Proc. Nat. Acad. Sci. USA
Vol. 71, No. 3, pp. 760–764, March 1974

A Preliminary Map of the Major Transcription Units Read by T7 RNA Polymerase on the T7 and T3 Bacteriophage Chromosomes*†

(*in vitro* T7 RNAs/gel electrophoresis/exonuclease cleavage/T3–T7 hybrid phages)

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Communicated by Paul Berg, October 19, 1973

ABSTRACT **Transcription of T7 DNA by T7 RNA polymerase *in vitro* gives rise to six major size classes of RNAs comprising seven major T7 RNA species. These RNAs are all read from the r-strand of T7 DNA and are not derived from the early (leftmost on the conventional genetic map) region of the molecule. When artificially shortened T7 DNA templates are transcribed, four (I, II, IIIb, and VI) of the seven species are found to be truncated or deleted. This indicates that all are terminated near the right end of the T7 DNA molecule, probably at a common termination site near 98.5%. (Map positions are all given in terms of percentage of total length measured from the left end of the molecule.) Since the approximate lengths of the transcripts are known, the promotor sites for T7 RNA species I, II, IIIb, and VI are tentatively mapped at 56, 64, 83, and 97% on the T7 chromosome. Only a single major T3 RNA is transcribed by T7 RNA polymerase; analysis of transcripts directed by shortened T3 DNA templates indicates it is analogous to T7 RNA species IIIb. Hence the promotor and terminator sites for T3 species IIIb are tentatively mapped at 83 and 98.5%, respectively, on the T3 chromosome. The major transcripts read by T7 RNA polymerase from T3–T7 hybrid phage DNAs vary, depending on which regions of the T7 chromosome are present. This provides an alternative method of mapping the strong T7 promotor sites on the T7 chromosome.**

Transcription of the T7 phage chromosome in the infected cell is mediated by two different polymerases (RNA nucleotidyltransferases, EC 2.7.7.6). The early region of the phage chromosome (leftmost on the conventional genetic map), is transcribed by the *Escherichia coli* RNA polymerase (1, 2) while the remainder of the molecule is transcribed by a T7-specific RNA polymerase (3, 4). The transcriptional specificity of both enzymes is preserved *in vitro* where transcription of isolated T7 DNA by *E. coli* RNA polymerase or T7 RNA polymerase gives rise to early or late mRNA species, respectively (3, 4). Thus both enzymes recognize specific signals on T7 DNA which govern RNA chain initiation (promoters) and RNA chain termination (terminators).

To locate the position on the T7 chromosome of the promoter and terminator sites read by T7 RNA polymerase we have characterized the RNA transcripts formed from T7 and T3 DNA *in vitro* (5). With T7 DNA as template, six bands are obtained by electrophoretic analysis on polyacrylamide gels (T7 bands I through VI), corresponding to six size classes of transcripts (molecular weights 5.5, 4.5, 2.0, 0.8, 0.4, and 0.2 × 10^6, respectively). The RNA species are formed in equimolar amounts except for species III (2 × 10^6), which is two times more abundant. Each of the T7 RNA species is initiated independently and contains GTP at the 5′ terminus. As a working model we postulated (5) that these RNAs are read from seven independent transcription units on the T7 chromosome, band III representing a mixture of two RNA species from separate units, IIIa and IIIb. With T3 DNA as template for T7 RNA polymerase only a single major transcript is formed corresponding in size to T7 species III.

To locate the sites for the initiation and termination of these transcription units on the T7 chromosome we have used two approaches: (*1*) transcription of DNA templates shortened at both ends by exonucleolytic cleavage and (*2*) transcription of DNAs obtained from genetically altered phages including hybrid phages between T3 and T7 and T7 deletion mutants.

MATERIALS AND METHODS

Procedures for the preparation and assay of T7 RNA polymerase (3, 6) and for the *in vitro* synthesis of RNAs (5) have been described elsewhere. Analysis of T7 RNAs by acrylamide–agarose gel electrophoresis (7) empolyed a modification (5) of the procedures of De Wachter and Fiers (8).

Recombinant (hybrid) phages containing regions of both T3 and T7 phages were provided by Drs. H. Beier and R. Hausmann (University of Freiburg, Germany). *E. coli* exonuclease III was the gift of Dr. I. R. Lehman (Stanford University) and *Aspergillus oryzae* nuclease S_1 was the gift of Dr. P. Berg (Stanford University). All DNA concentrations are given in terms of concentrations of nucleotide. Intact linear DNA from T7 or T3 phages (9) was shortened by exonuclease III digestion at 37° (10). Reaction mixtures (2 ml) contained 25 mM Tris·HCl buffer (pH 8.0), 0.25 mM of $MgCl_2$, 5 mM 2-mercaptoethanol, 0.45–0.6 mM T7 or T3 DNA, and 5.5 μg/ml of exonuclease III. After exonuclease III treatment, 0.9-ml aliquots of the reaction mixture were combined with 0.1 ml of concentrated S_1 buffer [300 mM sodium acetate buffer (pH 4.6), 5 mM $ZnCl_2$, 2 mM NaCl (11)] and 1.6 μg of S_1 enzyme was added. The mixture was incubated for 10 min at 50° and release of acid-soluble nucleotides from T7 [^{3}H]DNA was measured (12). Release of acid soluble nucleotides from unlabeled DNAs was measured as follows: 0.1-ml samples of the exonuclease III or S_1 digestion mixtures were added to 2 ml of ice cold 3.5% perchloric acid. After 10 min at 0°, the mixtures were centrifuged for 30 min at 10,000 × *g*. Absorbances of the supernatant were read at 260 nm in a Zeiss spectrophotometer. After exonuclease treat-

* This is paper V of a series. The preceding paper is "Characterization of T7 specific ribonucleic acid polymerase. IV. Resolution of the major *in vitro* transcripts by gel electrophoresis." (*J. Biol. Chem.*, in press).

† Reprints of this paper will not be available for distribution.

ment the resulting DNA samples were extracted twice with phenol and were dialyzed against a solution containing 0.05 M NaCl, 0.01 M Tris and 10^{-4} M EDTA.

RESULTS

1. Major RNA species transcribed by T7 RNA polymerase from shortened templates

Since T7 DNA is a linear molecule having a unique, nonpermuted nucleotide sequence (10) it is possible to delete a unique set of genetic sequences at the ends of the molecule by exonucleolytic cleavage. Transcription of such a shortened template should in principle lead to RNA products in which any RNA which terminates or is initiated in the deleted region is either shortened or completely deleted. We chose to shorten T7 DNA by exonucleolytic cleavage of the 3'-OH termini with *E. coli* exonuclease III (12, 13) followed by treatment with *A. oryzae* S_1 nuclease (11) to remove the 5'-*P*-terminated single-stranded regions. Preparations of both enzymes are available which have little or no endonuclease activity [although the specificity of T7 RNA polymerase is not highly sensitive to the presence of single-stranded interruptions in the template (6)]. The conditions used for exonucleolytic cleavage were chosen to favor a synchronous attack which would lead to a population of shortened DNA molecules of reasonably uniform length (10)‡.

Analysis of the RNAs transcribed by T7 RNA polymerase from shortened T7 DNA templates shows that four of the six major RNA bands are altered (Fig. 1). RNA species VI is no longer formed from either of the shortened T7 DNA preparations. RNA species I and II are shortened and approximately half of the RNA from band III is lost and appears as a shortened species (IIIb). Two very large minor T7 RNA species are reproducibly seen on these gels (apparent molecular weights 8×10^6 and 6.5×10^6). These also appear to be shortened when transcribed from shortened T7 DNA (Fig. 1). The extent to which species I, II, and IIIb are shortened is proportional to the extent of shortening of the DNA template (Table 1*A*). We have also analyzed the transcripts from more extensively shortened T7 DNA templates in which a total of 30% of the molecule has been excised from each end; these DNAs serve as effective templates only for synthesis of T7 RNA species IIIA, IV, and V.

When shortened T3 DNA is used as template for T7 RNA polymerase (Fig. 2) the single major RNA species formed is shortened, and again the extent of shortening depends on the extent of cleavage of the DNA template (Table 1*B*).

These data allow one to position the transcription units for species I, II, and IIIb on the T7 chromosome. Since deletions in the early region of the DNA molecule do not affect species I, II, or IIIb (see below) these species cannot originate from the left end of the DNA molecule. Moreover, all of the RNA

‡ A comparison of the widths of the original RNA bands with those from shortened DNA templates gives a rough measurement of the effective synchrony of exonuclease attack. Bands for the shortened transcripts I, II, and IIIb are increased in width by less than 30% for the 15-min-shortened DNA; for the 30-min-shortened DNA there is little further spreading of bands for species I and II while the width of species IIIb has increased by 70%. Even if all of the increase in band width in the latter case is due to heterogeneity, the actual variation in the total lengths of the DNA templates would be less than 2%. Hence, the relative sharpness of the RNA bands confirms our hope that the exonuclease attack is relatively synchronous.

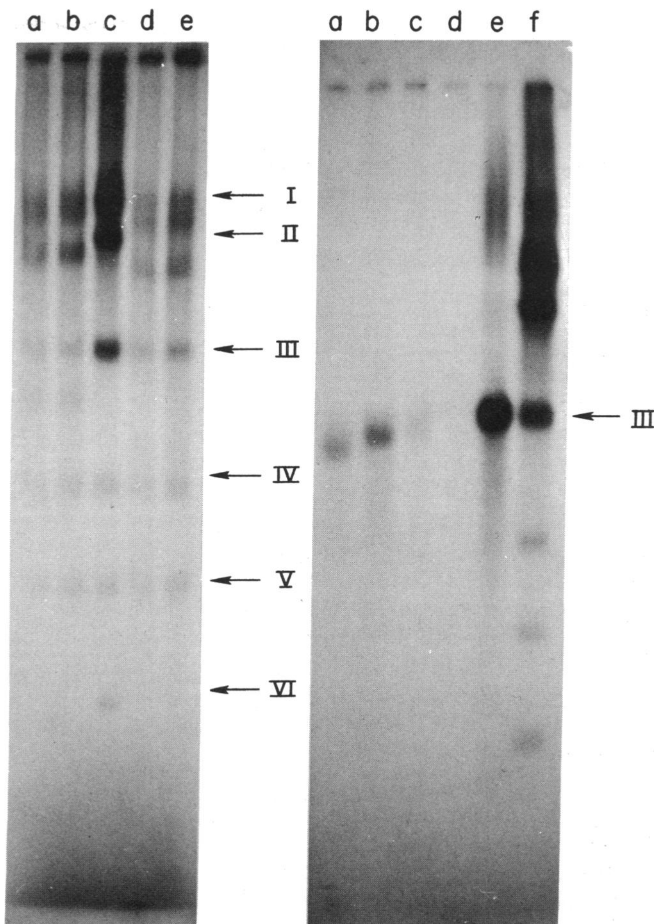

FIG. 1. (*left*) Electrophoretic analysis of T7 RNAs transcribed by T7 RNA polymerase from exonucleolytically shortened DNA. T7 RNAs were synthesized *in vitro* with T7 RNA polymerase (5) in a 10-min synthetic reaction at 37° with [^{32}P]ATP as labeled substrate; aliquots of the reactions were mixed with 0.25% sodium dodecyl sulfate and were placed directly on a 1.75% acrylamide–0.5% agarose gel for analysis. After electrophoresis gels were analyzed by autoradiography. *Tracks a, b:* T7 RNA transcripts from T7 DNA templates treated for 15 min with exonuclease III followed by S_1 nuclease. Transcription was carried out at 0.066 mM (*a*) or 0.13 mM (*b*) DNA nucleotide. *Tracks d, e:* T7 RNA transcripts from T7 DNA templates treated for 30 min with exonuclease III followed by S_1 nuclease. Transcription carried out at 0.055 mM (*d*) or 0.11 mM (*e*) DNA nucleotide. *Track c:* T7 RNA transcripts from intact T7 DNA template; transcription carried out at 0.29 mM DNA nucleotide.

FIG. 2. (*right*) Electrophoretic analysis of T3 RNAs transcribed by T7 RNA polymerase from exonucleolytically shortened DNA. T3 RNAs were synthesized and analyzed as described in legend to Fig. 1. *Tracks a–c:* T3 RNA transcripts from T3 DNAs treated for 60, 30, or 20 min, respectively, with exonuclease III followed by S_1 nuclease. DNA template concentrations during RNA synthesis were 0.6 mM each. *Tracks e and f:* RNA transcripts from intact T3 and T7 DNA templates, respectively. DNA template concentrations were 0.5 mM and 0.29 mM, respectively, during RNA synthesis.

synthesized by T7 RNA polymerase is complementary to the r-strand (3) and hence must be read from left to right on the conventional genetic map (Fig. 3; ref. 14). We conclude that species I, II, and IIIb must originate from overlapping transcription units at the right end of T7 DNA which are defined by promoter sites at about 56, 64, and 83%, respectively, on

the T7 r-strand and a common terminator site near the right end of the molecule (map positions on the T7 chromosome are all given in terms of percentage of the total length measured from the left end of the molecule). The major T3 transcript formed by T7 RNA polymerase, designated T3 species IIIb, also appears to be read from a transcription unit with a terminator at the right end of the DNA molecule and is thus analogous to T7 species IIIb. This region of the T3 DNA molecule is homologous in DNA nucleotide sequence to the corresponding region of the T7 DNA molecule (17).

A comparison of the amount of DNA removed from the templates in several experiments with the actual decrease in size of the RNA species (Table 1) suggests that a small and approximately constant amount of DNA (0.2×10^6) is removed in excess of the shortening of the RNA species. A simple explanation for this would be that the terminator for transcription is located inside the 5′ terminus of the r-strand (2). The present data locate this terminator at about 0.2×10^6 from the 5′ terminus of the r-strand (98.5%); however, there is a considerable uncertainty in this result.

The transcription unit for species VI cannot yet be positioned unambiguously. It is most likely that the promoter for species VI is located at about 96.7% and that this transcription unit shares a common terminator with species I, II, and IIIb at 98.5%. However, we cannot completely exclude the possibility that species VI is read from a separate unit located to the right of the terminator for I, II, and IIIb, which overlaps the terminal redundancy region [99.3–100% (10)]. The accurate positioning of the terminator for species I, II, and IIIb and of the species VI promoter should be possible with less extensively shortened T7 DNA templates.

2. Major RNA species transcribed from genetically modified templates

We have analyzed the T7 RNAs transcribed by T7 RNA polymerase with DNAs from several deletion mutants of T7. Deletions ΔH1, ΔH3, and LG 26 were used; these DNAs contain deletions extending from 2.9 to 7.0%, 3.9 to 7.6%, and 19.1 to 23.5%, respectively (15). This corresponds to a loss of about 0.5×10^6 daltons of the r-strand of T7 DNA for each deletion. Although these DNAs all gave rise to a shortened early RNA (18) when transcribed in control experiments with *E. coli* RNA polymerase, (with the exception of LG 26, in which the terminator for *E. coli* RNA polymerase is deleted) none of the major species transcribed by T7 RNA polymerase were detectibly altered as measured by acrylamide gel electrophoretic analysis. Thus T7 species IIIa, IV, and V are probably initiated at a site located to the right of 23.5%.

The finding that T7 RNA polymerase reads only a single major T3 transcription unit suggested that the transcription of recombinant (hybrid) phages containing both T3 and T7 DNA sequences might be useful in locating the T7 transcription units. Such recombinant phages have been isolated by Beier and Hausmann (Table 2). Ideally the presence or absence of the seven major T7 RNA species in the *in vivo* transcription pattern of a hybrid phage DNA by T7 RNA polymerase would indicate directly which of the seven major T7 promoter sites were contained on the hybrid chromosome. Transcription mapping of a collection of hybrid phages, taken together with DNA mapping information, should provide a valuable alternative approach to the position of the remaining major T7 transcription units on the T7 chromosome.

DNA preparations from three hybrid phages have been tested; the origin and possible physical structures of these DNAs are summarized in Table 2. While the genetic selection assures that the selected wild-type markers and corresponding genetic regions are from the appropriate parental phage, the exact position of the crossover events between the selected markers cannot be specified. Thus the positions of the T3–T7 crossover points shown in Table 2 are arbitrary. The leftmost region of each hybrid DNA molecule has been selected from the T3 parent, the rightmost from the T7 parent. In addition, since the terminal redundancy regions of T7 and T3 are different and must be homologous on any single molecule (17), a second recombinatory event has presumably occurred in each case distal to the outermost selected markers on one end to ensure that both ends are the same.

Transcription of *hybrid 12* shows all of the major T7 RNA species (Fig. 4); it must contain T3 sequences at the left end up to at least 17%. Since the T7 species VI promoter is located

TABLE 1. *Relationship between amount of DNA removed from ends of T7 or T3 DNA template and reduction in size of RNAs transcribed from the shortened template*

Duration of exonuclease treatment	ΔDNA $\times 10^{-6}$	ΔRNA $\times 10^{-6}$ Species I MW = 5.5×10^6	Species II MW = 4.5×10^6	Species IIIb MW = 2.0×10^6	ΔDNA − ΔRNA $\times 10^{-6}$
A. T7 DNA template					
15 min	0.79 ± 0.08	0.50 ± 0.30	0.60 ± 0.25	0.55 ± 0.10	0.24 ± 0.13
30 min	1.03 ± 0.10	0.80 ± 0.29	1.0 ± 0.23	0.95 ± 0.09	0.13 ± 0.13
B. T3 DNA template					
20 min	0.28 ± 0.03			0.13 ± 0.11	0.15 ± 0.11
30 min	0.44 ± 0.04			0.26 ± 0.11	0.18 ± 0.12
60 min	0.74 ± 0.07			0.49 ± 0.10	0.25 ± 0.12

ΔDNA is one-fourth of the total amount of DNA rendered acid-soluble during treatment by exonuclease III and S_1 nuclease and is expressed in molecular weight values. Error in ΔDNA was estimated from precision of the assay procedures. ΔRNA represents the apparent decrease in molecular weight of a given RNA species as determined by the increase in electrophoretic mobility observed upon acrylamide-agarose gel analysis. Error in ΔRNA was calculated from the precision with which T7 RNA bands can be located on gels, determined from repeated measurements (±4%). ΔDNA − ΔRNA represents the amount of DNA cleaved from each strand in excess of the amount to which the average corresponding RNA transcript is cleaved. MW is molecular weight.

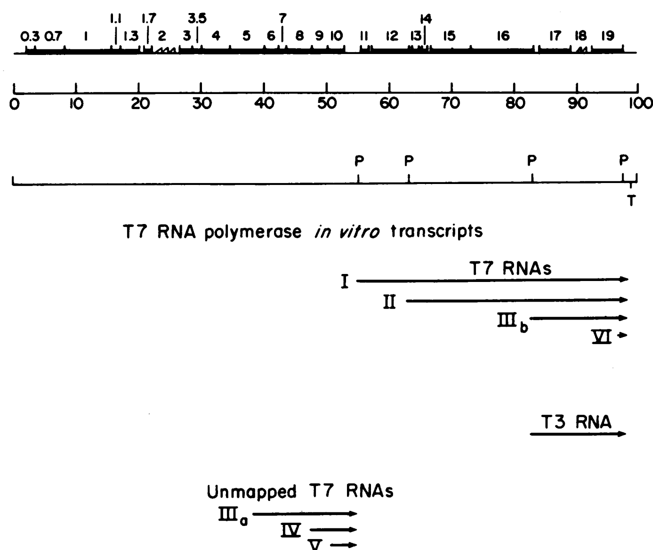

FIG. 3. Partial map of the major *in vitro* transcripts formed by T7 RNA polymerase on T7 and T3 DNAs. Distances indicated by heavy lines on the genetic map are the sizes of the designated T7 genes as calculated from the sizes of their known protein products (14). Jagged lines indicate that the size of the gene product is unknown. Positions of the T7 genes on the chromosome are assigned on the basis of data presented by Simon and Studier (15) and Hyman *et al.* (16). The symbol *P* represents a promoter (RNA chain initiation) site for T7 RNA polymerase, *T* represents a site at which RNA chain termination occurs. The scale is calibrated in % of total genome length. The numbers above the map represent the known phage genes (14).

very near to or even within the terminal redundancy region, the presence of species VI in the transcription product makes it unlikely that recombination has occurred to the right of that site to give a T3 terminal redundancy. Instead replacement of the left end of the molecule by a T7 terminus is most probable. DNA from *hybrid 6* contains T3 sequences at the left end possibly as far as gene 6 (40%). Transcription of *hybrid 6* DNA shows that it yields all of the major T7 species I–V but lacks species VI. The absence of the T7 species VI promoter on *hybrid 6* DNA probably reflects the presence of T3 terminal redundancy regions. The data for *hybrids 12* and *6* support our conclusion that promoters for species IIIA, IV, and V lie to the right of the early and leftmost late regions. Transcription of *hybrid 8* yields only T7 species IIIb and VI; this hybrid DNA contains T3 sequences possibly as far as 70%; this is well to the right of our location of the promoter for species II. Thus the replacement of a strong T7 promoter read by T7 RNA polymerase by the corresponding T3 promoter in a hybrid phage leads to the disappearance of the corresponding major T7 transcript.

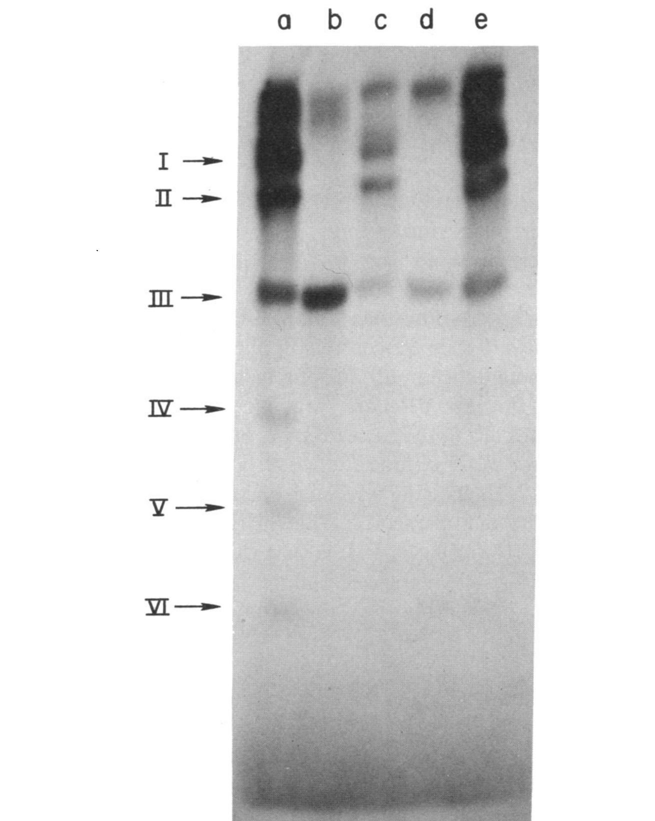

FIG. 4. Electrophoretic analysis of RNAs transcribed from T3–T7 hybrid phages by T7 RNA polymerase. RNAs were synthesized and analyzed as described in legend to Fig. 1. *Tracks a, b:* RNA transcripts from T7 and T3 DNA templates, respectively. *Tracks c, d, and e:* RNA transcripts from hybrid phages *6*, *8*, and *12*, respectively. Transcription carried out in all cases at a DNA template concentration of 0.3 mM.

DISCUSSION

Four of the seven major transcripts of T7 DNA synthesized by T7 RNA polymerase are altered when exonucleolytically shortened DNA templates are used. The transcription units which govern the synthesis of these species must be located at the right end of the T7 DNA molecule and share a common terminator which is probably located just inside the right end of the molecule. The positions of the promoter sites for these transcripts can be estimated from their molecular weights (5)

TABLE 2. *Genetic structure of T3–T7 hybrid phages*

Hybrid	Genetic selection			Probable structure
6	T3 am H47-am A1 (gene 6⁻, gene 15⁻)	×	T7 am H280-H13 (double gene 1⁻)	1 3 6 15 19
8	T3 am A1-am H1 (gene 15⁻, gene 19⁻)	×	T7 am H280-H13 (double gene 1⁻)	1 3 6 15 19
12	T3 am H283-am H1 (gene 3⁻, gene 19⁻)	×	T7 am H280-H13 (double gene 1⁻)	1 3 6 15 19

T7-T3 hybrids were selected as wild-type recombinants from crosses shown (unpublished experiments of H. Beier and R. Hausmann). Their probable structures were inferred from the genotypes of the parental strains and from the transcription data discussed in the text. . . . . .T3 DNA regions ____ T7 DNA regions.

and from the physical and genetic map of the T7 chromosome (14) (Fig. 3). Species I and II probably originate at the beginning of genes 11 (or 12) and 13 (or 14) respectively. The promoter for species IIIb is likely to be at the beginning of gene 17 since we assume that a promoter site will not be located within a gene and since species III is not large enough to contain all of the information for genes 16, 17, 18, and 19. This locates the promoter for species IIIb at about 83%; both this promoter site and the DNA sequences contained in species IIIb are homologous for T7 and T3 (ref. 17 and above).

It is interesting to note that a map of the positions of easily denatured, A + T rich sites on the T7 chromosome (20) shows a close correspondence in all but one instance with our tentative map of the late promoter and terminator sites. Easily denatured sites are found at 57, 63, 66, 86, 91, and 99% while promoters and terminators are located at 56, 64, 83, 97, and 99%. There is increasing evidence that promoter sites for bacterial RNA polymerase may be easily denatured A + T rich regions (21) and it is possible that this is a feature of sites read by T7 RNA polymerase as well.

We have not yet mapped the promoter and terminator sites for the major T7 species IIIa, IV, and V. This problem may be most easily approached by studying the transcription patterns of a collection of T3-T7 hybrid phages. Our results indicate that analysis of the transcription patterns of hybrid phages can be used to detect the presence of the major T7 promoter sites on the phage DNAa. Since physical maps of the hybrids can be obtained from heteroduplex mapping of the hybrids with T7 and T3 DNAs we anticipate that transcriptional analysis of hybrid DNAs will be very useful in mapping the remaining major T7 promoter sites.

The RNA species IIIa, IV, and V have an aggregate mass of 3.3×10^6 daltons which corresponds to a length equivalent to 25% of the r-strand. Since the late region of the T7 chromosome not transcribed into species I, II, IIIb, or VI represents at least 35% of the r-strand we conclude that a substantial portion of this region (at least 10%) is not read as part of any major transcript. This result is extended by our results using extensively shortened T7 DNA templates and using the deletion mutant LG 26 which suggest that the unmapped major T7 transcripts must originate from promoter sites to the right of 30%. It is known that T7 RNA polymerase acting *in vitro* with T7 DNA can transcribe mRNA for the ligase gene (gene 1.3, 17.1–20.2%) (ref. 19, and unpublished observations of R. C. Gesteland *et al.* and J. Broach and M. Golomb) and that this region is probably also transcribed by T7 RNA polymerase *in vivo* (14). Since none of the major T7 transcripts are derived from this region, it is likely that the promoters that govern this region are weak promoters which are read far less frequently by T7 RNA polymerase *in vitro*.

The late proteins from the 20 to 42% region of the chromosome (genes 1.7–6) have been designated class II proteins, and cease to be synthesized about 15 min after infection, while synthesis of class III proteins (genes 7–19) continues throughout infection (14). It is tempting to assume that these regulatory events are related to the transcription properties of the two regions. It may be that the class II protein region is read entirely from weak promoter sites, while the major T7 transcripts are all read from regions which specify class III proteins. In fact, an attractive possibility is that the remaining unmapped major transcripts (IIIa, IV, and V) all share a common termination signal near 56% and contain sequences only for the region from 41 to 56% which governs synthesis of the remaining class III proteins.

These results demonstrate that purified T7 RNA polymerase is highly selective in its recognition of specific promoter and terminator sites on the T7 DNA molecule *in vitro*. Confirmation that the specificity of this process duplicates that of site selection *in vivo* will require the demonstration that identical transcription units are read *in vivo*.

We thank Drs. H. Beier and R. Hausmann for their generous gift of T3-T7 hybrid phages; Dr. M. N. Simon for a very helpful discussion, and Drs. Beier, Hausmann, Simon, and F. W. Studier for the communication of unpublished data, and G. Kassavetis for the preparation of hybrid DNAs. This investigation was supported by United States Public Health Service Research Grant GM12010 from the National Institute of General Medical Sciences. One of us (M.G.) was supported by a USPHS Training Grant (GM01389) from the National Institutes of Health.

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