Bacteriophage T4 Gene Expression

EVIDENCE FOR TWO CLASSES OF PREREPLICATIVE CISTRONS*

(Received for publication, December 22, 1972)

PATRICIA Z. O'FARRELL AND LAWRENCE M. GOLD

From the Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80302

SUMMARY

The antibiotic rifampicin has been used in an investigation of the temporal regulation of gene expression during bacteriophage T4 infection of *Escherichia coli*. We have asked at what times after infection the transcription of specific cistrons becomes rifampicin insensitive. Gene expression has been studied using analyses of proteins on sodium dodecyl sulfate-acrylamide gels; thus translation has been used to assay for the appearance of specific transcripts.

Two types of promoters account for most prereplicative transcription; early promoters are recognized immediately after infection, whereas quasi-late promoters are recognized only after a delay of 1.5 min. Those transcripts previously defined as immediate early and delayed early RNAs initially appear to be under the control of early promoters; however, the quasi-late promoters may provide a second mode of regulation for several of those transcripts.

Infection of *Escherichia coli* with the bacteriophage T4 is marked by the orderly appearance of specific “classes” of phage-specific RNA (1–4). These classes were defined originally on the basis of the times at which specific transcripts first appeared relative to phage DNA replication; thus pre- and postreplicative RNAs were designated “early” and “late” (1–3). Recently the prereplicative RNAs have been reclassified using criteria in addition to kinetics. Those prereplicative RNAs which are synthesized immediately after infection, even in the complete absence of protein synthesis, are called immediate early RNAs (5); those prereplicative RNAs which first appear after a lag of about 2 min and whose synthesis apparently requires protein synthesis are called delayed early RNAs (5). A third prereplicative class, called quasi-late, is defined by low abundance prior to DNA synthesis and a high relative abundance at postreplicative times (5). All of the prereplicative RNA is synthesized asymmetrically from the same strand of the T4 DNA (6).

Owing to the striking inhibition of delayed early RNA accumulation brought about by chloramphenicol or puromycin (4, 5), much research has been focused on the transition between immediate early and delayed early transcription. In highly purified cell-free systems, T4 DNA may be transcribed by the *E. coli* RNA polymerase to yield, in the proper temporal order, both immediate early and delayed early RNAs (7, 8); promoter recognition occurs at sites adjacent to the immediate early genes (8). If the *E. coli* transcriptional termination factor ρ is added to these systems, delayed early RNAs are not synthesized (9, 10). These data have led to two alternative (but not mutually exclusive) models for the onset of delayed early transcription in vivo. An antiterminator model proposes that the product of an immediate early gene abolishes ρ factor activity, thereby allowing RNA polymerase to continue to the end of each early transcription unit (5, 10, 11). In a model utilizing new promoter recognition, ρ mediated terminations persist, but the product of an immediate early gene allows initiation of RNA synthesis beyond the ρ termination site (12–15). In either model the immediate early gene product which allows delayed early transcription is thought to be a protein, since chloramphenicol and puromycin restrict transcription to immediate early genes (4, 5). Consequently, Mobley et al. (11) suggested that most delayed early RNAs arise on polycistronic messages containing immediate early RNA; their data exclude the new promoter model as the primary mechanism for delayed early transcription. We argued also that delayed early RNAs arise in vivo as the promoter distal portions of polycistronic messengers (16–19). However, we believe that no T4-encoded protein is required or utilized in the transcription of most delayed early genes, since a coupled cell-free system derived from uninfected *E. coli* transcribes and translates both immediate and delayed early genes (17–19). A more complete argument in support of this view is developed in the accompanying paper (18).

Two pieces of evidence suggest that prereplicative RNA synthesis may occur under the control of promoters other than those recognized immediately after infection. Firstly, rIIB-encoded protein is required or utilized in the transcription of most delayed early genes, since a coupled cell-free system derived from uninfected *E. coli* transcribes and translates both immediate and delayed early genes (17–19). A more complete argument in support of this view is developed in the accompanying paper (19).

In order to investigate the control of prereplicative gene expression, we have utilized the antibiotic rifampicin (20). Rifampicin binds to the β subunit of the *E. coli* RNA polymerase and thereby prevents the initiation of transcription (27).
chain elongation is not affected by rifampicin (28). Since RNA synthesis in T4-infected E. coli is sensitive to rifampicin throughout the lytic cycle (29), we may ask at which times various classes of T4 transcripts become rifampicin refractory.

In the experiments reported below, we identify specific transcripts by analyzing their protein products on SDS-acrylamide gels (30, 31). We will discuss the validity of this approach below. Our experiments suggest that prereplicative RNAs may be subdivided into two classes: early and quasi-late. The quasi-late RNAs include those specific transcripts which first become labeled at 1 min after infection. The early RNAs include most of the immediate early and delayed early RNAs defined previously on the basis of experiments with the antibiotic chloramphenicol (5). Among the quasi-late genes are several which initially are controlled by early promoters; thus new promoter recognition for some genes provides a second mode of transcription.

MATERIALS AND METHODS

Preparation of Infected Cell Extracts—E. coli AS19 (32) and bacteriophage T4D were used for all of the experiments reported except where otherwise noted in the text. E. coli AS19 is a B strain with an increased permeability to rifampicin.4 Infections were carried out in M9 media plus tryptophan at 30°C (2, 31). Cells were irradiated with ultraviolet light prior to every infection (31). Control experiments indicated that the burst is reduced in irradiated cells; however, neither the temporal order of transcription and translation nor the relative yield of specific proteins is affected by the irradiation.4 Phage were added in one-fifth the final volume to appropriately concentrated cells such that the final concentration was 3 × 10^6 cells per ml, infected at a multiplicity of eight. Between 1 and 5 ml of infected cells were used for various experiments as described in the figure legends.

Mixed [3H]amino acids were used to label proteins under various conditions. We used sufficient levels of [3H]amino acids and, when necessary, unlabeled casamino acids, to ensure that radioactive amino acids were not limiting throughout the labeling period. Infected cultures were stopped by pouring the cells onto ice (in the presence or absence casamino acids). The preparation of radioactive samples for electrophoresis is described in the accompanying paper (31). The radioactive proteins in sample buffer for electrophoresis represent a 10-fold increase in final cell concentration. The volume of sample used for electrophoresis was 25 μl. Thus all figures containing gel patterns represent equal volumes of cells. Amino acid incorporation into protein was determined by removal of an aliquot from the samples and measuring acid-precipitable radioactivity (19).

SDS-Acrylamide Gel Electrophoresis—The procedure for electrophoresis is described in the accompanying paper (30, 31). The percentage of acrylamide used in the separating gel was 10% for all experiments except those shown in Fig. 5 and 8 in which the gel was 7.5% acrylamide. All of the figures of gels are autoradiograms prepared as described (31).

1 The abbreviation used is : SDS, sodium dodecyl sulfate.
2 Early transcription units are bounded Pr → Tr, where Pr is a promoter recognized by the host E. coli RNA polymerase (7, 8) and Tr is a termination site recognized directly by RNA polymerase (10). The promoter proximal portions of early transcription units encode immediate early RNAs, whereas the promoter distal portions encode delayed early RNAs (7, 8). Such early transcription units are arranged in tandem on the T4 genome (8).
3 D. Oppenheim, unpublished experiments.
4 Unpublished results from this laboratory.

Antibiotic Experiments—The experiments using the antibiotic rifampicin were performed at a final concentration of 200 μg per ml. This concentration was sufficient to prevent any T4-specific protein synthesis if added to cells at zero time along with phage. Chloramphenicol was used at a final concentration of 250 μg per ml for the experiment reported in Fig. 6.

Chemicals—Rifampicin (rimactane) was a generous gift from CIBA Pharmaceutical Company. Mixed [3H]amino acids were obtained from Schwarz-Mann (No. 3122-09). [methyl-3H]Thymidine was obtained from New England Nuclear (No. NET-027X).

Phage Mutants—T4D and the T4 mutants in genes 32, 44, and r119 were from the collection of R. H. Epstein. Other mutants were as described in the accompanying paper (31).

RESULTS

Temporal Patterns of T4-specific Protein Synthesis—We first asked in what order T4 genes are transcribed and their mRNAs translated after infection. Bacteria were exposed to ultraviolet light to preclude host RNA and protein synthesis (33, 34). Following infection, we pulse-labeled the phage proteins with [3H]amino acids using 1-min pulses at every minute for the first 22 min of infection. Amino acid incorporation was measured and the radioactive proteins were analyzed on SDS-acrylamide gels (Fig. 1, A and B). These experiments are different from similar ones published by Hasoda and Levinthal (33) only in that we possess a larger category of band assignments (31) and we utilize an electrophoresis system with increased resolution (30). We have diagrammed the times at which specific proteins first appear and the times at which those proteins are no longer synthesized (Fig. 1C). These patterns do not reveal a small number of protein classes; rather, the first 5 to 6 min of infection show the continuous appearance of new species. Furthermore, the time at which a protein first appears does not prejudice the time at which its messenger ceases to be translated. Under our conditions, T4 DNA synthesis is first detectable 7 min after infection. Those proteins which appear before that time, by definition, are products of prereplicative genes. During the interval between the onset of DNA replication at 7 min and the onset of late translation at about 12 min, no new proteins appear. During the first 2 min of infection fewer than 12 proteins are strongly labeled; we assume that those proteins are encoded for by genes which are adjacent to promoters recognized immediately after infection (P_L) and are therefore products of immediate early RNAs (5). Those assignments are rather premature due to considerations of the lengths of specific genes. We will present in a separate manuscript our detailed attempts at promoter mapping.

Effect of Rifampicin on Prereplicative Transcription and Translation—E. coli AS19 was infected with T4 at time zero; at 1 min post-infection, one-half of the culture was transferred into rifampicin. The accumulation of radioactive protein in each culture was measured (Fig. 2A). Rifampicin addition 1 min after infection decreased the apparent rate of protein synthesis to about half the rate measured in the control culture; furthermore, after about 16 min the rifampicin-treated culture no longer accumulated additional radioactive proteins. Cells treated with rifampicin 1 min after infection were also pulse-labeled at various times after infection with [3H]amino acids (Fig. 2B). The apparent rate of protein synthesis increased during the first 9 min of infection and subsequently decayed; the decay in protein-synthesizing capacity occurred slowly in this experiment and may not represent the rate of messenger translation but perhaps the rate of RNA transcription.

REFERENCES

The effects of rifampicin addition on protein synthesis (implicit in Fig. 2C) may be shown more dramatically through the use of continuously labeled cultures. Two T4-infected cultures were labeled with $^{14}$C-amino acids for the first 12 min after infection; one culture received rifampicin at 1 min post-infection. The radioactive proteins from these two cultures were analyzed on SDS-acrylamide gels (Fig. 3). At least 10 bands have greatly decreased intensities in the preparation from rifampicin-treated T4-infected cells. Among the T4 prereplicative genes whose expression is selectively inhibited by rifampicin addition at 1 min post-infection are the genes 43, 46, 45, rIIB, 32, and internal protein III. Bautz and his co-workers have presented evidence that the rIIB cistron is under the control of an independent promoter which is not recognized until about 2 min post-infection at 30° (15). Thus the class of cistrons represented by...
FIG. 2. The effect of rifampicin addition 1 min after infection on T4-specific protein synthesis. Cells were infected with T4 and simultaneously labeled with 14C-amino acids (1 μCi per ml; 10 ng of casamino acids per ml). At 1 min post-infection the cells were divided into a control culture (●) and a rifampicin-treated culture (○). At various times post-infection, aliquots of 1.0 ml were removed and the incorporation of 14C-amino acids into proteins was determined (A). In a separate experiment cells were infected with T4, and rifampicin was added to the entire culture at 1 min post-infection. Aliquots of this culture were pulsed with 14C-amino acids (0.8 μCi per ml) at the following times: 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 26, and 26 to 31 min post-infection. The rates of amino acid incorporation for an uninfected aliquot (●) and the various pulses after infection (○) are given as counts per min per min for 0.5 ml of cells (B). Several samples from B were run on SDS-gels and are shown in C.

Fig. 3. The effect of rifampicin addition at 1 min post-infection on T4-specific protein synthesis during continuous labeling. Cells were infected at zero time and rifampicin was added to one-half of the culture at 1 min post-infection. Radioactive amino acids (0.2 μCi per ml) were added successively to the cultures at zero time, 3, 6, and 9 min after infection; the infections were stopped at 12 min post-infection. The radioactive proteins were analyzed on SDS-gels. The arrows indicate those bands which are missing or present in reduced quantities in the culture to which rifampicin was added at 1 min post-infection.

The 10 rifampicin-sensitive bands in Fig. 3 may share a common regulation with the rIIA cistron. For the remainder of this paper we assume that these rifampicin experiments define a second class of promoters which we designate quasi-late (PQ). Under "Discussion" we will outline a defense for this specific interpretation.

Kinetics of Quasi-late Promoter Recognition—We now ask at what time after infection the second class of promoters is recognized. Since we know that no new prereplicative proteins are synthesized later than 6 min post-infection (Fig. 1), we can be certain that all prereplicative promoters are first recognized before that time. Therefore we have infected E. coli and added rifampicin to the infected cultures at various times after infection. Each of these cultures was continuously labeled for the first 12 min of the infection. The data for amino acid incorporation in such cultures and the SDS-polyacrylamide gel analyses are shown (Fig. 4, A and B). These preliminary experiments show that a short period of transcriptional initiations is sufficient to saturate the translational apparatus; that is, for rifampicin addition at 1 min and 2 min post-infection, amino acid incorporation is approximately 60% and 80% of the untreated control, respectively (see below). As a function of the time of
Time of Rifampicin Addition
(minutes after infection)

FIG. 4. T4-specific protein synthesis after rifampicin addition at various times post-infection. Cells were infected at zero time in the presence of \(1^\text{4C}\)-amino acids (0.8 \mu Ci per ml). At the times indicated, aliquots were transferred into rifampicin. The infections were stopped at 12 min after infection by pouring onto ice in the presence of casamino acids (10 mg per ml). Amino acid incorporation into protein is shown for two separate experiments (A, B); the data are for 0.25 ml of infected cells (A). Samples taken from the cultures which received rifampicin at every minute after infection (B) were run on SDS-gels (C). The autoradiogram also shows a control culture labeled for the first 12 min of infection in the absence of rifampicin.

In order to understand the implications of Fig. 4B, the results were re-examined quantitatively. T4-infected cells were again treated with rifampicin at various times post-infection; the labeling period was again continuous but was extended to 20 min (which allows complete translation of available mRNA (see Fig. 2B)). The radioactive proteins were analyzed on SDS-acrylamide gels; the band densities in the resulting autoradiograms were determined. We then plotted the relative accumulation of eight prereplicative proteins as a function of the time of rifampicin addition (Fig. 5, A and B). Clearly two classes of prereplicative proteins may be distinguished: some proteins (encoded by genes 52, rIIA, 39, and 293) accumulate in decreasing amounts if rifampicin is added after 2 min (Fig. 5B), whereas some proteins (encoded by genes 43, 46, 32, and rIIB) accumulate in increasing amounts if rifampicin is added at 2 min post-infection or later (Fig. 5A). Included in the second class is the gene 32 protein whose synthesis is completely inhibited by rifampicin addition during the first 12 min of infection. We will interpret these data (under "Discussion") by suggesting that some genes (such as rIIA, 39, 52, and 293) are regulated uniquely by promoters recognized immediately after infection (\(P_n\)'s), that some genes (such as 43, 46, and rIIB) are regulated both by \(P_n\)'s and quasi-late promoters (\(P_Q\)'s), and that some genes (such as 32) may be uniquely regulated by \(P_Q\)'s.
addition, we will argue that quasi-late promoter recognition first occurs at 1 1/4 min (see also below) post-infection, but that quasi-late transcripts probably account for only a small fraction of the prereplicative RNAs which accumulate in the first minutes after infection (5, 11). Lastly, we must include in any model building the possibility that T4 development occurs at mRNA excess.

We next examined the regulation of the rIIB cistron. The rIIB cistron may be transcribed polycistronically along with the rIIA cistron or under the control of a promoter site which lies between the rIIA and rIIB genes (15, 20). The rIIA cistron is regulated by a promoter recognized immediately after infection (Fig. 4B and 5B). Therefore, the ratio of the synthetic capacities for the rIIB and rIIA proteins, if rifampicin is added at various times after infection, should reflect the relative number of rIIB messengers initiated at each promoter by the time of rifampicin addition. To quantitate the ratio of rIIB protein to rIIA protein, we constructed a mutant lacking genes 32 and 44 (since the products of genes 32 and 44 have similar molecular weights to the rIIB protein (31)). Fig. 6A shows that in the absence of the gene 32 and 44 proteins, the rIIB protein resides in a unique position on an SDS gel. Using the double mutant (32-, 44-), we analyzed promoter recognition for the rIIA and rIIB cistrons. Rifampicin was added to infected cells at various times; each culture was labeled with 14C-amino acids from the time of infection until 20 min post-infection. The radioactive proteins were run on SDS gels and the amounts of rIIA and rIIB proteins were determined from densitometer tracings. The result of this experiment is quite striking. The ratio of rIIB to rIIA protein begins to rise steeply in cultures to which rifampicin is added at times later than 11/4 min post-infection (Fig. 6B). We suggest that this result vindicates the use of rifampicin as a probe for the timing of promoter recognition, since 2 min post-infection is precisely the time at which rIIA-independent rIIB mRNA can first be detected (15). Thus the ratio of rIIB:rIIA rises at the same moment that gene 32 expression becomes insensitive to rifampicin (Fig. 5A). We note that rifampicin addition during the first min of infection allows the rIIB protein to be synthesized in 2-fold molar excess of the rIIA protein. Thus the polycistronic transcript of the rIIA and rIIB region may be translated in a manner rather different from the equimolar translation of the polycistronic tryptophan operon of E. coli (37).

Rifampicin Sensitivity of T4 DNA Synthesis—We have used T4-specific DNA synthesis as an additional diagnostic probe for the recognition of quasi-late promoters. Since at least three quasi-late genes are required for efficient DNA replication (genes 45, 43, and 32 (38)), the time at which DNA synthesis becomes rifampicin insensitive could reflect the initiation of transcription at quasi-late promoters. Infected cells were treated with rifampicin at various times after infection; control cultures were treated with chloramphenicol at similar times. The cultures...
FIG. 7. T4 DNA synthesis after addition of rifampicin or chloramphenicol at various times post-infection. Cells were infected in the presence of [methyl-3H]thymidine (4 μCi per ml; 4 μg of thymidine per ml). At the times indicated, aliquots of 1.0 ml were transferred into rifampicin (●) or chloramphenicol (▲). The infections were stopped at 30 min post-infection by the addition of an equal volume of 1 N NaOH. The samples were allowed to sit in NaOH overnight; incorporation of tritiated thymidine into DNA was measured by standard techniques (3).

were labeled with [3H]thymidine for the first 30 min of infection, and the incorporation of radioactivity into DNA was measured. DNA synthesis first became rifampicin insensitive at about 3 min post-infection (Fig. 7). In the chloramphenicol-treated cultures, DNA synthesis did not become antibiotic insensitive until later than 6 min post-infection. Apparently 6 or 7 min of protein synthesis are required for T4 DNA synthesis to begin.

DNA synthesis first became rifampicin insensitive at about 3 min post-infection (Fig. 7). In the chloramphenicol-treated cultures, DNA synthesis did not become antibiotic insensitive until later than 6 min post-infection. Apparently 6 or 7 min of protein synthesis are required for T4 DNA synthesis to begin.

In a variety of experiments designed to measure when T4 DNA synthesis first begins, we have been unable to detect incorporation of tritiated thymidine into DNA by the end of the first 7 min post-infection (3). We suspect that in these experiments DNA synthesis reflects the presence of late promoters that do not become antibiotic insensitive until later.

Promoter Recognition during Postreplicative Transcription—Experiments were designed to ask at what time the late promoters first become rifampicin insensitive; these experiments are conceptually identical with those shown earlier. Rifampicin is added to infected cells at various times post-infection; these cultures are labeled continuously with 35S-amino acids for the first 7 min of infection (Fig. 8A). At about the 11th min after infection, late protein synthesis becomes insensitive to rifampicin (Fig. 8B). These facts are compatible with DNA-RNA hybridization data (5, 6), which suggest that late transcription may be detected at about the 11th min post-infection at 30°C. Sheldon has measured the time at which the synthesis of the gene 34 protein becomes insensitive to rifampicin (42); our data support her conclusions. Since her experiments were performed in cells made permeable to rifampicin by the procedure of Levine (43), the similarities in our data suggest that the permeability of rifampicin is not a complication in our experiments.

DISCUSSION

Rifampicin as a Probe for Classes of Gene Expression—Most of the experiments in this paper are dependent on the action of the antibiotic rifampicin (29). For our interpretations to be valid, we must know that rifampicin rapidly prevents the subsequent initiation of RNA synthesis in T4-infected E. coli AS19. In fact, we do not know precisely to what extent the rifampicin has blocked initiation events. We used as controls cultures to which rifampicin and bacteriophage were added simultaneously; never did such cultures transcribe and translate any bacteriophage genes (see for example, Fig. 4B and 8B). Since irradiated hosts were used for these experiments (31), we would have observed easily a tiny fraction of the normal synthetic rate in autoradiograms obtained after long exposures. These results suggest that by the time adsorption and DNA penetration have occurred, the RNA polymerase of the host must be completely inhibited by the antibiotic.

We assume for the remainder of the discussion that the sensitivity of RNA polymerase to rifampicin during T4 infection is not altered by changes in the uptake of the drug. Support for
this assumption may be derived from a comparison of our data for rifampicin-refractive synthesis of the gene 34 protein and similar data obtained by Sheldon (42). Sheldon's experiments were performed with cultures made permeable to rifampicin using the procedure of Leive (43); in her experiments the synthesis of the gene 34 protein became rifampicin insensitive at the same time as in our experiments (Fig. 5D). Lastly, the available data suggest that T4-induced modifications to the RNA polymerase (12-14, 21-25) do not decrease significantly the sensitivity of the enzyme to rifampicin (29).

**T4 Development May Occur under Conditions of Excess mRNA**—A striking observation may be made by examining the gel patterns and amino acid incorporation data shown in Figs. 4 and 8. If, as in Fig. 8, rifampicin is added at various times post-infection to cultures labeled continuously for 30 min, the translational apparatus is nearly saturated with that mRNA synthesized via initiations which occur during the first 2 min. Furthermore, those proteins which are translated under the regulation of promoters recognized later in infection (quasi-late and late) appear to be translated at the expense of proteins under the control of previously available promoters. Thus, in a 30-min labeling, substantially more protein encoded by genes 39, 52, and rIIA accumulates if rifampicin is added 2 min after infection than if the antibiotic is added much later. Quantitative data which are consistent with this interpretation are shown in Fig. 5, A and B. Although complicated interpretations for this phenomenon may be considered (44), we favor the notion that the addition of new transcripts to an excess pool of mRNA can cause "shutdown" of some gene expression. Thus new mRNAs could successfully compete with old transcripts for the limiting translational apparatus. We mention this aspect of T4 development here because P4 recognition against a background of excess mRNA must have a profound effect on the subsequent accumulation of quasi-late proteins (as in Fig. 5). We plan to explore this phenomenon further.

**Topology of Early Transcription Units**—The early transcription units (P5->T5) must encode the majority of the prereplicative proteins. When rifampicin is added to infected cells after 1 min of infection, all but a few of the prereplicative proteins are synthesized (Figs. 2 and 3); these include the delayed early proteins (5, 19). Among the T4 proteins synthesized in large quantities in a culture treated with rifampicin at 1 min post-infection is the rIIA protein; since the rIIA cistron is in the delayed early class (15, 20, 45), the rIIA does not inhibit selectively transcription of delayed early genes. This is consistent with data which suggest that the major delayed early species are generated via promoter recognitions that occur immediately after infection; most delayed early RNAs are the 3'-OH termini of polycistronic messengers containing immediate early species at the 5'-end (11, 17).

Our experiments utilizing a cell-free system derived from uninfected *E. coli* suggest that the majority of immediate early and delayed early genes can be expressed in vitro (10-19, 46, 47). Early gene expression requires no components from T4-infected cells other than T4 DNA; therefore, we may argue that the complete expression of early transcription units occurs without the mediation of transcriptional or translational factors altered during T4 infection (17, 19). Therefore the host RNA polymerase must be responsible for the recognition of early promoters (7, 8, 48).

The experiments reported in this paper, the experiments with cell-free systems (16-19), and the data demonstrating read-through from immediate early cistrons as the primary source of delayed early RNA in vivo (11) all provide strong confirmation of the topological model for the prereplicative portions of the T4 genome (proposed originally by Milanesi et al. (7, 8)). Tandemly arranged early transcription units (P5->T5) span the prereplicative regions; these transcription units must account for the bulk of the prereplicative transcripts which accumulate during the first 4 to 5 min of infection (5, 8, 19). If initiations at early promoters occur rapidly (5, 6), if the average early transcription unit is not longer than 8000 base pairs (10, 49), and if the RNA chain growth rate is between 1000 and 1500 nucleotides per min at 30°C (15, 50, 51), all of the early RNAs would first appear within the initial 5 min of infection (5, 11). This is in fact the case (5).

Lastly, early transcription units are frequently thought to contain termination signals dividing those units into promoter proximal (immediate early) and promoter distal (delayed early) segments (4, 5). The primary experiment in support of this concept is the exclusive transcription of immediate early regions during T4 infection of chloramphenicol-treated *E. coli* (4, 5). We have argued previously that chloramphenicol, by virtue of its ability to cause polarity (52, 53), can restrict apparent transcription to promoter proximal genes (17, 19, 54, 55). Until further experiments are reported which deal directly with the putative regulatory regions between immediate early and delayed early genes, we will assume that passive transcription of early transcription units provides an adequate description of most prereplicative RNA synthesis (19).

**Rifampicin Experiments Define a Second Class of Prereplicative Promoters**—Of the many possible interpretations for our data, the simplest is that the sensitivity of specific gene expression to rifampicin 1 min after T4 infection (see Fig. 3) reflects a delay in promoter recognition for those genes. Schmidt et al. (15) reported that rIIIB transcription first occurs under the control of a promoter adjacent to the rIIB cistron, and that rIIB specific mRNA can first be detected 2 min after infection. In support of those data, we find that the rIIB promoter is first recognized 1½ min post-infection (Fig. 6B), and that the rIIB protein is first detectable during a pulse of ²H₂O amino acids from 2 to 2½ min post-infection. The data in Figs. 3, 5, and 6B suggest that many cistrons display a rifampicin sensitivity analogous to the rIIB cistron, we believe that these cistrons are regulated as well by new promoters.

Support for this straightforward interpretation may be derived from two independent sources. Travas has shown that the RNA polymerase extracted from T4-infected cells during the prereplicative period has the capacity to initiate transcription at sites other than those recognized by the *E. coli* holoenzyme (12-14). In addition, Salser et al. described a prereplicative class of transcripts which has a low relative abundance during the first 5 min of infection and an increased relative abundance at later times (5). Each of the prereplicative proteins (the products of genes 43, 45, 46, 32, rIIB, internal protein III, as well as each of the four remaining unidentified bands of Fig. 3) which may be under the control of new promoters is synthesized at a higher rate 20 min post-infection than at any time during the first 5 min of infection (see Fig. 1); conversely, most of the prereplicative proteins are synthesized at decreased rates at
lates in general (5)) support a transcriptional explanation for late cistrons. Such complicated models seem premature at this time. However, it is clear that other, more fanciful, interpretations are possible. For example, our data could be explained by the rapid synthesis after infection of any post-transcriptional "factor" which increases the translational yield of the quasi-late cistrons. Such complicated models seem premature at this time. Furthermore, the DNA RNA hybridization data (concerning the rII B cistron specifically (15), and the quasi-lates in general (5)) support a transcriptional explanation for our experiments.

**Topology of Prereplicative Transcription Units—**Our terminology may now be reclarified and set into proper perspective. Early transcription units designate prereplicative regions bounded by a promoter and termination site, PE → TE; immediate early genes are proximal to PE sites, whereas delayed early genes are distal to those same PE sites (8). Quasi-late promoters may regulate genes which are included within or excluded from early transcription units; if a quasi-late promoter regulates a gene included within an early transcription unit, that gene may be an immediate early or delayed early gene (Fig. 9). Of the 10 protein bands under the control of quasi-late promoters, perhaps three do not accumulate at all if PE recognition is blocked by rifampicin (Figs. 2C and 3). Of these three bands, only one has been identified (the product of gene 32). Jayaraman has demonstrated that the prereplicative genes 40 and 41 are not transcribed in vitro by the E. coli RNA polymerase (59) ; perhaps these three genes are essentially dependent on PE recognition for any significant expression. Conversely, the rIIB, 43, and internal protein III cistrons are encoded within early transcription units; the rIIB protein and internal protein III are efficiently synthesized as well under the control of early promoters (17, 19). The rIIB cistron is promoter distal with respect to a PE site and is a delayed early gene (15, 20); the internal protein III gene is nearly adjacent to a PE site and is an immediate early gene (17, 45). Our experiments with rifampicin suggest strongly that the rIIB and internal protein III cistrons are regulated by quasi-late promoters in addition to early promoters. Thus, as depicted in Fig. 9, the early transcription units and the quasi-late transcription units must overlap to some (unknown) extent. If chloramphenicol functions as described above, T4 infection of chloramphenicol-treated E. coli would result in the accumulation of immediate early RNAs as well as (owing to overlap) RNAs subsequently controlled by PE/As. Chloramphenicol would restrict against the accumulation of delayed early RNAs and RNAs subsequently controlled by PE/Bs and PE/Cs. These facts will make the analysis of those components required for quasi-late promoter recognition in vitro a difficult task (12–14); in fact, only cistron-specific analyses of the products of cell-free RNA synthesis may be used to demonstrate rigorously quasi-late promoter recognition.

An additional aspect of PE recognition is implicit in Fig. 9. The quasi-late promoters may be used both to increase the transcriptional yield of specific prereplicative cistrons and also to insure that those transcripts continue to be synthesized if initiations at early promoters become infrequent. We note that each of the identified quasi-late cistrons (except gene 32) is thought to encode a catalytic function (60); therefore it is not clear what evolutionary constraints demanded the generation of this second transcriptional mode. However, the notion of overlapping transcription units is not original and has been discussed previously (61). In particular, we call attention to an extremely thoughtful discussion pertaining to such overlap in the prereplicative regions of the T4 genome (48); that discussion, although addressed to a slightly different point, could well have been written for the data presented in this manuscript.

We emphasize that we do not understand in detail what alterations of the host RNA polymerase are brought about by T4 infection, nor do we understand what role such alterations play in controlling promoter recognition. The host enzyme is altered soon after infection; the α subunit is adenylated (21, 25), the β and β′ subunits are altered in an unknown manner (22), and the σ factor no longer is bound to the core during purification (23). These changes occur quickly enough to cause quasi-late promoter recognition (21, 25), although no direct evidence has been presented that the two phenomena are related. We note that a new T4 mutation has been described which, under restrictive conditions, does not appear to allow the expression of the quasi-late cistrons.8 The RNA polymerase undergoes further alterations at later times, becoming associated after the

---

8 A cistron is considered to be under the control of a PE if the protein encoded by that cistron appears when rifampicin is added to the culture 1 min after infection. These conclusions are verified by performing an identical infection using an appropriate mutant in that gene.

---

8 T. Mattson, personal communication
9th min with the protein products of genes 33 and 55 (62). Since the products of genes 33 and 55 are required for post-replicative transcription (3, 63), it is reasonable that the addition of these proteins to the RNA polymerase is involved directly in late promoter recognition. We do not know whether RNA polymerase is altered at the complete expense of the previously functioning enzyme. If the enzyme is only partially altered at each conversion, then the infected cell continuously acquires additional capacities to interact with DNA, without losing (completely) previous promoter options. If these ideas are approximately correct, the infected cell continuously develops, the complexity of its genetic regulation continuously increases. We hope that a description of the temporal control of promoter recognition in vivo will be of value to those attempting cell-free transcription using components found in T4-infected cells.

Acknowledgments—We thank Dr. Joyce Silver for reading this manuscript and Tom Hill for helping with the experiments. We thank many of our colleagues in Boulder, especially the other members of our laboratory, Dr. David Hirsh’s laboratory, and Dr. Charles Yegian’s laboratory for helpful and enjoyable discussions. One of us (L.M.G.) thanks Drs. Richard Epstein and Ed Brody for their hospitality during pleasant visits to their laboratories.

REFERENCES

1. NYGAARD, A. P., AND HALL, B. D. (1964) J. Mol. Biol. 9, 125
Bacteriophage T4 Gene Expression: EVIDENCE FOR TWO CLASSES OF PREREPLICATIVE CISTRONS
Patricia Z. O'Farrell and Lawrence M. Gold


Access the most updated version of this article at http://www.jbc.org/content/248/15/5502

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/248/15/5502.full.html#ref-list-1