Proc. Nat. Acad. Sci. USA
Vol. 72, No. 12, pp. 4928–4932, December 1975
Biochemistry

Effects of bacteriophage T4-induced modification of *Escherichia coli* RNA polymerase on gene expression *in vitro*

(cell-free protein synthesis/ADP ribosylation/rifampicin/reconstitution/host switch-off/RNA nucleotidyltransferase)

REINHARD MAILHAMMER*, HUEY-LANG YANG†, GARY REINESS†, AND GEOFFREY ZUBAY†

* Max-Planck-Institut für Biochemie, 8033 Martinsried, West Germany; and † Department of Biological Sciences, Columbia University, New York, N.Y. 10027

Communicated by F. Lynen, October 7, 1975

ABSTRACT **After T4 bacteriophage infection of *E. coli* a complex series of events take place in the bacterium, including gross inhibition of host transcription and discrete changes in the classes of the genes of T4 that are transcribed. Accompanying these changes in the pattern of transcription one finds T4-induced changes in the RNA polymerase (EC 2.7.7.6; nucleosidetriphosphate:RNA nucleotidyltransferase). The effects of modified polymerase on transcription can be advantageously analyzed in a DNA-directed cell-free system for protein synthesis. In this system gene activity is measured indirectly by the amounts and types of proteins synthesized. In the DNA-directed cell-free system this modified polymerase, like normal polymerase, transcribes T4 DNA with a high efficiency but transcribes bacteriophage λ and host DNA very poorly. Polymerase reconstruction experiments show that modification of the α subunit of the RNA polymerase is sufficient for inhibition of host transcription. Host transcription is also inhibited *in vitro* by T4 DNA. This latter type of inhibition is presumed to involve competition between host DNA and T4 DNA for some factor essential for transcription.**

The T4-modified polymerase transcribes from T4 DNA many of the same genes as normal unmodified polymerase; it also shows a capability for transcribing certain "non-early" T4 genes which is enhanced in the presence of protein-containing extracts from T4-infected cells.

When T4 bacteriophage infects *E. coli* there is an abrupt change in metabolism which favors synthesis of progeny phage. At the level of transcription one finds an inhibition of the initiation of host mRNA synthesis, while completion of already initiated host mRNA molecules goes on. At the same time synthesis of a discrete set of T4 mRNAs, known as "immediate early," is begun. As infection progresses different classes of T4 RNAs are synthesized; these RNAs are known by names that signify their order of appearance such as "delayed early," "quasi-lates," "anti-lates," and "true lates" (1).

It is clear that these changes in the pattern of transcription must result from changes in the ability of RNA polymerase (EC 2.7.7.6; nucleosidetriphosphate:RNA nucleotidyltransferase) to recognize different classes of promoters. Analysis of host polymerase after T4 infection indicates chemical modification of the existing polymerase subunits and association with some new small polypeptides (2–4). The ability of normal and T4-modified polymerase to transcribe different classes of genes can be analyzed in a DNA-directed cell-free system for protein synthesis. Here gene expression is measured indirectly by determining the amount of gene-related protein that is synthesized (5). Normally this system is composed of DNA, a cell-free extract (S-30) containing all the macromolecular components necessary for RNA and protein synthesis, and all the salts and substrates necessary for the same. After 1 or 2 hr of incubation the cell-free synthesized proteins are quantitatively assayed. For the purposes of this particular study the S-30 extract was made from a rifampicin-sensitive strain. Normal or T4-modified polymerase was isolated from a rifampicin-resistant strain and introduced into the cell-free system in the presence of rifampicin. Under these conditions all transcription results from the added purified polymerase. In this way the difference in proteins synthesized in the presence of normal or T4-modified polymerase can be studied in isolation (see ref. 6 for a preliminary report of this work). The following is an accounting of our findings using this approach with a number of different DNA templates and some attendant observations.

Abbreviations: E_N, normal RNA polymerase; E_M, T4-modified RNA polymerase.

METHODS

Bacterial Strains Used to Make S-30 Cell-Free Extracts. All strains used are derivatives of strain 514, which was used in earlier investigations of β-galactosidase (EC 3.2.1.23; β-D-galactoside galactohydrolase) synthesis. The construction of these strains has been described (5), and only critical features are mentioned here. Except for Z19i^q, strains used contain a deletion of the *lac* operon including the *i* gene. Strain 440 used for λ*dtrp-lac* DNA-directed β-galactosidase synthesis is *trp* R. Strain Z19i^q was used for tRNA synthesis studies. For most other synthesis studies strain LG4 was used.

Source of DNA Used to Stimulate Cell-Free RNA or Protein Synthesis. DNAs from λ*plac* 5 and λ*dtrp-lac* were isolated from lysogenic strains. The details for growth and DNA isolation from these viruses have been described (5). Φ80*psu*$^+{}_{III}$ DNA was isolated from a virus grown and isolated by described procedures (7). T4 DNA was isolated from bacteriophage T4D$^+$ grown on *E. coli* B (8).

Bacterial Extracts Used for Cell-Free Synthesis. Growth of cells and preparation of S-30 extracts used for RNA and protein synthesis was as described (5). The S-100 extract used for tRNA synthesis was prepared in the same way except that, after preincubation, the extract was centrifuged at 45,000 rpm for 3 hr in a Spinco 50 rotor. The decanted supernatant was dialyzed against buffer III (5) and stored in liquid N_2 prior to use.

Conditions for Cell-Free Synthesis. Procedures used for synthesis in S-30 extracts have been described (5). For λ*plac* 5 DNA-directed β-galactosidase, 0.5 mM cyclic AMP was added. Rifampicin, when present, was used at a final concentration of 2 μg/ml. It was added to the incubation mixture before the addition of RNA polymerase or S-30 extract. The time for synthesis was usually 60 min, and the S-30 from strain LG4 was used unless otherwise stated. For β-galactosidase assay, 0.2 ml of the incubation mixture was added to 1.5 ml of *o*-nitrophenyl-β-galactoside solution. After 1:1 dilution the values for OD_{420} are given, calculated for a 1-hr test.

Transfer RNA from $\phi 80psu^{+}_{III}$ DNA was synthesized in an S-100 (an S-30 from which the ribosomes have been spun down) in the presence of 10^{-4} M isopentenylpyrophosphate (7). The RNA was labeled with 25 μCi of [^{3}H]UTP (22 Ci/mmol) per 0.15 ml of incubation mixture, purified by phenol extraction, and subjected to electrophoresis on an acrylamide gel. Incorporations in the 4S $tRNA^{Tyr}$ bands were compared.

Conditions used for RNA synthesis in the purified transcriptional system have been described (9). The only protein present in these experiments is purified RNA polymerase. Gross RNA synthesis was estimated as cold trichloroacetic acid-precipitable counts.

Preparation of Purified Polymerases and Sigma Factor Used in Synthesis. Rifampicin-resistant RNA polymerase was isolated from strain *AJ7*, rif-*rJ7* (10, 11). The σ subunit was purified from this enzyme by phosphocellulose chromatography followed by sucrose gradient centrifugation (12). For isolation of the modified RNA polymerase, strain *AJ7* was infected with T4 *am*N 82 at a multiplicity of infection of 8. Cells were collected 8 min after infection.

Preparation and Characterization of Reconstituted RNA Polymerases. Purified normal polymerase (E_N) and T4-modified polymerase (E_M) were separated into their subunits, α, β', β, and σ, by electrophoresis on a cellulose acetate gel in 6 M urea-containing buffer. Active enzymes were reconstituted in their original compositions and in compositions where the α subunits have been exchanged (13).

RESULTS AND DISCUSSION

Normal and T4-Modified Polymerase Recognize λ and T4 DNAs with Markedly Different Effectiveness. The goal of our studies was to compare the ability of normal and T4-modified polymerase to express different genes. Highly purified enzymes were isolated from a rifampicin-resistant strain as described in *Methods*. In a simple *in vitro* system for RNA synthesis composed of DNA, RNA polymerase, and the salts and substrates necessary for RNA synthesis (see *Methods* for details), the two enzymes show roughly comparable activity (see Table 1). As far as gross synthesis is concerned λ, T4, and calf thymus DNAs make almost equally active templates. *In vitro* systems of this type frequently give a false impression of template effectiveness because the RNA polymerase tends to recognize many places for initiation in addition to the true promoter sites. Hence gross RNA synthesis often is a poor measure of meaningful RNA synthesis. This difficulty is minimized, however, in the presence of S-30 or by measuring the translatability of the RNA synthesized in a cell-free system. For this purpose we have used a coupled DNA-directed cell-free system for RNA and protein synthesis in which the newly synthesized RNA is immediately translated into protein. This coupled system contains DNA, a cell-free extract (S-30) capable of carrying out RNA and protein synthesis, and all the necessary salts and substrates. Since the S-30 extract contains RNA polymerase, it would be difficult to discriminate between the transcripts made by it and those made by added polymerase unless special steps were taken. To study the transcripts of the added polymerase exclusively the endogenous polymerase is blocked by addition of 2 μg/ml of rifampicin. Purified normal (E_N) and T4-modified (E_M) enzymes were not affected by the drug since they were prepared from a rifampicin-resistant strain. Table 1 shows a comparison of the gross amounts of RNA and protein synthesized when normal and T4-modified polymerases are used with either λ or T4 templates. As far as gross RNA synthesis is concerned, T4 DNA is about three times as active as λ with either polymerase in the presence of S-30 extract. E_N is about three times more active than E_M. Because of the complications mentioned above we regard comparisons of gross peptide synthesis to be of greater significance. In parallel experiments gross peptide synthesis was measured. E_N is seven times as active as E_M when λ DNA is used. By contrast, when T4 DNA is used the two enzymes are about equally effective. With either polymerase, T4 DNA is a much more active template. The most significant conclusion to be drawn from these results is that T4-induced modification of *E. coli* polymerase drastically lowers the ability of the enzyme to make translatable RNA from a λ but not from a T4 template. Further experiments indicate that *E. coli* DNA behaves like λ DNA in such tests (data not shown). These gross analyses were done to get an overall picture. In subsequent studies specific gene products were assayed for quantitative variation.

Table 1. Gross RNA and protein synthesis as a function of template and polymerase

Synthesis conditions	Added polymerase	DNA λ	DNA T4	DNA Calf thymus
Purified transcriptional system [^{3}H]UTP	E_N	2380	3380	2140
	E_M	3700	4520	3840
S-30, rif, [^{3}H]-UTP (20 min incorporation)	E_N	4810	15720	
	E_M	1980	5610	
	—	460	730	
S-30, rif, [^{14}C]-Leu (1 hr incorporation)	E_N	790	4050	
	E_M	200	3090	
	—	100	230	

Synthesis and assay in the purified transcriptional system were as described in *Methods* with the following modifications: 6.5 μg of the indicated DNA was added to a 0.1-ml reaction mixture; either 8.8 μg of E_N or 10 μg of E_M was added as indicated. Synthesis and assay in the S-30 coupled system were as described in *Methods* with the following modifications: 27.5 μg of λ DNA or 32.5 μg of T4 DNA were added to a 0.5-ml reaction mixture which contained 16.5 μg of E_N or 18.7 μg of E_M; rifampicin (rif) was added at a final concentration of 2 μg/ml; and 5 μCi of [^{3}H]UTP or 0.1 μCi of [^{14}C]leucine was added. After synthesis was terminated, 50-μl samples were washed and radioactivity was determined. Results are reported as cpm.

T4-Modified Polymerase Is a Poor Catalyst for Synthesis of Certain Bacterial RNAs. The coupled system was used to measure the relative effectiveness of E_N and E_M to transcribe three different types of bacterial genes, the *lac* operon, the *trp* operon, and the Su^{+}_{III} tRNA gene. The experimental design here was very similar to the one described above except that different DNAs were used. For the study of the *lac* operon λ*plac* 5 DNA was used. This DNA contains the *lac* operon, and other studies have shown that about half the β-galactosidase synthesized is due to *lac* promoter recognition, the other half to some unknown site on the DNA (5). The results in Table 2 and Fig. 1A show that modification of the RNA polymerase reduces its effective-

Table 2. Activity of E_N and E_M on different operons

DNA	Rifampicin	Polymerase added	β-Galactosidase OD_{420}
λ*plac* 5	—	—	0.58
	—	E_N	0.96
	—	E_M	0.404
	+	—	0.010
	+	E_N	0.268
	+	E_M	0.020
λ*dtrp-lac*	—	E_N	0.277
	—	E_M	0.227
	+	E_N	0.157
	+	E_M	0.040

The rifampicin-resistant RNA polymerases were added in saturating amounts (E_N = 157 μg; E_M = 182 μg per 0.5-ml incubation mixture). Incubation mixtures were as described in *Methods*. The time for β-galactosidase assay was 60 min for λ*plac* 5 DNA and 260 min for λ*dtrp-lac* DNA.

ness for β-galactosidase synthesis by more than an order of magnitude. The diminished response of β-galactosidase synthesis to addition of small amounts of E_N seen in Fig. 1A is very reproducible. It may reflect a competitive inhibiting effect of the endogenous rifampicin-sensitive polymerase (about 30 μg) to the added rifampicin-resistant polymerase (about 6 μg) in the presence of rifampicin. At higher levels of added polymerase the response becomes linear until saturating levels are reached. This competition leads *in vivo* to the establishment of dominance of rifampicin sensitivity over rifampicin resistance (14). The possibility that the low activity of E_M is due to a deficiency of polymerase subunit σ was checked by adding increasing amounts of σ before synthesis. The addition of σ (see Fig. 1B) improved the activity about 50% when E_N was used but had little effect when E_M was used.

For studies of the *trp* operon, λ*dtrp-lac* DNA was used. In this DNA the *z* gene for β-galactosidase has been fused to the *trp* promoter-operator region so that *z* gene expression is under the control of the *trp* system (5). Use of this DNA instead of the normal *trp* operon is a convenience since β-galactosidase is an easy enzyme to assay. Once again one observes (Table 2) a drastic lowering in activity when E_M is compared to E_N. In this case the difference in enzyme synthesized is about 4-fold.

In an example of yet another type of bacterial gene, the tRNA transcript from Φ80*psu*$^+_{III}$ DNA was studied. The tRNA was synthesized by procedures described elsewhere (7) and analyzed by electrophoresis of the radioactive product synthesized in a cell-free system on an acrylamide gel which results in separation of the 4S tRNA (7) from other radioactive products. E_M is only one-fourth as effective as E_N for *su*$^+_{III}$ tRNA synthesis.

The results of this section show that when discrete bacterial gene products were analyzed, normal RNA polymerase appears to be 4 to 15 times more effective than T4-modified polymerase. Subsequent studies were carried out to determine which subunit(s) was responsible for this loss of ability to express bacterial genes.

Modification of α-Subunit of Polymerase Is the Significant Change Affecting *lac* Gene Expression. T4-induced polymerase modification is known to involve covalent attachment of one adenosine diphosphoribose residue to each α polypeptide (15). There may be further changes in the other polymerase subunits. In order to find out which of the

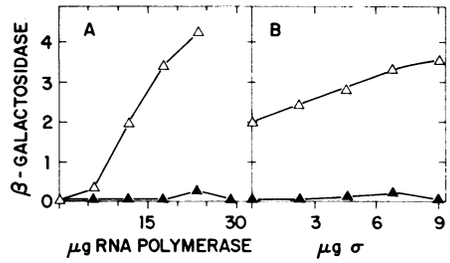

FIG. 1. Comparison of E_N and E_M in a λ*plac* 5 DNA-directed system for β-galactosidase synthesis without (A) or with (B) added extra σ subunit. Conditions for cell-free synthesis were as described in *Methods*. Rifampicin was present in all syntheses. In (A) 22.5 μg of λ*plac* 5 DNA was used in the presence of either E_N (△) or E_M (▲) added at the indicated concentrations. In (B) varying amounts of σ subunit were added to an incubation mixture containing either 12 μg of E_N (△) or 17 μg of E_M (▲). All incubation mixtures had a total volume of 0.5 ml.

changes affects gene expression, subunits of E_N and E_M were separated and reconstituted in their original compositions and in compositions where the α subunits have been exchanged (see *Methods*). These reconstituted enzymes were tested in the purified transcriptional system and in the coupled system with *plac* 5 DNA. In the purified transcriptional system, gross RNA synthesis was determined as radioactively labeled, cold trichloroacetic acid-precipitable product. After reconstitution the activity yield in gross RNA synthesis in the purified transcription system was 60% for E_N and 74% for E_M. The normal reconstituted polymerase shows the highest specific activity and the modified the lowest (see Fig. 2a). The maximum differences in specific activities seen with this crude assay is only about a factor of two. Much greater differences are seen when the activities of these enzymes are compared for DNA-directed β-galactosidase synthesis (Fig. 2b). The normal reconstituted polymerase shows the highest activity, the reconstituted polymerase with β′ and β subunits from modified polymerase is about 50% as active; both reconstituted polymerases with modified α subunits show more than a 10-fold reduction in activity. Thus, α modification is the primary factor affecting expression of the *lac* gene. Up to now it was only known that α subunits in addition to β, β′, and σ are required for reconstitution of the catalytically active holoenzyme. Our results show that the normal α subunit is essential for efficient transcription of the *lac* operon. It seems likely that the α subunit plays a major role in promoter recognition.

The large effect of α modification on host transcription *in vitro* leads us to believe that α modification would inhibit host transcription *in vivo*. However, the role of α modification in inhibiting host transcription has been seriously questioned by Horvitz (16). Horvitz has isolated phage mutants that do not carry out polymerase modification but that nevertheless grow normally and inhibit host transcription *in vivo*. Horvitz suggests that modification may be required for growth in some strains of *E. coli* and hence be selectively advantageous because it extends the normal host range of the phage. To this we might add that all of Horvitz's mutant tests were done at a rather high multiplicity of infection of eight, where one might expect inhibition of host transcription from other causes which are discussed below.

Expression of *lac* Operon *In Vitro* Is Severely Inhibited by T4 DNA. It is well known that host transcription *in vivo* is inhibited by at least two different mechanisms, one requiring protein synthesis and one that does not (8, 16–22) The latter mechanism is demonstrable by incubating cells

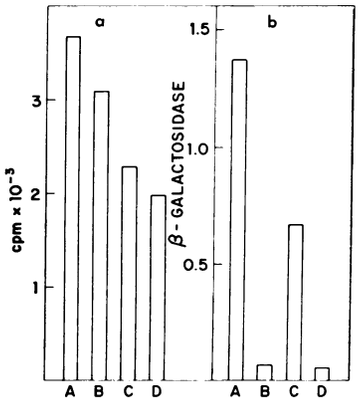

FIG. 2. Comparison of various reconstituted polymerases in the purified transcriptional system (a) and in the λ*plac* 5 DNA-directed system for β-galactosidase synthesis (b). A = $(\alpha_N)_2\beta_N\beta'_N\sigma_N = E_N$; B = $(\alpha_M)_2\beta_N\beta'_N\sigma_N$; C = $(\alpha_N)_2\beta_M\beta'_M\sigma_N$; D = $(\alpha_M)_2\beta_M\beta_M\sigma_N$. (a) 17 μg of the indicated RNA polymerase containing 21% σ were used; (b) 28 μg of the identical enzyme preparations were used. In no case was the amount of polymerase in excess, since the response in β-galactosidase synthesis was proportional to polymerase activity. Rifampicin was present in all syntheses, and the procedures for the syntheses and assays were as described in *Methods*.

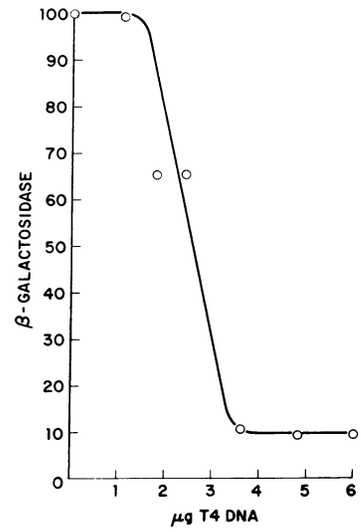

FIG. 3. Synthesis of β-galactosidase directed by λ*plac* 5 DNA as a function of the concentration of T4 DNA. Results are reported in %, 100 being the amount of β-galactosidase synthesized in the system with no added T4 DNA. 53 μg of λ*plac* 5 DNA was used in a 0.5 ml of incubation mixture. Strain 514 was used for S-30 preparation. No rifampicin or extra polymerase was present. Otherwise conditions used were as described in *Methods*.

with chloramphenicol before phage infection. Inhibition of host transcription under such conditions is incomplete and increases as the multiplicity of infection increases. It seems likely that direct competition between host and phage DNA for some factor(s) essential for transcription is involved. This possibility was explored *in vitro* by determining the inhibitory effect of increasing amounts of T4 DNA on DNA-directed β-galactosidase synthesis. It was found that synthesis of β-galactosidase directed by λ*plac* 5 DNA (106 μg/ml), in a normal S-30 without the addition of any purified polymerase or rifampicin, is almost completely inhibited by small amounts of T4 DNA (Fig. 3). As little as 7 μg/ml of T4 DNA brings about greater than 90% inhibition of β-galactosidase synthesis. In control experiments comparable amounts of λDNA produced no inhibition, so evidently the inhibiting effect is due to some special effect of the T4 DNA. This result *in vitro* might simulate the situation *in vivo* with respect to inhibition of host RNA synthesis.

In retrospect it is unfortunate that Horvitz did not estimate inhibition of host RNA synthesis on his modification mutants at a lower multiplicity of infection, where this type of competitive inhibition would be minimized. For this reason we feel that the importance of T4-induced α modification to phage and host metabolism is still unclear. To put it

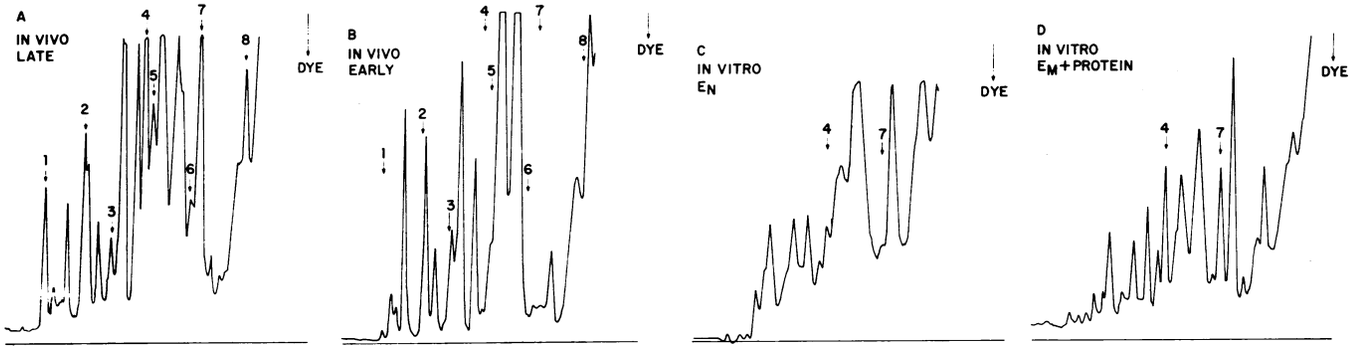

FIG. 4. Electrophoretic patterns of radioactively labeled proteins obtained from *in vivo* T4 "late" infected cells (A), *in vivo* T4 "early" infected cells (B), *in vitro* proteins synthesized in the presence of added E_N (C), and *in vitro* proteins synthesized in the presence of added E_M and protein-containing extract for T4-infected cells (D). Migration is from left to right. Dye front is indicated by vertical arrow. Preparation of *in vivo* labeled proteins from T4-infected cells was according to the procedures of O'Farrell and Gold (23). [^{3}H]Aminoacid mixtures were used to label proteins. (A) Label was added 15 min after infection and cells were collected after 30 min. (B) Rifampicin and ^{3}H-labeled amino acids were added 1 min after infection and cells were collected after 16 min. Protein-containing extract from T4-infected cells (D) was prepared as follows: strain LG4 at midlogarithmic phase was infected with T4 phage at a multiplicity of infection of 5. Growth was continued for another 35 min at 30°, and the cells were chilled and collected. Nucleic acid-free S-100 protein extract was prepared according to procedures described elsewhere (24). A 600-μg sample of this protein extract was added to 0.5 ml of incubation mixture. The procedures used for T4 DNA-directed protein synthesis (in C and D) were the same as used in other coupled syntheses (see *Methods*) except for the particulars mentioned here. T4 DNA (65 μg/ml) was used; 2 μg/ml of rifampicin and 33 μg/ml of either E_N or E_M was added. ^{3}H-Labeled aminoacid mixture, 1 mCi/ml, was added at 50 μCi/ml. Synthesis was for 2 hr at 37°. All ^{3}H-labeled proteins were analyzed by sodium dodecyl sulfate-acrylamide gel electrophoresis and fluorography. Electrophoresis was done by the method of Studier (25). The gel consisted of a 5% acrylamide stacking gel on top of a 10–20% linear gradient of acrylamide separating gel. Electrophoresis was performed at room temperature at 150 V for 1.7 hr. The gel was processed and fluorographed according to the procedure of Bonner and Laskey (26). The resulting fluorographs were measured by densitometry on a Gilford spectrophotometer.

another way, the unresolved question here is the extent to which α modification accounts for the type of inhibition of host transcription which requires phage protein synthesis.

T4-Modified Polymerase Transcribes Many Early T4 Genes with an Efficiency Similar to that of Normal Polymerase: Some "Non-Early" Genes Are Expressed with Greater Efficiency in the Presence of Modified Polymerase and Protein-Containing Extracts from T4-Infected Cells. As noted above, E_N and E_M are almost equally efficient in coupled systems for DNA-directed protein synthesis. Cursory examination by sodium dodecyl sulfate-acrylamide gel electrophoresis and fluorography of the radioactively labeled products also indicates great similarities in the types of proteins that are synthesized. Closer examination shows some differences. In order to determine the nature of these differences, the *in vitro* products have been compared with proteins made in T4-infected cells under conditions favoring either "early" or "late" phage protein synthesis. At a multiplicity of 5, strongly ultraviolet irradiated cells were infected with T4 phage. At 15 min after infection ^{3}H-labeled amino acids were added and synthesis was allowed to proceed for another 15 min before it was stopped. Such a labeling procedure should favor "late" phage proteins. A parallel batch of irradiated cells was treated with 200 μg/ml of rifampicin and ^{3}H-labeled amino acids at 1 min after infection. These cells were killed at 16 min after infection. According to the observations of O'Farrell and Gold (23), phage proteins that are synthesized under this rifampicin treatment are exclusively of the "early" type. Accordingly, any radioactive proteins present in the uninhibited preparation that are not present in the rifampicin-treated preparation must be of the "non-early" type and therefore must be "quasi-lates," "anti-lates," or "true lates." Comparison of these *in vivo* products by sodium dodecyl sulfate-acrylamide gel electrophoresis and fluorography reveals numerous "non-early" protein bands (compare Fig. 4A and B, bands 1–8). When E_N is used to program cell-free synthesis, these "non-early" proteins are barely detectable (Fig. 4C). By contrast, when E_M is used, small amounts of some "non-early" bands can be seen. If E_N is used in conjunction with protein-containing extract from late T4-infected cells the amounts of bands 4 and 7 are approximately tripled. Comparable protein extracts from uninfected cells show no stimulating effect (data not shown). Finally, when E_M is used in conjunction with similar T4 protein-containing extracts the amount of bands 4 and 7 is doubled again. Comparison of bands 4 and 7, both of the "non-early" type, shows an enormous overall increase when E_N is replaced by E_M and T4-protein extract is added (compare Fig. 4C and D). It is concluded that both E_M and protein-extract from late infected cells are required for optimal "non-early" protein synthesis of the type seen here. Clearly a full explanation of these results *in vitro* will require much more work. Nevertheless, it appears from the present results that more than the T4-modified polymerase is required for optimal synthesis *in vitro* of "non-early" T4 proteins. The factors from T4-infected cells that stimulate "non-early" T4 protein synthesis may include the small proteins that are sometimes associated with T4-modified polymerase (4) or other factors. Totally unexplained are the requirements for shutting off "early" T4 protein synthesis.

R.M. wishes to thank W. Zillig for stimulating discussions and a critical reading of the manuscript. We are also grateful for the helpful advice of John Dunn and William Crockett. Excellent technical assistance was provided by Ingelore Holz. The work was supported in part by the Deutsche Forschungsgemeinschaft and in part by a grant from the American Cancer Society (NP-12E) to G.Z.

1. Wu, R., Geiduschek, E. P., Rabussay, D. & Cascino, A. (1973) in *Virus Research*, eds. Fox, C. F. & Robinson, W. S. (Academic Press, New York and London), pp. 181–204.
2. Walter, G., Seifert, W. & Zillig, W. (1968) *Biochem. Biophys. Res. Commun.* **30**, 240–247.
3. Horvitz, H. R. (1974) *J. Mol. Biol.* **90**, 727–738.
4. Stevens, A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 603–607.
5. Zubay, G. (1973) *Annu. Rev. Genet.* **7**, 267–287.
6. Mailhammer, R., Zillig, W., Yang, H.-L. & Zubay, G. (1975) "Abstracts of the annual meeting H 110," in *Abstracts of the American Society of Microbiology*, p. 114.
7. Manley, J., Reiness, G., Zubay, G. & Gefter, M. (1973) *Arch. Biochem. Biophys.* **157**, 50–54.
8. Schachner, M., Seifert, W. & Zillig, W. (1971) *Eur. J. Biochem.* **22**, 520–528.
9. De-Crombrugghe, B., Sankar, A., Gottesman, M. & Pastan, I. (1973) *Nature New Biol.* **241**, 260–264.
10. Zillig, W., Zechel, K. & Halbwachs, H. J. (1970) *Hoppe Seyler's Z. Physiol. Chem.* **351**, 221–224.
11. Boyd, D. H., Zillig, W. & Scaife, F. J. G. (1974) *Mol. Gen. Genet.* **130**, 315–320.
12. Burgess, R. R. (1969) *J. Biol. Chem.* **224**, 6160–6167.
13. Palm, P., Heil, A., Boyd, D. H., Grampp, B. & Zillig, W. (1975) *Eur. J. Biochem.* **53**, 283–291.
14. Ilyina, T. S., Ovadis, M. J., Mindlin, S. Z., Gorlenko, Z. M. & Khesin, R. B. (1971) *Mol. Gen. Genet.* **110**, 118–133.
15. Goff, C. G. (1974) *J. Biol. Chem.* **249**, 6181–6190.
16. Horvitz, H. R. (1974) *J. Mol. Biol.* **90**, 739–750.
17. Kaempfer, R. O. R. & Magasanik, B. (1967) *J. Mol. Biol.* **27**, 475–494.
18. Kaempfer, R. O. R. & Magasanik, B. (1967) *J. Mol. Biol.* **27**, 453–468.
19. Ennis, H. K. (1970) *Virology* **40**, 727–733.
20. Adesnik, M. & Levinthal, C. (1970) *J. Mol. Biol.* **48**, 187–208.
21. Hayward, W. S. & Green, M. H. (1965) *Proc. Nat. Acad. Sci. USA* **54**, 1675–1678.
22. Nomura, M., Witten, C., Mantei, N. & Echols, H. (1966) *J. Mol. Biol.* **17**, 273–278.
23. O'Farrell, P. Z. & Gold, L. M. (1973) *J. Biol. Chem.* **248**, 5502–5511.
24. Yang, H.-L. & Zubay, G. (1974) *Biochem. Biophys. Res. Commun.* **56**, 725–731.
25. Studier, F. W. (1973) *J. Mol. Biol.* **79**, 239–248.
26. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.