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Revised Transcription Map of the Late Region of Bacteriophage T7 DNA

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The genes in the late region of the bacteriophage T7 genome are transcribed by a phage-specified RNA polymerase in two temporal classes (II and III). Through the use of an agarose-formaldehyde gel system that permits the resolution of high-molecular-weight RNAs, transcription of template DNA that had been cut with various restriction endonucleases, and transcription of isolated restriction fragments from the class II region, six previously unreported promoters utilized by the phage RNA polymerase in vitro have been mapped between 28 and 46% of the T7 genome. No evidence could be found for the existence of a promoter at 61% that had previously been reported to give rise to a diffuse band (species I RNA) in acrylamide-agarose gels.

During infection, the late genes of bacteriophage T7 are transcribed by a phage-specified RNA polymerase. Transcription of the late genes proceeds in two temporal classes: class II mRNA's are synthesized from 4 until 15 min after infection; class III mRNA's are synthesized from 6 to 8 min after infection until lysis (at 30 min) (10, 15). The locations of many of the class II promoters and all of the class III promoters have been reported previously (2, 3, 8, 11, 14, 17-19, 22, 24). In this report, we describe experiments to locate the remaining class II promoters, and we review previous results with the intention of presenting a more refined transcription map of T7 DNA. In addition, we have found that one class III transcription product (species I RNA), which had previously been observed as a diffuse band in low-percentage acrylamide-agarose composite gels (7), is an artifact resulting from the poor resolution of high-molecular-weight RNAs in this gel system.

(This work will be submitted as partial fulfillment of the requirements for the Ph.D. degree of A.D.C. at Rutgers University.)

MATERIALS AND METHODS

Phage and bacterial strains and preparation of DNA. All stocks of bacteriophage T7 were obtained from F. W. Studier and have been previously described (26). *Escherichia coli* B was from the laboratory of E. K. F. Bautz.

Unlabeled phage DNA and phage DNA uniformly labeled with ^{32}P were prepared as previously described (1, 13).

Cleavage of DNA and electrophoresis of fragments. Restriction endonucleases *Bgl*I, *Bgl*II, *Hpa*I, and *Eco*RI (23) were purchased from Bethesda Research Laboratories (Rockville, Md.). *Mbo*I (23) was purchased from New England Biolabs (Beverly, Mass.); *Mbo*I has the same specificity as *Dpn*II, previously used to map T7 DNA (16, 23). Conditions of cleavage were those recommended by the supplier. For double digestion, the first enzyme was inactivated by the addition of 10 mM EDTA followed by heating to 65°C for 5 min. The DNA was precipitated in the presence of 70% ethanol and 0.3 M sodium acetate, washed with 70% ethanol, dried in vacuo, and taken up in buffer suitable for cleavage by the second enzyme. DNA fragments were analyzed by electrophoresis through agarose gels (16).

Isolation of DNA from agarose gels. Individual restriction fragments were resolved by electrophoresis through 0.6% agarose gels and visualized by staining with ethidium bromide and long-wave UV irradiation. The desired bands were cut out, and fragments were eluted from the gel by electrophoresis (16) and further purified by DEAE-cellulose chromatography (29).

Complementary strands of DNA were separated by electrophoresis in agarose gels by the method of Hayward (9). The strands were visualized by ethidium bromide staining and UV irradiation, eluted from the gels by electrophoresis, and precipitated with ethanol.

Synthesis of RNA and analysis by gel electrophoresis. Unless otherwise indicated, all reactions contained, in a volume of 50 μl: 8 mM $MgCl_2$; 40 mM Tris-hydrochloride (pH 7.9); 5 mM dithiothreitol; 0.1 mM tetrasodium EDTA; 4 mM spermidine-hydrochloride; 5% (vol/vol) glycerol; 0.4 mM ATP, GTP, and CTP; 0.1 mM [α-^{32}P]UTP (specific activity, ca. 160 μCi/μmol); 2.5 μg of T7 DNA; and 10 U (5) of T7 RNA polymerase. After 10 min of synthesis, unlabeled UTP was added to a concentration of 1 mM, and incubation was continued for 3 min. Reactions were stopped by the addition of an equal volume of 2% (vol/vol) sodium dodecyl sulfate, 50 mM tetrasodium EDTA, and 100 μg of stripped yeast RNA per ml. RNA was precipitated overnight at −20°C by the addition of 0.3 M KCl and 2.5 volumes of ethanol, and the RNA pellets were dried in vacuo.

For gel analysis of high-molecular-weight RNAs (0.2×10^6 daltons and larger), RNA pellets were taken

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up in 20 μl of sample buffer (1.1 M formaldehyde, 0.1% sodium dodecyl sulfate, 2 mM tetrasodium EDTA, 0.1 M phosphate [pH 7.0]) and heated to 65°C for 5 min. After cooling, glycerol (5%) and bromophenol blue were added to the samples, which were then loaded into slots in a 0.8% agarose gel (3 mm thick) cast in the horizontal gel apparatus described by McDonnell et al. (16). Gel and electrophoresis buffer components were the same as in the sample buffer. Electrophoresis was carried out at 50 V for 6.5 h. Gels were dried in a Hoeffer gel dryer set at 55°C and exposed to X-ray film (Kodak SB-5).

For resolution of RNAs less than 0.2×10^6 daltons, dried RNA pellets were taken up in 25 μl of sample buffer (4 mM Tris-acetate [pH 7.5], 1% sodium dodecyl sulfate, 2 mM EDTA, 1% β-mercaptoethanol, 10% glycerol) and heated to 100°C for 2 min. After cooling, bromophenol blue was added, and samples were loaded onto a 4% polyacrylamide gel (27) containing: 40 mM Tris-acetate (pH 6.5), 0.1% sodium dodecyl sulfate, 2 mM EDTA, 0.05% *N,N,N',N'*-tetramethylethylenediamine, and 0.1% ammonium persulfate. Electrophoresis buffer was 40 mM Tris-acetate (pH 7.5), 0.1% sodium dodecyl sulfate, and 2 mM EDTA. Electrophoresis was carried out at 100 V for 2.5 h.

Transfer of RNAs to nitrocellulose filters and hybridization of ^{32}P-labeled DNA. Unlabeled RNAs transcribed from *Eco*RI-cleaved DNA were separated by electrophoresis in a 0.8% agarose gel in the presence of 1.1 M formaldehyde and transferred to nitrocellulose filters (Brian Seed and David Goldberg, personal communication; for details, see reference 1a). The filters were cut into strips and baked in vacuo at 80°C. Hybridization was carried out in a volume of 1 ml of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) plus 0.1% sodium dodecyl sulfate containing 5×10^3 cpm of ^{32}P-labeled, single-stranded DNA at 65°C for 16 to 20 h. The filters were extensively washed and exposed to X-ray film.

RESULTS

Previous investigators had reported that the class II region of T7 DNA is transcribed poorly by purified T7 RNA polymerase (7, 8, 14, 17). The inefficient transcription of the class II region in vitro, however, is due to the choice of reaction conditions; under appropriate conditions the class II region is transcribed as strongly as the class III region (13a, 15). Early attempts to identify and map the class II transcription products were only partially successful. By subjecting the in vitro products to electrophoresis in low-percentage acrylamide-agarose gels and then eluting bands from the gels for analysis by Southern blotting, it was found that the majority of RNAs that hybridize to DNA fragments from the class II region are very large ($>5 \times 10^6$ daltons) (14). These results indicate that transcripts which are initiated in the class II region are probably terminated at a common termination signal at 60.62 T7 units (see map, Fig. 6).

A number of class II promoters have subsequently been mapped either by transcription of isolated restriction fragments in vitro (11) or by analysis of recombinant plasmids (22; McAllister et al., manuscript in preparation). To analyze the high-molecular-weight transcription products that are expected to result from initiation at class II promoters, we have employed a gel system in which the RNAs are denatured by treatment with formaldehyde and run in low-percentage agarose gels (12). Under the conditions we have employed, the relationship of the square root of the molecular weight versus log distance migrated is linear over the range from 2×10^5 to 4.3×10^6 (data not shown). Despite the advantages of this gel system, most of the expected class II transcripts are still too large to be resolved (Fig. 1). The analysis of these high-molecular-weight RNAs is further complicated by the presence of "read-through" products which are a consequence of the failure of the T7 RNA polymerase to recognize the termination signal at 60.62 T7 units consistently (8, 14; see below).

To circumvent these problems, we have employed T7 DNA that was digested by a variety of restriction endonucleases as templates for RNA synthesis. Since all transcription of the T7 genome is from left to right relative to the map presented in Fig. 6 (28, 30; see below), the effect of cutting a template with a restriction enzyme will be to truncate RNA species that are initiated to the left of the cut site and that ordinarily span the restriction site. RNAs which arise from promoters that lie to the right of the restriction site are unaffected by cutting of the DNA template, as are those RNAs that are terminated before reaching the cut site.

The most useful enzyme for this work has been *Eco*RI. Although this enzyme does not cut wild-type T7 DNA, *Eco*RI does cut DNA from an amber mutant of T7 (T7 *am*28) at one position. This cleavage site was originally mapped by electron microscopic analysis at 46 T7 units (31). For the purpose of this investigation, it was desirable to map the *Eco*RI cleavage site more accurately. Double digestion of T7 *am*28 DNA with *Eco*RI and either *Hpa*I or *Mbo*I revealed that *Eco*RI cuts within *Hpa*I fragment O and *Mbo*I fragment A (Fig. 2). From the sizes of the resulting fragments, the cut site has been mapped at 45.78 T7 units.

Transcription of uncut wild-type and uncut *am*28 templates resulted in identical, complicated displays of transcripts (Fig. 1, lanes 3 and 4). When *Eco*RI-digested *am*28 DNA was used as template, the pattern was greatly simplified. The high-molecular-weight class II read-through products were eliminated, and class II transcripts normally terminating at 60.62 T7 units were prematurely terminated at the *Eco*RI

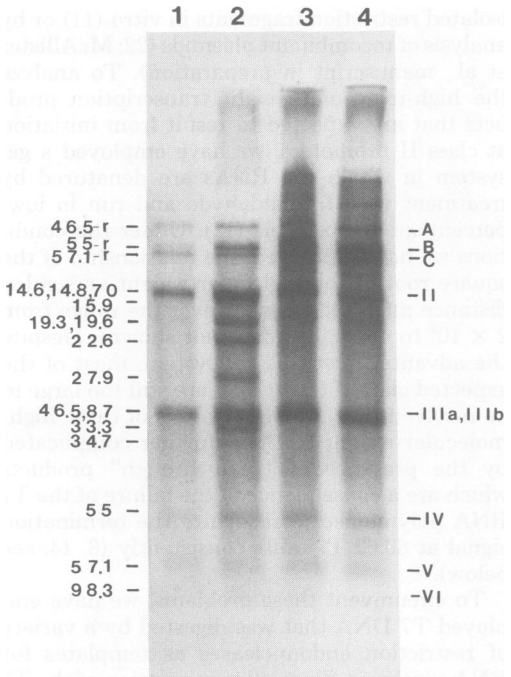

FIG. 1. *Resolution of class II RNAs in a 1.1 M formaldehyde–0.8% agarose gel. Uniformly* ^{32}*P-labeled RNAs were synthesized in vitro under standard conditions and analyzed by electrophoresis in a 0.8% agarose gel in the presence of 1.1 M formaldehyde. Template DNA was as follows: lanes 1 and 2, EcoRI-digested am28 DNA; lane 3, uncut am28 DNA; lane 4, uncut wild-type DNA. KCl (150 mM) was added to the reaction shown in lane 1 before the start of RNA synthesis. Due to the increased number of transcription products that may be identified by this analysis, it is no longer convenient to assign each RNA species a Roman numeral as had previously been done (7). Instead, we have identified each RNA species by its corresponding promoter location. Read-through products (resulting from failure to terminate at 60.62 units) are identified by the appropriate promoter location followed by "-r." The numbers on the left indicate the location of the promoter which gave rise to each RNA species; the letter and Roman numerals on the right identify the class III RNA species according to the previous nomenclature (7, 14).*

cleavage site, resulting in the appearance of several new, shortened RNA species (Fig. 1, lane 2). Since the leftmost class III promoter maps at 46.5 T7 units (24), class III RNA species are unaffected by *Eco*RI cleavage of the template. From the sizes of the shortened RNAs and the knowledge that they all terminate at 45.78 T7 units, we calculated that the promoters which give rise to these transcripts are located at 15.9, (19.3, 19.6), 22.6, 27.9, 33.3, and 34.7 T7 units (see map, Fig. 6). The promoters from 15.9 to 27.9 units correspond to locations of class II promoters that had been previously mapped (11, 22); the promoters at 33.3 and 34.7 are reported here for the first time. Two class II promoters at 14.6 and 14.8 units had also been mapped previously (18, 19, 22); however, the RNAs that arise from these promoters comigrate with the class III RNA species from the promoter at 70 units and were not resolved in this gel.

One of the features of T7 late transcriptional regulation in vitro is that transcription from class II promoters is uniformly more sensitive to inhibition by salt than transcription from class III promoters (13a). This feature is often useful in determining whether an RNA species arises from a class II or a class III promoter. When the KCl concentration in the reaction was increased from 0 to 150 mM, all of the class II RNA species disappeared from the pattern (Fig. 1, lane 1).

Transcription from isolated class II restriction fragments. In the experiments described above, both class II and class III RNAs were synthesized in the reactions and are represented in the autoradiograms. As a result, class

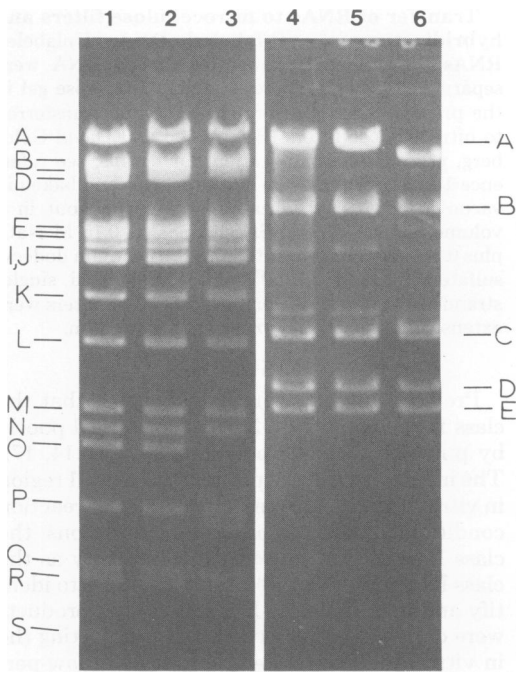

FIG. 2. *Mapping of the EcoRI cleavage site in T7 am28 DNA. The EcoRI cleavage site in am28 DNA was mapped by standard techniques of restriction analysis. Restriction fragments were separated by electrophoresis in 0.8% agarose gels and visualized by ethidium bromide staining and UV irradiation. Restriction digests were as follows: lane 1, HpaI digest of wild-type T7 DNA; lane 2, HpaI digest of am28 DNA; lane 3, HpaI-EcoRI double digest of am28 DNA; lane 4, MboI digest of wild-type T7 DNA; lane 5, MboI digest of am28 DNA; lane 6, MboI-EcoRI double digest of am28 DNA. The restriction fragments are identified by letter in the margin (16).*

II RNAs that arise from weak promoters or class II RNAs having sizes similar to those of the class III RNAs might go undetected. One way to avoid these problems is to analyze transcripts of isolated restriction fragments from the class II region of T7 DNA. When the isolated *BglII-EcoRI* fragment that includes the region from 28.76 to 45.78 T7 units (see Fig. 6) was transcribed in vitro, four additional transcripts were observed (Fig. 3, lanes 2 and 4). From their sizes, we calculate that the promoters which give rise to these RNA species are located at 32.3, 37.9, 40.8, and 44.4 T7 units. When the *BglI-EcoRI* fragment that extends from 33.8 to 45.78 units was transcribed, bands 32.3 and 33.3 were not observed (Fig. 3, lane 6). This verifies that the promoters for the latter transcription products lie to the left of 33.8.

Asymmetric transcription. Throughout the analysis above, we have made the assumptions that transcription of the T7 genome is asymmetric (i.e., only one strand of T7 DNA is transcribed), and that all transcription is from left to right (28, 30). To confirm this and to search for the possible existence of rightward-oriented promoters, unlabeled RNAs were resolved by gel electrophoresis, transferred to nitrocellulose filters, and hybridized to ^{32}P-labeled, separated strands of T7 DNA (Fig. 4). All transcripts hybridized to only one strand of T7 DNA, and this is the same strand of T7 DNA that is transcribed in vivo (9, 30).

Lack of species I RNA. In previous reports in which the T7 transcription products were analyzed by electrophoresis in polyacrylamide gels, a high-molecular-weight transcript designated as species I RNA was observed as a diffuse band near the origin of the gel. From its size and

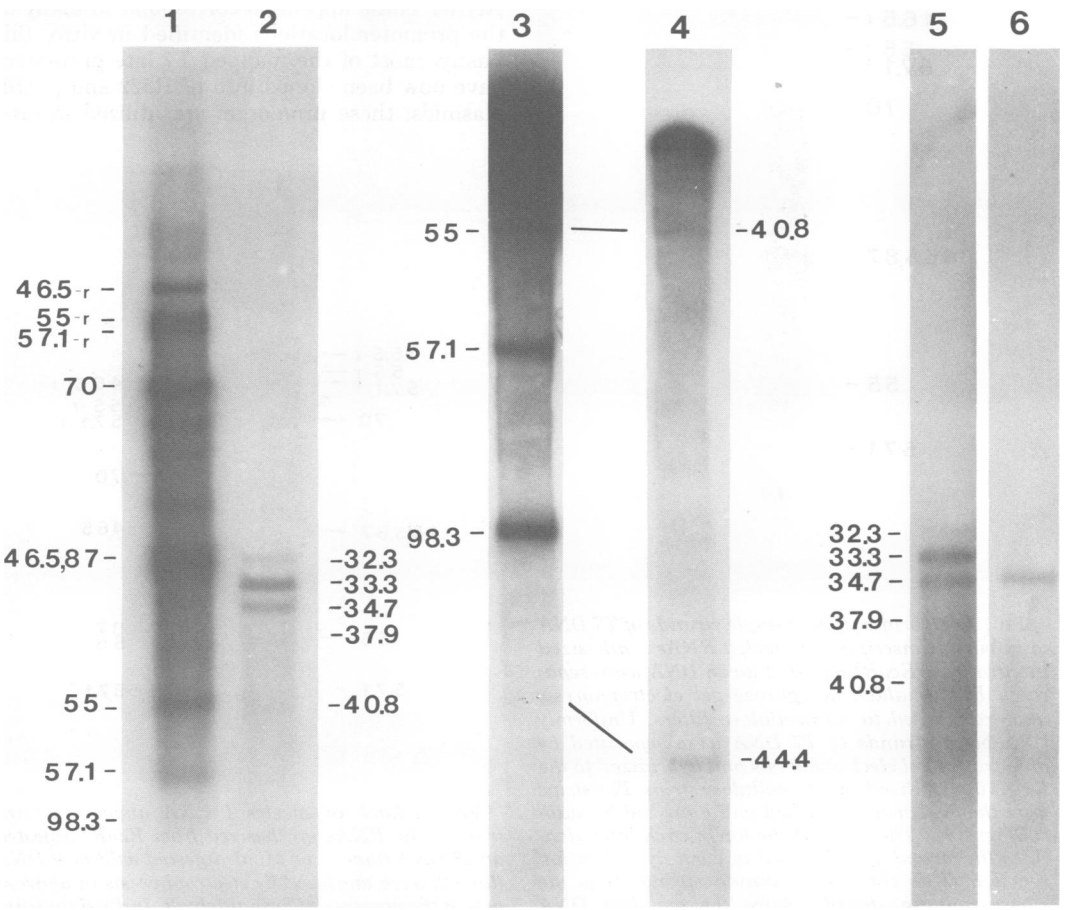

FIG. 3. *Transcription of isolated class II restriction fragments. ^{32}P-labeled RNAs transcribed from EcoRI-cut T7 am28 DNA (lanes 1 and 3), the isolated BglII-EcoRI fragment which spans the region from 28.76 to 45.78 units (lanes 2, 4 and 5), and the isolated BglI-EcoRI fragment (from 33.8 to 45.78 units) (lane 6) were analyzed by formaldehyde-agarose gel electrophoresis (lanes 1, 2, 5, and 6) or by 4% acrylamide gel electrophoresis (lanes 3 and 4). RNA species are identified by promoter position as in Fig. 1.*

apparent termination near the right end of the DNA molecule, it was concluded that this transcript was initiated at a major promoter near 61 units (8, 14). Transcription of T7 DNA that has been cut with either *Eco*RI or *Mbo*I should result in the synthesis of a well-resolved species I RNA. However, no band with the mobility expected of species I RNA was observed (Fig. 5). We conclude that the band previously designated as species I RNA is probably an artifact that results from the poor resolution of high-molecular-weight RNAs in polyacrylamide gels.

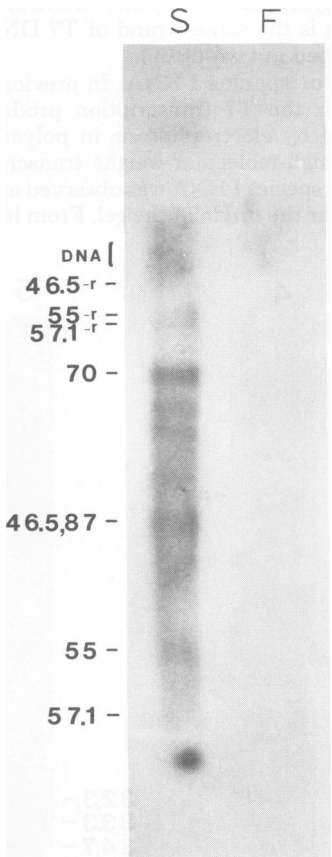

FIG. 4. *Hybridization of single strands of T7 DNA to in vitro transcripts. Unlabeled RNAs synthesized in vitro from EcoRI-digested am28 DNA were separated by formaldehyde-agarose gel electrophoresis and transferred to nitrocellulose filters. Uniformly ^{32}P-labeled strands of T7 DNA were separated by preparative gel electrophoresis and hybridized to the RNAs immobilized on nitrocellulose strips. The strips were then extensively washed and analyzed by autoradiography. The letter at the top of each lane identifies the strand of DNA used in each hybridization reaction (F is the faster-running strand; S is the slower-running strand). Since the template DNA present in the initial transcription reaction was not removed before transfer, each nitrocellulose strip contains unlabeled DNA (designated "DNA") which migrated in the region above the largest in vitro RNA.*

DISCUSSION

As a result of this work and that of previous investigations, a total of nineteen late promoters have now been mapped on the T7 genome (Fig. 6). A variety of experimental results suggest that these promoters are also utilized in vivo. (i) Because the primary transcripts are processed by RNase III in vivo, most of the large RNAs synthesized in vitro cannot be detected in infected cells containing RNase III; however, RNAs extracted from cells which do not contain RNase III appear to be equivalent to most of the class III in vitro transcripts (6). (ii) By translating RNAs from infected cells in a cell-free system, Pachl and Young (20) predicted the approximate promoter locations for several class II RNAs. These appear to correspond to many of the promoter locations identified in vitro. (iii) Lastly, most of the mapped T7 late promoters have now been cloned into pBR322 and pMB9 plasmids; these promoters are utilized in vivo

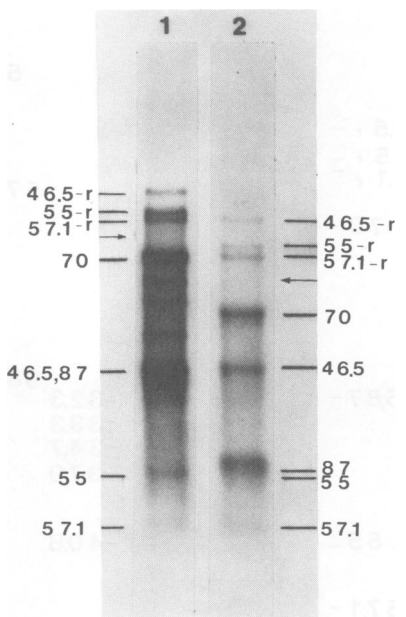

FIG. 5. *Lack of species I RNA among in vitro transcripts. RNAs synthesized from EcoRI-digested am28 DNA (lane 1) or MboI-digested wild-type DNA (lane 2) were analyzed by electrophoresis in agarose gels in the presence of formaldehyde. Individual transcripts are identified by promoter position. The expected locations of RNA bands originating from the putative species I promoter (at 61.4 T7 units) are marked by arrows.*

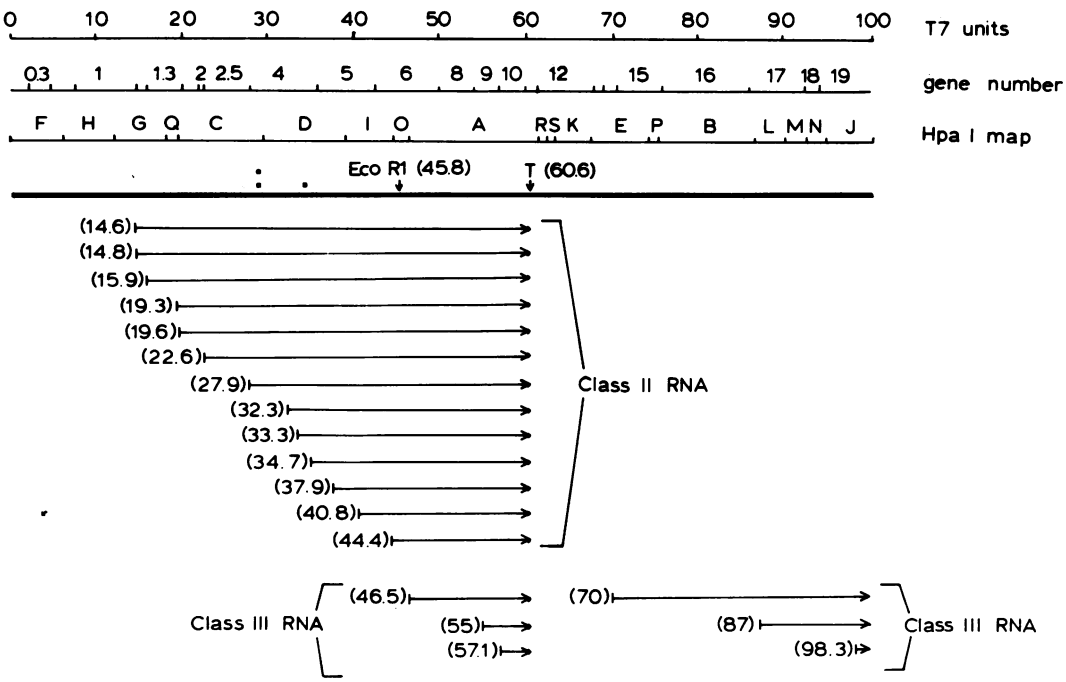

FIG. 6. *Revised transcription map of the late region of bacteriophage T7 DNA. A transcription map of the late region of T7 is presented. A partial genetic map (25) and HpaI restriction map of T7 (16) are included. The locations of the BglII site (:) (25), the BglI site (·) (25), the EcoRI site, and the internal termination site (T; mapped at 60.6 units by M. Rosa, personal communication) are indicated in the fourth line. The RNA products synthesized by the T7 RNA polymerase in vitro are represented by arrows indicating their origin and direction of transcription. Promoter locations, expressed as distances in T7 units from the left end of the genome, are given in parentheses before each arrow. For clarity, read-through products resulting from failure to terminate at 60.6 units are not presented. The locations of class II promoters to the left of 28 T7 units and the locations of the class III promoters have been previously reported (2, 3, 8, 11, 14, 17–19, 22, 24).*

even though they are located in plasmid DNA (McAllister et al., manuscript in preparation).

The accuracy of locations assigned to the promoters that we have mapped depends upon the reliability of the sizes assigned to the transcripts observed from each promoter. We estimate that the reliability is between 0.3 and 0.5 T7 units. The locations of the promoters at 33.3 and 34.7 units have subsequently been more precisely determined by cloning these promoters into the plasmid pBR322 and sequencing the cloned DNA (Carter and McAllister, manuscript in preparation). Future work will no doubt refine the locations assigned to the other promoters that we have mapped and may uncover additional promoters that have gone undetected in this analysis.

Some of the class II promoters that we have mapped are weak (those at 32.3, 37.9, 40.8, and 44.4 T7 units). Panayotatos and Wells (22) have also mapped a weak promoter at 15.9 units. The sequence of the latter promoter differs from other late promoters by changes at positions +4 and −5, and the authors have proposed that these nucleotides are crucial for promoter-polymerase contact (21, 22). Sequencing of the weak promoters that we have found may identify other important nucleotide positions.

Species I RNA, which had previously been identified as a diffuse band in acrylamide gels (8, 14), is apparently an artifact resulting from poor resolution of high-molecular-weight RNA in this gel system. Accordingly, we have eliminated this RNA from the transcription map presented in Fig. 6. In support of this, we note that Rosa (24) was unable to identify a promoter for species I RNA by hybridization of 5′-labeled in vitro transcripts to restriction fragments of T7 DNA. Furthermore, cloned DNA fragments of the region presumed to contain the species I promoter do not complement T7 mutants in genes 10 and 11 (4), nor do the recombinant plasmids exhibit T7 late promoter activity in vivo after T7 infection (McAllister et al., manuscript in preparation).

Read-through products which result from failure of the T7 polymerase to recognize the termination signal at 60.62 units are apparent in

many of the autoradiograms in this report. By densitometric analysis of the autoradiograms, we estimate that on a molar basis species 46.5-r, 55-r, and 57.1-r are about 6 to 10% as abundant as species 46.5, 55, and 57.1. Thus, termination failure must occur about 6 to 10% of the time. This frequency of read-through appears to be relatively independent of ionic strength (data not shown).

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ADDENDUM IN PROOF

Bands 37.9, 40.8, and 44.4 in Fig. 3 are very weak. In the absence of evidence to the contrary, we tentatively concluded that these bands arise from weak promoters at 37.9, 40.8, and 44.4 T7 units (Fig. 6). Fragments of T7 DNA that contain these putative promoters have now been cloned into pBR322. Screening of these plasmids for late T7 promoter activity does not provide support for the existence of these promoters. These bands may therefore be artifactual.

LITERATURE CITED

1. **Bailey, J. N., D. R. Dembinski, and W. T. McAllister.** 1980. Derivation of a restriction map of bacteiophage T3 DNA and comparison with the map of bacteriophage T7 DNA. J. Virol. **35**:176–183.

1a. **Bailey, J. N., and W. T. McAllister.** 1980. Mapping of promoter sites utilized by T3 RNA polymerase on T3 DNA. Nucleic Acids Res. **8**:5071–5088.

2. **Beier, H., M. Golomb, and M. Chamberlin.** 1977. Isolation of recombinants between T7 and T3 bacteriophage and their use in in vitro transcriptional mapping. J. Virol. **21**:753–765.

3. **Boothroyd, J. C., and R. S. Hayward.** 1980. New genes and promoters suggested by the DNA sequence near the end of the coliphage T7 early operon. Nucleic Acids Res. **7**:1931–1944.

4. **Campbell, J. L., C. C. Richardson, and F. W. Studier.** 1978. Genetic recombination and complementation between bacteriophage T7 and cloned fragments of T7 DNA. Proc. Natl. Acad. Sci. U.S.A. **75**:2276–2280.

5. **Chamberlin, M., J. McGrath, and L. Waskell.** 1970. New RNA polymerase from *Escherichia coli* infected with bacteriophage T7. Nature (London) **228**:227–331.

6. **Dunn, J. J., and F. W. Studier.** 1974. Processing, transcription, and translation of bacteriophage messenger RNAs. Brookhaven Symp. Biol. **26**:267–276.

7. **Golomb, M., and M. Chamberlin.** 1974. Characterization of T7-specific ribonucleic acid polymerase IV. Resolution of the major in vitro transcripts by gel electrophoresis. J. Biol. Chem. **249**:2858–2863.

8. **Golomb, M., and M. Chamberlin.** 1974. A preliminary map of the major transcription units read by T7 RNA polymerase on the T7 and T3 bacteriophage chromosomes. Proc. Natl. Acad. Sci. U.S.A. **71**:760–764.

9. **Hayward, G. S.** 1972. Gel electrophoretic separation of the complementary strands of bacteriophage DNA. Virology **49**:342–344.

10. **Hopper, J. E., G. Ko, and E. T. Young.** 1975. Comparative analysis of the in vivo and in vitro expression of bacteriophage T7 messenger RNAs during infection of *Escherichia coli*. J. Mol. Biol. **94**:539–554.

11. **Kassavetis, G. A., and M. J. Chamberlin.** 1979. Mapping of class II promoter sites utilized in vitro by T7-specific RNA polymerase on bacteriophage T7 DNA. J. Virol. **29**:196–208.

12. **Lehrach, H., D. Diamond, J. M. Wozney, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry **16**:4743–4751.

13. **McAllister, W. T., and C. L. Barrett.** 1977. Hybridization mapping of restriction fragments from the early region of bacteriophage T7 DNA. Virology **82**:275–287.

13a. **McAllister, W. T., and A. D. Carter.** 1980. Regulation of promoter selection by the bacteriophage T7 RNA polymerase in vitro. Nucleic Acids Res. **8**:4821–4837.

14. **McAllister, W. T., and R. J. McCarron.** 1977. Hybridization of the in vitro products of bacteriophage T7 RNA polymerase to restriction fragments of T7 DNA. Virology **82**:288–298.

15. **McAllister, W. T., and H.-L. Wu.** 1978. Regulation of transcription of the late genes of bacteriophage T7. Proc. Natl. Acad. Sci. U.S.A. **75**:804–808.

16. **McDonnell, M., M. N. Simon, and F. W. Studier.** 1977. Analysis of restriction fragments of T7 DNA and determination of molecular weights by electrophoresis in neutral and alkaline gels. J. Mol. Biol. **110**:119–146.

17. **Niles, E. G., and R. C. Condit.** 1975. Translational mapping of bacteriophage T7 RNAs synthesized in vitro by purified T7 RNA polymerase. J. Mol. Biol. **98**:57–67.

18. **Oakley, J. L., and J. E. Coleman.** 1977. Structure of promoter for T7 RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. **74**:4266–4270.

19. **Oakley, J. L., R. E. Strothkamp, A. H. Sarris, and J. E. Coleman.** 1979. T7 RNA polymerase: promoter structure and polymerase binding. Biochemistry **18**: 528–537.

20. **Pachl, C. A., and E. T. Young.** 1978. The size and messenger RNA activity of bacteriophage T7 late transcripts synthesized in vivo. J. Mol. Biol. **122**:69–101.

21. **Panayotatos, N., and R. D. Wells.** 1979. Recognition and initiation site for four late promoters of phage T7 is a 22 base pair DNA sequence. Nature (London) **280**: 35–39.

22. **Panayotatos, N., and R. D. Wells.** 1979. Identification, cloning and characterization of three late promoters at 14.6, 14.8 and 15.9% of T7 DNA. J. Mol. Biol. **135**:91–109.

23. **Roberts, R. J.** 1980. Restriction and modification enzymes and their recognition sequences. Nucleic Acids Res. **8**:63–80.

24. **Rosa, M.** 1979. Four T7 RNA polymerase promoters contain an identical 23 bp sequence. Cell **16**:815–825.

25. **Rosenberg, A. H., M. N. Simon, F. W. Studier, and R. J. Roberts.** 1980. Survey and mapping of restriction endonuclease cleavage sites in bacteriophage T7 DNA. J. Mol. Biol. **135**:907–915.

26. **Studier, F. W.** 1969. The genetics and physiology of bacteriophage T7. Virology **39**:562–574.

27. **Studier, F. W.** 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. **79**:237–248.

28. **Studier, F. W., and J. V. Maizel, Jr.** 1969. T7-directed protein synthesis. Virology **39**:575–586.

29. **Studier, F. W., A. H. Rosenberg, M. N. Simon, and J. J. Dunn.** 1980. Genetic and physical mapping in the early region of bacteriophage T7 DNA. J. Mol. Biol. **135**:917–937.

30. **Summers, W. C., and W. Szybalski.** 1968. Totally asymmetric transcription of coliphage T7 in vivo: correlation with poly G binding sites. Virology **34**:9–16.

31. **Tsujimoto, Y., and H. Ogawa.** 1977. *Eco*RI-sensitive mutation of T7 phage. Mol. Gen. Genet. **150**:221–223.