyacrylamide gels. Elution of the RNAs from the gel slices gave a single peak corresponding to 23S from the 50S subunits or 16S from the 30S subunits and also trace amounts of RNA of higher molecular weight. Each of these RNAs retained its ability to hybridize with the heavy strand of F1 phage DNA. Therefore, from this experiment it is not possible to know whether or not rRNA is coded by the F1 phage genome. The hybridization abilities of both 16S and 23S RNAs isolated from bulk RNA by electrophoresis and from ribosomal subunits may be due to contamination by F1 mRNA species. The rifampicin treatment has been used to eliminate the presence of mRNA associated with the ribosomal subunits in cells of *E. coli* (15).

Cells of *C. sporogenes* were labeled with [³H]uridine at 20 min after F1 infection and then treated with rifampicin for 30 min after the radioactive labeling period. Ribosomal subunits were isolated and the RNA from these subunits was found to be free from contaminating F1 mRNA, since a single band corresponding to 16S or 23S rRNA was obtained after electropho-

resis on polyacrylamide gels. This 23S and 16S rRNA did not hybridize with F1 phage DNA. In our efforts to remove F1 mRNA from *C. sporogenes* ribosomes, we also treated 70S ribosomes in vitro with puromycin (4, 19). However, we were unable to isolate ribosomes free from contaminating mRNA's, since in all our puromycin preparations various RNA species were identified, by polyacrylamide gel electrophoresis, in addition to the 23S or 16S rRNA species.

DISCUSSION

Immediately after infection, F1 phage synthesizes a class of RNA by using the RNA polymerase of its host, *C. sporogenes* (20). This class of RNA is transcribed exclusively from the heavy strand of F1 phage DNA in presence of CAM, i.e., in absence of phage-coded protein synthesis, and has been designated "immediate early" mRNA. Our results show that this class of mRNA consists of three different species of RNA ranging in molecular weight from 1.65×10^6 to 2.0×10^6 .

In absence of CAM, delayed early mRNA synthesis begins from about 5 min and continues until 10 to 15 min after infection. This newly synthesized RNA does not resemble immediate early mRNA since it was not competed by unlabeled CAM RNA in hybridization competition experiments (20). Gels of RNAs labeled between 3 and 8 min after infection in absence of CAM show the same three species that made up the immediate mRNA class but also contain two new ones of low molecular weight (0.45×10^6 and 0.35×10^6) that are not competed out by the CAM RNA. These low-molecular-weight RNAs probably belong to the class of delayed early mRNA that has been shown to account for 30 to 50% of the total mRNA synthesized from the heavy DNA strand between 5 and 15 min after infection. The early classes of F1 mRNA, immediate early and delayed early, consist of five different species, having a combined molecular weight of about 6.25×10^6 . The molecular weight of F1 DNA is 33×10^6 (20); therefore, early species can account for about 38% of the coding capacity of the double-stranded DNA, which is approximately 16.5×10^6 daltons.

Between 15 to 20 min, late mRNA appears and is continually synthesized. Late mRNA did not compete with either immediate early or delayed RNA; therefore, all late RNA species are newly transcribed. At 20 to 25 min, four species of mRNA are transcribed, ranging in molecular weights from 1.4×10^6 to 2.3×10^6 , from the heavy strand of F1 phage DNA. At 40

to 45 min, immediately before lysis, five species are observed; one of the high-molecular-weight species (1.5×10^6) seen at 20 min after infection is replaced by two of smaller molecular weight (0.4×10^6 and 0.5×10^6). Competition hybridization with unlabeled RNA synthesized at 20 to 22 min after infection and heavy-strand DNA showed previously (20) that RNAs synthesized at 40 min are similar to those made at 20 min; therefore, the two small-molecular-weight species represent some portion of the larger-molecular-weight species (1.5×10^6) present at 20 min.

F1 RNA transcription begins from the light strand of F1 DNA only late in infection, between 10 and 15 min, and only accounts for a small proportion of total transcription (20). Two RNA species are synthesized from the light strand, having molecular weights of 1.5×10^6 and 0.5×10^6 . Thus, the light strand of F1 DNA codes for mRNA species with a total molecular weight of 2.0×10^6 , whereas the heavy strand codes for transcription of a total molecular weight of 13×10^6 . The combined value of 15×10^6 is slightly less than the possible molecular weight of the RNA transcripts that could be coded for by the double-stranded F1 genome with a molecular weight of 33×10^6 . However, errors of about $\pm 5\%$ are inherent both in the measurement of the length of the F1 phage DNA molecules and the molecular weights of the RNA species. Thus, it is possible that each RNA species identified by polyacrylamide gel electrophoresis is transcribed from a different part of the F1 genome as individual transcripts. Alternatively, F1 mRNA species may be synthesized as polycistronic molecules and then broken down by endonuclease to form individual mRNA transcripts that are then translated. In the latter case, addition of all the molecular weights of the various species visible in gel electrophorograms would give too high an estimate for the total mRNA transcribed.

Throughout F1 infection, radioactive precursors are incorporated into 23S and 16S species, of which only a small percentage (less than 3%) hybridized with F1 DNA (Table 1). The remaining radioactivity therefore belongs to host rRNA, which is continually synthesized until lysis occurs. The inability of F1 phage to shut off host rRNA synthesis is in direct contrast to the virulent coliphage infections of T4 (1) and T7 (18). In the former case, host rRNA synthesis ceases in 1 to 1.5 min, whereas in the latter synthesis is shut off within 4 to 8 min after infection. The 23S and 16S RNAs present throughout F1 infection do not appear to be rRNA synthesized by uninfected cells in the

cultures, because [^3H]uridine was still found to be incorporated into rRNA when the multiplicity of infection was raised from 2 to 10.

One unusual feature of the RNA synthesized in F1-infected *C. sporogenes* is that 23S and 16S RNAs, either isolated from ribosomal subunits or fractionated from the bulk RNA, hybridized with the heavy strand of F1 DNA throughout the infective cycle. Host rRNA failed to compete with these RNA species that hybridized with F1 DNA. The percentage of hybridized was small: 0.4 to 3% of the RNA isolated from gels and 0.1 to 0.6% of the RNA isolated from ribosomal subunits. Treatment of the total RNA before electrophoresis with 50% dimethyl sulfoxide to destroy secondary structure did not change either the banding pattern or the hybridization results. Thus the two bands in the 23S and 16S regions do not appear to be artifact caused by aggregation of phage mRNA. In cells treated with CAM throughout infection, none of the 23S or 16S RNA hybridized with F1 DNA (Fig. 2), but when CAM was removed from the growth medium before infection by centrifugation, a small percentage of the 23S and 16S RNAs again hybridized with F1 DNA. Thus, phage-coded protein synthesis appears to be necessary for transcription of RNAs from the heavy strand of F1 DNA that band at the 23S and 16S RNA positions. The various F1 mRNA species synthesized at 20 min after infection have a half-life of approximately 8 min (Taylor and Guha, unpublished observation). They were allowed to decay over a 30-min period in the presence of rifampin. Ribosomal subunits isolated from rifampin-treated cells of *C. sporogenes* were thus free from contaminating mRNA, and RNA isolated from these subunits did not hybridize with F1 phage DNA. These contaminating species of mRNA may originate as degradation products of the higher-molecular-weight mRNA species of the F1 phage, and therefore the 23S and 16S RNAs hybridizing with F1 phage DNA do not represent rRNA's transcribed by the F1 phage genome. So far no attempt has been made to characterize them. The presence of endogenous mRNA in ribosomes of *C. pasteurianum* was also reported earlier (11). The absence of these mRNA species in CAM-treated cells may be explained by the breakdown of the ribosome-mRNA complex in presence of the drug, which has never been reported. Alternatively, CAM may prevent degradation of F1 phage mRNA molecules of high molecular weight that remain associated with the ribosomes or may decrease the efficiency of hybridization, thereby preventing detection of F1 mRNA species.

ACKNOWLEDGMENTS

This investigation was supported by the National Research Council of Canada (grant A-6215). D.E.T. holds a studentship from the Medical Research Council of Canada.

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