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Transcription of the Bacteriophage T4 Template. Obligate Synthesis of T4 Prereplicative RNA *in Vitro**

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ABSTRACT: T4 DNA is transcribable asymmetrically and selectively by *Escherichia coli* ribonucleic acid (RNA) polymerase to yield prereplicative transcripts. We have studied (a) the generality of this selectivity, and (b) the correlation of selectivity with asymmetry of transcription. RNA has been synthesized *in vitro* under a variety of conditions—with *E. coli* and *Micrococcus lysodeikticus* RNA polymerase, at low and high ionic strength, at enzyme or template excess, with Mn^{2+} or Mg^{2+}, with or without polyamine, with deoxyribonucleic acid (DNA) prepared in various ways, sheared or sonicated, with vegetative T4 DNA, and in water–tetramethylurea and water–dimethyl sulfoxide mixtures. None of these manipulations yield appreciable asymmetric synthesis of other than early transcripts. What *late* transcription occurs is concomitant with extensive breakdown of the asymmetry of transcription.

We conclude that selective prereplicative T4 transcription *in vitro* is an "intrinsic" property of bare viral DNA and complete bacterial polymerase and not due to a negative control element. Transcription of late portions of the viral template must be due to elaboration of positive transcription control elements.

When DNA extracted from T4 phage is used as a template for *Escherichia coli* RNA polymerase, transcription of the sections of the T4 DNA that code for the *late* gene products appears to be restricted (Khesin and Shemyakin, 1962; Geiduschek *et al.*, 1966). *In vitro* transcription of phages SPOl and λ by *E. coli* RNA polymerase can also be more or less confined to those parts of their genomes that are expressed early in development (Naono and Gros, 1966; Geiduschek *et al.*, 1968). On the other hand, *in vitro* systems for transcribing *late* T4 RNA can be devised (Snyder and Geiduschek, 1968). In understanding the significance of such *late* transcription *in vitro*, it is essential to know what limitations exist for restricted transcription of *early* RNA by bacterial RNA polymerase. For this reason, we have investigated *in vitro* T4 RNA synthesis with mature and vegetative T4 DNA templates under a wide range of conditions. We find that selective *in vitro* RNA synthesis of T4 prereplicative RNA is retained under the most varied circumstances, while *late* T4 RNA synthesis with purified *E. coli* RNA polymerase is accompanied by a generalized loss of specificity in transcription, as evidenced by the parallel synthesis of symmetric RNA (Geiduschek *et al.*, 1968). These findings reinforce the conclusion that some T4 gene product(s) must be a part of any transcription system for *in vivo* and *in vitro* synthesis of late RNA. The T4 gene 55 appears to provide one of these products (Pulitzer *et al.*, 1968; Snyder and Geiduschek, 1968; Pulitzer, 1970).

Materials and Methods

Preparation of E. coli B RNA Polymerase. E. coli B (mid log, minimal medium grown) were purchased from Grain Processing Co., Muscatine, Iowa. A number of enzyme preparations were either made exactly according to Chamberlin and Berg (1962), or with only slight modifications. Some preparations were made as described by Colvill *et al.* (1966), with centrifugation through a glycerol gradient (10–30%) substituting for DEAE-cellulose chromatography as a

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final purification step. In our hands, this procedure gave a broad distribution of enzymatic activity over the glycerol gradient.

Several *E. coli* RNA polymerase preparations were made with the following modifications, kindly communicated by P. Berg (1966).

The ammonium sulfate precipitate (fraction III) was back-extracted with *ca.* 45% saturated neutralized $(NH_4)_2SO_4$ before being dissolved in *ca.* 37% saturated $(NH_4)_2SO_4$.

Enzyme was then reprecipitated with 50% saturated $(NH_4)_2SO_4$ (fraction IIIa). Fraction IIIa was chromatographed on DEAE-Sephadex rather than DEAE-cellulose. Pooled fractions containing RNA polymerase activity were precipitated with an equal volume of saturated $(NH_4)_2SO_4$, dissolved in "freezing buffer" (0.01 M Tris, pH 7.9–0.001 M $MgCl_2$–0.1 M $(NH_4)_2SO_4$, pH 7.5–0.005 M reduced glutathione), divided into suitable aliquots, and frozen in liquid N_2. Upon thawing, the enzyme was immediately diluted with an equal volume of 0.01 M Tris, pH 7.9–0.01 M $MgCl_2$–0.0001 M EDTA–0.01 M β-mercaptoethanol; the enzyme is inactivated within 12 hr at 0° in freezing buffer.

The specific activity of the enzyme preparations used for RNA synthesis varied from 1000 to 5000 units/mg of protein. The modification of the Chamberlin–Berg purification which is outlined above has given good yields of RNA polymerase with specific activities varying from 3000 to 5000 units/mg of protein.

M. lysodeikticus RNA Polymerase. A preparation of *M. lysodeikticus* RNA polymerase purified from spray-dried cells (Miles Laboratories) according to Nakamoto *et al.* (1964) was the very generous gift of Dr. T. Nakamoto.

RNA Synthesis. With exceptions that are stated below, reaction conditions for T4 RNA synthesis were: Tris chloride, pH 7.5, 100 μmoles/ml; $MgCl_2$, 10 μmoles/ml; spermidine chloride, 1 μmole/ml; 3 unlabeled nucleoside triphosphates, each 1 μmole/ml; ^{3}H- or ^{32}P-labeled nucleoside triphosphate, 0.1 μmole/ml; T4 DNA, RNA polymerase, and temperature variable. Units of RNA polymerase activity are defined following Chamberlin and Berg (1962). One unit of enzyme is defined as incorporating 1 mμmole of labeled nucleoside triphosphate into trichloroacetic acid precipitable material in 1 hr using excess salmon sperm DNA as template.

Incorporation was measured at varying DNA:enzyme ratios with both the *E. coli* and *M. lysodeikticus* polymerases for 10 min at 37°. At constant DNA concentration such titration curves have an initial linear portion and ultimately reach a plateau of synthesis insensitive to further addition of enzyme. In this and the subsequent paper, we define DNA excess synthesis by the linear portion of such curves and enzyme excess by the plateau. Saturation is defined as a function of enzyme activity, rather than of protein concentration, and only for 10-min reaction time. It is known that saturation of enzyme binding and of RNA production during brief incubation are not necessarily concordant (Pettijohn and Kamiya, 1967).

Preparation of T4 Phage and DNA. Phage T4 were prepared by differential centrifugation or by separation between polyethylene glycol and dextran sulfate containing aqueous phases (Albertsson, 1965) according to Salser (private communication 1966) and then centrifuged on a CsCl step gradient (G. Kellenberger-Gujer, private communication 1965), dialyzed first against 0.1 M NaCl–0.01 M Tris-Cl, pH 7.5, and then against 0.01 M Tris-Cl, pH 7.5 (even the osmotically sensitive T4D$^+$ can be purified in this way).

The standard DNA preparation was by cold phenol extraction (Mandell and Hershey, 1962) and dialysis against 1 M NaCl–0.01 M Tris-Cl, pH 7.5, followed by 0.01 M Tris-Cl, pH 7.5. One sample of DNA was digested with 12.5 μg/ml of pancreatic RNase (boiled for 10 min to destroy DNase activity) at 37° for 15 min in 2X SSC, then reextracted with phenol to remove RNase and dialyzed to remove phenol. Another T4 DNA preparation was incubated at 37° for 60 min with 50 μg/ml of Pronase which had been self-digested for 2 hr at 37° just prior to use. It was then reextracted with phenol and dialyzed.

^{35}S-labeled T4 phage were prepared by infecting *E. coli* B in a $Na_2[^{35}S]SO_4$ supplemented, low sulfur medium. Phage were purified from the lysate as above. Three phenol extractions of these CsCl purified phage removed 99.8% of the trichloroacetic acid precipitable ^{35}S. If 7% of the ^{35}S is in internal protein (Hershey and Chase, 1952) then at least 97% of the internal protein ^{35}S is removed by three phenol extractions. To remove any remaining internal protein, the DNA was extracted with $CHCl_3$–isoamyl alcohol according to the procedure of Bachrach and Friedman (1967). Nine such extractions further reduced the trichloroacetic acid precipitable ^{35}S by about 40% (to 0.14% of the original phage preparation). $CHCl_3$ and isoamyl alcohol were extracted with fresh diethyl ether, which was removed by blowing N_2 over the DNA solution; the DNA was then dialyzed and used for RNA synthesis.

Isolation of Labeled Vegetative T4 Phage DNA. The method described by Frankel (1966a) was used with modification so that more concentrated DNA could be obtained. The following T4 phage strains were used to infect *E. coli* B^E or BB: T4D wild type, *am* N134 (gene 33), *am* BL292 (gene 55), the double mutant T4 *am* N134,BL292. The double mutant was a gift of J. Pulitzer. *E. coli* B^E or BB was grown from overnight culture to 5×10^8 cells/ml in M9S (Bolle *et al.*, 1968a) and infected with 5–10 phage/bacterium. Infection was at 37°, for the wild type and the amber mutant. Four minutes after infection, [^{14}C]thymidine (0.05 μCi/ml, 1.2 μg/ml) or [^{3}H]thymidine (*methyl*-H^3, 1 μCi/ml, 1.2 μg/ml) was added.

At various times between 12 and 20 min after infection, cells were chilled, centrifuged, and resuspended in 0.1 volume of M9S. An equal volume of lysis buffer (0.1 M NaCN–0.1 M EDTA, pH 8.5, 100 μg twice-crystallized lysozyme) was added and the suspension kept at 4° for 15–20 min, followed by transfer to 65° and a further 3-min incubation. Sodium dodecyl sulfate was added to 0.2–0.5% and the lysate was kept 8 min more at 65° (the subsequent steps were carried out at room temperature or 15°). Crude lysate (1–2 ml) was layered on top of a linear sucrose gradient (25 ml, 20–70% sucrose in 0.1 M NaCl–0.05 M EDTA, pH 8.5) and centrifuged at 23,000 rpm (SW 25 rotor, L2 Spinco ultracentrifuge), 12° for 1–3 hr. Fractions were collected by syphoning from the top of the gradient (the diameter of the collection tube was 3 mm to minimize shearing of the DNA) and radioactivity was determined on a small aliquot of each fraction. Any pelleted DNA was removed by washing the centrifuge tubes with 0.01 M Tris-Cl, pH 7.5. Recoveries of radioactivity were 75–100%. Fractions containing replicative DNA were pooled and further purified in either of the following ways.

PHENOL EXTRACTION. The sucrose-containing fractions were diluted with standard 0.1 M potassium phosphate buffer, pH 7.0, and extracted 2–3 times with buffer-saturated phenol, in the cold. The extracted DNA was dialyzed first against 1 M NaCl–0.01 M Tris, pH 7.5, then against 0.01 M Tris, pH 7.5, to remove all traces of phenol. Recoveries of radioactivity in the dialyzed DNA solution were 85–90%.

CsCl EQUILIBRIUM CENTRIFUGATION OF VEGETATIVE DNA. The sucrose-containing fractions were diluted into 0.01 M Tris-Cl, pH 7.5, and solid CsCl was mixed in very gently to give a final density of 1.7 g/ml. These solutions were overlayered with mineral oil, then centrifuged in a Spinco SW65 rotor for 24 hr at 48000 rpm, 15°. The DNA band was collected through a hole in the bottom of the tube and dialyzed against 0.01 M Tris-Cl, pH 7.5, prior to use. The approximate sedimentation coefficients of purified DNA preparations were determined by preparative band-centrifugation on CsCl (ρ = 1.507 g/cm^{-3}) using Cyanophage LPPl DNA (33.5 S) (Luftig and Haselkorn, 1967) as a marker. (We thank R. Luftig for a gift of this material.)

DNA–RNA Hybridization and RNA–RNA Duplex Formation. Hybridization–competition was done by the liquid annealing technique (Nygaard and Hall, 1964). Hybrids were formed during 6 hr at 60° in 2X SSC (0.30 M NaCl plus 0.03 M trisodium citrate), digested with RNase, and filtered on nitrocellulose membranes (Schleicher and Schuell B6) as described previously (Bolle *et al.*, 1968a). This is hybridization method I.

During the course of these hybridization–competition experiments, an annealing-dependent decrease in the ability of denatured DNA to stick to nitrocellulose filters was observed. This effect depends on the size of the denatured DNA and on the rate of filtration and washing of the hybrids. The following conditions have been found to eliminate the loss of DNA from the filter: T4 DNA is sheared by 5–7 passages through a 22-gauge needle, treated with 0.1–0.33 M NaOH for 10 min at room temperature, neutralized with 1.5 moles of NaH_2PO_4/mole of NaOH, and diluted to an appropriate concentration for annealing with 0.01 M Tris-Cl, pH 7.5. After the customary annealing and RNase treatment, samples are slowly filtered and washed (1–3 ml/min). This is hybridization method II.

The interpretation of hybridization competition curves has been discussed elsewhere (Bolle *et al.*, 1968a).

RNA–RNA duplex formation was done according to Colvill *et al.* (1965), with annealing in 2X SSC at 60° for 6 hr.

In vivo T4 RNA was prepared as described elsewhere (Bolle *et al.*, 1968a; method 2) and tested to ensure that all DNA had been removed by hot phenol extraction. Test competitions verified that all labeled and unlabeled *in vivo* RNA preparations had the hybridization–competition properties expected for either prereplicative or 20-min RNA (Bolle *et al.*, 1968a).

RNA Syntheses in Water–Tetramethylurea and Water–Dimethyl Sulfoxide Mixtures. An order of mixing reagents was adopted which avoids denaturation or precipitation of T4 DNA due to transient solvent inhomogeneities. T4 DNA was added slowly to the premixed other components of the reaction mixture with continuous gentle stirring. RNA synthesis was then started by adding RNA polymerase. T4 DNA and RNA polymerase were added in the smallest practicable volumes (otherwise the DNA would be subjected to a transient higher concentration of organic solvent before RNA synthesis). Organic solvent concentrations are v/v in the final reaction mixture.

TABLE I: Nomenclature of T4 Transcripts.

Post-replicative Modulation	Dependence of First Appearance on a Viral Protein[a]	Names, Salser *et al.* (1970)	This Work
Transcripts first appearing before replication			
Down	No	Preearly or early A and B	Immediate early (Ia)[b]
	Yes	Postearly or early C	Delayed early (Ib)[b]
Up	Not yet specified	Quasi-late[b]	Postreplicative[b] early (II)
Transcripts first appearing after replication and replication dependent			
		Late	Late (III)

[a] More precisely, dependent on protein synthesis in the infected cell. [b] Classes Ia, Ib, and II are also collectively called "early" (Hall *et al.*, 1963; Khesin and Shemyakin, 1962; also Bolle *et al.*, 1968a,b; Snyder and Geiduschek, 1968; Geiduschek *et al.*, 1966, 1968; Milanesi *et al.*, 1969).

Purification of in Vitro Synthesized RNA. Reactions were stopped by adding sodium dodecyl sulfate to 1%, followed by sodium acetate, pH 5.2, to 0.1 M. An equal volume of water-saturated phenol was added and the suspension was heated in a 68° bath and mixed for 1–4 min. Alternate heating and mixing were repeated three times more. The mixture was then chilled in ice and centrifuged (10 min, 8000*g*). The aqueous phase was removed, more phenol added, and the extraction repeated (usually two to three times more) until no white film (DNA) could be seen at the H_2O–phenol interface. (Tetramethylurea–water and dimethyl sulfoxide–water mixtures require an extra extraction, because the first extraction removes the organic solvent from the aqueous phase and only after this does DNA appear at the H_2O–phenol interface.)

Phenol-extracted RNA was dialyzed against 1 M NaCl–0.01 M Tris-Cl, pH 7.5, and then against 0.01 M Tris-Cl, pH 7.5. Recoveries of RNA varied from 50 to 75%.

Occasionally this procedure has proved inadequate for removing all the DNA, especially sheared or sonicated DNA, from *in vitro* RNA. Such preparations were extracted twice with hot phenol; the aqueous layer was then extracted with diethyl ether and the ether removed by blowing N_2 over the sample (under sterile precaution). The RNA was brought to pH 7 and 0.02 M $MgCl_2$ and digested with pancreatic DNase (Worthington, chromatographically purified, 5–30 μg/ml) for 30 min at 37°. Afterwards, the pH was again lowered to 5.2 with acetate buffer and two additional hot phenol extractions done to remove DNase.

TABLE II: Restricted T4 RNA Synthesis with Excess *E. coli* RNA Polymerase.[a]

Asymmetry[b] (μg/ml of [^{3}H]RNA)	% RNase Resistance after Annealing
5.4	5.5
0.27 + 840 μg/ml of unlabeled T4 *in vivo* 5-min RNA	4.5
0.27 + 550 μg/ml of unlabeled T4 *in vivo* 20-min RNA	7.0
0.27 + 560 μg/ml of *E. coli* rRNA	3.7
0.27 + 33 μg/ml of T4 RNA synthesized *in vitro*	4
0.27, heated and quenched just before RNase digestion	0.7

[a] RNA synthesis as described in text with 10 μg/ml of T4 DNA and 200 units/ml of glycerol gradient purified *E. coli* RNA polymerase for 30 min at 37°. Approximately 3 μmoles of ribonucleoside triphosphate incorporated per μmole (nucleotide) of DNA template. [b] Methods, DNA–RNA Hybridization and RNA–RNA Duplex Formation.

TABLE III

Hybridization–Competition[a]	Cpm Hybridized	%
5.4 μg/ml [^{3}H]RNA, 1.6[b] μg/ml denatured T4 DNA	1325	100
Omit DNA	(32)	
5.4 μg/ml [^{3}H]RNA + 2530 μg/ml T4 5-min *in vivo* RNA	78	5.9
5.4 μg/ml [^{3}H]RNA + 2130 T4 μg/ml 20-min *in vivo* RNA	48	3.6

[a] See footnote *b*, Table II. [b] The abundant labeled RNA species are in excess over their DNA complements. Trichloroacetic acid precipitable radioactivity (2.5%) is bound to DNA in the absence of unlabeled competing RNA.

Scintillation Counting. All counting was done in a Packard Tri-Carb scintillation counter. Trichloroacetic acid precipitates were filtered onto Whatman glass GF/C filters, dissolved in 0.5 ml of hydroxide of Hyamine, and counted in toluene–Liquifluor (Pilot Chemical Co.) scintillation fluid with external standardization to determine the counting efficiency. Nitrocellulose filters were dried and counted in toluene–Liquifluor scintillation fluid.

Miscellaneous Materials. Electrophoretically purified pancreatic RNase was from Worthington Biochemicals. It was heated to 100° for 10 min in 2X SSC before use for all digestions. T1 RNase, purchased from Sankyo Ltd. (Tokyo), was heated to 80° for 10 min in 2X SSC, pH 5.5, for all digestions. These pretreatments inactivate DNases but also lower enzyme activity and slightly raise backgrounds in RNA–RNA duplex assays over what may otherwise be obtained.

Names of T4 RNA Species. T4 RNA species can be subdivided into several classes on the basis of time of first synthesis, prerequisites, and postreplicative abundance changes (Salser *et al.*, 1970). When the temporal sequence of viral transcription was first discovered (Hall *et al.*, 1963, 1964; Kano-Sueoka and Spiegelman, 1962; Khesin and Shemyakin, 1962), the names "early" and "late" (Jacob *et al.*, 1957) were used for the prereplicative and postreplicative collections of viral transcripts and various prefixes and qualifications have been added since. In these papers, we shall follow the nomenclature suggested by R. H. Epstein which is explained in Table I.

Results

Some Parameters of in Vitro Transcription. The program of *in vivo* RNA synthesis after T4 infection has been described in some detail (Hall *et al.*, 1964; Bolle *et al.*, 1968a,b; Salser *et al.*, 1970) and this allows one to use *in vivo* T4 RNA to analyze *in vitro* synthesized T4 RNA. In the present work, we have asked to what extent *in vitro* RNA made under a variety of conditions contains T4 RNA species that are synthesized *only* late in infection. The analytical method involves hybridization competition of labeled *in vitro* synthesized RNA by unlabeled RNA isolated from *E. coli* B 5 min (early) and 20 min (late) after T4 infection at 30°. The proportion of *in vitro* RNA corresponding to late *in vivo* RNA is, for the most part, the only parameter discussed in this paper, although it is not the only one accessible to analysis. For instance, some *in vitro* preparations of T4 early RNA can, in addition, be shown to have *all* or *nearly all* the early species (Geiduschek *et al.*, 1966) while others have only *some* of these species (Milanesi *et al.*, 1969). Another analysis of hybridization–competition assays, discussed in a subsequent paper (Brody and Geiduschek, 1970), provides some information about relative abundances of different RNA species.

The *in vitro* T4 RNA first reported to contain only early species (Geiduschek *et al.*, 1966) had been synthesized with *E. coli* RNA polymerase purified to the $(NH_4)_2SO_4$ step of Chamberlin and Berg (1962). Polymerase further purified in the three ways specified in section IIA yielded early, asymmetric RNA on native T4 DNA templates with limiting amounts of enzyme; when the three corresponding preparations of *in vitro* RNA were analyzed by hybridization, competition with unlabeled 5-min RNA was almost complete, more complete than with unlabeled 20-min RNA.

It is possible that RNA polymerase binding sites of early (classes I and II) and late (class III) transcription units on T4 DNA might differ quantitatively but not qualitatively in their affinity for *E. coli* RNA polymerase (*cf.* Stead and Jones, 1967). To test this possibility, RNA was synthesized at high enzyme excess and was found to be predominantly confined to early species. The hybridization–competition experiment which shows this (Tables II and III) has been designed to detect even a small fraction of late species by saturating DNA with *in vitro* [^{3}H]RNA. In this way, DNA binding of major *in vitro* RNA species is less than proportional to their abundance in the [^{3}H]RNA and rare transcripts are bound out of proportion to their abundance. Even so, only a very small fraction of the annealed radioactivity is subject to competition by 20-min but not by 5-min *in vivo* RNA.

TABLE IV: Selective Early T4 RNA Synthesis on Various T4 DNA Templates.

Preparation of DNA	Competition of *in Vitro* ^{3}H RNA: Per Cent of Hybridized Label Not Competed for by Unlabeled *in Vivo* RNA in a DNA Excess Competition Experiment				Antimessenger: RNase Resistance with 5-min *in Vivo* RNA	
	5-min RNA		20-min RNA			
	μg/ml	%	μg/ml	%	μg/ml	%
Pronase[a]	2250	4.5	2550	9		
Pancreatic RNase[a]	1850	4.5	2150	8.5		
Chloroform–isoamyl[b] alcohol extraction	3200	7.7	1825	22		
T4* DNA[c]	2350	5	2580	13		
Sheared DNA: enzyme excess[d]	3970	4	3850	7	960	10
Sheared DNA: DNA excess[e]	3970	4	3850	9	960	8
Sonicated DNA: enzyme excess[f]	2490	15	2370	16	730	24

[a] RNA synthesis: 30°, 30 min, 0.13–0.15 μmole of RNA synthesized per μmole of DNA template. [b] RNA synthesis: 30°, 40 min, 0.10 μmole of RNA synthesized per μmole of DNA template. [c] RNA synthesis: 37°, 45 min, 1.2 μmoles of RNA synthesized per μmole of DNA template. [d] Sheared DNA: $s_{20,w}$ = 14.4 at 20 μg/ml in 0.9 M NaCl; RNA synthesis: 37°, 45 min, 1.5 μmoles of RNA synthesized per μmole of DNA template. [e] Same DNA as *d;* RNA synthesis: 37°, 45 min, 0.13 μmole of RNA synthesized per μmole of DNA template. [f] Sonicated DNA: $s_{20,w}$ = 8.0 at 20 μg/ml in 0.9 M NaCl; RNA synthesis: 37°, 45 min, 1.2 μmoles of RNA synthesized per μmole of DNA template.

Enzyme-excess preparations using DEAE-Sephadex-purified enzyme are also asymmetric and early (data not shown), as are a number of other enzyme-excess preparations which will be discussed below. We conclude that those regions of the T4 genome which are *only* transcribed late in infection are not merely transcription units whose binding-initiation sites have somewhat lower affinities for host RNA polymerase.

While more RNA polymerase can be attached to DNA at low than at high ionic strengths, much of the bound enzyme is in a transcriptively inactive form (Pettijohn and Kamiya, 1967; Sternberger and Stevens, 1966; Richardson, 1966). The connection between this excess binding and selective early RNA synthesis has been investigated by making RNA at lower ionic strength with Tris-Cl, pH 7.5, 14 μmoles/ml instead of 100 μmoles/ml; 2.5 μmoles/ml of $MnCl_2$ substituted for 10 μmoles/ml of $MgCl_2$; and spermidine chloride omitted. Both the rate of incorporation (*cf.* Pettijohn and Kamiya, 1967) and the size of the longest RNA molecules are decreased when RNA is made at low ionic strength; after 60-min CTP incorporation is only 78%, and the mean sedimentation constant is 82% of a control sample of RNA made under standard conditions (see Materials and Methods, RNA Synthesis). Both DNA-excess and enzyme-excess RNA preparations are early and asymmetric. There is a slight difference between the DNA-excess and enzyme-excess RNA: the latter contains more antimessenger and is slightly less completely competed for by 5-min *in vivo* RNA.

Variations in Mature T4 DNA Preparation and Source. Phenol extraction of T4 phage removes protein very efficiently from DNA (Mandell and Hershey, 1962). Nonetheless, such protein as remains (or other contaminants) might affect transcription of late genes. Phenol-purified T4 DNA was further subjected to Pronase or pancreatic RNase (see Experimental Procedure). *In vitro* RNA made from these DNA preparations is restricted in the usual way—it is prereplicative and asymmetric (Table IV). A proposal has been made that the T4 internal protein (Hershey and Chase, 1952; Minagawa, 1961) may be responsible for blocking transcription of late genes, and, moreover, that phenol extraction is not sufficient to remove most of the internal proteins from T-even DNA (Bachrach and Friedman, 1967). As outlined in Materials and Methods (Preparation of T4 Phage and DNA), ^{35}S-labeled T4 phage has been extracted with phenol and subsequently deproteinized with chloroform–isoamyl alcohol (Bachrach and Friedman, 1967). When RNA is made *in vitro* from this DNA one finds that the restriction pattern is not fundamentally altered (Table IV).[1] The restricted early RNA synthesis evidently does not result from blockage by internal protein or other phenol-insensitive macromolecules.

T4 DNA contains glucosylated hydroxymethylcytosine as a replacement for cytosine (Wyatt and Cohen, 1953; Sinsheimer, 1956; Jesaitis, 1957; Volkin, 1954). However, the RNA made *in vitro* from unglucosylated T4* DNA (Hattman and Fukasawa, 1963) is asymmetric and early (Table IV).

Sheared and sonicated T4 DNA were also tested for late transcription. Shearing was effected by passing native T4 DNA through a 30-gauge, 0.5-in. needle 10–15 times at 4°. *In vitro*

[1] Some variations in the abundance distributions of different prereplicative species are not excluded. However this may be, in part, a property of different enzyme preparations.

TABLE V: Selective Early T4 RNA Synthesis with Vegetative T4 DNA.[a]

Vegetative DNA Used as Template	Method of DNA Extraction	Antimessenger		Hybridization–Competition of *in Vitro* [^{3}H]RNA: Per Cent of Hybridized Label Not Competed for by Unlabeled *in Vivo* RNA in DNA Excess Competition			
		% RNase Resistance	Unlabeled *in Vivo* 5-min RNA, μg/ml	5-min RNA		20-min RNA	
				μg/ml	%	μg/ml	%
T4 wild type	Phenol	1.8	540	2340	1.7	2100	9.5
T4 wild type	Phenol	9.5	480	2400	4.9	2300	6.9
T4 wild type	CsCl			2400	3.9	2300	4.7
am BL292	Phenol	2.9	540	2340	0	2100	0.4
am N134,BL292	Phenol	11.7	480	2400	11.1	2300	4.8

[a] RNA syntheses were for 30–60 min at 30° with 10 μg/ml of DNA and 18–20 units of glycerol enzyme per μg of DNA: yield, 3.5, 0.5, 1.0, 3.2, and 0.25 μmoles of RNA per mole of DNA for lines 1 to 5, respectively.

RNA made with sheared DNA ($s_{20,w}$ = 14.4 at 20 μg/ml; molecular weight *ca.* 3 × 10^6) at DNA or RNA polymerase excess contained only early species (Table IV; the symmetry was slightly higher than that of RNA made from native, intact DNA; Geiduschek *et al.*, 1968). Both the rate and extent of RNA synthesis on sheared DNA templates of this molecular weight are lower than with intact DNA, for synthesis at both DNA excess and enzyme excess. This may be due to the presence of long *in vitro* transcription units which are broken by the shearing process (Milanesi *et al.*, 1970). Hybridization-competition of this RNA (Table IV) shows a result rather similar to that encountered with denatured DNA—about 15% of the label is poorly competed for by 5 *or* 20 min *in vivo* RNA (*cf.* Brody and Geiduschek, 1970). Apart from this there is no clear-cut removal of the restriction to early transcription.

Vegetative T4 DNA Templates for in Vitro RNA Synthesis. SEDIMENTATION OF VEGETATIVE DNA. Newly synthesized viral DNA from wild type infected cells, 12–14 min after infection at 37° (labeled from 4 to 12 or 14 min with [^{14}C]-thymidine), contains DNA that sediments much faster than mature phage DNA (Frankel, 1966a). We call this DNA "300S vegetative" DNA, although the actual sedimentation coefficient of this fraction has not been determined accurately by us and is subject to substantial variation.[2] Extracts obtained from mutant lysates in either maturation defective gene (gene 55 or 33) contain practically all their DNA in a form which sediments at least two to three times faster than T4 am^+ vegetative DNA prepared under the same conditions, as previously shown by Frankel (1966b). Typical 20–70% sucrose gradient patterns for wild type and mutant extracts are shown in Figure 1A. In contrast to the T4 am^+ vegetative DNA, the very rapidly sedimenting DNA of maturation defective conditional lethal mutations is relatively insensitive to manipulation. For example, after dialysis, it can be rerun on sucrose gradients and has the same sedimentation rate, while T4 am^+ vegetative DNA, under the same conditions, appears as material sedimenting like mature phage DNA. The sedimentation profiles in Figure 1A suggest that the double-mutant *am* N134,BL292 (genes 33,55) vegetative DNA sediments even faster than the DNA from either single mutant, and a briefer sedimentation, shown in Figure 1B, establishes this point more definitely. Here ^{14}C-labeled vegetative *am* N134 and [^{3}H]*am* N134,BL292 DNA are run in the same gradient. The DNA from the single mutant sediments as a broad peak; the DNA from the double mutant sediments as a relatively sharp peak slightly faster than the single-mutant DNA. (The recovery of radioactivity from all gradients is between 75 and 100%.)

RNA SYNTHESIS ON VEGETATIVE DNA TEMPLATES. Sucrose gradient purified vegetative DNA was further purified by one of the two methods described in Materials and Methods. Zone sedimentation on CsCl (ρ = 1.507 g/cm^{-3}) gave approximate sedimentation coefficients of 95 S for the phenol-extracted DNA and 75 S for the equilibrium density gradient purified material, both kinds of preparations appearing to be relatively homogeneous in sedimentation rate. The different DNA preparations were first tested for their ability to serve as templates for RNA synthesis with limiting amounts of *E. coli* RNA polymerase. In general, the phenol extracted vegetative DNAs are not such good templates for RNA synthesis as T4 mature DNA or CsCl-purified wild-type vegetative DNA (data not shown). All single amber mutant DNA gave approximately the same incorporation rates as vegetative T4 am^+ DNA. [^{32}P]-RNA for hybridization–competition was synthesized on these templates at enzyme excess for 30–60 min at 30°.

All RNA preparations were extracted at least three times with hot phenol, dialyzed extensively, and tested for their asymmetry (Table V). The hybridization–competition analysis of the *in vitro* RNA preparations (Table V) shows very effective competition by 5-min *in vivo* RNA; 20-min *in vivo* RNA also competes very well, but (except against [^{3}H]RNA made with *am* N134,BL292 vegetative DNA) less completely than 5 min *in vivo* RNA. More detailed analysis over a range of concentrations of unlabeled competitor RNA (data not shown) also shows an important common feature of competi-

[2] Sucrose gradients (20–70%) loaded with lysates of 5.10^9 or 10^{10} cells are very viscous, and all sedimentation coefficients assigned in the original sucrose gradients are only rough estimates.

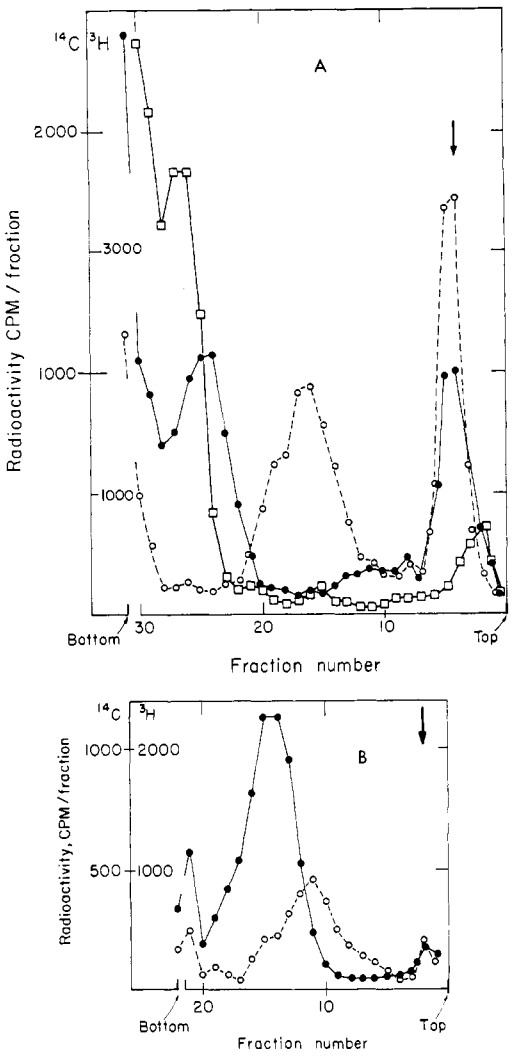

FIGURE 1: Sucrose gradient (20–70%) sedimentation of T4 vegetative DNA. Aliquots of lysates corresponding to 5 × 10^9–10^{10} T4 infected cells are centrifuged for 90 min, 15°, 70,000*g* (Spinco SW25 rotor at 22,000 rpm) in presence of ^{32}P mature T4 DNA marker (↓). (A) (○---○) ^3H-labeled T4 wild type DNA. (●---●) ^{14}C-labeled T4 *am* N134 DNA. As the single mutants *am* N134 and *am* BL292 gave approximately the same profile; only the *am* N134 pattern is shown. (□---□) ^3H-labeled T4 *am* N134, BL292 DNA. T4 wild-type and T4 *am* 134 [^{14}C]DNA were cosedimented as were T4 *am* 134 [^{14}C], and T4 *am* 134, 292 [^3H]DNA in another bucket of the same run. (B) Peak fractions of *am* N134,BL292 DNA from previous run were pooled, dialyzed extensively, and rerun for 1 hr, 22,000 rpm, 10°. (●—●) T4 *am* 134, 292 (^3H); (○---○) T4 *am* 134 (^{14}C).

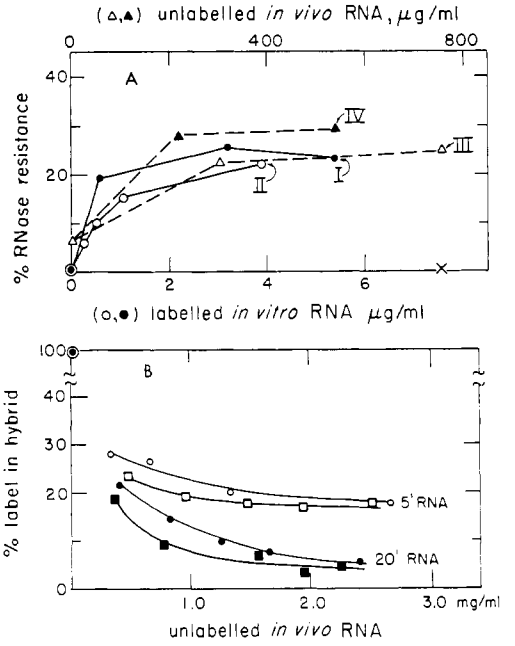

FIGURE 2: Properties of T4 RNA synthesized with *M. lysodeikticus* RNA polymerase. (A) Self-complementarity and antimessenger content. Curve I: (●) self-complementarity of RNA synthesized at DNA excess (RNA 1). Curve II: (○) self-complementarity of RNA synthesized at enzyme excess (RNA 2). (⊙) RNA 1 or 2 heat-denatured and quenched just before RNase digestion (plotted at zero RNA concentration). Curve III: antimessenger content of *in vitro* RNA 1 (enzyme excess, 0.26 μg/ml); conversion into RNase resistance by unlabeled T4 *in vivo* 5-min RNA (△). Curve IV: Same but RNA–RNA hybridization with unlabeled T4 *in vivo* 20-min RNA (▲). (×) Control; as above but no annealing, heated and quenched just before digestion. (B) Hybridization–competition. (○, ○) Hybridization–competition of RNA 1 with unlabeled T4 *in vivo* 5-min (○) and 20-min (●) RNA. Denatured T4 DNA (3.1 μg/ml), 0.11 μg/ml of ^3H *in vitro* RNA. Hybridization efficiency 7%; 100% hybridization = 710 cpm; 11 cpm bound to filter in absence of DNA. Analysis method I: (□,■) hybridization–competition of RNA 2 with unlabeled T4 *in vivo* 5-min (□) and 20-min (■) RNA. Denatured T4 DNA (10 μg/ml), 0.21 μg/ml of ^3H *in vitro* RNA. Hybridization efficiency 18%; 100% hybridization = 1026 cpm bound; 5 cpm bound to filter in absence of DNA. Analysis method II: conditions of synthesis: RNA 1, synthesis at DNA excess for 60 min in standard medium at 37°; yield: 0.4 μmole of RNA/μmole of DNA (nucleotide). RNA 2, synthesis at enzyme excess for 45 min in standard medium at 37°; yield: 5.2 μmole of RNA/μmole of DNA (nucleotide).

tion curves of these *in vitro* RNA samples made on vegetative DNA templates and RNA synthesized on mature T4 DNA (Brody and Geiduschek, 1970): 20-min *in vivo* RNA is a poorer competitor than 5-min *in vivo* RNA at all *in vivo* RNA concentrations. The only exception is that of RNA made with *am* N134,BL292 vegetative DNA (Table V, line 5) which is, for unexplained reasons, more symmetric. At low concentrations, 5-min *in vivo* RNA is also a better competitor than 20-min *in vivo* RNA for this *in vitro* RNA. The competition curves cross at higher competitor concentrations to yield the result shown in Table V, line 5. In line with considerations of the following paper we interpret this property of the *am* N134,BL292 RNA as due to a predominantly early (classes I and II) *in vitro* transcription and some nonspecific, symmetric transcription which is probably not an intrinsic property of this template. One can summarize the results of this section as showing that asymmetric RNA synthesized *in vitro* on vegetative T4 DNA templates with *E. coli* RNA polymerase is early RNA. A similar result was reported by Crouch and Hall (1967).

Transcription of T4 DNA by M. lysodeikticus RNA Polymerase. T4 DNA functions as a template at least as well for *M. lysodeikticus* RNA polymerase as for the *E. coli* enzyme (Figure 2). The synthesized RNA is, as previously reported, more symmetric than that made with the *E. coli* enzyme, but enzyme and DNA excess preparations are about equally symmetric. Figure 2A shows the antimessenger content and

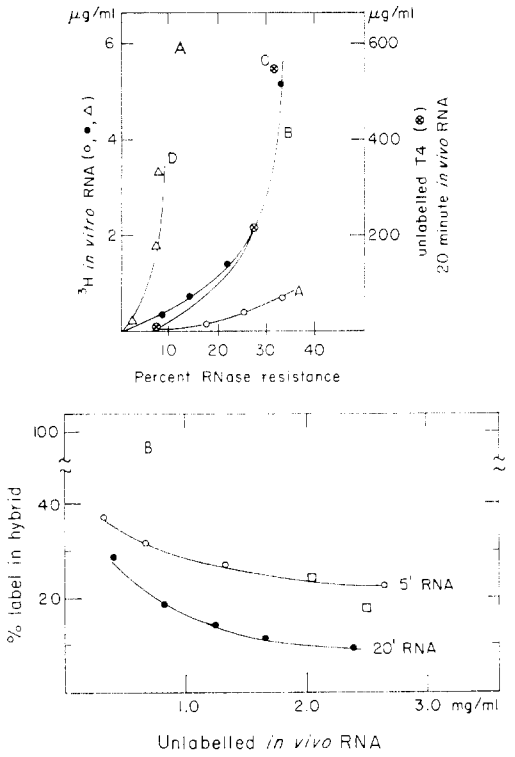

FIGURE 3: RNA synthesis with *M. lysodeikticus* RNA polymerase on denatured and sonicated T4 DNA templates. (A) Self-complementarity and antimessenger content. Curve A: (○) self-complementarity of RNA made with denatured DNA (RNA 1). Curve B: (●) self-complementarity of RNA made with sonicated DNA (RNA 2). Curve C: (⊗) antimessenger content of RNA 2: 0.36 μg/ml of *in vitro* RNA and varying quantities of T4 unlabeled 20-min RNA. Curve D: (△) self-complementarity of RNA synthesised in tetramethylurea–water mixture; see Figure 4. (B) Hybridization–competition. RNA 2: 10 μg/ml of T4 DNA, 0.27 μg/ml of *in vitro* [^{3}H] RNA. Hybridization efficiency 13%; 100% hybridization = 763 cpm; background 7 cpm. Analysis method II: (○) competition with 5-min RNA; (●) competition with 20-min RNA. RNA 1: 3.0 μg/ml of T4 DNA, 0.34 μg/ml ^{3}H *in vitro* RNA. Method I: not corrected for background; 100% hybridization (469 cpm) = 14% of trichloroacetic acid precipitable radioactivity; (□) competition with 5-min RNA. Conditions of synthesis: RNA 1, denatured DNA; 40-min synthesis at 50°; yield *ca.* 0.02 μmole of RNA per μmole of DNA (nucleotide); RNA 2, sonicated DNA ($s_{20,w}$ = 7.0); 45-min synthesis at 37°; yield 0.28 μmole of RNA per μmole of DNA (nucleotide).

self-complementarity of RNA synthesized at DNA excess and enzyme excess. Unlabeled 20-min *in vivo* RNA is a better competitor than 5-min RNA for these *in vitro* RNA preparations made with *M. lysodeikticus* enzyme (Figure 2B). The amount of label in late species is between 10 and 15%, compared with approximately 30–40% late RNA in labeled *in vivo* RNA 20 min after infection (Bolle *et al.*, 1968a). The competition of symmetric labeled RNA by unlabeled *in vivo* RNA must include the antimessenger since it is so extensive. We conclude that unlabeled *in vivo* RNA is able to complex labeled *in vitro* antimessenger and remove it from the annealing reaction to denatured DNA, since RNA–RNA duplexes pass through nitrocellulose filters (see Brody and Geiduschek, 1970, for further detail). The estimate of late RNA (10–15%) accordingly includes both sense and antisense species. Thus

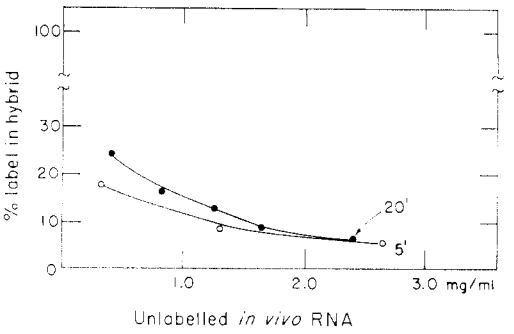

FIGURE 4: Hybridization–competition of T4 RNA synthesized with *M. lysodeikticus* RNA polymerase in 15% tetramethylurea: (○) competition with 5-min RNA; (●) competition with 20-min RNA; 0.08 μmole of RNA synthesized per μmole of DNA template (nucleotide) during 40 min at 37°. DNA excess hybridization–competition: hybridization efficiency 19%; 100% hybridization = 275 cpm; 6 cpm bound to filter in the absence of DNA. Analysis method II.

late T4 RNA synthesis is somewhat restricted with this preparation of *M. lysodeikticus* RNA polymerase, but not nearly to the same extent as with *E. coli* RNA polymerase.

In an effort to increase the proportion of late RNA without increasing the symmetry of *in vitro* RNA made from *M. lysodeikticus* RNA polymerase, many reaction conditions were manipulated, but without yielding the desired result. (a) RNA synthesized at DNA excess with sequential addition of *M. lysodeikticus* enzyme was slightly less symmetric (14% antilate RNA) than the related material described in Figure 2 and had, concomitantly, a lower proportion of late species (5% difference in competition by 2 mg/ml of late and prereplicative RNA, the former being the more complete competitor). (b) RNA synthesized with native T4 DNA and the relatively heat stable *M. lysodeikticus* RNA polymerase (Fox, 1964) at 50° is slightly less symmetric and contains less late RNA. (c) RNA synthesized at 50° with denatured or sonicated T4 DNA is substantially more symmetric (Figure 3A) but does not contain more late RNA (Figure 3B). Evidently the restriction to early T4 transcription is more difficult to disrupt than the asymmetry of transcription. With *E. coli* RNA polymerase, appearance of late RNA is also accompanied by an increase in RNA symmetry (Brody and Geiduschek, 1970). The implication of this result is that even the sites of "false" T4 RNA chain initiation by both enzymes are not randomly distributed on the T4 template. Accordingly, asymmetric synthesis with *M. lysodeikticus* RNA polymerase should be accompanied by a restriction to early species. We have made asymmetric RNA *in vitro* with the *M. lysodeikticus* RNA polymerase, and it fulfills this expectation. The conditions of synthesis are somewhat bizarre, but native T4 DNA and *M. lysodeikticus* RNA polymerase in 15% (v/v) tetramethylurea yield substantially asymmetric RNA (Figure 3A, curve D). As expected, this RNA is restricted to early species (Figure 4). It seems paradoxical that conditions similar to those that yield symmetric transcription with *E. coli* RNA polymerase (see below) should restrict the transcription with *M. lysodeikticus* RNA polymerase. It also seems paradoxical that one should be unable to explain a change of transcription specificity in terms of initiation (σ) factors. However, these two enzymes operate *in vivo* in rather different

internal milieux (Mitchell and Moyle, 1956; Epstein and Schultz, 1965). Tetramethyl urea, which lowers the dielectric constant of the reaction solvent, presumably promotes ion association at the same time that it tends to disorder the secondary structure of DNA (*cf.* Herskovits, 1963) and perhaps also that of the enzyme. The *M. lysodeikticus* polymerase is quite thermostable and perhaps more resistant to solvent denaturation. Clearly, further studies would be required to understand this intriguing relationship between two RNA polymerases. Our experiment is presented here in quite another context—that of the relationship of selective early transcription to asymmetry of the synthesized RNA.

T4 RNA Synthesis with E. coli RNA Polymerase in Me_2SO–Water Mixtures. Our attempts to force late T4 RNA synthesis *in vitro*, by manipulating the mature viral template and the host RNA polymerase, have included syntheses in water–Me_2SO mixtures. Me_2SO is known to be a relatively potent DNA denaturing solvent (Helmkamp and Ts'o, 1961; Herskovits, 1962). It is not a potent irreversible inactivating agent of proteins in general (Singer, 1962) or of RNA polymerase. Even Me_2SO concentrations that almost completely abolish RNA synthesis (Figure 5A) lead to only slow enzyme inactivation (data not shown). RNA synthesis can be sustained for hours in the presence of relatively high proportions of Me_2SO. The synthesized RNA is relatively symmetric under all the Me_2SO synthetic conditions that have been employed (Figure 5B). Such high symmetry suggests comparison with transcription on denatured DNA templates. Chain termination is relatively frequent when RNA is transcribed from single-stranded DNA (Maitra *et al.*, 1967). On the contrary, transcription in Me_2SO–water mixtures resembles transcription of double-helical DNA, in that termination of RNA chains is a relatively infrequent event (data not shown). Evidently, RNA chains are slowly propagated in Me_2SO–water solvents for a relatively long time. Hybridization competition of RNA synthesized in 25% and 35% Me_2SO shows the following result: a small fraction of this synthesized RNA is in competition with 20-min *in vivo* but not with 5-min *in vivo* RNA (Figure 5B). On the other hand, this relatively small proportion of late RNA synthesis is concomitant with substantial symmetry for all Me_2SO synthetic conditions that have been employed. These data fit a general relationship correlating late and symmetric T4 RNA synthesis *in vitro* (Brody and Geiduschek, 1970).

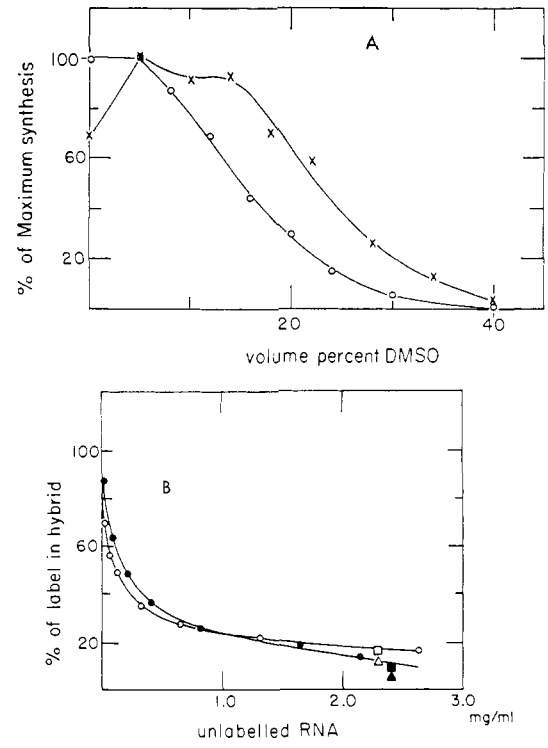

FIGURE 5: RNA synthesis in Me_2SO–water mixtures. (A) Enzyme activity as a function of solvent composition: (○) 15-min synthesis at 30°. The reaction mixture contained, per ml: 100 μmoles of Tris-Cl, pH 7.5, 1 μmole of ATP, UTP, and GTP, 0.1 μmole of [^{3}H]CTP, 10 μmoles of $MgCl_2$, 10 μmoles of β-mercaptoethanol, 20 μg of T4 DNA, and 30 units of *E. coli* RNA polymerase. (×) 60-min synthesis at 30°. The reaction mixture contained 100 μmoles of Tris-Cl, pH 7.5, 100 μmoles of ammonium acetate, 1 μmole each of ATP, GTP, and UTP, 0.1 μmole of [^{3}H]CTP, 5 μmoles of $MgCl_2$, 10 μmoles of β-mercaptoethanol, 20 μg of T4 DNA, and 6.5 units of RNA polymerase. (B) DNA excess hybridization–competition of T4 RNA synthesized in Me_2SO–water mixtures. The three RNA samples (A, B, C) are described in Table VI.

Discussion

There have been numerous reports of selective transcription of viral templates by bacterial and animal RNA polymerase (Geiduschek *et al.*, 1966, 1968; Naono and Gros, 1966; Furth *et al.*, 1968; Summers and Siegel, 1969). Yet such reports invariably have been fragmentary and have addressed themselves only to the question of whether selective transcription was possible. In the work reported here, we set ourselves the task of showing that the restriction of asymmetric T4 *in vitro* transcription by bacterial RNA polymerase to early RNA extends essentially to the entire range of experimental conditions. By implication, this restriction must be a fundamental property of the transcription mechanism of T4 DNA. We have been able to obtain some synthesis of *late* RNA *in vitro* by destroying the general specificity of the transcription reaction. Nevertheless even complete denaturation of T4 DNA yields relatively little late RNA synthesis. The implication is that late and early T4 RNA are initiated at sites that are very different.

Naturally, these experiments demonstrating the selectivity of early transcription do not of themselves specify how late regions of the genome are, in fact, transcribed. Nevertheless, a number of simple and otherwise plausible models have been ruled out by the present work and the accompanying paper. "Opening up" the template to various degrees by changes in temperature, solvent conditions, and denaturation of the template does not lead to synthesis that mimics late *in vivo* transcription. Neither can this change be effected by varying the proportions of RNA polymerase to DNA which is, in a formal sense, analogous to changing gene dosage.

Viral DNA replication has been shown to be required for T4 late RNA synthesis *in vivo* (Bolle *et al.*, 1968b). Nonetheless, purified replicating intracellular T4 DNA as a template for *E. coli* RNA polymerase still does not give late T4 RNA synthesis. Of course, the procedures used to purify vegetative DNA for RNA synthesis yield a material that, although it sediments more rapidly than mature T4 DNA and is most probably larger than mature T4 DNA (Frankel, 1968), is

TABLE VI: Description of Symbols in Figure 5.

Symbols[a]	Conditions of Synthesis	Yield (μmole of product/ μmole of template)	Temp (°C) and Time of Synthesis (hr)	Antimessenger Assays: % RNase resistance
A: ○, ●	25% Me_2SO and standard reaction mixture. DNA excess	0.18	30, 0.75	22% (0.08 μg/ml of [^{3}H]RNA with 600 μg/ml of unlabeled 5-min RNA); 32% (0.08 μg/ml of [^{3}H]-RNA with 600 μg/ml of unlabeled 20-min RNA)
B: □, ■	25% Me_2SO, 100 μmoles of Tris-Cl, pH 7.5, 1 μmole of GTP, ATP, and UTP; 0.1 μmole of [^{3}H]CTP, 5 μmoles of $MgCl_2$, 10 μmoles of β-mercaptoethanol/ml. DNA excess	0.27	30, 3	40% (0.15 μg/ml of [^{3}H]RNA, 510 μg/ml of unlabeled 5-min, and 435 μg/ml of unlabeled 20-min RNA)
C: △, ▲	35% Me_2SO, 100 μmoles/ml of ammonium acetate, rest as B	0.066	30, 3	39.4% (same mixture as above)

[a] Open symbols: competition by unlabeled 5-min RNA; closed symbols: competition by unlabeled 20-min RNA.

not as large as the native vegetative DNA (Figure 1; Frankel, 1966a; Huberman, 1969). Thus, in spite of the results reported here, one might cling to the notion that contiguity of the vegetative, catenated DNA constitutes the sole requirement for late transcription *in vitro*, but this seems intrinsically implausible. Selective transcription is a property of initiation which must occur at very many sites for the late cistrons (Salser *et al.*, 1970) and it is difficult to construct a model for the *in vitro* transcription of many units of $0.5–5 \times 10^3$ nucleotide pairs (as bare DNA) that depends on the intactness of 1000 times that many nucleotide pairs. *In vivo* experiments to be reported elsewhere (Diggelmann *et al.*, 1970) make such a hypothesis even less tenable. The experiments which we have described here show directly that the transcription restriction pattern observed with mature DNA holds for DNA ranging in size from 8 to 95 S.

It is known that at least two genes not concerned with DNA synthesis are involved in late messenger production *in vivo*. These are the two maturation genes, 33 and 55 (Epstein *et al.*, 1963; Bolle *et al.*, 1968b); the gene 55 product appears to be continuously required for expression of late genes (Pulitzer, 1970). *In vitro* late T4 RNA synthesis dependent on the gene 55 product has been demonstrated (Snyder and Geiduschek, 1968). Yet at least one subunit of the host RNA polymerase continuously functions for all viral transcription throughout phage T4 development (Haselkorn *et al.*, 1969). One might therefore postulate that the product of gene 55 (and 33) modifies newly replicated T4 DNA for transcription by the host RNA polymerase. The experiments showing vegetative DNA from T4 am^+ and *am* (genes 33, 55) infected cells equally incompetent for late transcription with the host polymerase argue against this model of the switch from early to late transcription.

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