The Regulation of Transcription in Bacteriophage T5-Infected *Escherichia coli*†

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ABSTRACT: The expression of bacteriophage T5-specific RNA and protein in infected cells is temporally separated into three classes: class I (preearly), class II (early), and class III (late). By immunoprecipitation techniques we have shown that T5 infection of cells leads to the synthesis of one class I polypeptide (11,000 daltons) and two class II polypeptides (90,000 and 15,000 daltons) capable of binding to the RNA polymerase of the host *Escherichia coli* cell. One of the class II polypeptides (90,000 daltons) is the product of gene C2, which is an essential gene product required for the initiation of class III RNA synthesis. The colicinogenic factor, ColIb, is a plasmid which prevents the normal synthesis of class II and class III bacteriophage T5-specific RNA in infected colicinogenic (ColIb$^+$) cells. In T5-infected colicinogenic cells, only the T5 class I polypeptide is found associated with the RNA polymerase. Mutants of T5, designated T5h^-, are capable of growth on both noncolicinogenic and Col1b$^+$ hosts. Extracts of T5h^- infected Col1b$^+$ cells were shown to lack a small class I polypeptide (12,000 daltons) as compared to T5-infected cells. The h^- mutation, however, has no effect on the levels of the class I T5 polypeptide of similar molecular weight which is bound to the RNA polymerase. One effect of the h^- mutation is to enhance the quantities of the two class II polypeptides bound to the enzyme.

Bacteriophage T5 and the closely related phage BF23 share several unique biological properties. The DNA of both phages is injected into the host cell by a two-step process. Attachment of the phage to a sensitive cell is followed by the injection of only 8% of the total phage DNA, designated the first step transfer portion (fst)[1] (Lanni, 1960). This limited portion of the genome must be transcribed and translated to yield phage specific class I RNA and protein before the remainder of the genetic material can be taken within the host cell (McCorquodale and Lanni, 1964).

T5 or BF23 infection of *Escherichia coli* cells that contain the Col1b factor (Col1b$^+$ cells) results in the normal synthesis of only the earliest (class I) T5-specific RNA and proteins (Moyer et al., 1972). The synthesis of phage-specific class II RNA and protein initiates normally. However, both types of macromolecular synthesis terminate prematurely several minutes later (R. C. Herman and R. W. Moyer, submitted to *Virology*). No class III RNA or protein is synthesized in infection Col1b$^+$ cells and the infection is abortive. Spontaneous phage mutants (designated h^-) selected for their ability to grow on Col1b$^+$ cells have been isolated (Strobel and Nomura, 1966; Nisioka and Ozeki, 1968; Mizobuchi et al., 1971). The mutation which allows the phage to grow on both colicinogenic and noncolicinogenic strains of *E. coli* is located within the fst portion of the genome and permits a normal infection and allows the transcription of all three classes of RNA and the subsequent formation of all three classes of protein. The primary defect in macromolecular synthesis in the abortive infection of Col1b$^+$ cells appears to involve transcription (Moyer et al., 1972). In an attempt to understand the mechanism by which the Col1b plasmid interrupts continued phage transcription we have undertaken a general study of the regulation of phage-specific RNA synthesis in T5-infected *E. coli*.

Bacteriophage T5, like T4, relies on the RNA polymerase of the host cell for transcription of all viral RNA (Beckman et al., 1972a; Haselkorn et al., 1969). Stevens (1972) has found four T4-specific proteins associated with *E. coli* RNA polymerase purified from T4-infected cells. Two of these proteins are the products of genes 33 and 55 (Horvitz, 1973; Stevens, 1974) which by genetic studies have been implicated in the regulation of T4 RNA synthesis in vivo (Snustad, 1968; Guha et al., 1971). Our approach to detect T5-specific polypeptides which interact with RNA polymerase has been to assume that any such polypeptides would coprecipitate with RNA polymerase when the enzyme itself is isolated by immunoprecipitation. Precipitation of RNA polymerase by the addition of specific antibodies has been used by Greenleaf et al. (1973) to detect a new 70,000-dalton polypeptide bound to the RNA polymerase of *Bacillus subtilis* during sporulation.

Materials and Methods

Preparation of Phage. T5*st*(0), a heat stable deletion mutant of T5$^+$ was used for all experiments and is referred to throughout the text simply as T5. T5h^-, a mutant of T5*st*(0) that overcomes the Col1b factor-mediated restriction, was selected as a spontaneous mutant able to grow on *E. coli* K12 W3110*thy*$^-$ (Col1b) (designated as Col1b$^+$

† From the Department of Biochemistry, Columbia University, New York, New York 10032. *Received October 25, 1974.* This work was supported by Research Grant AI-10302 from the National Institute of Allergies and Infectious Diseases and by Grants E637 and VC-142 from the American Cancer Society. C.S. and B.D. were supported by Training Grant GM00255 from the National Institute of General Medical Sciences to the Department of Biochemistry. R.W.M. is supported by a Career Development Award GM34354 from the National Institute of General Medical Sciences.

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[1] Abbreviations used are: fst, first step transfer portion of T5 DNA; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; Col1b$^+$, *E. coli* cells that contain the colicinogenic factor Col1b; EDTA, ethylenediaminetetraacetic acid.

cells throughout this paper). High titer stocks of both phages were prepared in T-MGM medium (Moyer and Buchanan, 1970) and purified by the procedure described by Bujard and Hendrickson (1973) except that the CsCl steps were omitted. Basic T-MGM medium contains per liter: maleic acid (5.8 g), Tris (12 g), KCl (2 g), $NaH_2PO_4 \cdot H_2O$ (0.18 g), Na_2SO_4 (0.12 g), $MgSO_4 \cdot 7H_2O$ (0.025 g), glucose (5 g), and NH_4Cl (4 g) at a final pH of 7.3. The medium was further supplemented with 5 μg/ml of thymine and adjusted to 2 m*M* $CaCl_2$. The host bacterium used for preparation of high titer stocks was *E. coli* K12 W3110*thy*$^-$.

Growth and Preparation of Cells for Infection. E. coli K12 W3110*thy*$^-$ and *E. coli* K12 W3110*thy*$^-$ (Col1b) are the noncolicinogenic or colicinogenic (Col1b$^+$) strains used in all the experiments reported here. Cells were grown in T-MGM medium to a concentration of 5×10^8 cells/ml and were harvested at 4° by centrifugation at 5000*g* for 10 min. The cells were resuspended at 0° at a concentration of 1×10^{10} cells/ml in T-MGM buffer (T-MGM lacking glucose and NH_4Cl) that contained 5 μg/ml of thymine and 2 m*M* $CaCl_2$. Phage was added to a final multiplicity of infection of 10 at 0° in an equal volume of T-MGM resuspending buffer. The infected cells were placed on ice for 20 min followed by a 5-min incubation at 37°. Viral growth was initiated by diluting the infected cells tenfold into T-MGM prewarmed to 37°. The final concentration of infected cells is 5×10^8 cells/ml.

Labeling of T5 Proteins for Subsequent Separation on SDS Polyacrylamide Slab Gels and Autoradiography. At various times after dilution of infected cells into prewarmed T-MGM, 5-ml samples were removed and incubated for 2 min at 37° without aeration with 5 μCi of a mixture of ^{14}C-labeled amino acids (specific activity >10 Ci/mol) (CFB-104 Amersham). Labeling was terminated by pouring the cells onto 5 g of ice with sufficient chloramphenicol to yield a final concentration of 100 μg/ml. The cells were pelleted by centrifugation at 5000*g* for 5 min at 0°. Each pellet was resuspended in 0.25 ml of 0.05 *M* Tris chloride (pH 6.8) that contained 1% SDS (w/v), 1% mercaptoethanol, 10% glycerol, and 0.005% Bromophenol Blue (w/v), boiled for 2 min, and incubated overnight at 37°. Samples of 10 μl were applied to 10 cm, 15% SDS polyacrylamide slab gels and the proteins were separated by electrophoresis at 35 V for 19 hr at room temperature using the Tris-glycine buffer as described by Studier (1972). After electrophoresis, the slab gels were stained with a solution of 0.25% (w/v) Coomassie Blue (Schwartz/Mann), 9% acetic acid, and 45.4% methanol for 2 hr at room temperature. The slab gels were destained by diffusion with several changes of a solution that consisted of 7.5% acetic acid and 5% methanol. The destained gels were dried under vacuum and subjected to autoradiography using Kodak No-Screen Medical X-ray film as described by Fairbanks et al. (1965).

Preparation of Rabbit Antiserum Directed against DNA-Dependent RNA Polymerase from E. coli. E. coli RNA polymerase holoenzyme was assayed and purified from *E. coli* B through the DEAE-cellulose step according to the procedure described by Burgess (1969). The RNA polymerase was further purified by chromatography on a calf thymus DNA-cellulose affinity column (Bautz and Dunn, 1971). The homogeneous enzyme [2 mg in 1 ml of 10 m*M* Tris · HCl, 10 m*M* $MgCl_2$, 0.1 m*M* dithiothreitol, 0.1 m*M* EDTA, and 5% glycerol (w/v) at a final pH of 7.9] was mixed with an equal volume of complete Freund's adjuvant. One toe pad on each foot of a young adult rabbit was injected with 0.05 ml of this suspension at 1 week intervals for 4 weeks. After the last injection, 25 ml of blood from alternate ears was collected weekly for 6 weeks. The blood samples were incubated at 25° for 1 hr, stored at 4° overnight, and then centrifuged at 43,000*g* for 30 min. The resulting crude serum was heated for 30 min at 56° and stored at −20°.

Labeling of Proteins for Precipitation with Antisera Prepared against E. coli RNA Polymerase. Uninfected cells were labeled in 20-ml samples with $Na_2{}^{35}SO_4$ in T-MGM medium that was modified to contain reduced amounts of sulfate. The $MgSO_4$ was replaced by $MgCl_2$ and the final Na_2SO_4 concentration was lowered from 0.12 g/l. to 0.0025 g/l. of $Na_2{}^{35}SO_4$ (40 μCi/ml, 50 Ci/mol) (New England Nuclear) was added to an exponentially growing culture at a density of 1×10^8 cells/ml at 37°. Growth was continued until the cell concentration reached 6×10^8 cells/ml at which time 10 mg/ml of unlabeled Na_2SO_4 was added to the culture. The cells were grown an additional 20 min, harvested by centrifugation, then either stored or subsequently infected.

For labeling with ^{3}H amino acids, leucine (0.5 μg/ml) and lysine (0.75 μg/ml) were first added to the T-MGM medium. Then 20-ml samples of infected cultures, at a concentration of 5×10^8 cells/ml, were pulse labeled for 3-min periods at 37° by exposure to 25 μCi/ml each of [4,5-^{3}H]leucine (>40 Ci/mmol) and [4,5-^{3}H]lysine (40 Ci/mmol) (Schwarz/Mann). To terminate the labeling, unlabeled leucine and lysine were each added to a final concentration of 0.2 mg/ml and incubation was continued for an additional 1.5 min. Chloramphenicol (Calbiochem) was then added to a final concentration of 100 μg/ml to terminate the infection. Pilot experiments have shown that both radioactive leucine and lysine are linearly incorporated throughout the 3-min pulse labeling period. After harvesting, all labeled samples were stored at −20°.

Extraction and Immunoprecipitation of E. coli RNA Polymerase. Since the preparation of extracts for immunological precipitation requires at least 1 g of cells for each sample, identically grown unlabeled infected or uninfected carrier cells were prepared from 750 ml of cells at 5×10^8 cells/ml and added to each labeled sample. All steps in the extraction procedure were performed at 4° and phenylmethanesulfonyl fluoride (Calbiochem.), a protease inhibitor, was present in all solutions at 0.3 mg/ml. A 6-ml amount of buffer (0.05 *M* Tris · HCl, 0.01 *M* $MgCl_2$, 0.2 *M* KCl, 0.1 m*M* dithiothreitol, 0.1 m*M* EDTA, and 5% glycerol at a final pH of 7.5) was added to each gram of cells. The cell suspensions were sonicated for a total of 2 min in 30-sec bursts. After sonication, pancreatic deoxyribonuclease (100 μg/g of cells) was added and the sonicated extract was incubated for 30 min. Cellular debris and ribosomes were removed by centrifugation in the Spinco 50Ti rotor (50,000 rpm for 90 min) and an equal volume of saturated $(NH_4)_2SO_4$ (pH 7.0) was added to the supernatant. After 30 min the precipitate was collected by centrifugation (40,000*g* for 20 min). The precipitates were resuspended by gently shaking overnight at 4° in 1 ml of buffer A (0.01 *M* Tris · HCl (pH 7.9), 1.0 m*M* $MgCl_2$, 0.1 m*M* EDTA, 0.1 m*M* dithiothreitol, and 5% glycerol). The samples were then clarified by centrifugation at 40,000*g* for 20 min prior to antibody precipitation.

RNA polymerase was precipitated by adding 50 μl of antisera to 300 μl of the clarified extract and incubating the mixture overnight at 4°. The precipitate was collected by

centrifugation at 40,000*g* for 20 min and was then washed four times with 2 ml of cold 0.9% NaCl (w/v). Each sample was then resuspended in 0.2 ml of dissolving buffer containing 0.02 *M* Na_2HPO_4, 6 *M* urea, 1% SDS (w/v), 0.5 *M* 2-mercaptoethanol, and 10% glycerol (w/v). The samples were then heated at 90° for 5 min and stored under liquid nitrogen.

SDS Polyacrylamide Gel Analysis of Labeled Proteins Precipitated with Antisera. The radioactive proteins precipitated with antisera were separated on SDS polyacrylamide gels as described by Weber and Osborn (1969). The 10-cm gels consisted of 0.1% SDS, 10% acrylamide, and 0.27% *N,N'*-methylenebisacrylamide in 0.1 *M* sodium phosphate (pH 7.2). The electrophoresis buffer contained 0.1% SDS and 0.1 *M* sodium phosphate (pH 7.2). Bromophenol Blue (0.04%) was added as a tracking dye and 50-μl samples were layered on each gel. Electrophoresis was performed at 2 mA/gel for 1 hr and then 7 mA/gel for 7.5 hr. Under these conditions the tracking dye migrated three-quarters of the way through a 10-cm gel. The protein bands were fixed and visualized by staining with Coomassie Blue as described earlier under Materials and Methods. The gels were then frozen and sliced into 1-mm segments. Each 1-mm segment was incubated overnight at 37° with shaking in a scintillation vial with Omnifluor scintillation fluid (New England Nuclear) that contained protosol (114 ml/l.) (New England Nuclear).

Since all T5-infected samples were prepared from cells identically grown and prelabeled with $Na_2{}^{35}SO_4$, a constant amount of ^{35}S label could either be applied or calculated for each gel. In this way, the amounts of ^3H-labeled T5-specific protein from a standard number of cells could be directly compared. The ^3H and ^{35}S counts obtained from each sample gel were corrected for channel spillover and are plotted as indicated in the individual figures.

Results

Immunological Isolation of E. coli RNA Polymerase. For these experiments, we used antisera prepared against host RNA polymerase holoenzyme purified from uninfected noncolicinogenic cells. In order to determine both the specificity of the antibody preparations and to assay for possible alterations of the polypeptide subunits of the host RNA polymerase which might occur as a result of T5 infection, exponentially growing cells were exposed to $Na_2{}^{35}SO_4$ in order to label all the proteins of the uninfected host cell. A portion of the prelabeled cells was removed and the remainder were infected with T5 and incubated for 12 min at 37°. The cells were harvested, and cellular extracts were prepared as described under Materials and Methods. The RNA polymerase was precipitated from each sample by the addition of the antisera and analyzed on SDS polyacrylamide gels. By 12 min after infection all three classes of T5-specific RNA have been formed; however, no alterations were detected in either the sizes or the relative amounts of the subunits of the host RNA polymerase (Figure 1B), compared with the enzyme from the uninfected cell (Figure 1A). It is of interest to note that at 12 min after T5 infection, an apparently intact host σ factor is still precipitable by the antisera. However, since the antisera was prepared to the RNA polymerase holoenzyme, the σ subunit is likely to precipitate whether or not it is associated with the core enzyme. The data presented in Figure 1 suggest that the antigen-antibody precipitates are free of contaminating host protein indicating that very little nonspecific precipitation

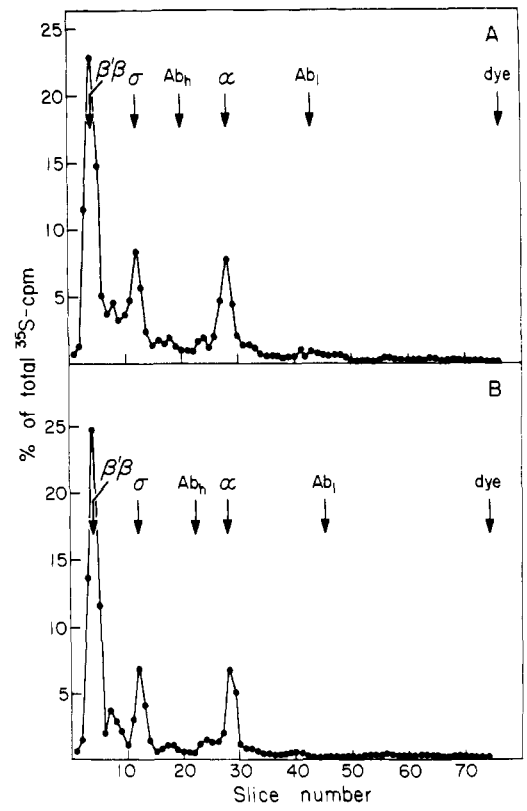

FIGURE 1: SDS polyacrylamide gel profiles of RNA polymerase from solubilized antigen-antibody precipitates. [^{35}S]RNA polymerase was prepared from (A) uninfected *E. coli* K12 W3110*thy*$^-$ and (B) *E. coli* K12 W3110*thy*$^-$ cells 12 min after T5 infection. The labeled subunits were separated on SDS polyacrylamide gels as described under Materials and Methods. Data are presented as the percent (%) of the total ^{35}S cpm applied to each gel. A total of 49,000 ^{35}S cpm and 50,000 ^{35}S cpm were applied to the gels represented in panel A and panel B, respectively. The electrophoretic mobilities of the $\beta\beta'$, σ, and α subunits of the RNA polymerase are indicated by arrows on the basis of coelectrophoresis of purified enzyme with each sample. Ab_h and Ab_l refer to the locations of the heavy and light chains of the immunoglobulin molecules. The molecular weights of these polypeptides are: $\beta\beta'$, 160,000; σ, 96,000; Ab_h, 55,000; α, 41,000; and Ab_l, 25,000.

occurs. The small peak between the $\beta\beta'$ bands (which do not resolve on these gels) and the σ band is a protein band designated as τ. The τ component is a protein that copurifies with RNA polymerase and is found in variable amounts in most RNA polymerase preparations (Burgess et al., 1969).

An experiment similar to that shown in Figure 1 was performed with ColIb$^+$ cells which were prelabeled with $Na_2{}^{35}SO_4$ prior to T5 infection. The RNA polymerase complex which precipitated before or after T5 infection yields gel patterns identical with those shown in Figure 1 (data not shown). These results suggest that the interruption of T5 transcription by the plasmid is not due to a plasmid induced degradation of the host enzyme or to a ColIb factor protein, present prior to infection, that can strongly interact with RNA polymerase either before or as a consequence of T5 infection.

Detection of Phage Specific Proteins That Associate with E. coli RNA Polymerase in Infected Noncolicinogenic Cells. In order to detect the presence of any new phage polypeptides which may associate with the *E. coli* RNA polymerase during T5 infection, a double labeling technique was used to differentiate between the host components and the phage specific proteins of the RNA polymerase. Expo-

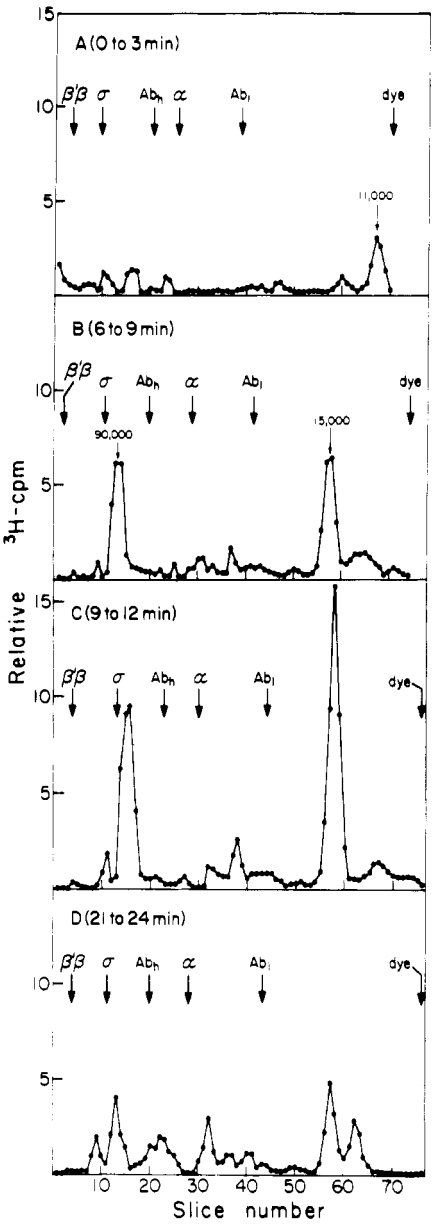

FIGURE 2: SDS polyacrylamide gel profiles of T5-specific ^{3}H-labeled proteins bound to RNA polymerase. Unstarved, ^{35}S-prelabeled *E. coli* K12 W3110*thy*$^-$ cells were infected with T5 at a multiplicity of infection of 10 and pulse-labeled with [^{3}H]leucine and [^{3}H]lysine for 3-min periods at (A) 0–3 min, (B) 6–9 min, (C) 9–12 min, and (D) 21–24 min after infection. The label, extraction, immunoprecipitation, and gel analysis procedures are described under Materials and Methods. Although the immunoprecipitated proteins are double-labeled, only the ^{3}H-labeled bands (corrected for label crossover) are shown with the arrows indicating the marker positions as described in Figure 1. To allow comparison between individual gel samples, the corrected amount of ^{3}H label in each gel slice was normalized to a selected sample as described under Materials and Methods. An equivalent amount of radioactivity derived from prelabeled RNA polymerase could be recovered from immunoprecipitates with an equal number of uninfected or infected cells for up to at least 30 min after infection. The total amount of ^{3}H cpm applied to each gel is (A) 52,000 cpm; (B) 46,810 cpm; (C) 75,350 cpm; and (D) 42,100 cpm.

nentially growing host cells were labeled with $Na_2{}^{35}SO_4$, and the labeling was terminated by the addition of an excess of unlabeled sodium sulfate. The prelabeled culture was then infected with T5 and the phage proteins were labeled with [^{3}H]leucine and [^{3}H]lysine at various times during the infectious cycle.

Since T5 infection results in an almost immediate cessation of host protein synthesis, only phage specific proteins are labeled with the tritiated amino acids. The RNA polymerase was then immunologically precipitated from the infected doubly labeled cell extracts. The ^{35}S-labeled subunits of the host enzyme and any associated ^{3}H-labeled T5 specific proteins that coprecipitated were separated on SDS polyacrylamide gels. The ^{35}S-labeled subunits of the host RNA polymerase served a dual purpose. First, they provide internal molecular weight standards in the gel analysis, and second, they facilitate the standardization of the relative amount of ^{3}H label in each sample per amount of ^{35}S-labeled subunits.

Between 0 and 3 min after infection, only class I T5-specific proteins are synthesized. Analysis by polyacrylamide gels of the RNA polymerase precipitated by the antisera from T5-infected cells labeled between 0 and 3 min after infection shows a ^{3}H-labeled protein which migrates near the Bromophenol Blue tracking dye (Figure 2A). Although this band of low apparent molecular weight (approximately 11,000) is found in somewhat variable amounts in repeat experiments, its appearance is dependent on T5-infection since this polypeptide is not present in control uninfected cells (Figure 2 and unpublished results).

The synthesis of class II T5 proteins begins at 5 min after infection, whereas the synthesis of class III proteins is initiated 9 min after infection. During these latter stages of infection, two additional phage proteins of approximately 90,000 and 15,000 daltons are found associated with the RNA polymerase by the antibody precipitation procedure. Both polypeptides are first detected in cells labeled between 6 and 9 min after infection, after class I protein synthesis has diminished, and are maximally detected (or bound) between 9 and 12 min after infection (Figure 2B and C). However, in cells labeled from 21 to 24 min after infection, reduced amounts of the 90,000- and 15,000-dalton proteins are present (Figure 2D). Since the amount of ^{3}H-labeled phage-specific proteins shown in Figure 2 has been normalized to allow quantitative comparison of all gel samples, the kinetics of appearance and disappearance of the 90,000- and 15,000-dalton proteins associated with the RNA polymerase suggests that these proteins probably represent class II phage polypeptides. The much smaller peaks of radioactivity observed on these gels occur in different regions in repeat experiments and are probably contaminants.

The Effect of Prior Cell Starvation on the Size of a T5 Polypeptide Bound to RNA Polymerase. T5 attachment to cells is a fairly slow process, and in order to maintain synchrony during infection, the cells are usually starved in buffer for 20–30 min at 37° prior to the addition of phage. However, if cells are starved prior to infection, the amount of the 90,000 dalton T5 polypeptide which is associated with RNA polymerase is greatly diminished and a new band of 45,000 daltons appears (Figure 3). The results of many separate experiments suggest that the increase in the amount of the 45,000-dalton band is proportional to the loss observed in the band of 90,000 daltons. In contrast, starvation causes no significant alterations in the host subunits of the RNA polymerase. We conclude that the appearance of the 45,000-dalton polypeptide in prestarved cells may occur by cleavage of the 90,000 dalton polypeptide by a host proteolytic activity induced by the starvation procedure. Therefore, in all the experiments reported in this paper, an infection procedure described under Materials and Methods was adopted which eliminates starvation but which still maintains synchrony throughout the infectious cycle.

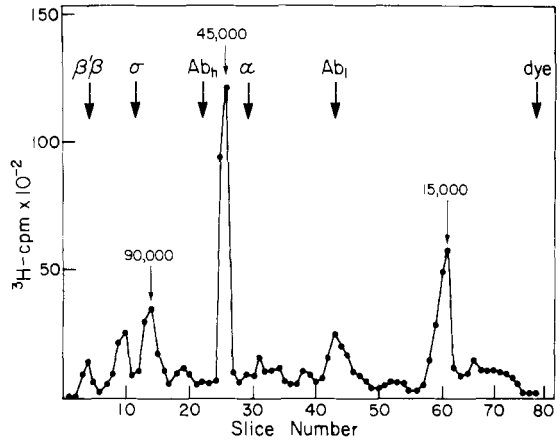

FIGURE 3: SDS polyacrylamide gel profile of ^{3}H-labeled T5-specific polypeptides bound to RNA polymerase. *E. coli* K12 W3110*thy*$^-$ cells, prestarved for 30 min, were infected with T5 and pulse labeled with [^{3}H]leucine and [^{3}H]lysine between 6 and 9 min after infection. The extracts were prepared and treated with antisera as described under Materials and Methods. The data are plotted as ^{3}H cpm per gel slice. A total of 31,000 ^{3}H cpm was applied to the gel.

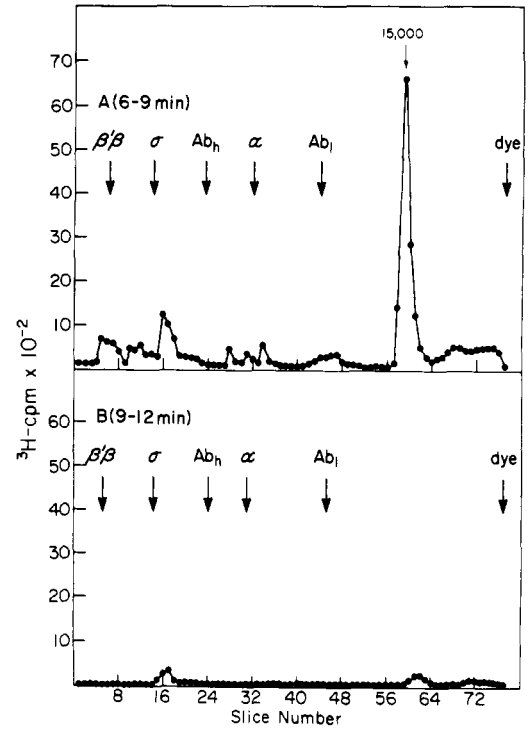

FIGURE 4: SDS polyacrylamide gel profiles of ^{3}H-labeled *T5amC2*-specific polypeptides bound to RNA polymerase. *E. coli* K12 W3110*thy*$^-$ cells, prelabeled with $Na_2{}^{35}SO_4$, were infected with T5*amC2* and pulse labeled with [^{3}H]leucine and [^{3}H]lysine. The extracts were prepared and treated with antisera as described under Materials and Methods. After solubilization of the antigen-antibody precipitates, amounts of [^{35}S]RNA polymerase equivalent to those applied to gels in Figure 3 were analyzed and the data treated as in Figure 3: (A) T5*amC2* infected cells pulse labeled 6–9 min after infection; (B) T5*amC2* infected cells pulse labeled 9–12 min after infection.

Antibody Precipitation Studies on Cells Infected with T5 Mutants Defective in Gene C2. No specific function can be assigned to any of the individual phage proteins that associate with RNA polymerase on the basis of the antigen–antibody precipitation experiments alone. However, the fact that these polypeptides are found bound to the polymerase renders them potentially capable of altering the transcriptional specificity of the enzyme. Two mutants of T5 have been described which fail to carry out significant synthesis of phage-specific class III RNA and protein. One mutant is known to be defective in gene *D15* which codes for a 5′-exonuclease (Chinnadurai and McCorquodale, 1973). The nuclease is necessary for the introduction of nicks or gaps into newly replicated T5 DNA, a step which is thought to render the DNA "competent" for late transcription (Frenkel and Richardson, 1971). The second mutant is defective in gene *C2* and likewise fails to synthesize significant amounts of T5 class III RNA and proteins. The biological properties of the *C2* mutant have led to the prediction that the product of the *C2* gene may be a putative T5 σ factor (Chinnadurai and McCorquodale, 1974).

We have, therefore, assayed labeled extracts of cells infected with T5*amC2* for the presence of the three phage polypeptides associated with RNA polymerase that we have described. Between 0 and 3 min after infection with T5*amC2* one class I polypeptide is associated with the polymerase as observed in the control (data not shown). In infected cells labeled between 6 and 9 min after infection the smaller, 15,000-dalton polypeptide is bound in quantities comparable to that found in wild T5 infected cells (Figure 4A). However, very little of the 90,000-dalton polypeptide is bound to the polymerase (compare Figure 4A and Figure 2B). By 9–12 min after infection with T5*amC2* neither of these phage proteins are detected in association with the polymerase (Figure 4B).

In addition to the very low synthesis of class III RNA and protein, one of the phenotypic properties of the T5*amC2* mutants is the premature termination of the synthesis of class II RNA and protein by 15 min after infection (Chinnadurai and McCorquodale, 1974). The absence of the 15,000-dalton polypeptide from cells labeled between 9 and 12 min after infection may be the result of even earlier termination of the synthesis of this polypeptide prior to the general premature termination of all class II protein synthesis. However, the 90,000-dalton polypeptide is never detected bound to RNA polymerase immunoprecipitated from cells infected with the *amC2* mutant, even between 6 and 9 min after infection, a time when the synthesis of the 15,000 dalton companion phage polypeptide is unaffected. These results suggest that the product of the *C2* gene could be a T5 specific σ factor protein of about 90,000 daltons. The possibility exists, however, that the action of the *C2* gene is indirect and is required for either the synthesis or the function of the 90,000-dalton polypeptide that we have described.

A Proposal for the Nomenclature of E. coli RNA Polymerase Complexes That Contain T5-Specific Polypeptides. In order to more concisely describe putative T5-directed modifications of the host RNA polymerase, we have utilized, in part, the suggestions of Stevens (1974). *E. coli* RNA polymerase containing a bound T5 polypeptide chain of 90,000 daltons will be designated RNAP(T5)(90). Likewise, RNAP(T5)(11), RNAP(T5)(15), or RNAP(T5)(90)(15) will be used to designate host RNA polymerase that contains bound T5 polypeptides of 11,000 daltons, 15,000 daltons, or two polypeptides of 90,000 and 15,000 daltons, respectively. Since the method of detection of these polypeptides is mostly by radioactivity measurements, this nomenclature is only used when a form of the enzyme can be unambiguously described. No *absolute* stoichiometry between phage and host subunits has been determined by us and none is intended in this nomenclature.

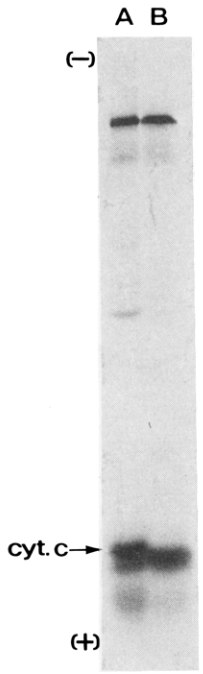

FIGURE 5: Separation of T5 and T5h^- proteins labeled 0–3 min after infection by electrophoresis on 15% SDS polyacrylamide gels. The procedures for electrophoresis and the subsequent autoradiography of the dried gels is described under Materials and Methods; (A) T5-infected *E. coli* K12 W3110*thy*$^-$ and (B) T5h^- infected *E. coli* K12 W3110*thy*$^-$. The electrophoretic mobility of nonradioactive equine cytochrome *c* (12,000 daltons) was determined after staining with Coomassie Blue and is indicated by the arrow. Only radioactive phage bands are visualized in the autoradiogram shown here.

The Pattern of T5-Specific Proteins Associated with RNA Polymerase in Infected Col Ib$^+$ Cells. Since the Col Ib plasmid appears to interfere with T5 development at the level of transcription, it was of interest to analyze the RNA polymerase from T5-infected Col Ib$^+$ cells for the presence of the three T5 polypeptides. Equivalent quantities of RNAP(T5)(11) are formed between 0 and 3 min after infection in infected Col Ib$^+$ cells compared to T5-infected noncolicinogenic cells, but at later times only very small amounts of the other two binding proteins are detected (data not shown). In Col Ib$^+$ cells only class I RNA and protein are synthesized in normal quantities and there is only a very limited synthesis of class II and no synthesis of class III protein (Moyer et al., 1972; R. C. Herman and R. W. Moyer, submitted to *Virology*). Therefore, the small amount of RNA polymerase containing the 90,000- and 15,000-dalton T5 polypeptides is undoubtedly related to the generally limited biosynthesis of the class II proteins. These observations on T5-infected Col Ib$^+$ cells support our hypothesis that RNAP(T5)(11) is a class I derived species of RNA polymerase and that the subsequent modification of the enzyme by the 90,000- and 15,000-dalton polypeptides represent a further alteration of the polymerase by class II proteins.

Observation on the Pattern of Class I Proteins Formed by T5 and T5h^- Mutants. The phage mutation which allows T5 growth on Col Ib$^+$ cells is located within the fst (class I) portion of the viral genome (Beckman et al., 1972b). Mizobuchi and McCorquodale (1974) compared the electrophoretic mobilities in nondenaturing polyacrylamide gels of the class I proteins formed by either BF23 or the BF23h^- mutants. They have noted that the presence of the h^- mutation results in differences in the quantities of several of the class I protein bands. BF23 induces the extensive synthesis of protein bands designated 1c and 1d but very little synthesis of protein band 1e, while BF23h^- induces very small amounts of protein bands 1c and 1d but large amounts of protein band 1e. The authors concluded that proteins 1c and 1d might be oligomeric forms of protein 1e and that the putative oligomers were responsible for the arrest of phage growth.

We have examined the labeled class I phage proteins in both T5 and T5h^- infected cells after treatment of the proteins with mercaptoethanol and SDS to disrupt any aggregates prior to analysis on SDS polyacrylamide gels. We have found that the T5h^- mutant does not synthesize one small class I polypeptide that is found in the T5 parental strain (Figure 5). This difference has been observed in both infected noncolicinogenic and colicinogenic host cells. The phage specific polypeptide present in T5 but not T5h^- infected cells has a molecular weight of about 12,000 as judged by its comigration with cytochrome *c* on SDS polyacrylamide gels.

The failure of the T5h^- mutant to synthesize a class I polypeptide present in cells infected with T5 wild strains, is an observation which could be consistent with the findings of Mizobuchi and McCorquodale (1974) if the missing polypeptide normally promoted the oligomer formation. The *lack* of synthesis of a polypeptide by the T5h^- mutant is consistent with the genetic observation that T5h^- mutants are recessive to T5 in a mixed infection of Col Ib$^+$ cells since the mixed infections are abortive (Nisioka and Ozeki, 1968; Beckman et al., 1972b; Szabo and Moyer, unpublished results). The recessive character of T5h^- mutants, moreover, would be predicted if the T5h^- mutant failed to synthesize a product involved in the restriction process.

The Effect of the h Gene on the T5-Specific Proteins Associated with RNA Polymerase. T5h^- mutants allow all the T5 genes to be transcribed equally well in both Col Ib$^+$ and noncolicinogenic infected cells. These mutants also fail to synthesize a class I polypeptide of 12,000 daltons, which is presumably involved, together with a plasmid product in the ultimate antagonism of continued T5 transcription. Therefore, it was of interest to examine the pattern of T5h^- specific proteins associated with RNA polymerase utilizing antigen–antibody precipitation experiments identical with those described for T5-infected noncolicinogenic cells. In T5h^- infected cells labeled from 0 to 3 min after infection, RNAP(T5)(11) is formed in amounts equivalent to those found for other T5-infected cells (Figure 6A). Likewise, beginning at 6–9 min after infection the 90,000- and 15,000-dalton polypeptides become associated with the polymerase (Figure 6B). However, as the T5h^- infection proceeds, a significant difference develops in the amount of the 90,000-dalton polypeptide bound to the polymerase compared to T5-infected noncolicinogenic cells under identical conditions. Between 6 and 9 min after infection nearly identical amounts of the 90,000 dalton species is found bound to the polymerase in both T5 and T5h^- infected noncolicinogenic cells (Figures 2B and 6B). By 9–12 min the amount of the larger peptide in T5h^- infected cells is more than double and by 21–24 min after infection is about five times greater than that found for a culture of T5-infected noncolicinogenic cells. However, no significant differences are found in the relative amounts of the 15,000-dalton polypeptide bound to the polymerase in T5 and T5h^- infected non-

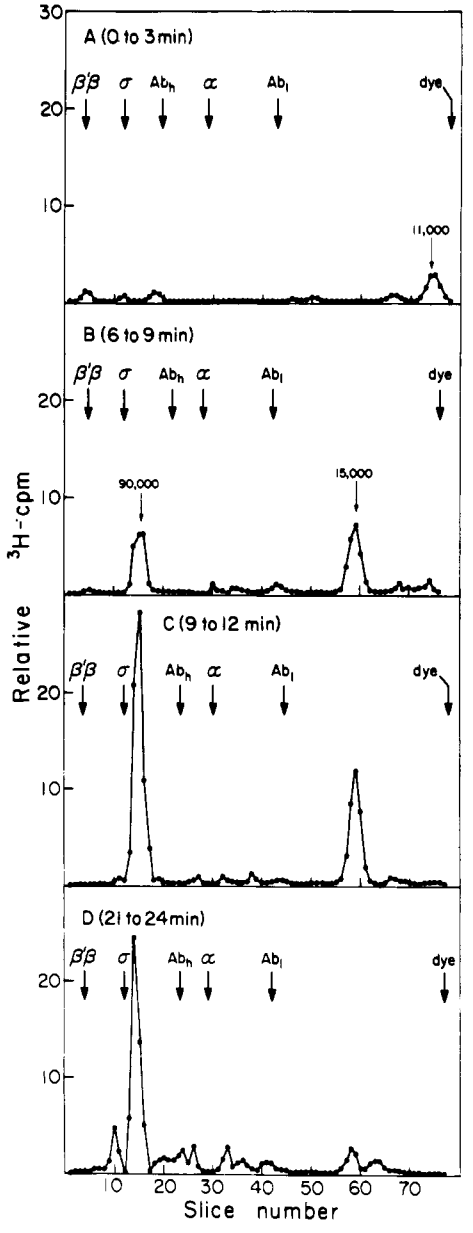

FIGURE 6: SDS polyacrylamide gel profiles of ^{3}H-labeled T5h^- specific protein bound to RNA polymerase. ^{35}S-prelabeled *E. coli* K12 W3110*thy*$^-$ cells were infected with T5h^- and pulse labeled with [^{3}H]leucine and [^{3}H]lysine from (A) 0–3 min; (B) 6–9 min; (C) 9–12 min; and (D) 21–24 min. The extracts were prepared and the data were treated as described in the legend for Figure 2. The total amount of ^{3}H cpm applied to each gel was (A) 19,800 cpm; (B) 64,700 cpm; (C) 110,100 cpm; and (D) 74,000 cpm.

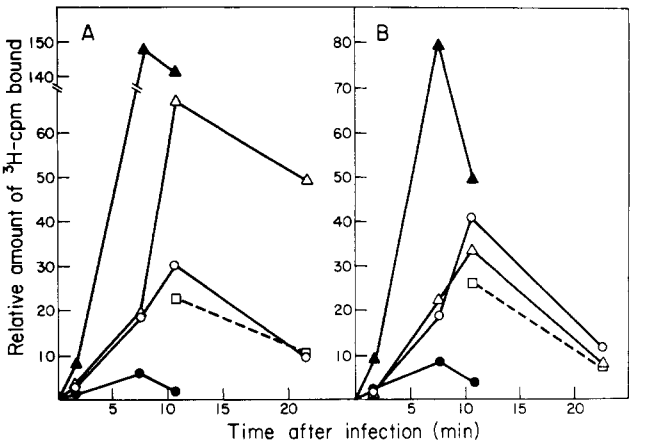

FIGURE 7: The kinetics of appearance of the class II T5-specific proteins which bind to the host RNA polymerase. Data which were obtained from the experiments described in Figures 2 and 6 were employed in this figure. Identical SDS polyacrylamide gel analysis was also performed for *E. coli* K12 W3110*thy*$^-$ (Col Ib) cells infected with T5 or T5h^- and pulse-labeled with [^{3}H]leucine and [^{3}H]lysine at 9–12 min and 21–24 min after infection. Extracts were prepared and analyzed on SDS polyacrylamide gels as described under Materials and Methods. The relative amounts of the 90,000-dalton protein (A) and the 15,000-dalton protein (B) were obtained by a summation of the ^{3}H cpm present in each band. The points in the figure refer to the midpoint of each pulse period. (○—○) T5-infected *E. coli* K12 W3110*thy*$^-$ cells; (●—●) T5-infected *E. coli* W3110*thy*$^-$ (Col Ib) cells; (△—△) T5h^- infected *E. coli* K12 W3110*thy*$^-$ cells; (▲—▲) T5h^- infected *E. coli* K12 W3110*thy*$^-$ (Col Ib) cells; (□—□) T5 and T5h^- infected *E. coli* K12 W3110*thy*$^-$ cells.

colicinogenic cells (Figures 2 and 6). The kinetics of appearance of the T5-specific proteins bound to RNA polymerase described in these experiments have been compiled and are presented in Figure 7.

In addition, RNA polymerase has been assayed for the presence of the three T5 proteins in cultures of T5h^- infected Col Ib$^+$ cells and in cultures of noncolicinogenic cells mixedly infected with T5 and T5h^-. Infection of Col Ib$^+$ cells with T5h^- results in a pronounced increase of both class II proteins bound to polymerase compared to either T5h^- or T5 infected cultures of noncolicinogenic cells (Figure 7). The amount of the class I 11,000-dalton protein was again unaltered (data not shown).

T5h^- mutants are recessive to wild type T5 since a mixed infection of Col Ib$^+$ cells yields no progeny phage. However, a mixed infection of noncolicinogenic cells yields equal numbers of both T5 and T5h^- progeny. The amount of the 90,000-dalton polypeptide in mixedly infected noncolicinogenic cells is similar to that seen in T5 rather than T5h^- single infected cells (Figure 7). Thus the total amount of this protein bound to RNA polymerase in T5h^- infected cells is reduced by coinfection with wild type T5.

In general, the effect of the h^- mutation on the three T5 polypeptides associated with RNA polymerase appears to be only a quantitative one. Under all conditions tested the amount of RNAP(T5)(11) appears to be unaffected and therefore this class I polypeptide does not appear to be the one deleted by the h^- mutation. Of the two class II polypeptides which bind to RNA polymerase, the level of binding of the larger of these seems to be enhanced by the h^- mutation irrespective of the host cell. Enhanced levels of binding of the smaller polypeptide is seen only in colicinogenic hosts.

Discussion

The synthesis of the three classes of T5-specific RNA depends on the RNA polymerase of the host cell (Sirbasku and Buchanan, 1971; Beckman et al., 1972). The temporal control of T5 transcription seems analogous to the regulation of T4 transcription in which phage-induced changes in the host RNA polymerase are necessary for the synthesis of the different classes of T4 RNA and for the shutoff of host transcription (Schachner and Zillig, 1971; Walter *et al.*, 1968). In the case of T4, the β,β' and α subunits of the host enzyme are conserved throughout infection (Goff and Weber, 1970; Stevens, 1972). However, after T4 infection it appears that the σ activity is changed (Seifert et al., 1969; Crouch et al., 1969; Bautz et al., 1969; Travers, 1970) and that several new phage polypeptides become associated with

the host enzyme (Stevens, 1972; Stevens and Crowder, 1974). Two of these peptides are probably the products of genes 33 and 55 (Stevens, 1972; Horvitz, 1973), which have been shown to be involved in late T4 RNA synthesis (Bolle et al., 1968; Snustad, 1968; Pulitzer, 1970; Pulitzer and Geiduschek, 1970; Snyder and Geiduschek, 1968; Guha *et al.*, 1971).

A search was initiated for T5-directed modifications of the host RNA polymerase which might play a role in the regulation of viral transcription. Although our goal was initially to examine the properties of purified enzyme preparations, it became obvious from both our own studies and those of others, that a rapid assay of the subunit composition of the RNA polymerase in infected cells was first essential to define any potential species of enzyme modified by phage polypeptides. For example, losses of unstable regulatory subunits from RNA polymerase complexes during the purification of the enzyme have been documented. Brown and Cohen (1974) found that a λ phage-induced protein of 72,000 daltons, which associates with RNA polymerase to stimulate transcription of λ DNA, is lost or inactivated during purification. Stevens (1974) has described similar observations with certain of the T4-associated polypeptides of RNA polymerase. The immunoprecipitation of RNA polymerase provides a very rapid assay of subunit composition and should minimize losses due to proteolysis or protein instability.

The evidence from our immunoprecipitation studies suggests that at least three phage-specific polypeptides associate with the host enzyme during the course of infection and may serve to regulate transcription. A T5 class I polypeptide of 11,000 daltons is the first phage protein found associated with the enzyme, forming RNAP(T5)(11). One possible role for the class I polypeptide might be to enhance transcription of class II RNA sequences from repaired rather than native DNA. Class II RNA is synthesized from 5 to 20 min after infection and is initially transcribed from the native, nicked form of parental DNA. In vitro, this form of T5 DNA serves as an excellent template for the synthesis of class II RNA with purified enzyme from both uninfected (Pispa and Buchanan, 1971) and infected *E. coli* (C. Szabo and R. W. Moyer, manuscript in preparation). However, repair of the T5 DNA begins at 6 min and yields fully repaired duplex molecules by 10 min after infection (Herman and Moyer, 1974). Therefore, the bulk of class II RNA is synthesized from a repaired T5 template, a process which may be facilitated by the class I T5 polypeptide of 11,000 daltons. Studies on the in vitro transcription of repaired T5 DNA should be very useful as an approach to answer this question.

Two class II polypeptides of 90,000 and 15,000 daltons have also been found to be associated with the *E. coli* RNA polymerase. Immunoprecipitation of RNA polymerase from cells infected with T5*amC2* mutants yields little or none of the 90,000-dalton class II polypeptide. These observations, together with those of Chinnadurai and McCorquodale (1974), who showed an absolute requirement of the *C2* gene product for the initiation of class III RNA synthesis, suggest that the *C2* gene product is a T5 specific σ factor of 90,000 daltons. Both this σ factor and the 5′-exonuclease produced by gene *D15* (Chinnadurai and McCorquodale, 1973) are needed for the initiation of class III or late T5 transcription.

An analysis of immunoprecipitates of RNA polymerase from cells starved prior to infection has shown that starvation induces a rapid degradation of the 90,000-dalton phage subunit. One product of this degradation is a 45,000-dalton polypeptide which, however, remains immunoprecipitable and hence is still presumably bound to the host enzyme. Although the starvation-induced degradation appears to involve only this polypeptide, the biological significance of this selectivity, if any, is not yet clear. We have also noted the complete removal of the 90,000-dalton polypeptide from RNA polymerase by DEAE-cellulose chromatography during routine purification of the enzyme (C. Szabo and R. W. Moyer, manuscript in preparation).

The β,β′ and α subunits of the host enzyme appear to be conserved during T5 infection, since no gross changes in these polypeptides have been observed in either noncolicinogenic or Col1b$^+$ cells as a result of infection. The fate and the role of the host σ factor following T5 infection is not yet clear. We have presented evidence for the existence of a new phage σ factor, yet the analysis of the RNA polymerase immunoprecipitated from either T5-infected noncolicinogenic cells late in infection or from infected Col1b$^+$ cells after all in vivo transcription has ceased suggest that the host σ factor is not destroyed during infection of either cell type.

In part, our attempts to determine how T5 transcription is regulated were initiated by our interest in the interruption of T5 transcription that occurs in Col1b$^+$ cells. In this regard, we have made several relevant observations. (1) SDS polyacrylamide gels of the host RNA polymerase subunits from infected and uninfected Col1b$^+$ cells are identical with those from noncolicinogenic cells. (2) The Col1b factor itself does not seem to specify any components which bind to the host RNA polymerase. (3) Only one T5 class I polypeptide of 11,000 daltons is found associated with RNA polymerase in T5-infected Col1b$^+$ cells. (4) SDS polyacrylamide gel analysis of T5-infected cell extracts indicates, as expected, that neither class II polypeptide is found bound to RNA polymerase in appreciable quantities in infected Col1b$^+$ cells. (5) The h^- mutation in T5 eliminates a class I phage polypeptide of 12,000 daltons and alleviates the premature termination of class II transcription to allow growth of T5 in Col1b$^+$ cells. The protein deleted by the h^- mutation, however, is not the class I polypeptide bound to RNA polymerase. Our studies have shown that equivalent amounts of the class I polypeptide of similar size (11,000 daltons) is found bound to RNA polymerase in T5h^- infected cells. (6) The h^- mutation also has an effect on the amount of the 90,000-dalton class II polypeptide found associated with RNA polymerase. T5h^- infection of noncolicinogenic cells leads to a twofold increase in the amount of this protein precipitated by antisera at 9–12 min and a fivefold increase between 21 and 24 min. T5h^- infection of Col1b$^+$ cells, however, results in a further large increase of both class II proteins precipitated, particularly at 6–9 min. Although the Col1b factor appears to prevent the normal synthesis of both class II binding proteins during wild type T5 infection, the plasmid enhances the amount of polypeptides which can be precipitated with antisera during T5h^- infection.

The increased amounts of these proteins bound to the polymerase may be the result of their increased affinity to RNA polymerase or to an increase in the total amount of the protein synthesized. These alternatives are not easily resolvable by immunoprecipitation experiments alone. Experiments to directly measure the rate of synthesis of the 90,000-dalton class II polypeptide in infected cells after

analysis on SDS polyacrylamide gels are hampered by the presence of the product of T5 gene *D9* (DNA polymerase) which has a nearly identical molecular weight (Chinnadurai and McCorquodale, 1974; Steuart et al., 1968). Preliminary experiments with T5*amD9* and T5h^-*amD9* mutants, in which the *D9* gene product is eliminated through amber mutation, suggest that the rate of synthesis of the 90,000-dalton T5 occurs normally for at least 10 min and implies that the h^- mutation affects the binding between phage and host components of the polymerase.

If a ColIb product interacts with or modifies the host RNA polymerase, it alone is not sufficient to block T5 transcription. Only with the further involvement of the *h* gene product can T5 growth be arrested. Since there is no evidence that either of these products binds to RNA polymerase, each in turn may serve as catalysts in the modification of the enzyme and this modified polymerase would then be unable to transcribe the in vivo template. Since T5 transcription in infected ColIb$^+$ cells ceases at a time when the nicks in the infectious T5 parental DNA are being repaired, it is possible that the changes brought about by the *h* gene and the ColIb factor prevent the utilization of repaired T5 DNA as a template for transcription. Experiments which measured the in vivo repair of the single-stranded interruptions in the T5 DNA (R. C. Herman and R. W. Moyer, submitted to *Virology*) suggested that the T5 DNA is ligated in vivo by a T5-specific DNA ligase, a class II protein. If translation of class II RNA is blocked, repair of the T5 DNA is impaired with the result that transcription continues in infected ColIb$^+$ cells. This last observation is consistent with the suggestion that transcription of repaired DNA in infected ColIb$^+$ cells is blocked and also implicates a T5 class II protein, perhaps a DNA ligase, in addition to the phage *h* gene product in the ColIb plasmid directed restriction. The ColIb factor clearly affects phage macromolecular synthesis at the level of transcription. However, because of the complexity of host cell–plasmid interactions, in our view it is still too early to state that T5 transcription is directly inhibited by the ColIb factor rather than as a consequence of a plasmid directed modification elsewhere within the cell.

Acknowledgment

The gift of antisera directed against *E. coli* RNA polymerase holoenzyme from Drs. B. Dharmgrongartama and P. R. Srinivasan is gratefully acknowledged. The authors thank Dr. R. C. Herman for many helpful discussions and Dr. D. J. McCorquodale for his generous gift of T5*amC2*.

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