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Phage T4-modified RNA polymerase transcribes T4 late genes *in vitro*

(asymmetric transcription/positive regulation/virus development/streptolydigin/cellophane)

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ABSTRACT **Initiation of T4 late RNA synthesis has been achieved in an *in vitro* system prepared from *Escherichia coli* cells infected with wild-type or maturation-defective mutant T4 phage. The system uses a cellophane membrane as a mechanical support for concentrated cell lysates and for added streptolydigin-resistant RNA polymerases. Transcriptional activity and selectivity of added RNA polymerases are tested while endogenous RNA polymerase activity is inhibited by streptolydigin. T4-modified RNA polymerase is required for substantial stimulation of T4 late RNA synthesis.**

The control of gene expression during the development of T4 and other phage is mainly exerted at the transcriptional level. It is useful to oversimplify the transcription program in terms of three main classes of T4 genes that are expressed at characteristic times during the T4 infectious cycle: (*i*) transcription of early genes, which starts within the first minute after infection; (*ii*) middle (frequently called "quasi-late") transcription which follows at about 2 min (at 30°); and (*iii*) transcription of the late genes, which almost exclusively code for structural and assembly proteins of the virus and starts about 9 min after infection. Some, but not all, early and middle genes are also transcribed later in the lytic cycle. Lates gene expression, *in vivo*, depends on concurrent T4 DNA replication and on the function of several low molecular weight T4-specific proteins that bind to RNA polymerase. These T4 proteins are gene products 33, 45, and 55 and two additional proteins with molecular weights of 15,000 and 10,000. The presence of hydroxymethylcytosine instead of cytosine in the T4 DNA template is also normally necessary for synthesis of late proteins (for review, see ref. 1).

Some of this regulatory complexity has been recognized for many years (2–4), yet its biochemical analysis has been greatly hampered by the lack of efficient *in vitro* systems. Purified RNA polymerases from uninfected or T4-infected *Escherichia coli* either transcribe T4 early genes (in the presence of σ) or transcribe intact T4 DNA very poorly (in the absence of σ). Even isolated vegetative T4 DNA does not permit transcription of late genes with purified RNA polymerases. Crude systems from T4-infected cells have been reported to synthesize late RNA *in vitro* (5–8). Various properties of these systems, and of another *in vitro* system using mature T4 DNA (9), have discouraged their use for deeper analysis of the late transcription process.

We have developed an *in vitro* system that yields a high fraction of late transcripts, initiates late RNA chains, and is active for at least an hour (ref. 10, and unpublished data). When isolated from appropriate genetic backgrounds, the system can also be used for *in vitro* complementation assays of purified components. Here we describe the complementation of T4 late transcription by added T4-modified RNA polymerase. These experiments open the way to a functional analysis of the different subunits of T4-modified RNA polymerase and of certain aspects of DNA template "competence" (1).

MATERIALS AND METHODS

Solutions and Chemicals. Streptolydigin (stl) was a gift of The Upjohn Co., Kalamazoo. Ribonucleoside and deoxyribonucleoside triphosphates were from P-L Biochemicals; [^{3}H]UTP and [^{14}C]thymidine were from Schwarz/Mann.

Bacterial and Phage Strains. *E. coli* B^E (su$^-$) was used as the standard host strain. Streptolydigin-sensitive (stl^s; e.g., strain DPR 4) and streptolydigin-resistant (stl^r; e.g., strain DPR 4-10) mutants of *E. coli* B^E were isolated essentially according to Schleif (11) and their RNA polymerases were tested as described (12). stl blocks RNA chain elongation (11).

The following T4 maturation-defective mutants were used: *am* N134-*am* C18 (double mutant in gene 33, designated below as 33$^-$); *am* BL 292 (gene 55); and the triple mutant *am* N134-*am* C18-*am* BL292. T4 *am* M41 (gene *e*, lysozyme), which shows a completely normal development but does not lyse the host cell, was used as "wild type."

RNA Polymerases. Holoenzyme was prepared from *E. coli* DPR 4-10 (stl^r) by a combination of described methods (13, 14). It had a specific activity of 53.0 units/mg with calf thymus DNA and a T4/calf thymus DNA specific activity ratio of 2.2. [One unit of RNA polymerase is the quantity of enzyme that incorporates 1 nmol of ATP into RNA in a standard assay (15) in 1 min.] Core enzyme was prepared by the method of Berg *et al.* (16). The enzyme had a specific activity of 6.2 (calf thymus) units/mg and a T4/calf thymus DNA specific activity ratio of 0.3. Only β, β', and α subunits could be seen on stained 10% polyacrylamide gels when 2 μg of enzyme was electrophoresed in a 5 × 0.8 mm channel. T4-modified RNA polymerase was isolated from log phase DPR 4-10 cells infected with T4 e$^-$ (8 plaque forming units per cell; 15 min at 37°), by essentially the methods that had been applied to the holoenzyme. The specific activity was 17.9 (calf thymus) units/mg and the T4/calf thymus DNA specific activity ratio was 0.25. Only four phage-coded subunits, β, β', α, and a trace of σ, were detected on gel electrophoresis when 3 μg of enzyme was run in a 9 × 0.8 mm channel. All RNA polymerases were stored in TMA-

Abbreviations: stl, streptolydigin; stl^s, stl-sensitive; stl^r, stl-resistant; SSC, standard saline–citrate (0.15 M NaCl/0.015 M Na citrate, pH 7); CM RNA, chloramphenicol RNA, a RNA sample from *E. coli* infected with T4 for 5 min (30°) in the presence of chloramphenicol. gp n, protein coded by gene n; MD, designation of maturation (late transcription)-defective mutants; T4 n$^-$, T4 *am* mutant in gene n; c.e., cell equivalents; *r*-transcription and *r*-transcript refer to the process and product of RNA synthesis that uses the T4 DNA *r* strand as template; PA RNA, a preannealed mixture of "early" and "late" RNA from T4-infected *E. coli*.

glycerol buffer (0.025 M Tris acetate, pH 7.4/5 mM Mg acetate/10 mM ammonium chloride/1 mM mercaptoethanol/50% glycerol) at −20°. These stocks also contain residual $(NH_4)_2SO_4$ in the range of 0.1–0.2 M.

Preparation of the *In Vitro* System. The procedure is based on the method of Schaller *et al.* (17). *E. coli* B^E were grown in 25 ml of Penassay broth at 37° in a rotary shaker bath (generation time, 25 min) to $OD_{650} = 0.15$ (0.6×10^8 cells per ml), infected with T4 e^- (10 plaque forming units per cell), and supplemented with [2-^{14}C]thymidine (0.01 μCi/ml, 53 Ci/mol). Survivors (usually less than 0.1%) were determined after 1 min. At 18 min after infection, cells were rapidly chilled, centrifuged, resuspended in 1 ml of TGNO buffer (per liter, 0.5 g of NaCl, 8 g of KCl, 1.1 g of NH_4Cl, 0.2 g of $MgCl_2$, 12.2 g of Tris·HCl, pH 7.4, 0.8 g of pyruvic acid, 0.16 mM Na_2SO_4, and 2 mM $CaCl_2$), recentrifuged, and resuspended in 62 μl of TGNO buffer.

One microliter of lysozyme solution (Worthington Biochemical Co., Freehold; 1 mg/ml in 0.1 M potassium 4-morpholinopropanesulfonate, pH 6.5) was spread onto a cellophane disc (Kalle Einmach Cellophan, W. Germany) on agar plate A (at 5°). [Agar plates A and B were as in ref. 17 except that Noble agar (Difco) and 10 mM $MgCl_2$ were used.] One microliter of the cell suspension (approx. 1.5 to 3×10^7 cells) was applied per disc and spread out. After 30 min at 5°, 1 μl of RNA polymerase (or TMA-glycerol buffer) was applied and the disc was transferred to agar plate B. Plate B was kept closed at 5° for 30 min, then air (at 5°) was blown over the discs to evaporate all "excess" liquid (this was judged visually).

***In Vitro* RNA Synthesis.** The cellophane discs were removed from the agar plate (5°) and placed on a 50-μl drop of incorporation mixture (1 mM ATP/CTP and GTP at 65 μM each/[5-^{3}H]UTP (12 Ci/mol), and unlabeled UTP in varying proportions at a total concentration of 65 μM/dATP, dCTP, dGTP, and dTTP at 20 μM each/1.25 mM uridine/170 μM thymidine/50 μM dithiothreitol/2.5 mM phospho*enol*pyruvate/100 μM NAD^+/20 mM K 4-morpholinopropanesulfonate, pH 7.4/100 mM KCl/5 mM $MgCl_2$). Drops of incorporation mixture were placed in a closed plastic petri dish kept on a thermostated brass plate at 25°. The temperature, measured in the drops, was 23°–24°. Standard incorporation time was 30 min. The reaction was stopped by transferring the disc into a tube with 1.5 ml of stop solution (1% sodium dodecyl sulfate/0.05 M EDTA/10% saturated $Na_4P_2O_7$/denatured salmon sperm DNA at 17.5 μg/ml; pH 7.0). The sample was heated in boiling water for 2 min, chilled, precipitated with 1.5 ml of 10% Cl_3CCOOH, filtered through a nitrocellulose membrane, washed with 2.5% Cl_3CCOOH and 70% ethanol, dried, and assayed for radioactivity in a toluene-based scintillation liquid. Radioactivity incorporated into RNA was normalized for 10^8 cell equivalents by using the internal standardization provided by the [^{14}C]thymidine incorporated into DNA. (The latter was determined by precipitating a sample of the bacterial culture of known optical density immediately after harvesting.) If the *in vitro* synthesized RNA was to be characterized by hybridization experiments, [^{3}H]UTP (10 Ci/mmol) was used and RNA synthesis was stopped by placing the disc in 0.5 ml of 0.1 M EDTA/0.25% sodium dodecyl sulfate (pH 7.3), boiling for 2 min, adding sodium acetate (pH 5.2) to 0.1 M, extracting with water-saturated phenol at 70°, and dialyzing against 0.1 × standard saline–citrate (SSC; 0.15 MNaCl/0.015 MNa citrate, pH 7)/1 mM EDTA.

Analysis of RNA. Synthesis of late T4 RNA *in vitro* was characterized by hybridization to the separated strands of T4 DNA (18) and by RNA·RNA duplex formation (19). Because almost all early RNA is transcribed off the *l* strand, while the overwhelming part of late RNA is transcribed from the *r* strand of T4 DNA, hybridization of an RNA sample to the separated T4 DNA strands, in excess, yields a measure of the ratio of early to late transcripts, *provided that the symmetry of transcription is low.* The percentage of RNA hybridized to the T4 DNA *r* strand was calculated as: (cpm hybridized to $r \times 100$)/(cpm hybridized to l + cpm hybridized to r). The hybridization efficiency was always greater than 70%. The symmetry of *in vivo* and *in vitro* RNA was assayed by RNA·RNA duplex formation with an excess of unlabeled T4 *in vivo* RNA. Two unlabeled RNA probes were used.

The simpler probe consisted of RNA extracted 5 min after infection of *E. coli* B^E at 30° with wild-type T4 phage in the presence of chloramphenicol (CM RNA) at 100 μg/ml. This RNA contains no antimessage (19) and consequently serves as a probe for detecting the *in vitro* synthesis of RNA that is complementary to immediate early mRNA.

The more sensitive unlabeled RNA probe (PA RNA) was prepared by annealing 6.4 mg of RNA extracted 5 min after infection of *E. coli* B^E with T4 e^- phage at 30° (5 min *in vivo* RNA) with 14.9 mg of 20 min *in vivo* RNA for 4 hr at 70° in 3.6 ml of 2 × SSC. The PA RNA probe sequesters *in vivo* antimessage in RNA·RNA duplexes and is therefore suitable for detecting *in vitro* synthesis of RNA that is complementary to most (or all) T4 mRNA. RNA·RNA duplexing samples contained 2.6 ng of *in vitro* [^{3}H]RNA per ml, no added RNA, and PA RNA or CM RNA at 590 or 925 μg/ml, respectively. Annealing and digestion of unduplexed RNA was as described in the legend of figure 2 of ref. 19. Samples were precipitated with Cl_3CCOOH immediately after addition of $Na_4P_2O_7$ and denatured salmon sperm carrier DNA. After filtration through GF/C filters (Whatman) and washing with 2.5% Cl_3CCOOH and then with 70% ethanol, samples were dried, digested with 0.1 ml of H_2O and 0.9 ml of NCS tissue solubilizer (Amersham/Searle) for 30 min at 50°, and assayed for radioactivity in toluene-based scintillation fluid.

RESULTS

The conditions for complementation of true late transcription in this *in vitro* system evolved from numerous experiments, only some of which are presented here; a detailed description of the system and its properties will be published elsewhere. The goal of the experiments here was to characterize *de novo* RNA synthesis initiated by various kinds of exogenous stl^r RNA polymerases while the endogenous RNA polymerase of the system was inhibited by stl.

The determination of the optimal concentration of stl is shown in Fig. 1. stl inhibited [^{3}H]UTP incorporation by endogenous (stl^s) RNA polymerase almost as completely as did EDTA or withholding of three of the four ribonucleoside triphosphates (data not shown). However, the stl^r RNA polymerase also was considerably inhibited at higher stl concentrations. We chose a stl concentration (40 μg/ml) at which the endogenous stl^s RNA ploymerase was greatly inhibited (90–95%) but the exogenous RNA polymerase still retained about 60% of its activity. Different concentrations of stl up to 200 μg/ml did not affect the r/l ratio of endogenous T4 transcription (data not shown).

To demonstrate complementation of late T4 transcription *in vitro*, cellophane disc cell-free systems were prepared from *E. coli* infected either with T4 wild type or with T4 maturation-defective mutants (in genes 33 or 55). The latter permit T4 DNA replication but are late transcription-defective. The T4 wild type-derived system had a high level of endogenous

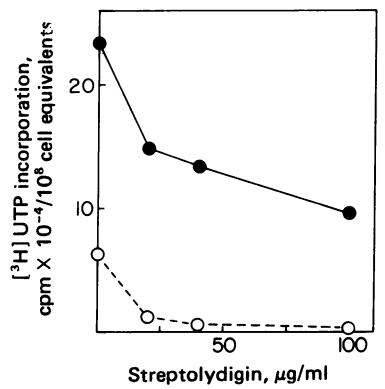

FIG. 1. Inhibition of endogenous and exogenous RNA polymerase activity by stl. The cell-free system was prepared from *E. coli* B^E infected with T4 *am* BL292 (gene 55). TMA-Glycerol (○) or 0.64 μg of stl^r T4-modified RNA polymerase (●) was added to each disc. The incorporation mixture contained the concentrations of stl shown; 1900 cpm corresponds to 1 pmol of UMP incorporated into RNA. *In vitro* reaction time was 30 min. Background (2200 cpm/10^8 cell equivalents for the endogenous activity; 4900 cpm/10^8 in the presence of added RNA polymerase) has not been subtracted. The average cell equivalents per disc was 1.6×10^7.

transcription of T4 late genes and was relatively poorly stimulated by exogenous T4-modified RNA polymerase (Table 1). The stimulatory effect was much greater in systems derived from cells infected with T4 MD mutants (Table 2). Most of the experiments were therefore carried out with *in vitro* systems from T4 maturation-defective mutant-infected cells.

Relative Rate of Late RNA Synthesis Varies with Amount of Added RNA Polymerase. The degree of complemented late transcription depended significantly on the amount of T4-modified RNA polymerase applied to each disc (Fig. 2). RNA synthesis in the presence of stl was almost entirely dependent on exogenous stl^r RNA polymerase. Two different *in vitro* systems, derived from T4 e^- and T4 55^- infected cells, were tested. Total and *r*-strand-specific RNA synthesis showed a different response to the addition of RNA polymerase in the two systems. The highest proportion of *r*-transcription was obtained with 0.3–1.5 μg—that is, with limiting quantities of RNA polymerase per disc. For the complementation experiments that follow, 0.64 μg of RNA polymerase was therefore chosen as the standard amount. The average number of cell equivalents per disc was 1.7×10^7 for the T4 55^- system and 1.5×10^7 for the T4 e^- system. If one assumes that there are 7000 β subunits in each *E. coli* cell (20), then 0.64 μg per disc represents an approximately 6-fold excess of exogenous over endogenous RNA polymerase subunits.

Host Holo- and Core-RNA Polymerases Do Not Stimulate T4 Late Transcription. The effect of various RNA polymerases in these different cell-free systems is shown in Tables 1 and 2. Each system was complemented with stl^r RNA polymerases in the absence and presence of stl. A sample of stl^s T4-modified RNA polymerase (a gift of R. Mailhammer) was also tested. Total and *r*-strand-specific RNA synthesis is given for each combination. In all three systems, T4-modified RNA polymerase caused the greatest stimulation of *r*-transcription (Table 1, lines 1–4, column 5; Table 2, lines 1–4, columns 5 and 10). This was true for both stl^r and stl^s T4-modified enzymes. The unmodified host core enzyme had lower total activity than the T4-modified enzyme (Tables 1 and 2, lines 3, 4, 9, and 10, column 3; Table 2, column 8) and generated a much lower proportion of *r*-transcript. Thus, T4-modified enzyme provides an 8- to 10-fold stimulation of *r*-transcription in the T4 55^- and 33^-, 55^- systems relative to *E. coli* polymerase core (Table 2,

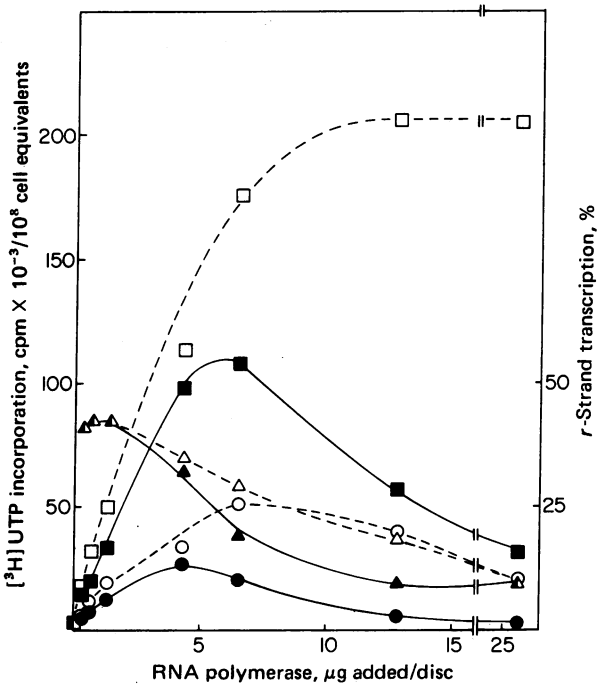

FIG. 2. Total and *r*-strand specific transcription as a function of RNA polymerase concentration. Cell-free systems from T4 *am* M41 (gene *e*)- and T4 *am* BL292 (gene 55)-infected *E. coli* B^E were prepared. Various amounts of T4-modified RNA polymerase, in 1 μl of TMA-glycerol, were added to top of the discs. The incorporation mixture contained stl at 40 μg/ml. Solid lines and solid symbols represent the T4 e^- system; broken lines and open symbols represent the T4 55^- system. □, ■, Total RNA synthesis (cpm/10^8 cell equivalents); ○, ●, *r*-strand specific RNA synthesis (cpm/10^8 cell equivalents); △, ▲, *r*-strand specific RNA synthesis (%); 375 cpm corresponds to 1 pmol of UMP incorporated into RNA. The symmetry of the various RNA samples, measured as RNA·RNA duplexes formed with PA RNA, varied between 5.0 and 7.5%. The endogenous RNA synthesis activity of the T4 e^- system was 43,500 cpm/10^8 cell equivalents (56% *r*-transcript) in the absence and 2840 cpm/10^8 cell equivalents in the presence of stl (40 μg/ml). For the T4 55^- system the numbers were 11,000 (8% *r*-transcript) and 1400 cpm/10^8 cell equivalents, respectively. There were, on the average, 1.5 and 1.7×10^7 cell equivalents per disc in the T4 e^- and T4 55^- systems, respectively.

lines 1, 3, 7, and 9, columns 5 and 10; data in *italics*). In fact, it is remarkable that the *E. coli* core enzyme generates so high a proportion of *l*-transcript when added to a cell extract whose T4 DNA is known to contain many breaks and gaps. This effect is most likely due to an interaction of the exogenous *E. coli* core enzyme with the endogenous σ protein. *E. coli* holoenzyme greatly boosted *l*-transcription but had little effect on *r*-transcription (Tables 1 and 2, lines 1, 2, 7, and 8). In each complementation system, whether in the presence of absence of stl, *E. coli* holoenzyme generated less than 3% *r*-transcript (line 7 minus line 1; line 8 minus line 2; columns 3 and 5 of Tables 1 and 2; columns 8 and 10 of Table 2). This value is significantly above the background of the assay, which is determined by the purity of the isolated DNA *r* strands and by the stringency of the hybridization conditions. However, it is not significantly above the level of *r*-transcription in conventional *in vitro* RNA systhesis with mature or vegetative T4 DNA. The level of *r*-transcription *in vivo* in the absence of functional T4 gene products 33, 45, and 55 is also of the order of 2–4%. We have therefore not pursued the analysis of this low level of *r*-transcription by *E. coli* holoenzyme although it may ultimately provide information that is relevant to the regulation of late transcription. The relative stimulation of *r*-transcription in the

Table 1. Complementation of cell-free system derived from T4 *am* M41 (gene e)-infected cells with different RNA polymerases and properties of reference RNAs

1	2	3	4	5	6	7	8
Additions		Total transcription,	*r* transcription		Asymmetry, % RNA·RNA duplex with		
RNA polymerase	stl	cpm/10^8 c.e.*	%	cpm/10^8 c.e.*	No RNA	CM RNA	PA RNA
1. None	+	1,860	50	*930*	1.6	6.7	5.9
2. None	−	21,340	44	9,390	2.4	6.7	6.1
3. T4-mod. (stl^r)	+	13,480	35	*4,720*	1.5	5.1	6.4
4. T4-mod. (stl^r)	−	35,360	42	14,850	2.6	6.6	6.5
5. T4-mod. (stl^s)	+	3,600	47	*1,690*	2.1	4.0	4.8
6. T4-mod. (stl^s)	−	24,550	48	11,800	2.1	5.0	7.3
7. *E. coli* holo (stl^r)	+	49,240	5	*2,460*	1.5	1.9	4.4
8. *E. coli* holo (stl^r)	−	103,680	9	9,330	0.2	0.6	4.3
9. *E. coli* core (stl^r)	+	8,900	20	*1,780*	0.8	2.1	8.1
10. *E. coli* core (stl^r)	−	28,330	38	10,770	2.8	3.0	7.4
11. *In vivo* RNA (17–19 min, 37°)	—	—	64	—	1.0	3.0	3.0
12. Symmetric *in vitro* RNA	—	—	48	—	2.0	14.0	35.0

The *in vitro* system was prepared from *E. coli* B^E infected with T4 e^-. TMA-glycerol, T4-modified RNA polymerases, *E. coli* holo-, or *E. coli* core-RNA polymerases were added to the discs, as indicated (1 μl; 0.64 μg of RNA polymerase). stl was present in the incorporation mixture, where indicated, at 40 μg/ml. Total RNA synthesis was measured by incorporation of [^{3}H]UTP; 380 cpm corresponds to 1 pmol of UMP incorporated into RNA. The average c.e./disc was 1.5×10^7. Symmetric *in vitro* RNA was synthesized with RNA polymerase II from *Saccharomyces cerevisiae*, with heat-denatured T4 DNA as template.

* c.e., cell equivalents.

T4 wild-type system, which already has a high level of endogenous *r*-transcription, was low compared to the late transcription-defective systems (compare lines 1–4, Tables 1 and 2). The RNA product was, in all cases, largely asymmetric (compare all other data concerning asymmetry with Table 1, lines 11 and 12).

DISCUSSION

It has been clear for some time that the regulatory genes of phage T4 late transcription code for RNA polymerase-binding proteins (21–24). Yet the lack of a suitable *in vitro* system has hampered the molecular analysis of this positive transcriptional regulation. As we show here, the cellophane disc system provides a way of demonstrating the T4 late gene-selective properties of T4-modified RNA polymerase. The properties of this transcription system and comparisons with other systems will be presented elsewhere. The pertinent properties of the *in vitro* system in the context of this work are: (*i*) it retains the viral late transcription characteristics of the phage T4-infected cells from which it is prepared. *r* and *l* strand-specific T4 RNAs are synthesized in the T4 wild type-derived endogenous system with approximately equal rates; (*ii*) the *in vitro* rate is almost constant for 50 min, and reinitiation of both early and late RNA chains occurs; (*iii*) late transcription does not depend on concurrent T4 DNA replication *in vitro* but does depend on prior T4 DNA replication *in vivo;* and (*iv*) the measured RNA synthesis occurs *in vitro* rather than in unlysed cells.

Stimulation of early T4 transcription in this system is easily achieved by adding various RNA polymerases or σ protein. However, as we have already emphasized, only the RNA polymerase from T4-infected *E. coli* effectively stimulates T4 late transcription. The latter enzyme contains the T4-specific subunits: gene product 33, gene product 55, and a 15,000 molecular weight subunit and a small amount of a 10,000 molecular weight subunit (21, 25). The α subunits of the enzyme are ADP-ribosylated. The T4-modified enzyme also transcribes the T4 *l* strand when it is added to the cellophane disc system. We have not yet analyzed the nature of these transcripts. Whether they are mainly early or middle transcripts, whether different *l*-transcripts are generated by different RNA polymerases, and whether endogenous σ is required for the *l*-transcription remain to be determined.

The dependences of maximal total and maximal *r*-strand-specific RNA syntheses on the amount of added RNA polymerase differ in the T4 e^- and T4 55^- derived cell-free systems. The fraction of *r*-transcription declines with increasing amounts of RNA polymerase in both systems, but the decay is more marked in the T4 e^- system. This suggests interesting possibilities regarding the enzymology of late transcription. The decline presumably reflects a lower availability of late transcription units, relative to early ones, to a vast excess of exogenous RNA polymerase, particularly in the T4 wild-type case. Our notions of "availability" of transcription units are not well defined, but it is obvious that the obstruction to RNA chain elongation by stl-inactivated, endogenous RNA polymerase might limit "availability" of transcription units. If that were the case, then the difference between complementation of wild-type and 55^- endogenous systems by T4-modified RNA polymerase would imply that, in the late transcription-defective cells, late transcription units are less obstructed by RNA polymerase. This would be most easily understood if gene product 55 *either* affected initiation, *or* if it affected attenuation of late RNA chains, accompanied by RNA polymerase release, at promoter-proximal sites. However, more complex models, in which our findings would be mechanistically indecisive, cannot yet be excluded.

The *in vitro* system that we have described here is interesting for two reasons: first, it allows one to study the enzymology of T4 late transcription and probably some aspects of DNA template "competence"; second, it provides, as do *in vitro* systems with bacteriophage SP01-modified RNA polymerases (26, 27), one of the first opportunities to study the ways in which the concurrent transcription of different gene classes is regulated by interchangeable sets of RNA polymerase subunits.

Table 2. Complementation of cell-free systems derived from T4 *am* BL292 (gene 55) and T4 *am* C18-N134-BL292 (genes 33, 55)-infected cells with different RNA polymerases

		T4 55⁻ system					T4 33⁻, 55⁻ system				
1	2	3	4	5	6	7	8	9	10	11	12
Additions		Total transcription,	*r* transcription		% RNA·RNA duplex formation with		Total transcription	*r* transcription		% RNA·RNA duplex formation with	
RNA polymerase	stl	cpm/10^8 c.e.*	%	cpm/10^8 c.e.*	CM RNA	PA RNA	cpm/10^8 c.e.*	%	cpm/10^8 c.e.*	CM RNA	PA RNA
1. None	+	1,310	13	*170*	5.3	4.1	3,210	13	*417*	2.4	2.1
2.	−	14,200	6	850	3.4	4.0	23,090	5	1,150	2.3	3.9
3. T4 (stl^r)†	+	31,470	38	*11,960*	5.4	6.3	26,520	38	*10,080*	3.3	5.5
4.	−	50,500	32	16,160	4.6	5.4	39,680	24	9,520	2.6	4.4
5. T4 (stl^s)†	+	1,700	—	—	4.0	3.1	2,740	22	600	4.4	3.3
6.	−	27,100	33	9,000	4.1	7.9	26,190	23	6,000	2.8	4.3
7. Holo (stl^r)‡	+	68,080	2	*1,360*	1.3	3.8	66,060	2	*1,320*	0.5	3.1
8.	−	128,000	2	2,560	1.4	3.8	137,650	2	2,750	0.5	3.1
9. Core (stl^r)§	+	14,580	9	*1,310*	6.4	9.0	17,550	9	*1,580*	3.5	6.8
10.	−	29,420	8	2,350	4.5	8.2	28,320	5	1,420	2.3	5.1

The *in vitro* systems were prepared from *E. coli* B^E infected with T4 *am* BL292 (gene 55) or *am* C18-N134-BL292 (genes 33 and 55); 380 cpm corresponds to 1 pmol of UMP incorporated. The average c.e./disc was 1.5×10^7. All other procedures were as described in the legend to Table 1. In the absence of added unlabeled *in vivo* RNA, the [^{3}H]RNA synthesized in the 55⁻ system gave 0.4–1.6% RNA·RNA duplexes; the RNA synthesized in the 33⁻, 55⁻ system gave 0.1–1.0% RNA·RNA duplexes.

* c.e., cell equivalents.

† T4-modified RNA polymerases.

‡ *E. coli* holo RNA polymerase.

§ *E. coli* core RNA polymerase.

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1. Rabussay, D. & Geiduschek, E. P. (1977) in *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. R. (Plenum Press, New York and London), Vol. 8, pp. 1–196.
2. Hall, B. D., Green, M. H., Nygaard, A. P. & Boezi, J. A. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **28**, 201–204.
3. Khesin, R. B., Shemyakin, M. F., Gorlenko, J. M., Bogdanova, S. L. & Afanasieva, T. P. (1962) *Biokhimiya* **27**, 1092–1105.
4. Wiberg, J. S., Dirksen, M.-L., Epstein, R. H., Luria, S. E. & Buchanan, J. M. (1962) *Proc. Natl. Acad. Sci. USA* **48**, 293–302.
5. Hall, B. D. & Crouch, R. (1968) in *The Biochemistry of Virus Replication*, eds. Laland, S. G. & Fröholm, L. O. (Universitetsforlaget, Oslo), pp. 49–59.
6. Snyder, L. R. & Geiduschek, E. P. (1968) *Proc. Natl. Acad. Sci. USA* **59**, 459–466.
7. Jörstad, K., Haarr, L. & Nygaard, A. P. (1973) *Abstracts, Ninth International Congress of Biochemistry*, Stockholm.
8. Maor, G. & Shalitin, C. (1974) *Virology* **62**, 500–511.
9. Travers, A. A. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 241–252.
10. Rabussay, D. & Geiduschek, E. P. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 1231.
11. Schleif, R. (1969) *Nature* **223**, 1068–1069.
12. Rabussay, D. & Zillig, W. (1969) *FEBS Lett.* **5**, 104–106.
13. Zillig, W., Zechel, K. & Halbwachs, J. (1970) *Hoppe Seyler's Z. Physiol. Chem.* **351**, 221–224.
14. Burgess, R. R. & Jendrisak, J. J. (1975) *Biochemistry* **14**, 4634–4638.
15. Burgess, R. R. (1969) *J. Biol. Chem.* **244**, 6160–6167.
16. Berg, D., Barrett, K. & Chamberlin, M. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York and London), Vol. 21, pp. 506–519.
17. Schaller, H., Otto, B., Nüsslein, V., Huf, J., Herrmann, R. & Bonhoeffer, F. (1971) *J. Mol. Biol.* **63**, 183–200.
18. Wu, R. & Geiduschek, E. P. (1975) *J. Mol. Biol.* **96**, 513–538.
19. Geiduschek, E. P. & Grau, O. (1970) in *RNA Polymerase and Transcription*, ed. Silvestri, L. (North Holland Publishing Co., Amsterdam and London), pp. 190–203.
20. Chamberlin, M. J. (1974) *Annu. Rev. Biochem.* **43**, 721–775.
21. Stevens, A. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 603–607.
22. Horwitz, H. R. (1973) *Nature New Biol.* **244**, 137–140.
23. Ratner, D. (1974a) *J. Mol. Biol.* **88**, 373–383.
24. Ratner, D. (1974b) *J. Mol. Biol.* **89**, 803–807.
25. Stevens, A. (1976) in *RNA Polymerases*, eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 617–627.
26. Tjian, R. & Pero, J. (1976) *Nature* **262**, 753–757.
27. Duffy, J. J. & Geiduschek, E. P. (1977) *Nature*, **270**, 28.