# *IN VITRO SYNTHESIS OF T4 LATE MESSENGER RNA**

By Loren Snyder and E. Peter Geiduschek

DEPARTMENT OF BIOPHYSICS, UNIVERSITY OF CHICAGO

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The temporal sequence of gene action that constitutes the "early" and "late" stages of the viral latent period is one of the most striking features of bacteriophage development. Such timing is achieved by having the expression of a part of the viral genome contingent on the fulfilment of certain prerequisites. Since contingent gene expression (and its restriction) is the essence of all developmental sequences, the basic mechanisms controlling the expression of viral "late" genes may have a much wider significance. In phage T4-infected *E. coli* B, the expression of certain viral genes depends on: (*a*) the synthesis of viral DNA and (*b*) the expression of at least one other viral gene; the action of gene 55 is required and that of gene 33 is necessary for effective expression at the right time. (The existence of additional viral genes controlling late transcription directly or indirectly is not excluded.) When conditions (*a*) and (*b*) are not met, the transcription of an entire class of messages does not occur.[1] The question of what mechanisms might be responsible for these requirements is put into sharper focus by the properties of mature T-even viral DNA as template for *in vitro* transcription with *E. coli* RNA polymerase. Early T-even messenger is selectively and asymmetrically synthesized, but conditions for asymmetrically transcribing late messenger have not thus far been found.[2, 3] It has, accordingly, been postulated that late messenger synthesis is dependent on the action of one or more positive control substances.[4]

We report our initial findings on late T4 messenger RNA synthesis in three related *in vitro* systems. Two of these represent little more than crude lysates of cells that are actively synthesizing late messenger *in vivo* at the time of lysis. The third system requires *in vitro* complementation of fractions isolated from *T4* amber mutant-infected cells which are not separately synthesizing late messenger.

*Materials and Methods.*—(a) *Bacteria, phage, media:* *E. coli* $B^E$ and CR63 were the nonpermissive ($su^-$) and permissive ($su_I^+$) strains infected with T4D (wild-type) and the following amber mutants: *am* N122 (gene 42), *am* BL292 (gene 55), and the double mutant *am* N122-BL292 (the kind gift of J. Pulitzer).[5] All experiments were performed at 30°C in *M9S* medium.[6] Cells were grown to a density of 4–6 $\times$ $10^8$/ml and infected with 5 $\pm$ 2 phage per bacterium.

(b) *Preparation of labeled (H³-uridine) and unlabeled RNA* from infected cells followed with only minor variations, a method published elsewhere (method II of ref. 8). Viral messenger and stable host RNA were not further fractionated. The properties of early and late RNA were controlled by hybridization competition, as described elsewhere.[3, 7]

(c) *Hybridization-competition* analysis was by the nitrocellulose-filter liquid method.[7, 8] Pertinent details have been published elsewhere.[6] Radioactivity retained on the filters was counted in toluene with "Liquifluor" (Pilot Chemical Corp.) scintillator.

(d) *Antimessenger* content was measured by annealing labeled *in vitro* synthesized RNA with unlabeled early and late *in vivo* RNA as described elsewhere.[9]

(e) *Mature viral DNA* was extracted with phenol[10] from CsCl purified phage. *T4* phage were grown in *E. coli* $B^E$ and T4* (nonglucosylated) phage were grown in *E. coli* W4597

(UDPG$^-$). Phage were purified by precipitation with polyethylene glycol and dextran sulfate,[11] followed by velocity centrifugation in a CsCl step gradient. Vegetative viral DNA was prepared from infected cells by a method based on the work of Frankel,[12] details of which will be published elsewhere.[13] RNA polymerase, purified according to Chamberlin and Berg,[14] was the gift of H. Diggelmann.

(f) *RNA incorporation in vitro:* The incubation mixture contained, per ml, 40 $\mu$moles Tris acetate, pH 8.0, 20 $\mu$moles $MgCl_2$, 6 $\mu$moles $\beta$-mercaptoethanol, 1 $\mu$mole uridine triphosphate (UTP), 1 $\mu$mole cytidine 5′-triphosphate (CTP), 2 $\mu$moles adenosine 5′-triphosphate (ATP), and 0.1 $\mu$mole $H^3$-labeled guanosine 5′-triphosphate (GTP), as well as various quantities of extracts from infected cells in their solvents as noted below. Incubation was for 15 min at 30°C. When incorporation of radioactive label was to be determined, the reaction was stopped with trichloroacetic acid (TCA). The chilled precipitate was centrifuged, redissolved in cold 0.25 *M* NaOH, reprecipitated with TCA, collected on glass filters, dissolved in "Hyamine" (Packard Instrument Co.), and counted in toluene-Liquifluor with external standardization to determine counting efficiency. When RNA was to be prepared, synthesis was terminated with sodium dodecyl sulfate, RNA was extracted with hot phenol[3] and dialyzed.

(g) *Protein concentration* was determined according to Lowry *et al.*[15]

(h) *Lysates of infected cells and cell fractions:* (1) *Cleared lysate:* Infected cells were poured onto frozen M9 salts, centrifuged, and taken up at a concentration of $5 \times 10^{10}$/ml in 0.05 *M* KCl, 0.01 *M* Mg acetate, 0.01 *M* Tris acetate, pH 7.8. Lysozyme was added to 300 $\mu$g/ml, the cells were lysed by freezing and thawing four times, and drawn up and down a long-nosed pipette several times (step I). The step I lysate was centrifuged for 15 min at 390,000 *g*, 0°C (60,000 rpm Spinco SW65 rotor), and the supernatant was used immediately for nucleotide incorporation.

(2) *Pellet fraction:* Infected bacteria were centrifuged, resuspended at a density of $5 \times 10^{10}$/ml in 0.05 *M* KCl, 0.005 *M* $MgCl_2$, 0.005 *M* $\beta$-mercaptoethanol, $5 \times 10^{-5}$ *M* ethylenediaminetetraacetate (EDTA), 0.01 *M* Tris chloride, pH 7.5 ("resuspension buffer"), and lysed as above. The step I lysate was centrifuged for 15 min at 0°C and 20,000 *g* (13,000 rpm Sorvall centrifuge) to remove unlysed cells and some cellular debris (step I*a*). One or 2 ml of the resulting supernatant were layered onto a 26-ml, 5–20% sucrose gradient in resuspension buffer and centrifuged 3 hr at 3°C, 83,000 *g* (24,000 rpm, Spinco SW25 rotor). Two-ml fractions were collected by siphoning from the top of the gradient. The material which pelleted through the gradient—*the pellet fraction*—was taken up in 1 ml of resuspension buffer.

(3) *Concentrated top supernatant fraction:* Ten ml of step I lysate in resuspension buffer ($10^{12}$ infected cells) were centrifuged for 4.5 hr at 390,000 *g*, 3°C (60,000 rpm Spinco SW65 rotor). The supernatant was brought to 70% saturation with $(NH_4)_2SO_4$. The precipitate was collected, redissolved in 0.5 ml resuspension buffer without prior washing, and dialyzed against the same solvent.

*Experimental Results.*—(1) *Late messenger synthesis in cleared cell lysates of cell infected with phage T4 and T4 am BL292 (maturation defective, gene 55):* Cleared *E. coli* $B^E$ prepared 18 minutes after infection (at 30°C) with T4 wild-type or T4 *am* BL292 incorporate radioactivity into RNA that can be submitted to hybridization-competition analysis. Lysates of T4 wild-type infected cells consistently incorporate label into polynucleotides not competed by unlabeled T4 early RNA but competed by T4 late RNA. The proportion of the hybridized RNA which is of this type is 8–15 per cent, which is considerably less late messenger synthesis than occurs *in vivo* in the cells from which the cleared cell lysate has been made.[6] Less rigorous manipulation or addition of a denatured heterologous DNA such as salmon DNA yields higher proportions of late T4 messenger.[16] For reasons that become evident further on, we have not thus far devoted attention to maximizing late messenger synthesis in this fraction. Comparable

experiments with cleared cell lysates of T4 *am* BL292 (gene 55)-infected cells do not yield late RNA in proportions that can be detected by hybridization-competition. Thus the cleared cell lysates exhibit nucleoside triphosphate incorporation into T4 RNA that is consistent with the properties of the *in vivo* systems from which they are prepared.[17]

(2) *Properties of pellet fractions of T4 and T4 am BL292 infected cells:* When a step I*a* lysate (see *Methods*) of T4-infected *E. coli* B (18 min, 30°C) is centrifuged into a sucrose gradient, the distribution of newly synthesized DNA and of RNA polymerase is that shown in Figure 1*A*. A rapidly sedimenting "pellet" fraction contains much of the DNA (as well as T4 mRNA and protein; data not shown) but no cells or particles resembling intact cells are observed, either in the phase microscope or in uranyl acetate negatively strained preparations in the electron microscope. (We thank R. Luftig for the electron micrographs.) The RNA polymerase activity of the pellet is not substantially stimulated by added DNA. Much RNA polymerase activity remains in the supernatant along with varying proportions of viral DNA. Ribonucleotide incorporation of supernatant fractions is strongly stimulated by added DNA. *M. lysodeikticus* DNA (28 mole % adenine-thymine base pairs compared to 50 and 65 mole % in *E. coli* and T4 DNA, respectively) also changes the relative incorporation of CTP and UTP dramatically for the supernatant fractions but little, or not at all, for the pellet. This suggests that the polymerase activity of the pellet is tightly bound to its (viral) DNA template. Labeled RNA has been synthesized *in vitro* with the pellet fraction of such T4 gradients and subjected to hybridization-competition analysis. Approximately 15–25 per cent of the label is incorporated into poly-

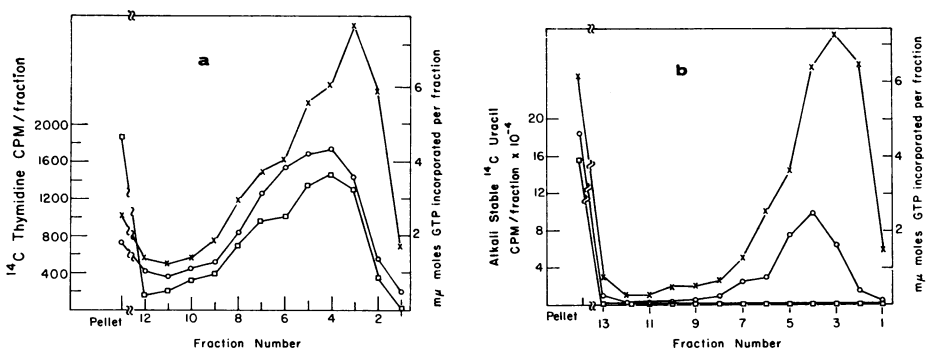


FIG. 1.—Sucrose gradient centrifugation of step I*a* lysates of T4-infected *E. coli* B$^{E}$ prepared 18 min after infection. One to 2 ml of lysate are centrifuged into 5–20% sucrose and collected as described in *Methods*. Sedimentation is from right to left. (*a*) One ml T4D step I*a* lysate (derived from $5 \times 10^{10}$ infected cells) layered on gradient. (*b*) Two ml T4 *am* BL292 lysate (derived from $10^{11}$ infected cells) layered on gradient. This contained 73% of the C$^{14}$-uracil incorporated into DNA; the rest had been centrifuged out of the lysate in making fraction I*a*.

O: RNA synthesis assayed without added DNA; 0.2 ml of each gradient fraction incubated in 0.5 ml standard assay for 15 min at 30°C.

×: RNA synthesis as above but including 100 μg/ml salmon sperm DNA.

□: Radioactivity incorporated into DNA. (*a*) C$^{14}$-thymidine added 2 min after infection. (*b*) C$^{14}$-uracil added 3 min after infection. Alkali-stable, acid-precipitable radioactivity is recorded.

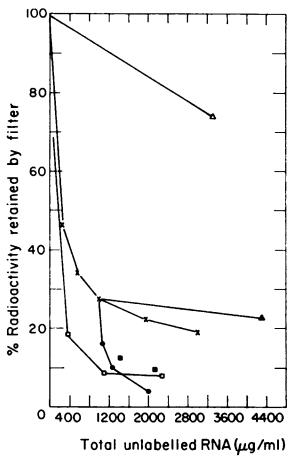


FIG. 2.—Hybridization-competition analysis of RNA synthesized with pellet fractions of wild-type and *am* BL292-infected *E. coli* $B^E$. Each sample contained 10 μg/ml denatured T4 DNA, *in vitro* synthesized RNA, and varying quantities of unlabeled RNA.

Δ, ▲, ×, ●: RNA synthesized with T4 pellet. 3.0 mμmoles $H^3$-GTP were incorporated in 15 min at 30°C/ml reaction mixture containing pellet fraction derived from $6 \times 10^{10}$ lysed cells. 100% hybridization corresponds to the radioactivity hybridized in the absence of competing unlabeled RNA–757 cpm, which is 15% of the TCA-precipitable input. Background binding to filters in the absence of added DNA is 23 cpm. Total RNA concentrations shown on abscissa.

Δ: Effect of unlabeled RNA from uninfected *E. coli* on hybridization efficiency.

×: Competition with early (5 min) T4 *in vivo* RNA.

●: Competition with 1000 μg/ml early and varying quantities of late (20 min) RNA.

▲: Competition with 1000 μg/ml early and 3,400 μg/ml uninfected RNA.

□, ■: RNA synthesized with *am* BL292 pellet. 0.4 mμmoles $H^3$-CTP were incorporated in 15 min at 30°C/ml reaction mixture containing pellet fraction derived from $2 \times 10^{10}$ lysed cells. 100% hybridization = 315 cpm, which is 13% of the TCA-precipitable input. Background without DNA 1 cpm.

□: Competition with early T4+ *in vivo* RNA.

■: Competition with 1140 μg/ml early and varying quantities of T4 late RNA.

nucleotide assignable as late messenger (Fig. 2). The T4 RNA synthesis is very asymmetric; less than 10 per cent of the label can be converted to an RNase-resistant form by hybridization with a large excess of unlabeled late or early *in vivo* RNA (Table 2, expt. 1 compared with expt. 3).

The properties of RNA prepared with a pellet fraction from cells infected with the maturation defective mutant *am* BL292 (gene 55) present a strong contrast. The partition of components on a sucrose gradient is shown in Figure 1*B*. Newly synthesized viral DNA is not found in the supernatant fraction. This is an interesting property of the gene 55 mutant. It is perhaps due to the fact that the vegetative DNA of this mutant is in a more rapidly sedimenting and mechanically less fragile form [12, 13] so that DNA fragments are less readily detached from the pellet fraction. RNA is synthesized asymmetrically with the pellet fraction of this mutant, but does not, by the criterion of hybridization-competition with T4 early and late unlabeled RNA, contain detectable proportions of late messenger (Fig. 2).

Two additional properties of these fractions are of interest: (1) RNA incorporation by pellets is stimulated by *E. coli* RNA polymerase. With sufficient added enzyme to increase the basal nucleotide incorporation 200-fold, late messenger synthesis is completely suppressed (Table 1, expt. 1). (2) The supernatant fractions of T4 sucrose gradients incorporate labeled nucleotides into T4-homologous RNA but we have thus far had no success in making detectable proportions of late RNA (Table 1, expt. 2).

(3) *De novo late T4 messenger synthesis by in vitro complementation:* The properties of the pellet fraction of *am* BL292-infected cells, and of gene 55 mutants *in vivo*, suggest an alternative approach to *in vitro* late messenger synthesis: its induction in the *am* BL292 pellet fraction would demonstrate *de novo* late tran-

TABLE 1. *Analysis of RNA synthesized in vitro: Hybridization-competition.*

| Expt. | RNA synthesis | RNA Concentration (μg/ml) $H^3$ *in vitro* RNA equivalent[a] | Unlabeled *in vivo* RNA Early | Unlabeled *in vivo* RNA Late | Competition[b, c] Cpm bound[d] | Competition[b, c] Per cent |
|---|---|---|---|---|---|---|
| 1 | Pellet T4 18 min a.i. + RNA polymerase[e] | 0.29 | — | — | 1115 | (100) |
| | | | 935 | — | 39 | 3.5 |
| | | | 1870 | — | 22 | 2 |
| | | | 935 | 263 | 25 | 2 |
| | | | 935 | 1050 | 23 | 2 |
| 2 | Supernatant of T4 sucrose gradient[f] | 0.13 | — | — | 801 | (100) |
| | | | 1041 | — | 55 | 7 |
| | | | 2776 | — | 30 | 4 |
| | | | 1041 | 105 | 53 | 7 |
| | | | 1041 | 1310 | 21 | 3 |
| 3 | *am* BL292 pellet + *am* N122 top supernatant[g] | 0.10 | — | — | 402 | (100) |
| | | | 1080 | — | 93 | 23 |
| | | | 2160 | — | 77 | 19 |
| | | | 1080 | 1000 | 7 | 0 |
| 4 | As in expt. 3, + *E. coli* RNA polymerase to increase incorporation 2.2-fold[h] | 0.23 | — | — | 1280 | (100) |
| | | | 1080 | — | 173 | 13.5 |
| | | | 2160 | — | 126 | 10 |
| | | | 1080 | 1000 | 30 | 2.3 |

[a] RNA concentrations calculated assuming that the specific activity of RNA is determined by the added nucleoside triphosphate only, without dilution by nucleotides or RNA contained in the cell fractions.

[b] All hybridizations with 10 μg heat-denatured T4 DNA/ml.

[c] Hybridization efficiencies (cpm hybridized to DNA in the absence of competitor × 100/cpm TCA-precipitable) for expts. 1–4 were 27, 18, 27, and 37%, respectively.

[d] Background counts (retained by filter in the absence of T4 DNA) for expts. 1–4 were 0, 0, 8, and 0 cpm, respectively.

[e] Two hundred units[16] of *E. coli* RNA polymerase added to 0.4 ml resuspended pellet (equivalent source: $4 \times 10^{10}$ infected cells; 10 mg protein) in 1 ml.

[f] RNA synthesized with fraction 3 of a gradient like that shown in Fig. 1*a*, which contained approximately 15% of the RNA-incorporating activity of the whole gradient, assayed in the presence of salmon DNA (incorporation in that assay was stimulated fivefold by 100 μg/ml added salmon DNA). No exogenous DNA was added to make the *in vitro* RNA; in 15 min at 30°C, 0.44 mμmoles/ml $H^3$-UTP were incorporated.

[g] RNA synthesized with 0.2 ml resuspended T4 pellet (equivalent source: $2 \times 10^{10}$ infected cells; *ca.* 5 mg protein), 0.4 ml concentrated *am* N122 top supernatant (5.2 mg protein) per ml of assay medium. In 15 min at 30°C, 0.64 mμmoles $H^3$-GTP were incorporated. Pellet and concentrated top supernatant were preincubated for 5 min at 30°C.

[h] As (*g*) but containing sufficient *E. coli* RNA polymerase to increase incorporation to 1.4 mμmoles $H^3$-GTP. Pellet and concentrated top supernatant were preincubated for 5 min.

scription. *In vivo* experiments on temperature-sensitive gene 55 mutants indicate that the product of this gene has a direct effect on late messenger synthesis.[18] *am* BL292 pellets might, accordingly, contain everything required for late messenger synthesis except the gene 55 product, for which a source must be found. The experiment described below is based on three assumptions about the gene 55 product: (*a*) It is relatively small—less rapidly sedimented than *E. coli* RNA polymerase. (*b*) It would be made, at least to some extent, even in mutants that do not replicate their DNA and consequently do not make viral late proteins (this second assumption seems reasonable because, in its kinetics of phenotypic rescue by 5 fluorouracil, the gene 55 *am* mutant BL292 closely resembles the behavior of early gene amber mutants[18–21]). (*c*) It might be most easily found in "soluble" form in cells infected with mutants that do not replicate DNA.

*Concentrated top supernatant fraction* has accordingly been prepared from *am*

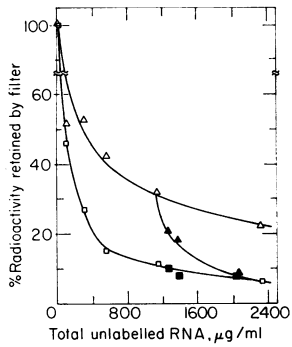


FIG. 3.—Hybridization-competition analysis of RNA synthesized in the complementation systems. Each sample contained 10 μg/ml denatured T4 DNA.

Δ, ▲: RNA synthesized with *am* BL292 pellet fraction and *am* N122 concentrated top supernatant. The low-ionic-strength mix contained 4.1 mg supernatant protein and pellet fraction derived from $2 \times 10^{10}$ infected cells. In 15 min reaction at 30°C, 0.33 mμmoles $H^3$-GTP were incorporated. 100% hybridization = 213 cpm, which is 19% of the TCA-precipitable input. Background in the absence of added DNA is 3 cpm.

Δ: Competition with T4 early (5 min) *in vivo* RNA.

▲: Competition with 1150 μg/ml early RNA and varying quantities of late (20 min) RNA.

□, ■: RNA synthesized with *am* BL292 pellet fraction and *am* N122-BL292 concentrated supernatant. 0.79 Mμmoles $H^3$-GTP were incorporated in 15 min at 30°C per ml reaction mixture containing 4.8 mg supernatant protein and pellet fraction derived from $2 \times 10^{10}$ infected cells. 100 % hybridization = 252 cpm, which is 31% of the TCA-precipitable input. Background without added DNA is 0 cpm.

□: Competition with T4 early *in vivo* RNA.

■: Competition with 1150 μg/ml early RNA and varying quantities of late RNA.

N122 (gene 42)-infected cells. The product of gene 42 is the enzyme deoxycytidylate hydroxymethylase. Cells infected with gene 42 amber mutants make no viral DNA and no late messenger. RNA polymerase can be centrifuged out of the *concentrated top supernatant* fraction of *am* N122, the remaining RNA polymerase activity being very low when assayed on a variety of templates including T4 and T4* (nonglucosylated) viral DNA, T4 and T4 *am* BL292 vegetative DNA, and salmon DNA.

*am* BL292 pellets, briefly preincubated with *am* N122 *concentrated top supernatant*, yield asymmetrically synthesized late messenger RNA (Fig. 3 and Table 2, expt. 2). If the effect of the *concentrated top supernatant* in eliciting late messenger synthesis from the pellet fraction is due to the action of gene 55, then a gene 55-gene 42 double mutant should not possess this stimulating activity. In fact, late messenger cannot be detected with *concentrated top supernatant* prepared from *E. coli* $B^E$ infected with *am* N122-*am* BL292 (genes 42, 55) (Fig. 3). Evi-

TABLE 2. *Analysis of in vitro synthesized RNA: Antimessenger content.*

| Expt. | RNA synthesis | $H^3$-RNA μg/ml equivalent[a] | Concentration Unlabeled *in vivo* RNA (μg/ml) Early | Late | Per cent RNase resistant[b] |
|---|---|---|---|---|---|
| 1 | Pellet fraction T4 lysate 18 | 0.1 | — | — | 5.8 |
| | min a.i.[c] | 0.1 | 500 | — | 8.6 |
| 2 | *am* BL292 Pellet + *am* N122 | 0.04 | — | — | 2.7 |
| | top supernatant[d] | | — | 490 | 6.5 |
| | | | — | 1460 | 8.2 |
| 3 | RNA from infected cells | 18.0 | — | — | 3.0 |
| | labeled 17–20 min a.i. | | — | 456 | 3.8 |
| | | | — | 1370 | 4.1 |

[a] See Table 1, footnote (*a*).

[b] Boiled pancreatic RNase (12.5 μg/ml) and 1.25 μg/ml boiled T1 RNase. Digest 15 min at 37°C in 2 SSC and precipitate with TCA in presence of 50 μg carrier DNA.

[c] The experiment was done on a different RNA preparation from that whose properties are shown in Fig. 2*a*. The difference in competition by 2 mg/ml early and late RNA was 17%.

[d] Sample of Fig. 3.

dently, the stimulating activity of the *concentrated top supernatant* is associated with gene 55.

Five experiments on four different *am* BL292 pellet and *am* N122 *concentrated top supernatant* preparations have given results similar to those shown in Figure 3; the pellets are stimulated to late messenger synthesis by the supernatant fraction, and differences in competition by early and late *in vivo* RNA (at concentrations of 2 mg/ml) are 8–22 per cent, with an average of 17 per cent. On the other hand, increased proportions of *concentrated top supernatant* beyond those used in the experiment shown in Figure 3 have not increased the proportion of late messenger in the synthesized RNA. Added *E. coli* RNA polymerase increases the total nucleotide incorporation and concomitantly lowers the proportion of late RNA in the synthesized product (Table 1, expts. 3 and 4).

The *in vitro* complementation experiment which yields T4 late messenger synthesis is based on conjectures about the control of T4 development that have been set forth. While the successful outcome of the experiments does not establish the validity of these conjectures, this is one direction that future work can take: these findings appear to provide an *in vitro* assay of a product associated with the functioning of T4 gene 55, which gene is also required for late transcription *in vivo*. The ability of gene 42 amber-infected cells to provide the gene 55-associated product suggests that its synthesis is not *absolutely* DNA-dependent. It is nondialyzable, precipitable by $(NH_4)_2SO_4$, and appears to have little or no RNA polymerase activity detectable on purified viral or other DNA.

However, a part of the secret of late transcription control in T4 development, particularly the DNA synthesis prerequisite of late messenger synthesis, is probably locked up in the pellet fraction. We have little to say on this subject at this point. It is intriguing that only the polymerase activity contributed by the pellet has been activated for late messenger synthesis by the *top supernatant fraction*, that the proportion of late messenger synthesis that can be elicited by the *top supernatant fraction* is limited, and that added polymerase appears not to be able to contribute capacity to synthesize late messenger. This raises the not entirely anticipated possibility that the enzyme which yields late messenger in the pellet fraction is *in situ* on late transcription units, yet inactive. Perhaps only a fraction of the late transcription units are in this state.[22]

These experiments apparently provide a direct demonstration, at the level of transcription, of the action of a control element of transcription. The control in which T4 gene 55 participates determines the expression of a large number of genes whose polypeptide outputs undoubtedly vary greatly and which surely constitute many transcription units. The gene 55 product appears to function as a *positive* control element of these transcription units; at least it helps to establish transcription of regions of a template which are evidently not transcribable by host RNA polymerase alone. The control clearly is multifaceted since it involves DNA synthesis as well (and not merely to allow synthesis of the gene 55 product). Replication-coupled controls on transcription are of widespread occurrence, and this provides added impetus to our further attempts to understand their mode of action in this instance.

*Summary.*—T4 late messenger RNA has been synthesized *in vitro*. In one of

three *in vitro* incorporation systems, late mRNA synthesis can be made to depend on the addition of a factor that is correlated with the functioning of T4 gene 55, a gene that also plays a role in controlling the expression of viral late genes *in vivo*. These experiments accordingly provide a direct *in vitro* demonstration, at the level of transcription, of the action of a control element of viral development.

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[5] Abbreviations: *am*, amber; su, suppressor; Tris, tris (hydroxymethyl) aminomethane; SSC, 0.15 *M* NaCl, 0.015 *M* Na citrate; RNase, ribonuclease; UDPG, uridine diphosphate glucose; a.i., after infection; mRNA, messenger RNA; T4 refers to the wild-type, T4D $am^+$.

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