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Roles of the Early Genes of Bacteriophage T7 in Shutoff of Host Macromolecular Synthesis

WILLIAM T. McALLISTER* AND CORNELIA L. BARRETT

Department of Microbiology, College of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, New Jersey 08854

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Through the use of phage mutants in which various combinations of the early genes are active, and in which late gene expression is blocked, we have examined the roles of each of the five early gene products of bacteriophage T7 in regulating the synthesis of host RNA and proteins. At least two independent transcriptional controls operate during bacteriophage T7 development. The product of gene 0.7, acting alone, leads to a rapid (by 5 min) shutoff of host transcription. In the absence of gene 0.7 function, and in the absence of the phage-specified RNA polymerase, a delayed shutoff of host-dependent transcription begins at approximately 15 min after infection. This secondary control element requires either a functional gene 0.3 or gene 1.1. In the absence of any early gene products, host shutoff is not observed until much later in infection (>30 min). The delayed manner in which the products of genes 0.3 and 1.1 exert their effect suggests that their mode of action is indirect. Under conditions in which the late genes are transcribed (inefficiently) by the host RNA polymerase, gene 1.1 is observed to stimulate the synthesis of lysozyme (the product of a late phage gene). In contrast, when the late genes are transcribed by the phage-specified RNA polymerase (the product of gene 1), the kinetics of synthesis of the phage RNA polymerase itself, and of lysozyme, are not affected by the deletion of genes 0.3, 0.7, 1.1, and 1.3. We conclude that under these conditions, the products of these genes are required neither for regulation of expression of the late genes nor for the shutoff of early phage gene expression.

After infection of *Escherichia coli* by bacteriophage T7, synthesis of most host RNA and proteins ceases in about 8 min (40). As host shutoff does not require the expression of the late viral genes (6), a number of mechanisms have been proposed to account for the manner in which early phage products interfere with host macromolecular synthesis (for reviews, see 22, 36, 38, 42). Evidence has been presented demonstrating that early gene products inhibit transcription by the host RNA polymerase (6, 7, 28, 31) and suggesting that viral products may also interfere with the translation of host mRNA (7, 18, 31). In addition, it has been suggested that products of early phage genes might be required for the efficient translation of certain late viral mRNA's (24).

During intracellular development, the DNA of bacteriophage T7 is transcribed by two different RNA polymerases. Whereas the five early genes (genes 0.3 to 1.3, see Fig. 1) are transcribed by the bacterial RNA polymerase, the late genes are transcribed by a phage-specified RNA polymerase which is the product of gene 1 (8, 43). In cells infected with gene 1^- mutants, the expression of the phage genome is therefore limited to the early region (20, 36). Through the use of T7 mutants in which all, none, or various combinations of the early genes are active, and in which late gene expression is blocked, we have determined the contribution of each of the early phage products to host shutoff. The results of these experiments have furthered our understanding of the mechanisms by which bacteriophage T7 regulates the expression of host and phage genes during infection.

MATERIALS AND METHODS

Bacterial and phage stocks. *E. coli* B (35) was from the collection of E. K. F. Bautz. *E. coli* C (1) and O11′ (35) were obtained from F. W. Studier. The *lac* transducing phage λ*plac*5cI857*S*7 ($i^-z^+y^-$) was isolated from *E. coli* strain CSH66 of the Cold Spring Harbor collection as described by Miller (23). All stocks of bacteriophage T7 were from the collection of F. W. Studier, with the exception of C5, LG3, which was isolated in this laboratory as a single-plaque revertant from C5, *am*342a, LG3. The nomenclature used to describe all mutants is that of Studier (35, 37, 38); deletion mutants are identified by their code number, whereas amber mutations are

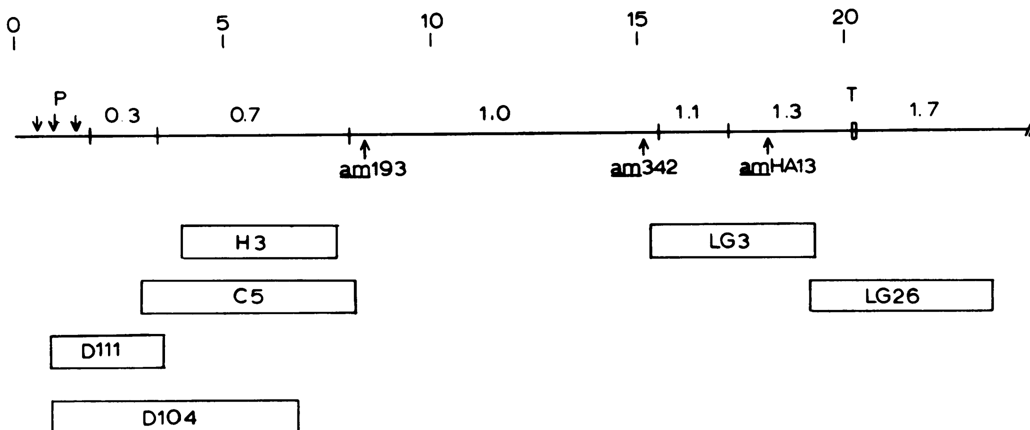

FIG. 1. *Early region of bacteriophage T7. Transcription by the host RNA polymerase is initiated at a promoter region (P) at the left end of the genome and proceeds through the five early genes (numbered sequentially: 0.3, 0.7, 1, 1.1, and 1.3) until the terminator (T) at the end of the early region is reached. Distances from the left end of the DNA molecule are given in T7 units above the line. The boxes below the line indicate the regions of DNA that are missing in the deletion mutants used in this study (33, 39). Locations of point (amber) mutations are given by the arrows (35).*

designated by the prefix *am*. Multiple mutants are designated by the code numbers of the single mutations from which they were constructed (e.g., C5, *am*342a, LG3). Where it is sufficient to indicate the phenotype of the virus, those genes that are defective are indicated by a minus superscript [e.g., (0.3^-, 1^-) phage]; all other genes are assumed to be nondefective.

Phage stocks were propagated and purified through discontinuous gradients of CsCl as described by Studier (35).

Enzyme assays. (i) Host enzymes (β-galactosidase). Bacteria were grown to a concentration of 3×10^8 cells per ml at 30°C in M9 medium (35) supplemented with 0.5% (wt/vol) Casamino Acids in place of glucose. Phage were added (10 phage particles per cell), and 15 s later the culture was induced to synthesize β-galactosidase by the addition of isopropyl-β-D-thiogalactoside to a concentration of 10^{-3} M. At intervals thereafter, samples were removed and assayed for β-galactosidase activity as described by Miller (23).

(ii) Phage-specific enzymes. Bacteria (*E. coli* C) were grown to a density of 3×10^8 cells per ml at 30°C in M9 medium and infected at a multiplicity of 10 phage particles per cell. Samples (2 ml) of the culture were poured over an equal volume of crushed, frozen buffer (0.02 M Tris-hydrochloride [pH 7.5]-0.005 M $MgCl_2$-0.002 M NaN_3-400 μg of chloramphenicol per ml), harvested by centrifugation, and washed once with 5 ml of ice-cold buffer (0.02 M Tris-hydrochloride [pH 7.5]-0.05 M $MgCl_2$-0.022 M NH_4Cl). The pellet was resuspended in 0.2 ml of buffer (0.01 M Tris-hydrochloride [pH 8.0]-0.22 M NH_4Cl-0.001 M dithiothreitol-0.005 M EDTA [tetrasodium salt]-5% [vol/vol] glycerol-100 μg of bovine serum albumin per ml) (32). The samples were frozen and thawed three times, centrifuged at $8,000 \times g$ for 2 min to remove cell debris, and stored on ice.

Phage-specific RNA polymerase was assayed at 37°C in reaction mixtures (0.1 ml) containing: 0.04 M Tris-hydrochloride, pH 7.9; 0.02 M $MgCl_2$; 0.01 M 2-mercaptoethanol; 0.1 mM EDTA; 0.4 mM ATP, GTP, and CTP; 0.2 mM [^{3}H]UTP (specific activity, 10 μCi/μmol); 10 μg of bovine serum albumin; 6 μg of T7 DNA; 2 μg of rifampin; and 20 μl of cell extract (8). After a 5-min preincubation period, synthesis of RNA was initiated by the addition of substrate and DNA. Twenty minutes later, the reaction was terminated by the addition of 1 ml of ice-cold 5% (wt/vol) trichloroacetic acid. The samples were filtered through glass-fiber filters (Whatman GF/C), which were dried and counted in a toluene-based scintillation fluid.

Lysozyme activity in the infected cells was determined by two methods. In the first method, lysozyme was assayed by its ability to cause a decrease in the optical density of a suspension of EDTA-sensitized *E. coli* (10). Substrate bacteria (*E. coli* B) were grown in T-broth (35) at 37°C to a concentration of 10^9 cells per ml and treated with EDTA as described by Greenblatt (16). Ten microliters of cell extract was added to 0.6 ml of sensitized cells at room temperature, and the decrease in optical density at 600 nm was monitored at 20-min intervals. Values given have been corrected for the rate of spontaneous lysis of the culture and for the proportion of cells resistant to lysis.

Lysozyme activity was also determined by the release of radioactivity from labeled cell wall material which had been fixed to filter-paper disks (15). Fifty microliters of cell extract was incubated with the filter in a reaction volume of 0.5 ml. After 2 h at 37°C, duplicate samples (100 μl) of the incubation fluid were removed to a scintillation mixture (4) and counted.

Hybridization assays. Bacteria (*E. coli* B) grown at 30°C in M9 medium supplemented with 0.5% Casamino Acids in place of glucose were induced to synthesize β-galactosidase by the addition of isopro-

pyl-β-D-thiogalactoside to a concentration of 10^{-3} M. Ten minutes after induction, the culture was infected at an input multiplicity of 10 phage particles per cell. At intervals thereafter, 2-ml samples of the culture were incubated for 2 min with 50 μCi of [^{3}H]-uridine. Uptake of label was terminated by mixing the sample with an equal volume of buffer containing 1% sodium dodecyl sulfate in a boiling-water bath (5). The sample was extracted twice at 60°C with phenol saturated with 0.02 M sodium acetate, pH 6.0. RNA was precipitated from the aqueous phase by the addition of 2 volumes of ethanol, dried in vacuo, and resuspended in 0.4 ml of 2× SSC–0.1% sodium dodecyl sulfate (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate).

Portions of the sample were counted in scintillation fluid to determine the total amount of RNA recovered, and duplicate portions (100 μl) of each sample were hybridized with DNA that had been denatured and immobilized on a nitrocellulose filter (14). Hybridization was carried out in 2 ml of 2× SSC–0.1% sodium dodecyl sulfate at 67°C for 18 to 20 h. Filters were treated with RNase (pancreatic, 20 μg/ml for 1 h at room temperature, followed by 10 min at 37°C), washed extensively with 2× SSC, dried, and counted in a toluene-based scintillation fluid. Values given have been corrected for background by subtracting the amount of label associated with filters having no bound DNA.

DNA was isolated from bacteriophage T7 and from λ*plac*5 as described by Miller (23). DNA from *E. coli* was isolated by the procedure of Bovre and Szybalski (3).

Pulse-labeling techniques. Bacteria grown in M9 medium at 30°C to a density of 3×10^8 cells per ml were infected at a ratio of 10 to 15 phage particles per cell. At intervals after infection, 0.1-ml samples of the culture were mixed with [^{3}H]uridine and ^{14}C-labeled amino acids (final concentrations, 10 and 2 μCi/ml, respectively). After 2 min of incubation at 30°C, the incorporation of label was terminated by spotting duplicate 50-μl portions of the sample onto filter-paper disks (Whatman 3MM, 2.5-cm diameter), which had previously been soaked in 10% trichloroacetic acid and dried (31). The filters were immediately immersed in ice-cold 10% trichloroacetic acid and subsequently batch-washed in 10% trichloroacetic acid and acetone, dried, and counted in a toluene-based scintillation fluid. Discriminator settings on the counting instruments were such that <14% of the ^{14}C counts were detected in the tritium channel.

The synchrony of infection for each sample was monitored by determining the number of uninfected bacteria at 3 min after infection. In all experiments reported here, less than 5% of the bacteria remained uninfected at this time.

Materials. Isopropyl-β-D-thiogalactoside and *o*-nitrophenyl-β-D-galactopyranoside were purchased from Sigma Chemical Co., St. Louis, Mo. Rifampin, ^{14}C-labeled amino acids (reconstituted protein hydrolysate, no. 3122-09), and [^{3}H]UTP were purchased from Schwarz/Mann, Orangeburg, N.Y. [^{3}H]uridine was purchased from New England Nuclear Corp., Boston, Mass. The unlabeled ribonucleoside triphosphates were purchased from Calbiochem, La Jolla, Calif. All chemicals used were reagent grade. [^{3}H]diaminopimelic acid was purchased from Amersham/Searle, Arlington Heights, Ill.

RESULTS

Kinetics of host shutoff in T7-infected cells. In cells infected with wild-type phage or with gene 1^- mutants, synthesis of host RNA ceases by about 4 to 6 min after infection. Previous work by others has demonstrated that this early shutoff of host transcription requires the product of gene 0.7: after infection with (0.7^-, 1^-) phage, synthesis of RNA by the host RNA polymerase continues at the preinfection level for up to 15 min after infection (7, 31). It had not been determined, however, whether the gene 0.7 product acts alone or in concert with other early phage products.

Despite the high level of RNA synthesis in cells infected with (0.7^-, 1^-) phage, synthesis of the host enzyme β-galactosidase is shut off with near-normal or only slightly delayed kinetics (7, 31). This observation suggested that there might be an additional phage function(s) that regulates the translation of host mRNA.

We have determined the kinetics of β-galactosidase synthesis in *E. coli* B after infection with mutants of T7 that are defective in a number of early genes (Fig. 2). In agreement with earlier work (7, 31), the ability of cells to synthesize β-galactosidase is greatly diminished after infection with either (1^-) or (0.7^-, 1^-) phage and is completely blocked by 10 min after infection. A significant delay in the shutoff of β-galactosidase synthesis is observed after infection with mutants that, in addition to being defective in genes 0.7 and 1, are also defective in genes 1.1 and 1.3. In these cases, β-galactosidase continues to be synthesized as late as 20 min after infection. Deletion of genes 1.1 and 1.3 without a defect in gene 0.7 does not prolong host enzyme synthesis, indicating that the contribution of the former phage functions to host shutoff can only be measured in the absence of gene 0.7 function.

To examine their roles in more detail, we have determined the effect of the T7 early genes when expressed individually or in various combinations. Since many of the mutants used in these experiments are defective in gene 0.3, and since gene 0.3 function is required to overcome host DNA restriction systems (39), the experiments were performed in a strain of *E. coli* lacking a restriction-modification system (*E. coli* C [1]).

Cultures of *E. coli* C were infected with bacteriophage T7 and simultaneously induced to synthesize β-galactosidase. At intervals after

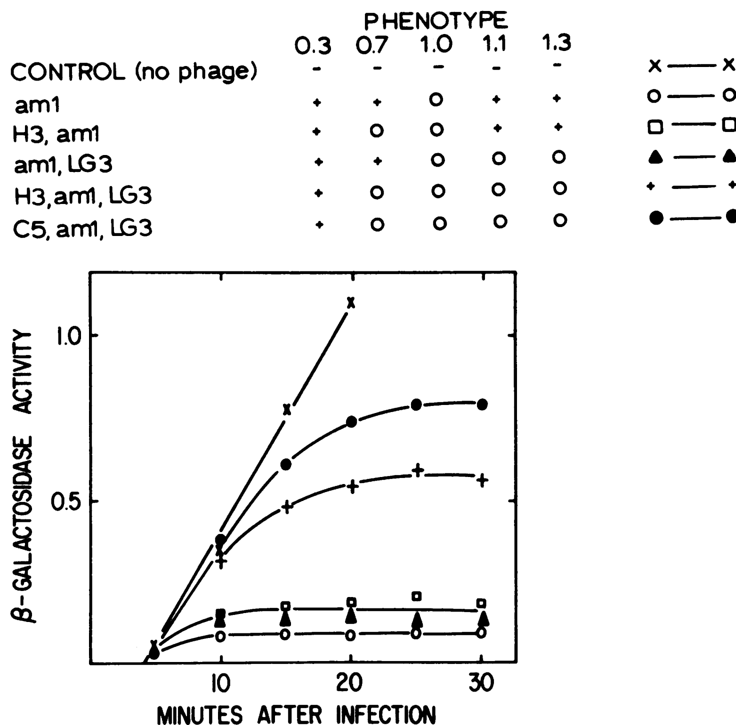

FIG. 2. Induction of β-galactosidase during infection. Bacteria (E. coli B) were grown to a concentration of 3 × 10⁸ cells per ml at 30°C in M9 medium supplemented with 0.5% (wt/vol) Casamino Acids in place of glucose. Phage were added (10 phage particles per cell), and 15 s later the culture was induced to synthesize β-galactosidase by the addition of isopropyl-β-D-thiogalactoside to a concentration of 10^{-3} M. At intervals thereafter, samples were removed and assayed for β-galactosidase activity (expressed as change in optical density at 420 nm per h per 0.1 ml of culture). In the key above the figure, the presence or absence of a functional gene product in cells infected with the viruses indicated is represented by + or 0, respectively.

infection, the rates of RNA and protein synthesis (measured as incorporation of [^{3}H]uridine or ^{14}C-labeled amino acids, respectively) and the levels of β-galactosidase in the infected cultures were determined (Fig. 3, Table 1).

Cells infected with phage in which none of the early genes are functional show little decrease in their ability to synthesize β-galactosidase, at least for the duration of this experiment. Synthesis of RNA and protein in these cells begins to drop off at about 15 to 20 min after infection but is not nearly so inhibited at this time as in cells infected with other T7 mutants.

Rapid shutoff of host-dependent RNA synthesis, and of β-galactosidase synthesis, is observed after infection with a T7 mutant in which gene 0.7 is the only active early gene. This result demonstrates that this gene product, acting alone, is sufficient to shut off host RNA synthesis.

Interestingly, although the products of genes 0.3 and 1.1 by themselves do not lead to an early shutoff of β-galactosidase synthesis, the combination of these two gene functions blocks the synthesis of the host enzyme almost as effectively as the product of gene 0.7. This result is all the more striking in that shutoff in this case occurs in the absence of an early inhibition of net RNA synthesis in the infected cells. Expression of either gene 0.3 or 1.1 alone results in a delayed block in the synthesis of β-galactosidase, and this appears to be co-temporal with a delayed inhibition of total RNA synthesis.

Thus far, it has not been possible to isolate mutants of T7 in which ligase (gene 1.3) is the only functional early gene (McAllister, unpublished observations; Studier, personal communication). Although the role (if any) of this gene in host shutoff cannot be directly determined, we have not detected any contribution of this gene product to host shutoff when measured in combination with other early genes (Table 1).

The effect of late phage products on host gene expression can be determined by infecting cells with a T7 mutant in which gene 1 (the phage RNA polymerase) is the only active early gene

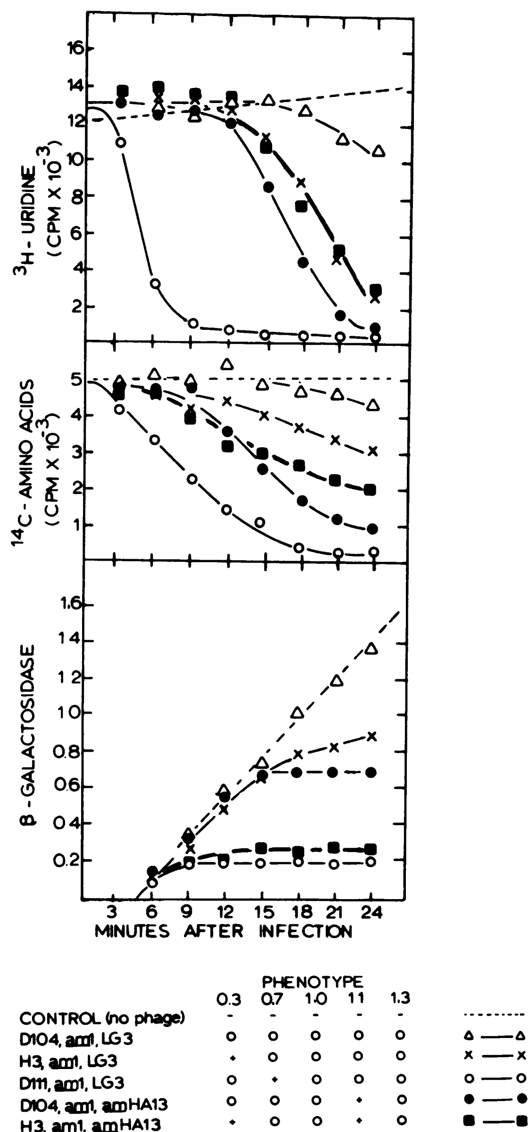

FIG. 3. *Effect of early genes of bacteriophage T7 on macromolecular synthesis in E. coli C. Cultures of E. coli C were infected with bacteriophage T7 (as indicated) and induced to synthesize β-galactosidase as described in the legend to Fig. 2. At intervals after infection, the activity of β-galactosidase in each culture was determined, and a portion (0.1 ml) of the culture was mixed with [³H]uridine and ¹⁴C-labeled amino acids (final concentrations, 10 and 2 μCi/ml, respectively). After 2 min of incubation at 30°C, incorporation of label was terminated by spotting 50 μl onto 3MM filter-paper disks that had been previously soaked in 10% trichloroacetic acid and dried. The filters were washed in 10% trichloroacetic acid and acetone and counted in a toluene-based scintillation fluid as described in the text. The key below the figure indicates the phenotype of each of the viruses tested.*

(Table 1). Here it is observed that β-galactosidase synthesis is shut off with kinetics nearly identical to those observed after infection with wild-type T7. Clearly then, whereas late genes are not required to shut off host gene expression, they are nearly as effective in doing so as the early genes. The involvement of late genes in host shutoff is not surprising, since a few of these functions (such as the products of genes 3.0 and 6.0) participate in the degradation of host DNA (36). Other, more direct mechanisms by which late genes might be involved are not ruled out.

RNA synthesis in infected cells. In cells infected with T7 phage defective in both genes 0.7 and 1, synthesis of the host enzyme β-galactosidase is terminated with near-normal kinetics, even though transcription by the host RNA polymerase is not shut off until later in infection (see above). In view of this observation, we asked whether all classes of RNA continue to be synthesized under these conditions, or whether the synthesis of some RNAs (such as *lac* mRNA, for example) is specifically repressed. The rates of RNA synthesis observed after infection of *E. coli* B with various mutants of T7 are presented in Fig. 4, where panel A represents the rate of overall RNA synthesis determined by precipitation of a sample of recovered RNA and panels B and C indicate the amount of newly synthesized RNA able to hybridize to a particular DNA probe immobilized on nitrocellulose filters.

Cells infected with phage having a functional gene 0.7 exhibit an early and complete cessation of RNA synthesis by the host RNA polymerase (panel A). This is true for the synthesis of T7-specific mRNA (panel D) as well as for the synthesis of cellular RNA (panels B and C). The gene 0.7 product therefore controls not only host RNA synthesis, but the synthesis of early phage mRNA as well.

In the absence of gene 0.7 function, synthesis of RNA by the host polymerase is only slowly inhibited (panel A). Although synthesis of host-specific RNA (including *lac* mRNA) is depressed under these conditions (panels B and C), significant amounts of host RNA continue to be made as late as 15 min after infection. This is noteworthy, because in cells infected with (0.7^-, 1^-) phage, synthesis of β-galactosidase ceases by about 10 to 15 min after infection (Fig. 1). Genes 1.1 and 1.3 are observed to have relatively little effect on the synthesis of host RNA (compare the effects of H3, *am*1 and H3, *am*1, LG3 in Fig. 4B and C).

Elimination of gene 0.7 leads to a threefold stimulation in the maximal rate of T7 RNA synthesis (Fig. 4D). The apparently lower rate

TABLE 1. *Shutoff of RNA and protein synthesis in T7-infected cells*

Genotype	Phenotype[a]							Time of shutoff (min)		
	0.3	0.7	1	1.1	1.3	T	1.7	RNA[b]	Protein[b]	β-Galactosidase[c]
WT[d]	+	+	+	+	+	+	+	ND[e]	ND	9
*am*193	+	+	0	+	+	+	+	6	7	10
D104, *am*193, LG3	0	0	0	0	0	+	+	>24	>24	>24
H3, *am*193, LG3	+	0	0	0	0	+	+	21	24	>17
D111, *am*193, LG3	0	+	0	0	0	+	+	5	9	7
D104, LG3	0	0	+	0	0	+	+	ND	ND	9
D104, *am*193, *am*HA13	0	0	0	+	0	+	+	18	16	12
D104, *am*193	0	0	0	+	+	+	+	14	12	12
D104, *am*193, LG26	0	0	0	+	0	0	0	12	10	ND
H3, *am*342, *am*HA13	+	0	0	+	0	+	+	20	18	9
H3, *am*193	+	0	0	+	+	+	+	19	19	9

[a] The presence or absence of a functional gene product in cells infected with the viruses indicated is represented by + or 0, respectively. T indicates the presence or absence of the termination signal at the end of the early region. Gene 1.7 is located immediately to the right of the termination signal (see Fig. 1).

[b] The rates of RNA and protein synthesis in cultures of *E. coli* C infected with the viruses indicated were determined at intervals after infection, as described in the legend to Fig. 3. For purposes of comparison, the time of shutoff of RNA and protein synthesis is arbitrarily defined as the time at which the rate of synthesis of these macromolecules in the infected cultures is depressed to a level of one-half that observed in the control (uninfected) culture.

[c] The intercept of plateau level with the control curve (see Fig. 2 and 3).

[d] WT, Wild type.

[e] ND, Not determined.

of T7 RNA synthesis observed in the absence of genes 1.1 and 1.3 may be somewhat misleading: the LG3 deletion used to eliminate these genes removes about 25% of the early region (33), thus diminishing the amount of template available for transcription by the host RNA polymerase.

Effect of early genes on the kinetics of phage enzyme synthesis. The delayed manner in which genes 0.3 and 1.1 exert their individual effects on host gene expression (see Fig. 3) suggests that these gene products act in an indirect fashion, perhaps by stimulating the production of some other (late) phage protein. In most of the experiments described above, the phage RNA polymerase was inactive due to an amber mutation in gene 1. Under these conditions transcription of the late region is dependent upon the host RNA polymerase. Although there is a transcriptional termination signal at the end of the early region which discourages read-through by the host polymerase into the late genes (20, 33; see Fig. 1), it is not completely effective in vivo, and therefore a low-level production of late phage mRNA results (21, 36).

The effect of the early phage genes on synthesis of a late phage enzyme (lysozyme, the product of gene 3.5 [36]) was determined as described in Table 2. In a wild-type infection, and in infections in which gene 1 is active, a high level of lysozyme activity is observed by 10 min after infection. In contrast when gene 1 is not active, lysozyme activity is not observed until 20 min after infection, and significant amounts of this enzyme are detected only in cases where gene 1.1 is active. Elimination of the termination signal at the end of the early region by means of deletion LG26 (see Fig. 1) results in a marked enhancement in the production of lysozyme.

Morrison and Malamy (24) have proposed a model for the regulation of T7 gene expression in which the product of an (unknown) early gene is required for efficient translation of mRNA from certain late genes (including the gene for lysozyme). The kinetics of phage enzyme synthesis in the absence of early genes other than gene 1 were examined in more detail in the experiment described in Fig. 5. It is apparent that the phage RNA polymerase and lysozyme are synthesized with identical kinetics regardless of the activity of genes 0.3, 0.7, 1.1, and 1.3. We therefore conclude that when transcription of the late genes is mediated by the phage RNA polymerase, none of the other early gene products is required for late enzyme synthesis, nor do they affect the time at which synthesis of early enzymes is turned off.

DISCUSSION

The single most effective genes of bacteriophage T7 in the negative control of host macromolecular synthesis is gene 0.7. Acting by it-

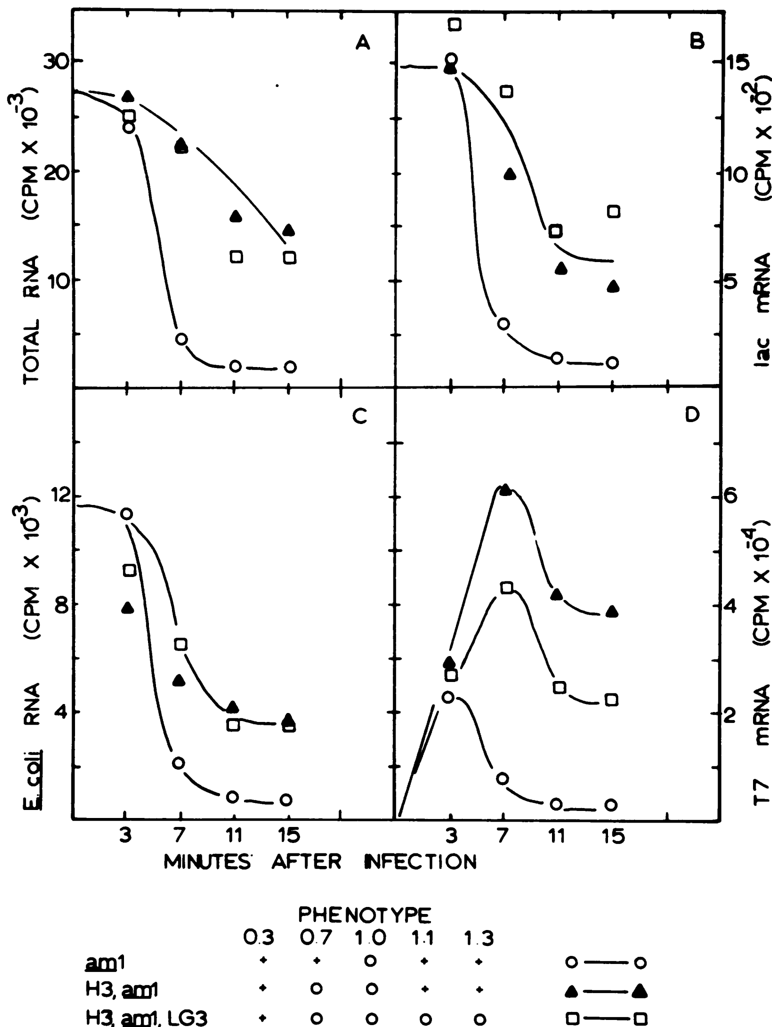

FIG. 4. *Rates of RNA synthesis in T7-infected cells. A culture of E. coli B was induced to synthesize lac enzymes by the additon of isopropyl-β-D-thiogalactoside to a concentration of 10^{-3} M. Ten minutes after induction, the culture was infected with bacteriophage T7 (as indicated), and at intervals thereafter samples of the infected culture were incubated with [^{3}H]uridine (25 μCi/ml) for 2 min. Incorporation of label was terminated, and total RNA was extracted from the cells by the hot sodium dodecyl sulfate-phenol method of Bremer and Yuan (5). Portions of the sample were counted for the determination of total RNA recovered (A), and duplicate portions were hybridized with DNA that had been immobilized on nitrocellulose filters. Data presented in B through D represent RNase-resistant radioactivity bound to: (B) λplac DNA, 10 μg/filter; (C) E. coli DNA, 100 μg/filter; (D) T7 DNA, 10 μg/filter.*

self, the product of this gene causes a rapid shutoff of the host RNA polymerase at about 4 min after infection and a gradual shutoff of most protein synthesis beginning shortly thereafter (see Fig. 3). The product of this gene has been identified as a protein kinase (an ATP:protein phosphotransferase), which phosphorylates a number of host proteins during infection (30). Among the host proteins phosphorylated are the β and β' subunits of the host RNA polymerase (45) and a few (unidentified) ribosomal proteins (29, 30). A dual role for this gene product (i.e., a direct negative control of both host transcription and translation) would be consistent with its known enzymatic activity. However, the observation that host protein synthesis is shut off by this gene product in a delayed manner (Fig. 3, middle panel) and the fact that ribosomes from T7-infected cells are functional in vitro (2, 9, 26, 44) make a direct role for gene 0.7 in the control of translation unlikely.

TABLE 2. *Lysozyme activity in T7-infected cells*

Genotype	Phenotype[a]							Relative lysozyme activity[b]	
	0.3	0.7	1	1.1	1.3	T	1.7	10 min after infection	20 min after infection
WT	+	+	+	+	+	+	+	1.0	1.0
D104, *am*193, LG3	0	0	0	0	0	+	+	0.01	0.05
D104, *1m*193, *am*HA13	0	0	0	+	0	+	+	0.01	0.49
D111, *am*193, LG3	+	0	0	0	0	+	+	<0.01	0.02
D104, *am*193, LG26	0	0	0	+	0	0	0	0.01	0.84
D104, LG3	0	0	+	0	0	+	+	1.1	1.09
*am*193	+	+	0	+	+	+	+	<0.01	0.06
H3, *am*193, *am*HA13	+	0	0	+	0	+	+	0.05	0.32

[a] The presence or absence of a functional gene product in cells infected with the viruses indicated is represented by + or 0, respectively. T indicates the presence or absence of the termination signal at the end of the early region. Gene 1.7 is located immediately to the right of the termination signal (see Fig. 1).

[b] Lysozyme activity in infected cells was measured as the ability of cell extracts to solubilize labeled cell wall material that had been affixed to filter-paper disks (as described in the text). To facilitate comparison between different experiments, results have been normalized to the level of activity observed in cells infected with T7$^+$ (wild type [WT]). This value ranged from 5×10^3 to 12×10^3 cpm, depending upon the time at which samples were collected and the batch of filters used. A more detailed analysis of lysozyme production in WT-infected cells is presented in Fig. 5.

Herrlich et al. (18) have reported the isolation of an inhibitor from T7-infected cells that prevents the appearance of the bacteriophage T3 enzyme *S*-adenosylmethioninease (SAMase) in an in vitro translation system programmed with bacteriophage T3 mRNA; these authors have attributed this inhibition to a translational repressor induced by T7. By UV inactivation studies, the T7 gene coding for this inhibitor has been mapped near gene 0.3 (18). In vivo, however, the product of gene 0.3 is found to exert relatively little effect on host protein synthesis (Fig. 3). An alternative explanation for these observations is that the T7 gene 0.3 product inhibits SAMase activity directly. SAMase is the product of gene 0.3 of bacteriophage T3 (17, 41), and it has been shown that this gene product, like that of gene 0.3 of bacteriophage T7, is required to overcome host restriction (39, 41). Bacteriophage T7, however, does not induce SAMase activity during infection (13). The inhibition of SAMase activity in the in vitro system might therefore result from an interaction between two functionally related but enzymatically dissimilar proteins in a multimeric complex, rather than from interference with translation per se. Evidence for such an interaction in vivo has come from experiments by Hausmann (17) in which the induction of SAMase activity during T3$^+$ infection was found to be inhibited by co-infection with enzymatically incompetent phage, either T3 *sam*$^-$ or T7. The degree of inhibition observed depended upon the relative proportion of the incompetent phage among the input virus particles, and not upon their absolute multiplicity.

In cells infected with (0.7$^-$, 1$^-$) phage, a delayed inhibition of host-dependent transcription commences at about 12 to 15 min after infection. This delayed inhibition is observed after infection with phage having either a functional gene 0.3 or 1.1 (Fig. 3). The delayed manner in which these genes exert their effect on RNA synthesis suggests that they might act in an indirect fashion, perhaps by stimulating the low-level synthesis of a late phage product (see below). Ponta et al. (28) have also observed the delayed shutoff of host transcription and, by UV inactivation experiments, have determined that the T7 gene responsible for this effect is located about 20 to 30% from the left end of the DNA molecule (see Fig. 1). An inhibitor of transcription in vitro by *E. coli* RNA polymerase has been isolated from wild-type T7-infected cells, but is not found in cells infected with gene 1$^-$ mutants (19, 27). This inhibitor may be responsible for the delayed shutoff in host transcription that we have observed.

We have demonstrated that under conditions in which the late phage genes are transcribed by the host RNA polymerase, gene 1.1 stimulates the production of certain late phage enzymes (i.e., lysozyme, see Table 2). The function of gene 1.1 during bacteriophage T7 development is not known. Although the origin of DNA replication has been mapped in this region (12), no role has yet been assigned to the gene 1.1 product, and the gene is dispensable in

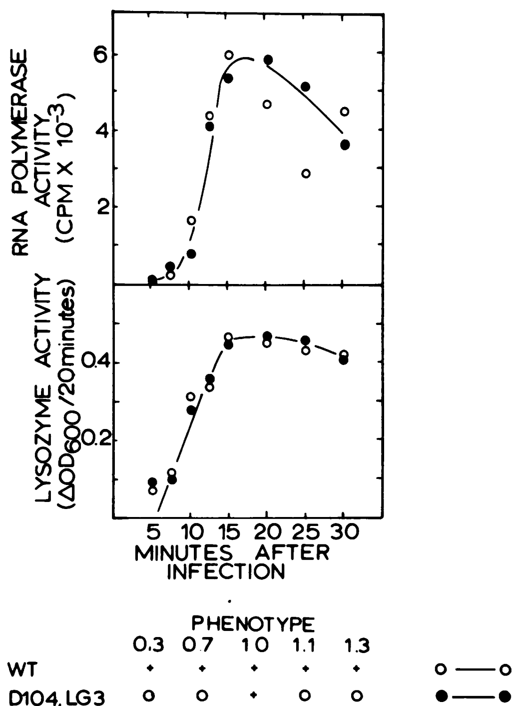

FIG. 5. *Regulation of synthesis of phage-specific enzymes by early genes. Bacteria (E. coli C) were grown to a density of 3 × 10⁸ cells per ml in M9 medium and infected at a multiplicity of 10 phage particles per cell with either T7⁺ or D104, LG3. At intervals after infection, samples of the culture were harvested by centrifugation, and the cells were lysed by three cycles of freezing and thawing. Portions of the sample were assayed for phage-specific RNA polymerase activity (upper) or lysozyme activity (lower). Phage RNA polymerase is the product of gene 1; lysozyme is the product of gene 3.5 (a late gene). Lysozyme activity was determined by the decrease in optical density of an EDTA-sensitized preparation of E. coli C (see text).*

all laboratory hosts tested thus far (37, 38). A trivial explanation for the apparent dependence of late enzyme synthesis on gene 1.1 activity could be that a significant level of transcription of the late genes is carried out by active phage RNA polymerase molecules which are produced in the cell despite the amber mutation in gene 1. The LG3 deletion used to eliminate gene 1.1 may eliminate a promoter for the phage RNA polymerase located near the left end of gene 1.1 (34), and it could be argued that the effect of the LG3 deletion stems from elimination of this late promoter, rather than from elimination of gene 1.1 itself. This explanation does not appear to be likely, since the product of gene 0.7 (which inhibits the host RNA polymerase, but not the phage RNA polymerase) abolishes the stimulatory effect of gene 1.1 on late enzyme synthesis (Table 2).

The effect of gene 1.1 on the synthesis of late enzymes is only observed under conditions in which transcription of the late region is mediated by the host RNA polymerase (i.e., under suboptimal conditions). When the late genes are transcribed by the phage RNA polymerase, the kinetics of synthesis of lysozyme are not affected by deletion of genes 0.3, 0.7, 1.1, and 1.3 (Fig. 5). Thus, there is no evidence to support a role for these genes in regulation of late phage gene expression during normal infection.

In contrast to the delayed manner in which genes 0.3 and 1.1 exert their individual effects on host macromolecular synthesis, the combination of these two phage genes during infection results in a rapid shutoff of β-galactosidase synthesis (Fig. 2 and 3). This effect is particularly interesting because of the observation that host mRNA is synthesized at the same rate whether gene 0.3 alone or genes 0.3 and 1.1 together are functional (Fig. 4). Ponta et al. (26) have recently observed that the gene 0.3 product causes a marked change in the intracellular concentration of monovalent cations. We have also observed this effect (McAllister and Wu, in preparation) and, in addition, have observed that permeability changes in T7-infected male (F′) cells are stimulated by gene 1.1 (McAllister and Barrett, unpublished observations). The combination of two permeability lesions might sufficiently alter the intracellular ionic balance so as to affect the translational capacity of the cell.

One of the early observations that suggested a block in the translation of host mRNA in T7-infected cells was that synthesis of host enzymes (such as β-galactosidase) is inhibited after infection with (0.7^-, 1^-) phage, despite a continued synthesis of RNA by the host RNA polymerase (7, 31). We have measured the rate of total RNA synthesis, and the rates of synthesis of specific classes of mRNA (such as *lac* mRNA and T7 mRNA) in (0.7^-, 1^-)-infected cells (Fig. 4). At times during which the total RNA synthetic capacity of the infected cell is slowly declining, the synthesis of host-specific mRNA appears to be preferentially inhibited. Nevertheless, significant amounts of *lac* mRNA continue to be made during intervals in which synthesis of β-galactosidase is inhibited. From these data, we cannot rule out the possibility that bacteriophage T7 might discriminate against the synthesis of host proteins at some stage after transcription.

When one compares the rates of T7 and host mRNA synthesis in (0.7^-, 1^-)-infected cells, it is apparent that as the synthesis of host mRNA

decreases, synthesis of T7 mRNA increases (Fig. 4C and D). The change in the utilization of host and phage DNA as template by the host RNA polymerase might also be due to alterations in the intracellular environment as a result of infection. It has been shown that transcription of T7 DNA by *E. coli* RNA polymerase is initiated at three closely spaced promoters near the left end of the molecule (38). These promoters are functionally distinct, and the efficiencies with which they are utilized depend markedly upon conditions of ionic strength and temperature (11). One could imagine that this feature of T7 DNA has evolved to reflect changes in intracellular conditions occurring after infection.

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