In Vitro Transcription of T4 Deoxyribonucleic Acid by Escherichia coli Ribonucleic Acid Polymerase

SEQUENTIAL TRANSCRIPTION OF IMMEDIATE EARLY AND DELAYED EARLY CISTRONS IN THE ABSENCE OF THE RELEASE FACTOR, RHO*

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SUMMARY

T4 DNA was transcribed in vitro by the DNA-dependent RNA polymerase from Escherichia coli. When the termination factor, rho, was present, all of the RNA made was 2000 nucleotides in length and was completely immediate early. When rho was absent, the entire early region of the T4 chromosome was transcribed as a series of polycistronic messengers, either 4300 nucleotides or 7100 nucleotides long. The product made, in the absence of rho, was 40% immediate early and 60% delayed early. Transcription of the delayed early cistrons, in the absence of rho, was due to continued transcription past the point at which rho causes release.

Experiments were done at 18°C at which initiation alone was allowed to proceed for several minutes by adding only ATP and GTP. The reaction mixture was then completed and rifampicin was added to inhibit further initiation. RNA synthesis continued for 1400 sec after the brief period of initiation. During the initial 400 sec of reaction, only immediate early sequences were synthesized. Delayed early sequences alone were made at later times. Transcription of the e and rII cistrons was studied. The e cistron was transcribed between 1300 and 1400 sec after initiation. The rII A and B cistrons were transcribed sequentially. Transcription of the rII A cistron occurred between 700 and 1100 sec after initiation but the B cistron was not transcribed until 1100 to 1400 sec of reaction. The e and rII transcripts were parts of RNA molecules that were 7100 nucleotides in length. The rII region was transcribed solely as a polycistronic messenger in vitro but only 20 to 30% of the rII transcripts made in vivo were polycistronic.

appears during the initial 2 min of infection and does not require the prior synthesis of phage-specific proteins. The delayed early family requires prior synthesis of phage proteins and does not appear until later than 2 min of infection (1, 2).

In vitro studies have shown that only immediate early cistrons are transcribed if the host initiation factor, σE. coli, and release factor, rho, are present (3, 4). Early in T4 infection, a new σ factor appears, σT4, which permits transcription to begin at delayed early cistrons (3, 4). The operation of these subunits seemed to explain adequately the control of early T4 RNA synthesis in vivo.

However, evidence has been presented that suggests the existence of another control element instrumental in the in vivo transcription of early cistrons. In the case of T4, the e gene, which is not under the control of an early promoter (5), can be transcribed during the early period of phage infection (5). This premature transcription of the e gene requires prior synthesis of phage protein (6). In the case of phage λ, the N gene does not appear to code for a new σ factor but the product of this gene allows transcription to extend further into the left arm of λ DNA (7).

Therefore, it appears as if an antiterminator protein is synthesized soon after phage infection and that this protein somehow obviates the host cell chain termination mechanism. Since rho cannot mediate release in the case of T4 delayed early cistrons, the apparent function of the antiterminator is to allow RNA polymerase molecules that have initiated at immediate early cistrons to transcribe beyond the point at which rho would normally cause release to take place.

Conceptually, at least, in vitro transcription of T4 DNA by E. coli RNA polymerase, in the absence of rho, should mimic the in vivo situation for these enzyme molecules when the antiterminator is present. To this end, it has been shown that E. coli RNA polymerase, in the absence of rho, transcribes two discrete size classes of T4 RNA in vitro (8). Roughly 70% of the product is 4300 nucleotides long and the remainder is 7100 nucleotides in length. We now ask to what extent the early region of the T4 chromosome is transcribed when rho is absent.

The data indicate that (a) probably the entire early region of the T4 chromosome is transcribed, (b) immediate early and delayed early cistrons are adjacent on the T4 DNA, (c) the order

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During the first 10 min of phage infection, two distinguishable families of T4 RNA are synthesized. The immediate early family
of appearance is immediate early → delayed early, and (d) each RNA molecule contains no more than one immediate early transcript but either one or several delayed early transcripts. In most respects, these results confirm already published data (9–11). We also demonstrate that the e and rII cistrons are transcribed in the absence of rho. All three cistrons appear to be associated with those RNA molecules that are 7100 nucleotides in length.

MATERIALS AND METHODS

Purification of E. coli DNA-dependent RNA Polymerase and Rho—DNA-dependent RNA polymerase (EC 2.7.7.6) was purified to greater than 90% purity by the glycerol gradient method (12). Rho was purified according to the method of Roberts (13) and was estimated to be greater than 80% pure.

Purification of DNA-dependent RNA Polymerase from T4-infected Cells—E. coli BB was grown in 7-liter batches to 1 × 10^9 cells per ml of growth medium. The growth medium was 3 × D (14) and the temperature was 30°C. Phage T4 was added to give a multiplicity of 5 and 2 min were allowed for phage adsorption. For pulse labeling of in vitro RNA, [G-3H]uridine (20 Ci per ml) was added.

Purification of RNA from Phage-infected Cells—After various times of infection or pulse labeling, the culture (175 to 200 ml) was poured over an equal volume of finely crushed frozen medium. The cells were centrifuged at 10,000 × g for 5 min and resuspended in proteolytic buffer (50 mM Tris (pH 7.9), 500 mM sucrose, 10 mM EDTA, and 150 μg per ml of benzotetreated lysozyme). After 5 to 7 min at 0°C, the protoplasts were collected by centrifugation at 5,000 × g for 5 min. Lysing buffer (50 mM Tris (pH 7.9), 10 mM NaCl, 1 mM Na2 citrate, and 1.5 mM sodium lauryl sulfate) and 150 μl of diethylpyrocarbonate (18) were added. After 10 min, the lysate was warmed to 37°C for 5 min and then chilled to 0°C. Saturated NaCl (0.5 volume) was added. The resultant precipitate was removed by centrifugation at 10,000 × g for 45 min and discarded. The remainder of the procedure was identical with the one outlined for in vitro RNA.

Purification of rII and e RNA—Specific messengers were purified to greater than 95% purity by three passages through T4 DNA-nitrocellulose columns (19). DNAs containing deletions rI72 and rI8 (20, 21) were used to purify rII and rI/B RNA, respectively. DNA carrying deletion eG19 was used to obtain e RNA (5, 6).

Molecular Weight Determination—[3H]T4 RNA was added to a solution of E. coli 4S, 5S, 16S, and 23S RNA (total unlabeled RNA concentration = 500 μg per ml) and precipitated with ethanol as described above. The precipitate was dissolved to its original concentration in 0.1 M sodium phosphate (pH 7.7), 1.1 M HCHO, and heated at 65°C for 5 min. Aliquots (0.2 ml) of the formaldehyde-treated RNA were layered onto 5-ml linear sucrose gradients (5 to 20% in 0.1 M sodium phosphate (pH 7.7), 1.1 M HCHO) and centrifuged at 50,000 rpm (SW 65L Ti rotor) at 4°C. Samples were collected from the bottom of the tube into 1.0 ml of distilled water. After the absorbance at 260 nm had been determined, the samples were individually precipitated with cold 10% trichloroacetic acid. Molecular weights were calculated with the formula of Boedtker (22).

DNA-RNA Hybridization—Unless otherwise stated, each assay tube contained 5 μg of labeled RNA, varying amounts of unlabeled competitor RNA, and 10 μg of alkali-denatured renatured T4 DNA (28). The ionic condition was 5 × SSC. The total volume was 0.75 ml.

After incubation at 65°C for 5 hours, the contents of each tube were filtered through 13-mm Schleicher and Schuell B6 nitrocellulose filters. The filters were washed with 100 ml of 5 × SSC (50 ml on each side of filter) and incubated for 90 min with boiled pancreatic RNase (30 μg per ml in 2 × SSC), dried, and counted.

For the experiments reported here, unless otherwise noted, the efficiency of hybridization was 20%. In the absence of competitor RNA, 30,000 cpm of [3H]RNA were retained on the filter and 10,000 cpm of [3H]-labeled in vitro RNA were retained.

The abbreviations used are: SSC, 150 mM NaCl, 15 mM Na2 citrate, pH 7.5; 5 × SSC, 2 × SSC, 5 and 2 times respectively the standard concentration of SSC; GG-RNA, RNA synthesized in vitro by the glycerol gradient-purified enzyme; GG ρ-RNA, RNA synthesized in vitro by the glycerol gradient-purified enzyme in the presence of the release factor rho.

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FIG. 1. Zone centrifugation of T4 RNA synthesized in vivo and in vitro. a, T4 RNA from cells (●) 2, (○) 5, and (△) 10 min after infection. The cells are labeled from zero time until the time given above. T4 RNA was detected by hybridization with T4 DNA. Total RNA was determined in parallel runs. The percent total RNA that hybridized with T4 DNA after 2, 5, and 10 min of infection was 2.2, 6.7, and 17.3, respectively. b, T4 RNA synthesized in vitro in presence (○) and absence (△) of rho. In both cases, 20.2% of the total RNA hybridized. For centrifugation, 0.2 ml (~20,000 cpm) of T4 RNA (in 10 mM Tris-Cl (pH 7.9), 10 mM KCl) was layered onto a 5-ml linear sucrose gradient (5 to 20% in 10 mM Tris-Cl (pH 7.9), 10 mM KCl) and centrifuged at 35,000 rpm (Sw 39 rotor) for 8 hours at 4°C. The sedimentation coefficients were based on Escherichia coli 4 S, 5 S, 16 S, and 23 S RNA run in a parallel tube.

TABLE I

Molecular weights of GG-p-RNA and GG-RNA

The sedimentation coefficients are based on the sedimentation rates of Escherichia coli 4 S, 5 S, 16 S, and 23 S RNAs. The average number of nucleotides per chain (N) is based on a mean mononucleotide residue weight of 350.

When the DNA is eliminated, only 30 to 40 cpm were retained on the filter.

Measurement of Radioactivity—The scintillation medium contained 4 g of 2,5-diphenyloxazole (PPO) and 0.1 g of 1,4-bis[2-(5-phenyloxazole)]benzene (POPOP) per liter of solution. The solvent was freshly distilled toluene. The samples were counted through three cycles in a Beckman scintillation counter.

RESULTS

Size Distribution of T4 RNA Synthesized in Vivo and in Vitro—Fig. 1a shows that the size distribution of T4 RNA made in vivo was not constant throughout the phage infection process.

After 2 min of infection, all of the T4 RNA sedimented as a narrow band at approximately 11 S. Later in infection (5 and 10 min), the majority of the T4 RNA molecules migrated as a broad band between 15 and 20 S. Of the T4 RNAs made in vitro, GG-RNA sedimented at 25 to 30 S whereas GG-p-RNA moved at 11 S (Fig. 1b). The molecular weights of in vitro RNA were determined and are given in Table I. Judging from the sedimentation coefficient of in vivo RNA vis-à-vis in vitro RNA, it can be estimated that 2 min in vivo RNA was 2000 nucleotides in length and the majority of 5- and 10-min RNA was 2500 to 3500 nucleotides in length. Therefore, the bulk of the T4 RNA present during the early period of phage infection was considerably shorter than GG-RNA.

Effect of Rho on Regions of T4 Chromosome Transcribed in Vitro—Fig. 2A shows hybridization competition experiments between GG-p-RNA and GG-RNA. Unlabeled GG-RNA competed completely with labeled GG-p-RNA but the reciprocal experiment showed that unlabeled GG-p-RNA competed only 40 to 41% with labeled GG-RNA. Consequently, the absence of rho allowed transcription of much larger regions of the T4 chromosome. Fig. 2B gives hybridization experiments between GG-p-RNA and 15 S-GG-RNA and 18 S-GG-RNA. The two size classes of GG-RNA species were purified by formaldehyde treatment followed by sucrose gradient centrifugation. The peaks were dialyzed extensively into 10 mM Tris-Cl (pH 7.9), 10 mM KCl to remove the formaldehyde. All RNAs were degraded to the size of GG-p-RNA by treatment with 0.1 M NaOH at 20°C for 1.5 to 3.0 min.
RNA cross-competed only to the extent that GG-\(p\)-RNA competed with either size class. As shown below, GG-\(p\) RNA is equivalent to immediate early RNA. Therefore, 15 S- and 18 S-GG-RNA molecules may share only immediate early transcripts.

To obtain an idea of when in the phage infection process RNA molecules were made that were identical with either GG-\(p\)-RNA or GG-RNA, phage-infected cells were pulse-labeled at various times and the RNA was isolated. Hybridization competition experiments were then performed between the pulse-labeled in vivo T4 RNA and pulse-labeled in vitro T4 RNA. •, GG-\(p\)-RNA as competitor; O, GG-RNA as competitor. The amount of pulse-labeled RNA that was T4 RNA was determined by exhaustive hybridization with T4- and 1 DNA strands (16). Between 0 and 2 min only 15% of the pulse-labeled RNA was T4 RNA; 2 to 5 min, 38%; 5 to 10 min, 72%; and, beyond 10 min, 85 to 95% (cf. References 24 and 25). The RNAs all were treated with 0.1 M NaOH at 20°C until their average size was the same as GG-\(p\)-RNA.

Left: FIG. 3. Hybridization competition between in vitro T4 RNA and pulse-labeled in vivo T4 RNA. •, GG-\(p\)-RNA as competitor; O, GG-RNA as competitor. The values are averages of three determinations.

Right: TABLE II

| Labeled RNA          | Competitor RNA | Competition % |
|----------------------|----------------|---------------|
| Immediate early ......| GG-\(p\)-RNA   | 99.9          |
| Immediate early ......| GG-RNA         | 99.9          |
| Early .................| GG-\(p\)-RNA   | 40 ± 2        |
| Early .................| GG-RNA         | 99.9          |
| Early .................| Immediate early| 99.9          |
| Early .................| Immediate early| 40 ± 1        |
| Early .................| Early          | 99.9          |

In Vivo Time of Appearance of Immediate Early and Delayed Early RNA—Most likely, the transcription of delayed early cistrons in vitro, in the absence of rho was due to continued transcription beyond the point at which rho would normally cause release. If such were the case, one would expect a time lag between the initiation of transcription and the appearance of delayed early sequences. To illustrate this, experiments were done in which initiation alone was allowed to proceed by incubating the DNA and enzyme with only ATP and GTP. After several minutes, [\(^{3}\)P]ATP, GTP, and UTP were added to complete the reaction mixture along with rifampicin to inhibit further initiation (26-29). The RNA was extracted at frequent intervals and tested for the presence of immediate early and delayed early RNA sequences.

Fig. 4 shows the kinetics of incorporation of [\(^{3}\)P]ATP into acid-insoluble material. Two distinct linear rates of incorporation were observed: one which was T4 RNA was determined by exhaustive hybridization with T4- and 1 DNA strands (16). Between 0 and 2 min only 15% of the pulse-labeled RNA was T4 RNA; 2 to 5 min, 38%; 5 to 10 min, 72%; and, beyond 10 min, 85 to 95% (cf. References 24 and 25). The RNAs all were treated with 0.1 M NaOH at 20°C until their average size was the same as GG-\(p\)-RNA.

Fig. 4. Incorporation of [\(^{3}\)P]ATP into acid-insoluble material after a brief period of initiation. Reaction mixtures were low ionic strength and prepared as described under "Materials and Methods." After incubation at 18°C with 0.5 mM each ATP and GTP, 0.5 mM each [\(^{14}\)C]ATP, CTP, and UTP and 10 \(\mu\)g per ml of rifampicin were added simultaneously. After various time intervals, 50-\(\mu\)l aliquots were removed and acid-precipitated as described elsewhere (8). • and \(\times\) represent repeats of the same experiment.
The linear rate of incorporation observed in Fig. 4. The change in GTP (15 Ci per mole) was present during the brief initiation period. The RNA was purified, formaldehyde-treated, and centrifuged. These controls showed that short polynucleotide chains, 50 to 100 nucleotides in length, were made during the initiation period. Generally, stocks of nucleoside triphosphates, as supplied by the manufacturer, are cross-contaminated. This probably explains the slight amount of propagation that occurred.

The reaction mixtures were identical with those given in Fig. 4. The RNA was heated with formaldehyde. Each gradient received 3500 to 4500 cpm of [U-14C]AMP and the recovery is 89%. Control experiments were done in which [U-14C]ATP or [U-3H]GTP (15 Ci per mole) was present during the brief initiation period. The RNA was purified, formaldehyde-treated, and centrifuged. These controls showed that short polynucleotide chains, 50 to 100 nucleotides in length, were made during the initiation period. Generally, stocks of nucleoside triphosphates, as supplied by the manufacturer, are cross-contaminated. This probably explains the slight amount of propagation that occurred.

The reaction stopped at 1400 sec. Fig. 5 shows that 70% of the RNA chains stopped growing after they were 4300 nucleotides long but the remainder continued to grow until a size of 7100 nucleotides was achieved. This probably explains the change in linear rate of incorporation observed in Fig. 4. The change in sedimentation coefficient with time indicated a chain elongation rate of five nucleotides per sec.

Hybridization competition experiments (Fig. 6) showed that only immediate early RNA sequences were present during the initial 400 sec of reaction. Past 400 sec, delayed early sequences appeared. When the reaction stopped, 59% of the product was immediate early, and 41% was immediate early. Considering the elongation rate and the time of appearance of delayed early sequences, it was estimated that delayed early cistrons were no more than 2000 nucleotides from an immediate early promoter.

In Vitro Transcription of rII and e Cistrons—One of the lines of evidence pointing to the existence of the antiterminator protein is the early transcription of the e cistron (6), a late function (5). Therefore, transcription of the e cistron was expected under the present in vitro conditions, provided that transcription in the absence of rho truly reflected the in vivo situation when rho is inhibited by the antiterminator. Since the rII cistrons are delayed early, their transcription was also expected since GG RNA appeared to contain transcripts of all of the delayed early cistrons.

The time sequence of e gene transcription is shown in Fig. 6. The e cistron was not transcribed until 1300 to 1400 sec after initiation. Therefore, the e cistron transcript seemingly constituted the last 500 nucleotides of an 18 S-GG-RNA molecule.

Transcription of rII region began at 700 sec and continued until the end of the reaction (Fig. 7). Between 700 and 1100 sec, all of the rII RNA belonged to the A cistron. The B cistron was not transcribed until later than 1100 sec. The relative amount of rIIA RNA decreased after 1100 sec, implying that the rIIA cistron was not transcribed after that time. The results also indicated that the A and B cistrons were transcribed sequentially. From these data, it was estimated that the entire rII region was 3500 nucleotides long. The A cistron was 2000 nucleotides in length and the B cistron was 1500 nucleotides long. These estimates agreed reasonably well with other estimates (6,32). Like the e cistron transcript, rII RNA apparently was associated with an 18 S-GG-RNA molecule.

To confirm the assignments of rII and e RNAs to 18 S-GG-RNA molecule, unlabeled GG ρ RNA, 15 S GG RNA, and 18 S-GG-RNA were used to compete the [3H]labeled specific RNAs.
Fig. 7. In vitro time of appearance of rII, rIIA, and rIIB RNAs after a brief period of initiation. •, rII; O, rIIA; C, rIIB. The experimental conditions were identical with those outlined in Fig. 4. The amount of rII RNA was determined by the cross-linked DNA-RNA hybrid method described in Fig. 6. The hybridization mixture contained 5 μg of GG RNA and 400 μg of a cross-linked DNA-RNA hybrid that bound only rII RNA.

Fig. 8. Hybridization competition between GG-p-RNA, GG-RNA, rII RNA, and e RNA. A, hybridization competition between [3H]e RNA and unlabeled (O) e RNA; ▲, GG-p-RNA; X, 15 S-GG-RNA; •, 18 S-GG-RNA. The competitor RNAs were degraded to the size of e RNA by treatment with 0.1 M NaOH at 20°C for 5 to 10 min. B, hybridization competition between [3H]rII RNA and unlabeled (O) e RNA; X, 15 S-GG-RNA; •, 18 S-GG-RNA; ▲, GG-p-RNA; X, 15 S-GG-RNA; and •, O, C, 18 S-GG-RNA, O, rIIA, C, rIIB, •, rII. The RNAs were all degraded to the size of e RNA by mild alkali treatment. (Fig. 8). The results show that only 18 S-GG-RNA competed with either rII or e RNAs.

Size Distribution of e RNA and rII RNA Synthesized in Vitro and in Vivo—When T4 RNA was hybridized with eG19 DNA, the remaining RNA sedimented as a single homogeneous boundary at 5 S (Fig. 9). This was equivalent to a molecular weight of $4.3 \times 10^6$ (1200 nucleotides), $7.1 \times 10^5$ (2000 nucleotides), and $1.1 \times 10^6$ (3000 nucleotides), respectively. Peaks I and II each represented 35 to 40% of the total rII RNA while Peak III constituted 20 to 30%. Only Peak III was observed if in vitro T4 RNA was hybridized with r1272 DNA. Peak I alone was obtained when either in vivo or in vitro T4 RNA is hybridized with r882 DNA.

On the other hand, rII RNA showed a more complicated picture. When in vivo T4 RNA was hybridized with r1272 DNA, three distinct peaks were observed (Fig. 9). Peak I sedimented at 9 S, Peak II at 11 S, and Peak III at 13.5 S. These sedimentation coefficients correspond to molecular weights of $4.3 \times 10^6$, $7.1 \times 10^5$, and $1.1 \times 10^6$ (3000 nucleotides), respectively. Peaks I and II each represented 35 to 40% of the total rII RNA while Peak III constituted 20 to 30%. Only Peak III was observed if in vitro T4 RNA was hybridized with r1272 DNA. Peak I alone was obtained when either in vivo or in vitro T4 RNA is hybridized with r882 DNA.

These data, plus the experiments shown in Table III, allowed the following assignments to be made. Peaks I and II were monocistronic transcripts of the rIIA and rIIA cistrons, respectively. Peak III contained transcripts of the entire rII region in which the A and B cistrons were present in equimolar quantities.

The size estimates of the rIIA cistron obtained by zone centrifugation and the data in Fig. 7 were identical. However, zone centrifugation provided a slightly lower estimated size for the rIIB cistron and the rII region than did the data in Fig. 7. Considering the fact that sedimentation coefficients are only
The data presented in this paper show that essentially the entire early region of the T4 chromosome was accessible to DNA-dependent RNA polymerase molecules that initiated at immediate early sites in the absence of rho. Since GG-RNA actually contained two discrete size classes of RNA, the early region was transcribed as a series of polycistronic messengers either 4300 or 7100 nucleotides long. These two size classes seemingly contained the same immediate early sequence but different delayed early sequences.

If each of the two size classes of GG-RNA contained one immediate early transcript per molecule, GG-RNA would be expected to be 41% immediate early. The observed value was 40 to 41% so it was concluded that each GG-RNA molecule contained no more than one immediate early transcript.

An estimate of the number of delayed early transcripts per GG-RNA molecule was obtained by assuming that all delayed early cistrons were comparable in size to the rII cistrons. With this assumption in mind, each 15 S GG-RNA molecule contained only one to two delayed early transcripts and each 18 S GG-RNA molecule had three to four delayed early transcripts.

The early genes of T4 occupy nearly 25% of the genetic map (32, 33). Assuming that they occupy a similar proportion of the T4 DNA, as many as 54,000 base pairs could be devoted to the early genes. Considering the relative contributions of 15 S and 18 S species in GG-RNA, seven 15 S GG-RNA molecules and three 18 S GG-RNA molecules would be required to transcribe the entire early region.

With the estimates made above for the number of delayed early and immediate early transcripts per GG-RNA molecule, the early region of T4 DNA could contain as many as 10 immediate early cistrons and 16 to 26 delayed early cistrons. Should an antiterminator mechanism account for a sizeable proportion of delayed early transcription, at some time during infection, such a high number of immediate early cistrons relative to the number of delayed early cistrons may be necessary. Obviously, these numbers must be viewed as tentative until gene titration studies have been done. We are currently engaged in such studies.

Milanesi et al. (11) reported that no more than 38% of their in vitro product was delayed early. They suggested that some of their enzyme molecules traversed more than 1 unit of transcription and re-entered immediate early cistrons. Our data were inconsistent with such a mechanism. It would appear that none of the enzyme molecules re-entered immediate early cistrons under the experimental conditions used here. The above-mentioned authors also suggested that immediate early and delayed early cistrons are interdigitated on the T4 chromosome. Our estimate of the numbers of immediate early and delayed early cistrons would allow one to speculate that generally immediate early cistrons are separated by no more than two delayed early cistrons.

It is not definitely known whether product release occurs in vivo while the termination mechanism of the host is nullified by the antiterminator. During T4 infection, only a portion of the rII transcripts were polycistronic (Fig. 10 and Reference 6). Therefore, it would appear as if termination can occur at the junction separating the A and B cistrons. Since the rII region was transcribed only as a polycistronic messenger in vitro, perhaps the known modifications of the core enzyme upon T4 infection (37) are associated with recognition of different “passive” release points. It would be of interest to examine in vitro transcription of T4 DNA by using the T4 core enzyme.

It is not known what relative contributions σT4 and the antiterminator make to delayed early transcription. The data in
Fig. 1 revealed no in vivo RNAs that were the same size as GG-RNA. There are three plausible explanations for this. (a) The antiterminator mechanism actually accounts for little delayed early transcription. (b) A phage-specific termination mechanism is established soon after infection. (c) RNA molecules the same size as GG-RNA cannot be made in vivo because degradation of these polycistronic messengers commences prior to their completion.

One of the predictions of the antiterminator mechanism is that the resultant polycistronic messengers be translatable into functional proteins. It will be of interest to examine the in vitro translation of GG-p-RNA and GG-RNA in systems known to give functional products (38, 39).

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REFERENCES
1. Grasso, R. J., and Buchanan, J. M., Nature, 224, 882 (1969).
2. Salser, W., Bolle, A., and Epstein, R., J. Mol. Biol., 49, 271 (1970).
3. Travers, A. A., Nature, 223, 1107 (1969).
4. Travers, A. A., Nature, 225, 1009 (1970).
5. Kasai, T., and Bautz, E. K. F., J. Mol. Biol., 41, 401 (1969).
6. Schmidt, D. A., Maranitis, A. J., Kasai, T., and Bautz, E. K. F., Nature, 235, 1012 (1970).
7. Szymbalski, W., in Proceedings of the First Lepetit Colloquium on RNA Polymerase and Transcription, North-Holland Publishing Company, Amsterdam, 1970, pp. 209-217.
8. Witmer, H., Biochim. Biophys. Acta, 245, 29 (1971).
9. Geduscheck, E. P., Snyder, L., Colvill, A. J. E., and Sarnat, M., J. Mol. Biol., 19, 541 (1966).
10. Milanese, G., Brody, E. N., and Geduscheck, E. P., Nature, 221, 1014 (1969).
11. Milanese, G., Brody, E. N., Grau, O., and Geduscheck, E. P., Proc. Nat. Acad. Sci. U. S. A., 66, 181 (1970).
12. Burgess, R. R., J. Biol. Chem., 244, 616 (1969).
13. Robertus, J. W., Nature, 224, 1168 (1969).
14. Travers, A. A., and Bautz, E. K. F., J. Mol. Biol., 205, 291 (1953).
15. Gupta, A., and Szymbalski, W., Virology, 34, 605 (1968).
16. Thomas, C. A., and Abelison, J., in G. L. Cantoni and D. R. Davie (Editors), Procedures in nucleic acid research, Harper and Row Publishers, Inc., New York, 1966, pp. 559-568.
17. Anderson, E. H., Proc. Nat. Acad. Sci. U. S. A., 32, 120 (1946).
18. Solymosy, F., Fedorcsak, I., Gulyas, A., Farkus, G. L., and Ehrenberg, L., Fur. J. Riechm., 5, 520 (1968).
19. Bautz, E. K. F., and Hall, B. D., Proc. Nat. Acad. Sci. U. S. A., 48, 400 (1962).
20. Benner, S., Proc. Nat. Acad. Sci. U. S. A., 41, 341 (1955).
21. Benzer, S., Proc. Nat. Acad. Sci. U. S. A., 47, 403 (1961).
22. Boedtker, H., J. Mol. Biol., 36, 61 (1968).
23. Studier, F. W., J. Mol. Biol., 11, 373 (1966).
24. Kennell, D., J. Virol., 6, 208 (1970).
25. Landy, A., and Spergelman, S., Biochemistry, 7, 1585 (1968).
26. Sippel, A., and Hartmann, G. Biochim. Biophys. Acta, 107, 218 (1966).
27. Tocchini-Valentini, G. P., Marino, P., and Colvill, A. J., Nature, 223, 276 (1968).
28. Werli, W., Knusel, F., and Stachel, M., Biochim. Biophys. Acta, 107, 215 (1968).
29. Hall, B. D., Nygaard, A. F., and Green, M. H., J. Mol. Biol., 9, 143 (1964).
30. Summers, W. C., and Szymbalski, W., J. Mol. Biol., 26, 107 (1967).
31. Stent, G. S., Molecular biology of bacterial viruses, W. H. Freeman, San Francisco, 1963.
32. Hayes, W., The genetics of bacteria and their viruses, John Wiley and Sons, Inc., New York, 1965.
33. Richardson, J. P., Progr. Nucl. Acid. Res. Mol. Biol., 9, 75 (1969).
34. Burgess, R. R., J. Biol. Chem., 244, 616 (1969).
35. Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. F., Nature, 221, 43 (1969).
36. Bautz, E. K. F., and Dunn, J. J., Biochim. Biophys. Res. Commun., 34, 240 (1969).
37. Gelbard, D. H., and Hayashi, M., Nature, 228, 1162 (1970).
38. Gesteland, R. F., Salser, W., and Bolle, A., Proc. Nat. Acad. Sci. U. S. A., 65, 2036 (1967).
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