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Generation of bacteriophage T3 mRNAs by post-transcriptional processing by RNase III

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Abstract. The primary transcripts synthesized *in vitro* from a T3 DNA template by *Escherichia coli* RNA polymerase and by T3 phage-specific RNA polymerase have been characterized with regard to cleavage by RNase III and the size of the products of the cleavage reaction have been compared with those of *in vivo* T3 RNAs. It has been observed that the large RNA molecule synthesized in *vitro* by *Escherichia coli* RNA polymerase from the early region of T3 DNA are cleaved at specific sites by *Escherichia coli* RNase III to produce all the early mRNAs normally observed in T3-infected cells. In contrast, evidence presented here shows that some of the late T3 mRNAs are generated as direct products of transcription of late regions of T3 DNA by T3 RNA polymerase without mediation of RNase III, while many other late T3 mRNAs are formed by RNase III cleavage of two of the high molecular weight T3 RNA polymerase transcripts. These *in vitro* data appear to be in good agreement with the observed sizes of late T3 mRNAs formed *in vivo* in T3-infected RNase III-deficient and RNase III$^+$ *Escherichia coli* cells.

Keywords. RNA processing; RNase III; RNA nucleotidyl transferase; T3 promoter.

Introduction

It is now well established that the expression of the virulent linear double-stranded DNA bacteriophages T3 and T7 in infected *Escherichia coli* cells occurs in two distinct stages. First, the early genes located in the leftmost 19–20% of the DNA ('early' or class I region) are transcribed by the host *E. coli* RNA polymerase, which terminates transcription at a site located at the end of the early region. The early region includes gene 1, which codes for a new phage specific RNA polymerase that transcribes the remaining 80% (.'late'or class II and class III genes) of the phage DNA (Chamberlin *et al.,* 1970; Dunn *et al.,* 1971, 1972; Maitra, 1971; Chakraborty *et al.,* 1974; Chamberlin and Ryan, 1982). Both T3 and T7 genes are transcribed from the same strand of DNA (Summers and Siegel, 1970; Dunn *et al.,* 1972; Chakraborty *et al.,* 1974) and, according to the conventional genetic map of the phages, transcription proceeds from left to right (Summers, 1972; Beier and Hausmann, 1973; Hausmann, 1973; Studier and Movva, 1976).

In our laboratory, we have been involved in detailed characterization of various aspects of the transcription process in bacteriophage T3-infected cells. Recently, availability of a detailed restriction map of T3 DNA has allowed determination of the locations and nucleotide sequences of the major T3 RNA polymerase promoters on the T3 genome (Adhya, 1981; Adhya *et al.,* 1981; Bailey *et al.,* 1983, 1984;

Abbreviations used: M_r, Molecular weight; SDS, sodium dodecyl sulphate.

Sarkar *et al.,* 1985). These include all the promoters located at the class III late region as well as a number of promoters in the class III region. These results along with the observation that there are two terminators for T3 RNA polymerase—one located at 58·5 T3 map unit while the other at 100 map unit have allowed construction of a transcriptional map for T3 RNA polymerase (Golomb and Chamberlin, 1977; Bailey and McAllister, 1980).

In vitro, T3 RNA polymerase, upon transcribing its specific template T3 DNA, yields 8 major discrete RNAs (designated I-VIII) with molecular weight (M_r) of approximately 6·2, 4·7, 4·0, 2·8, 1·8, 0·9, 0·52 and 0·21 × 10^6, respectively (Chakraborty *et al.,* 1977; Golomb and Chamberlin, 1977). Comparison of the sizes of the *in vitro* T3 RNA polymerase transcripts with *in vivo* late T3 mRNAs indicates that several late mRNAs produced in T3-infected cells do not correspond to any *in vitro* T3 RNAs, and no RNAs as large as the 3 largest *in vitro* RNA species I, II and III are observed in T3-infected cells (Chakraborty *et al.,* 1977). In contrast, inclusion of purified *E. coli* RNase III in T3 RNA polymerase reaction mixtures resulted in the generation of shorter RNA products similar in size to those synthesized *in vivo* (Majumder *et al.,* 1977). These results suggested that RNase III is involved in the generation of late T3 mRNAs, but did not answer the question whether all or some of the transcripts are processed by RNase III.

In the present communication, we have carried out a systematic study of the effect of RNase III on each of the T3 RNA polymerase transcripts. The results indicate that only the high M_r transcripts, I, II, III as well as transcript VIII are processed by RNase III while species, V, VI and VII are not cleaved by RNase III under any ionic conditions. The effect of RNase HI on *E. coli* RNA polymerase products of T3 DNA in the generation of T3 early mRNAs has also been investigated.

Experimental procedures

Enzymes

Homogeneous T3 RNA polymerase was isolated as described (Chakraborty *et al.,* 1973). *E. coli* RNA polymerase holoenzyme was purified by a modification of the published procedure (Maitra and Hurwitz, 1967). A unit of either polymerase activity is equivalent to 1 nmol of UMP incorporated in 15 min at 37°C under conditions previously specified (Chakraborty *et al.,* 1973; Maitra and Hurwitz, 1967). One picomole of T3 RNA polymerase is equivalent to 15 units. Both preparations were free of detectable RNase (including RNase III), DNase and nucleoside triphosphatase activities by criteria described previously (Chakraborty *et al.,* 1973). RNase III was prepared from *E. coli* MRE-600 by a modification of the published procedure (Robertson and Dunn, 1975). The modification involved further purification of the DEAE-cellulose fraction by chromatography on a DNA-agarose column and then on a phosphocellulose column followed by centrifugation in a 10-30% glycerol gradient. The final specific activity of the RNase III preparation was 60,000– 70,000 units/mg of protein when assayed with poly(I) poly(C) as substrate under assay conditions described (Bishayee and Maitra, 1976). The purified enzyme was free of dectectable endo and exoribonuclease activities as assayed on T3 RNA polymerase transcript ' band V ' (M_r l·8 × 10^6).

Isolation of in vivo T3 RNAs

Late *in vivo* [^{32}P]-RNA was isolated from T3 phage-infected cells as follows: *E. coli* SY106(20 ml) growing at 37°C in a low phosphate medium (Landy *et al.,* 1976) was irradiated with ultraviolet light to suppress host RNA synthesis (Rosenberg *et al.,* 1974) and then infected with phage T3 at a multiplicity of infection of 10. After 10 min of infection, carrier-free $^{32}P_i$(2 5 μCi/ml) was added to the infected bacterial culture and infection was allowed to proceed for an additional 4 min, whereupon the 'pulse' was terminated by the addition of 20 mM potassium phosphate buffer, pH 6·8. The cells were then poured onto crushed ice containing 0·2 M NaN_3, 20 mM Tris-HCl, pH 7·4 and 5 mM $MgCl_2$. The cells were then harvested in a Sorvall centrifuge at 4°. From the compact pellet of cells, RNA was isolated by the diethylpyrocarbonate method of Summers (1970).

For preparation of *in vivo* early T3 [^{32}P]-RNA, chloramphenicol (200 μg/ml) was added to bacterial cultures 5 min before infection with T3 and infection was then carried out for 8 min in the presence of $^{32}P_i$ (10 0 μCi/ml) in the culture medium.

In vivo experiments related to effects of RNase III on T3 late transcription were carried out using the following host strains: *E. coli* BL15, an F$^-$ derivative of A19 (RNase III$^+$), and BL107, an F$^-$ derivative of AB301–105 (RNase III$^-$), and *rnc* 105 (RNase III$^-$) . Since wild-type T3 does not grow on these strains (derived from *E. coli* K 12), a mutant T3 phage that grows on *E. coli* K 12 was used in these experiments. Both the RNase III$^-$ host and the mutant T3 phage was kindly provided by Dr. B. W. Studier of Brookhaven National Laboratory.

Preparation of in vitro T3 RNA synthesized by T3 RNA polymerase and by E. coli RNA polymerase

Reaction mixtures (0·1 ml) contained 50 mM Tris-HCl, pH 7·8, 1 mM $MgCl_2$, 50 mM KCl, 4 mM dithiothreitol, 20 nmol of T3 DNA (expressed as deoxynucleotide residues), and 40 nmol each of ATP, GTP, UTP and CTP; UTP was labelled with either ^{3}H or with ^{32}P in the α-phosphate position (2–5 × 10^5 cpm/nmol). The reaction was initiated by the addition of either T3 RNA polymerase (0·4–1 pmol) or *E. coli* RNA polymerase (5 pmol). After incubation at 37°C for 15 min, reactions were terminated by adding EDTA and sodium dodecyl sulphate (SDS) to a final concentration of 25 mM and 0·1 %, respectively. Labelled RNA products were isolated by phenol extraction followed by precipitation with 2·5 vol. of ethanol at – 20°C. It was then chromatographed on CFII cellulose to remove residual precursors and DNA, following the procedure of Franklin (1966) as modified by Robertson and Hunter (1975). Both the unprocessed and RNase III-processed RNAs elute from CFII cellulose at positions expected for single-stranded RNA. Purified RNAs were stored as ethanol precipitates at – 20°C.

Electrophoretic analysis of RNAs

Labelled RNAs were analyzed by electrophoresis either on slab gels of 2% polyacrylamide-0·5% agarose as described (Chakraborty *et al.,* 1977; Majumder *et al.,* 1977) or in 12-cm long cylindrical gels in the presence of 6 M urea by an adaptation

of the procedure of Floyd *et al.* (1974) as described (Chakraborty *et al,* 1977). In all electrophoretic analysis performed, [^{32}P]-labelled 23S and 16S *E. coli* rRNA were run in a separate lane to provide M_r markers. The M_r values of all observed RNA species were estimated from their electrophoretic mobilities relative to 23S and 16S rRNA markers.

Isolation of individual T3 RNA polymerase transcripts from Polyacrylamide gels

For isolation of individual T3 RNA polymerase transcripts from gels, T3 RNA polymerase transcripts were synthesized under standard reaction conditions with 2 pmol of T3 RNA polymerase and [α-^{32}P]-UTP (2 × 10^6 cpm/nmol). [^{32}P]-labelled RNA was purified as above, then subjected to electrophoresis for 12 h at 70 V on 2% Polyacrylamide [acrylamide/bisacrylamide (20:1)/0·5% agarose slab gels (13·5 × 10 × 0·4 cm)] as described (Majumder *et al.,* 1979). After electrophoresis, RNA bands were visualized by autoradiography, cut fro the gel, and eluted electrophoretically into dialysis bags using a buffer that contained 20 mM Tris-HCl, pH 7·4, 40 mM sodium acetate, 1 mM EDTA, 0·1% SDS at 150 V for 10 h. RNAs were extracted with phenol, and precipitated with 2·5 vol. of ethanol. A typical separation of T3 RNA polymerase transcripts is shown in figure 1. Transcripts I and II, which can be separated on 6 M urea cylindrical gel (Chakraborty *et al.,* 1977), move with nearly identical mobilities in the non-denaturing gel system described here.

Results

Effect of RNase III on individual T3 RNA polymerase transcripts

In vitro transcription of T3 DNA by T3 RNA polymerase yields several discrete RNA species. Polyacrylamide gel electrophoretic analysis of these products in 6 M urea in cylindrical gels resolves 8 discrete size classes of RNA, designated I-VIII, of *Mr,* 6·2, 4·7, 4·0, 2·8, 1·8, 0·8, 0·52 and 0·21 × 10^6, respectively (Chakraborty *et al.,* 1977; Majumder *et al.,* 1977). (2% acrylamide — 0·5% agarose slab gels do not resolve species I and II as shown in figure 1). Since RNA species I, II and III formed *in vitro* were larger than any *in vivo* T3 RNA species (Majumder *et al.,* 1977), we determined whether RNase III cleaved these high *Mr* products. The influence of RNase III on other *in vitro* transcripts, V, VI, VII and VIII were also investigated. For this purpose, the individual T3 RNA polymerase transcripts; labelled with [α-^{32}P]-UTP, were purified electrophoretically as described in 'materials and methods'. The effect of RNase III on the size ofeach transcript was then ascertained. As shown in figure 2, under conditions optimum for 'primary' site cleavage (Dunn, 1976) by RNase III, namely concentrations of 5-10 mM Mg^{2+} and 50– 100 mM KCl in reaction mixtures, T3 RNA species (I + II) and III were cleaved by RNase III to give rise to several discrete size classes of RNA species, each ofwhich appears to co-migrate with an apparent M_r similar to an *in vivo* late T3 mRNA. It is noteworthy that most of the RNase III cleavage products generated from both species are of similar M_r. This is expected since the transcriptional map of T3 genome demonstrates that T3 RNA species II and III originate from promoters located at 56 and 65%, respectively, on

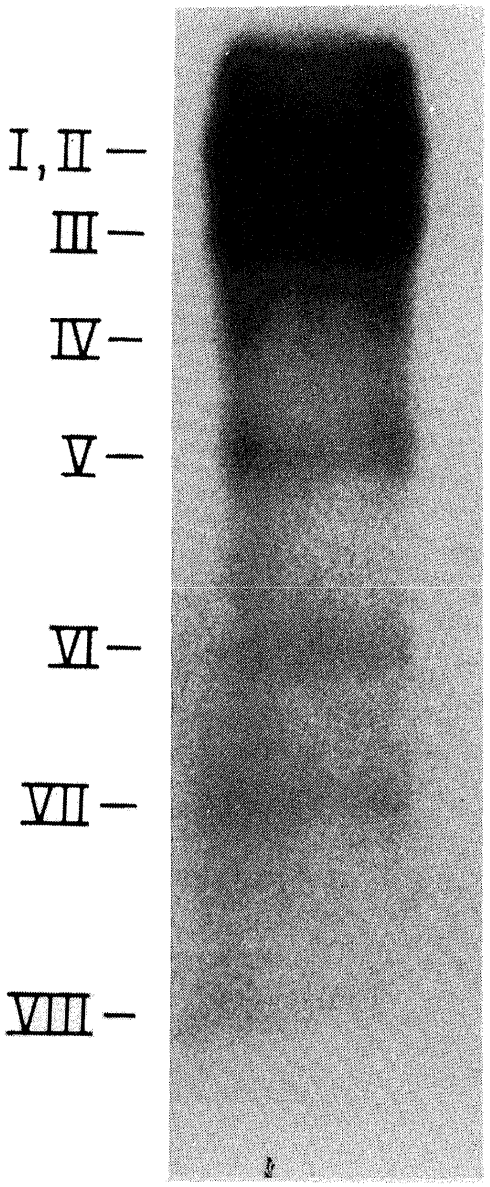


Figure 1. Autoradiogram of a polyacrylamide/agarose gel of [^{32}P]-labelled T3 RNA polymerase transcripts. The synthesis and electrophoretic separation of T3 RNA polymerase transcripts, labelled with [α-^{32}P]-UTP, were carried out as described in 'methods'. After completion of the run, [^{32}P]-labelled RNA species were detected by autoradiography.

the standard T3 genetic map and share a common terminator at approximately 100% (Beier *et al.,* 1977). Thus these two RNA species transcribe overlapping transcription units.

In contrast to site specific cleavage of T3 RNA species II and III by RNase III,

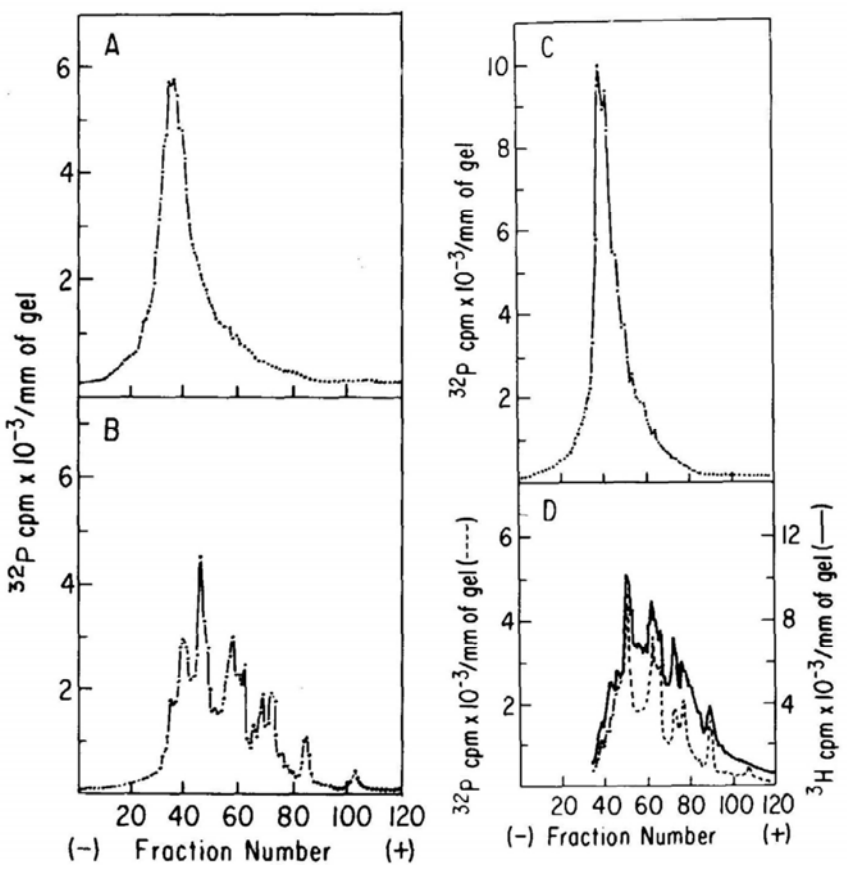


Figure 2. Effect of RNase III on T3 RNA polymerase transcripts (I + II), and III. Reaction mixtures (50 μl) contained 25 mM Tris-HCl buffer (pH 7·8), 5 mM $MgCl_2$, 100 mM KCl, 2 mM dithiothreitol, approximately 300,000 cpm of isolated [^{32}P]-labelled T3 RNA polymerase transcript I+ II (figure 2A and B) or transcript $_{III}$ (figure 2C and D). Reaction mixtures B and D also contained 2 μg of RNase III. Following incubation at 37°C for 10 min, the reaction was terminated by the addition of EDTA and SDS to final concentrations of 50 mM and 0·1 %, respectively, followed by dialysis against 10 mM sodium phosphate buffer (pH 6·8) for 2 h. For reaction mixtures A, B and C, an aliquot of the dialyzed material (20 μ1) containing 150,000 cpm of [^{32}P]-radioactivity was subjected to get electrophoresis in 12 cm long cylindrical gels by an adaptation of the procedure of Floyd *et al.* (1974) as described (Chakraborty *et al.,* 1977). Electrophoresis was carried out at 15°C at 5 mA per gel for 7 h. For reaction mixture D, a similar aliquot of the dialyzed *in vitro* RNA products was mixed with *in vivo* late [^{3}H]-RNA (approximately 300,000 cpm of radioactivity) and the mixed sample was analyzed by electrophoresis as above. The gels were sliced into 1 mm fractions with an automatic Gilson gel slicer and the radioactive content of each slice was determined in 10 ml of Bray's solution in a liquid scintillation spectrometer. (A), (C), No RNase III added: (B), (D), RNase III added.

RNA species V, VI and VII were not cleaved by RNase III under the above conditions of the cleavage reaction (figure 3). T3 RNA species VIII were found to have an RNase III cleavage site giving rise to two RNA species of apparent M_r 170,000 and 40,000 (data not shown). The minor T3 RNA species IV, because of low yield of recovery by elution from the gel, was not tested in the cleavage reaction.

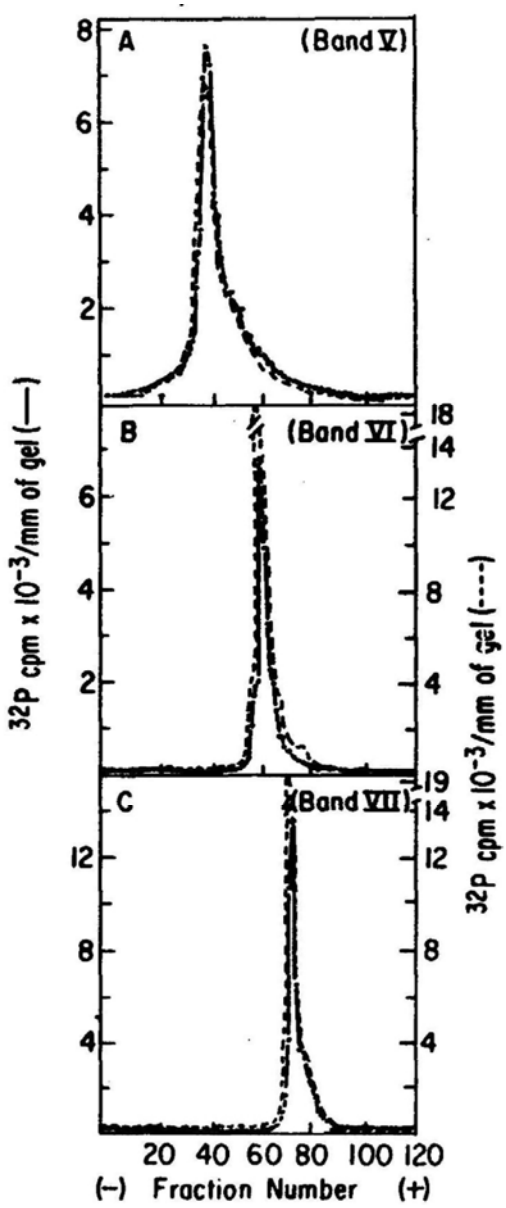


Figure 3. Effect of RNase III on isolated T3 RNA polymerase transcripts V, VI and VII. Three sets of reaction mixtures, A, B and C were prepared; each set contained two separate reaction mixtures, one contained 1 μg of RNase III while the other did not contain RNase III. Each reaction mixture (100 μl) was prepared as described in the legend to figure 2 except that each of the [^{32}P]-labelled T3 RNA polymerase transcript V, VI, or VII was added as substrate for RNase III action. Following termination of the reaction, RNA products were analyzed on 12 cm cylindrical gels in 6 M urea as described in the legend to figure 2. (A), Transcript V; (B), transcript VI; (C), transcript VII;(●–●),No RNase III added; (●- - -●),, 1 μg of RNase III added.

Synthesis of late T3 mRNAs in RNase III—Deficient strains

Further confirmation that RNase III cleavage of the primary transcripts synthesized by T3 RNA polymerase is part of the normal pathway for the production of some of the late T3 RNAs came from analysis of *in vivo* transcripts synthesized in TS-infected host mutants deficient in RNase III. Since RNase III-deficient mutants were *E. coli* K 12 strains and wild-type T3 phage does not grow on such strains, it was necessary to use for these experiments a mutant T3 phage that grows on such strains (Studier and Movva, 1976).

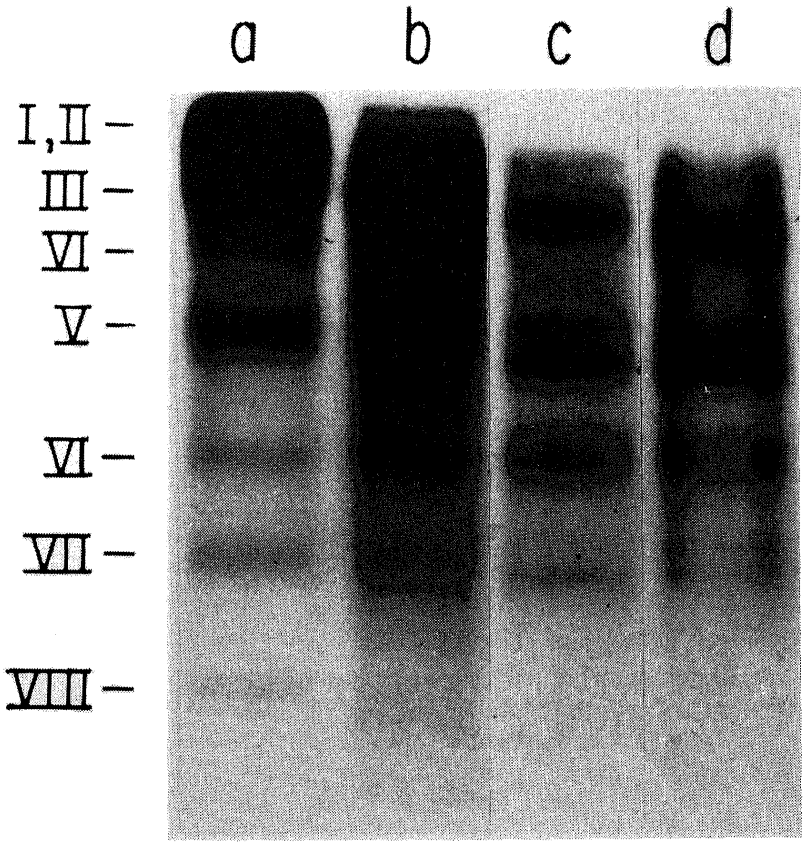
As shown in figure 4, late RNAs produced in T3-infected *E. coli* deficient in RNase III are of similar size as T3 RNA polymerase transcripts II and III. In addition, T3 RNA species V, VI and VII also have corresponding *in vivo* counterparts (figure 4, lanes a and b). Furthermore, treatment of T3 late RNAs isolated from T3-infected RNase III-deficient mutants with RNase III *in vitro* produces RNA transcripts similar in mobility to late T3 mRNAs synthesized in RNase III^+ cells (figure 4, lanes c and d). It should be noted that *in vitro* species VIII has no *in vivo* counterpart in RNase III^+ or RNase III^- cells.

Involvement of RNase III in the generation of T3 early mRNAs

Early in the infection of *E. coli* by phage T3, *E. coli* RNA polymerase holoenzyme initiates transcription at one of a group of several promoters located at the genetic left end of the T3 genome to transcribe the early genes, 0·3, 0·7, 1, 1·1 and 1·3, respectively, and terminates at a site located at about 20 T3 map units (Koller *et al.,* 1974). Although termination at this site occurs quite efficiency *in vivo* (Issinger and Hausmann, 1973), *in vitro* the polymerase reads through this termination site to copy a part of the late region. The presence of *rho* protein in the *in vitro* transcription system restricts RNA polymerase to transcribing the early genes only (Dunn *et al.,* 1972; Chakraborty *et al.,* 1974; Neff and Chamberlin, 1978; Adhya *et al.,* 1979).

The first strong *E. coli* RNA polymerase promoter located at the left end of T3 DNA is between 0·89 and 1·69 map units, as judged by restriction fragment transcription with *E. coli* RNA polymerase (Adhya *et al.,* 1981). Initiation of transcription from this promoter and termination at 20 map units should yield a transcript of approximately M_r value $2{\cdot}5 \times 10^6$. As shown in figure 5 (lane a), transcription of intact T3 DNA by *E. coli* RNA polymerase, under defined *in vitro* conditions, yielded a single RNA species of M_r $2{\cdot}5 \times 10^6$, corresponding to asymmetric transcription of the entire early region of T3. In contrast to the results obtained *in vitro,* analysis of the early RNAs produced after infection of *E. coli* by wild-type and deletion mutants of T3 identified 5 T3 early mRNAs (Studier and Movva, 1976; Adhya, 1981; see also figure 5, panel B). Each mRNA corresponds to each of the early genes 0·3, 0·7, 1, 1·1 and 1·3. The observed *Mr* values for the early RNAs are: $1{\cdot}1 \times 10^6$ for gene 1; $0{\cdot}56 \times 10^6$ for gene 0·7; $0{\cdot}41 \times 10^6$ for gene 1·3 and $0{\cdot}21 \times 10^6$ each for genes 0·3 and 1·1 (Studier and Movva, 1976).

It was of interest to determine whether, as in the case of T7 (Dunn and Studier, 1973), cleavage by RNase III is part of the pathway for manufacture of early T3 mRNAs.When RNase III was included in the *in vitro E. coli* RNA polymerase reaction, several new discrete RNA bands appeared on the gel (figure 5A, lanes b



Figured 4. T3 late RNAs made after infection of RNase III$^+$ and RNase III$^-$ hosts. BL15. an F$^-$ derivative of A19 (RNase III$^+$), and BL107, an F$^-$ derivative of AB301–105 (RNase III$^-$), were irradiated with UV to supress host RNA synthesis and then infected with a mutant T3 phage that grows on *E. coli* K 12 in the presence of $^{32}P_i$ as described in Methods. Samples were removed after 12 min of infection and [^{32}P]-RNA was isolated by diethylpyrocarbonate method of Summers (1972). Isolated *in vivo* [^{32}P]-RNA samples were electrophoresed on vertical slab gels alongside [^{32}P]-labelled *in vitro* T3 RNA polymerase transcripts. In a separate experiment, an aliquot of *in vivo* [^{32}P]-RNA isolated from T3-infected BL107 (RNase III $^-$) cells was treated *in vitro* with 2 μg of RNase III and run in a similar slab gel. The conditions of gel electrophoresis were as described in 'methods'. After electrophoresis, gels were analyzed by autoradiography. Lane a, [^{32}P]-labelled *in vitro* T3 RNA polymerase transcripts; lane b, T3 late [^{32}P]-RNA isolated from RNase III$^-$ cells (BL107); lane c, T3 late [^{32}P]-RNA from RNase III$^+$ cells (BL15); lane d, T3 late [32P]-RNA from RNase III-deficient (BL107) cells incubated with 2μg of RNase III under standard reaction conditions. As noted above, the experiment in lane (d) was run in a separate gel. In this gel, in parallel lanes (not shown), [^{32}P]-labelled *in vitro* T3 RNA polymerase transcripts as well as [^{32}P]-labelled *in vivo* late mRNAs isolated from T3-infected RNase III$^+$ cells were run as controls. RNA band patterns in these control lanes were similar to those observed in lanes (a) and (c), respectively. The molecular weight values of all RNA species were calculated from their electrophoretic mobilities relative to [^{32}P]-labelled 23S and 16S *E. coli* rRNA markers which were run in a parallel lane (not shown).

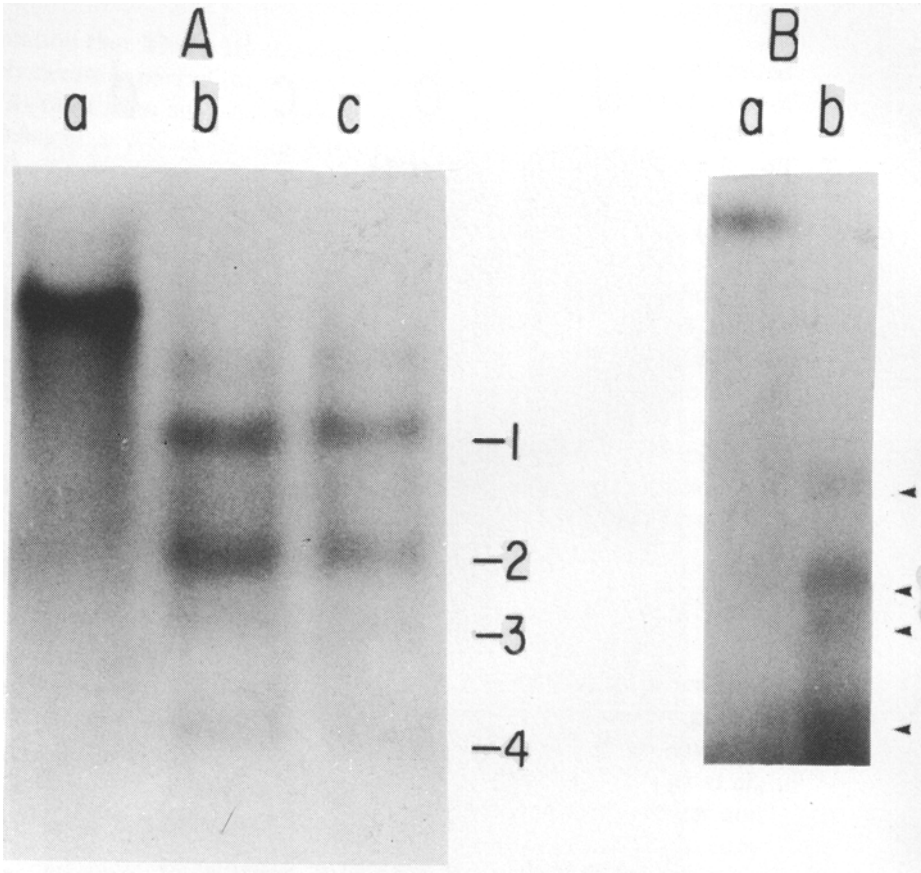


Figure 5. T3 early RNAs synthesized *in vitro* and *in vivo*. Labelling of RNAs is described in 'methods' while analysis of RNAs by gel electrophoresis was carried out as described in the legend to figure 4. *In vitro* RNAs, labelled with [α-^{32}P]-UTP, were transcribed by purified *E. coli* RNA polymerase from a T3 DNA template and subsequently purified on columns of cellulose CFII. as described in 'methods'. *In vitro* transcripts thus isolated were incubated with various concentrations of RNase III for 15 min at 37 in 20 mM Tris-HCl (pH 7·8), 5 mM $MgCl_2$, 100 mM KCl, 1 mM dithiothreitol. *In vivo* RNAs were labelled with $^{32}P_i$ after infection in the presence of chloramphenicol; the host had been irradiated with UV to supress synthesis of host RNAs. Considerable label was incorporated *in vivo* into material of low M_r that migrates near the front. This material tends to obscure small RNAs on these gels. Panel A: *In vitro E. coli* RNA polymerase transcripts of T3 DNA: lane (a), transcript alone, no RNase III: (b) and (c), transcript +1 μg and 2 μg of RNase III, respectively; Bands 1–4 represent RNA species of *Mr* 1·25. 0·56, 0·41 and 0·21 × 10^6, respectively. Panel B: *In vivo* early RNA isolated from T3-infected *E. coli* SY106 cells labelled with $H_3^{32}PO_4$ from (a) 0-4 min, (b) 0– 8 min. after infection in the presence of 200 μg/ml of chloramphenicol. The arrows represent the positions of the early RNAs. The calculated *Mr* values of these RNAs are 1·3. 0·6, 0·4 and 0·21 × 10^6, respectively. The intense smear at the bottom of the gel probably represents unincorporated $^{32}P_i$.

and c). The major species correspond in size to early *in vivo* T3 mRNA (figure 5). Several minor species were also observed; these may arise by RNase III cleavage at secondary sites (Dunn, 1976). RNase III therefore appears to be at least one factor involved in generation of T3 early mRNAs *in vitro*.

Discussion

In the present study, we show that like the T7 system, (Dunn and Studier, 1977) the pathway for production of early T3 RNAs appears to involve endonucleolytic cleavage by RNase III of a $2{\cdot}5 \times 10^6$ large precursor RNA synthesized by *E. coli* RNA polymerase from T3 DNA template. In contrast, RNase III appears to be involved in the generation of only some of the late T3 mRNAs, while some other late T3 mRNAs are made as direct products of transcription of the late regions of T3 DNA by T3 RNA polymerase. Among the 6 major T3 RNA polymerase transcripts examined for *in vitro* cleavage by RNase III, transcripts II, III and VIII are cleaved at specific sites by RNase III. While species VIII has no *in vivo* counterpart, *in vitro* cleavage of species II and III generates RNA species that appear to co-migrate in gel electrophoresis with late mRNAs isolated from T3-infected cells. These *in vitro* data appear to be in good agreement with the *in vivo* observations that late T3 mRNAs isolated from RNase III-deficient strains contain high M_r RNA products which are of similar size as *in vitro* T3 RNA species II and III. Furthermore, the large high M_r products isolated from RNase III-deficient strains can be processed *in vitro* with purified RNase III to produce normal size T3 late mRNAs. As expected, each of the *in vitro* T3 RNA species V, VI and VII which are not cut by RNase III in *vitro,* appears to have an in *vivo* mRNA counterpart both in Tuninfected RNase III^+ and T3-infected RNase III^- cells.

In the present study the identity of *in vivo* and *in vitro* RNA products have been inferred on the basis of molecular size analysis in Polyacrylamide gels. It will be important to map the RNase III cleavage sites on T3 genome and to show that *in vitro* RNA products formed by RNase III cleavage arise from the same region T3 DNA as the corresponding *in vivo* RNA. Recently, during the course of sequencing of class III T3 RNA polymerase promoters, two putative RNase III cleavage sites have been mapped around 45·0 and 64·8 T3 map units (Basu *et al.,* 1984). Furthermore, the present study has not excluded the possibility that the products of RNase III cleavage are processed further *in vivo.* It will be necessary to show that mRNAs produced *in vivo* have the same base sequences at their ends as the RNAs of similar sizes formed after RNase III cleavage.

The biological function of specific cleavage of T3 and T7 RNAs by RNase III is unknown. Unlike eukaryotic mRNAs, most prokaryotic mRNAs are polycistronic; some are monocistronic. Bacteriophage T3 and T7 mRNAs are however unique in that most of the RNAs are transcribed into a polycistronic mRNA, each from a separate transcription unit, which is subsequently cleaved at specific sites to yield mRNAs for the individual genes. Since T3 and T7 may have developed from a common ancestor, it is significant that despite the divergence of the two phage DNA sequences, RNase III cleavage sites in both the early and late mRNA sequences have been retained in both phage systems. Presumably, RNase III cleavage of polycistronic mRNAs in T3 and T7-infected cells offers some selective advantage for the phage at least on its natural hosts. Dunn and Studier (1975) have however concluded that such cleavages are not essential for growth of T7 since the phage is able to grow in RNase III-deficient strains, and all but a few T7 proteins are made at essentially the same rate in RNase III deficient as in RNase III^+ strains. The exceptions are the gene 0·3 protein, an early protein, and a few late proteins. Cleavage of the early mRNAs by RNase III greatly stimulates the production of the 0·3 protein both *in vivo* and in cell-free protein synthesizing systems (Dunn and Studier, 1975). We have

likewise observed that T3 grows normally in RNase III-deficient host strains although the lysis period is somewhat lengthened in these strains as compared to RNase III^+ cells. These observations lend support to the view that cleavage by RNase III are not required for growth of T3 and T7 in *E. coli.* The possibility, however, exists that such cleavage may be essential for good growth of these phages in some of the hosts upon which T3 and T7 grow in nature.

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