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Regulation of transcription of the late genes of bacteriophage T7

(T7 RNA polymerase/lysozyme/changes in cell permeability)

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ABSTRACT **The transcription program of bacteriophage T7 *in vivo* was analyzed by hybridizing T7 mRNAs, labeled at intervals after infection, to *Hpa* I restriction fragments of T7 DNA. Transcription of the late genes is temporally regulated: class II genes are transcribed between 4 and 16 min after infection; most class III genes are transcribed from 8 min after infection until lysis. Genes 8–10 are transcribed as both class II and class III genes. The rate of T7 RNA synthesis decreases sharply at 10 min after infection. The rapid decrease in the rate of T7 RNA synthesis and the shutoff of class II RNA synthesis were not observed in cells infected with phage defective in gene 3.5 (lysozyme). Although the decrease in the rate of T7 RNA synthesis is independent of DNA replication, the failure to shut off class II RNA synthesis normally in 3.5⁻-infected cells may reflect the role of T7 lysozyme in DNA replication. *In vitro*, the regions of T7 DNA transcribed by the phage RNA polymerase were found to be dependent upon ionic conditions.**

The bacteriophage T7 proteins are synthesized in three classes during infection: class I proteins from 4 until 8 min after infection, class II proteins from 6 until 15 min after infection, and class III proteins from 6–8 min after infection until lysis (1, 2). Although the regulation of expression of the class I (early) genes has been studied in some detail (2–4), the mechanism(s) by which expression of the class II and class III genes are regulated is poorly understood. Hopper *et al.* (5) have demonstrated that the time-dependent appearance of the T7 late proteins *in vivo* can be duplicated in a cell-free system programmed with mRNA isolated from T7-infected cells at intervals after infection. Thus, the control of synthesis of these proteins is dependent upon the availability of functional class II and class III mRNAs and does not appear to involve modification of the ribosomes or other translational factors.

Transcription of the class II and class III genes requires the synthesis of a phage-specified RNA polymerase, the product of gene 1 (a class I gene) (6, 7); no other phage genes are required for the expression of the class II or class III genes (2). *In vitro*, the purified phage RNA polymerase transcribes both the class II and class III regions of T7 DNA but synthesizes much more class III RNA than class II RNA (8–10). It had been suggested that a preferential synthesis of class III mRNA *in vivo* might account for the shutoff of class II protein synthesis midway during infection: as the more abundantly produced class III mRNAs accumulate in the infected cell, they would successfully compete for the available translational capacity of the cell (9). Preliminary evidence, however, indicated that the class II RNAs are synthesized to a much greater extent *in vivo* than *in vitro* (10).

In this study we characterized the transcription program of bacteriophage T7 *in vivo* by hybridizing T7 mRNA, labeled at intervals after infection, to restriction fragments of T7 DNA. We found that transcription of the late genes of bacteriophage T7 is temporally regulated: the class II and class III RNAs are synthesized at different times and in different amounts. In part, regulation of T7 late RNA synthesis is mediated by phage lysozyme.

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MATERIALS AND METHODS

Bacteria and Phage. All mutants of bacteriophage T7 used in this study were generously provided by F. W. Studier and have been described (11–14). *Escherichia coli* B was from the collection of E. K. F. Bautz.

Labeling and Isolation of T7 mRNA. Bacteria propagated at 30° in B2 medium (15) to a concentration of 4–5 × 10^8 cells per ml were infected at an input multiplicity of 10–15 phage particles per cell. At intervals after infection, 3-ml portions of the culture were withdrawn and incubated with $^{32}PO_4$ (60–100 μCi/ml) for 4 min. Incorporation of label was terminated by mixing the sample with sodium azide (0.02 M) and an equal volume of ice. The cells were harvested by centrifugation (12,000 × *g*, 5 min) and extracted with phenol at 60° in the presence of 0.5% sodium dodecyl sulfate and 0.02 M sodium acetate, pH 5.2 (16). The RNA was further purified by cellulose/ethanol chromatography (17) and taken up in 0.2 ml of 2× standard saline-citrate/0.1% (wt/vol) sodium dodecyl sulfate.

Hybridization of T7 mRNA to Restriction Fragments of T7 DNA. *Hpa* I restriction fragments of T7 DNA were resolved by electrophoresis in agarose gels (18), denatured *in situ*, and transferred to strips of nitrocellulose filters by a modification (19) of the technique of Southern (20). The filter strips, each bearing about 1 μg of T7 DNA, were incubated with RNA samples in 1 ml of hybridization fluid (2× standard saline-citrate/0.1% sodum dodecyl sulfate) for 18–20 hr at 67°. Each hybridization reaction received 10^5 cpm of labeled RNA; control experiments have demonstrated that, under these conditions, DNA is in excess in all hybridization reactions (19). At the end of the hybridization period the strips were washed, treated with RNase, and exposed to x-ray film (19).

RNA Polymerase Assays. ^{32}P-Labeled T7 RNAs were synthesized at 37° in reaction mixtures (0.25 ml) containing: 40 mM Tris·HCl, pH 7.9; 50 mM KCl; 10 mM 2-mercaptoethanol; T7 DNA, 100 μg/ml; bovine serum albumin, 100 μg/ml; 0.4 mM GTP and CTP; 0.2 mM UTP; 0.2 mM [α-^{32}P]ATP (82.5 μCi/μmol); 75 units of T7 RNA polymerase; and $MgCl_2$ as indicated. After incubation for 20 min, RNA was isolated as described (10).

RESULTS

Kinetics of T7 RNA Synthesis. A map of T7 DNA showing the positions of the known T7 genes, the cleavage sites for the restriction endonuclease *Hpa* I, and the regions of the genome corresponding to the class I, class II, and class III genes is presented in Fig. 1. To determine the times at which the various

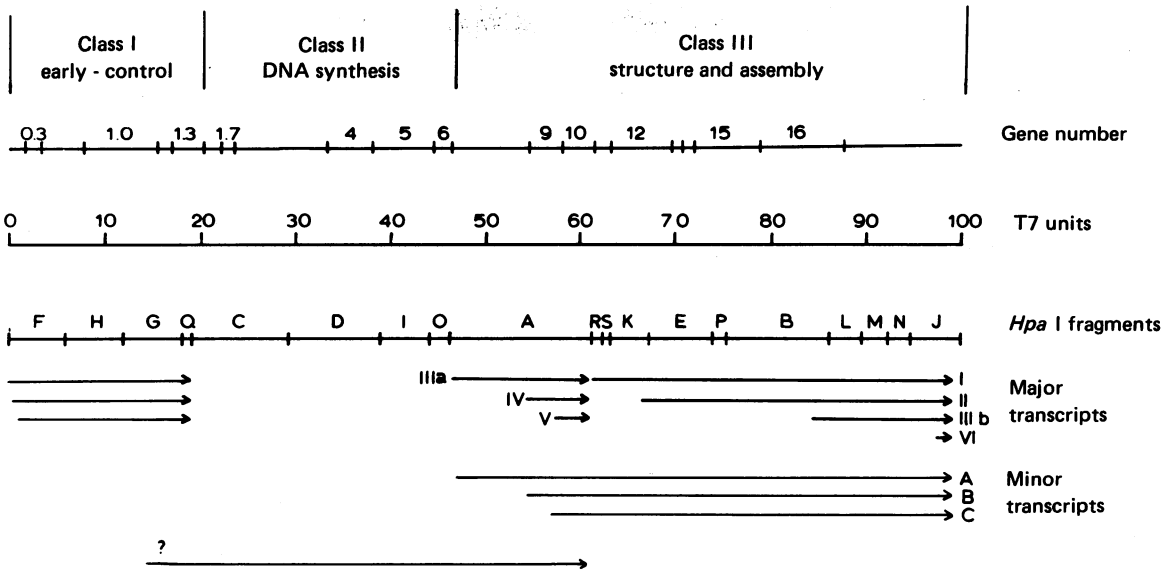

FIG. 1. Transcription map of bacteriophage T7. At the top, the T7 genes are identified by gene number and grouped according to their function and observed time of expression (2, 4). Distances from the left end of the genome are given as percentage of total genome length (T7 units); the alignment of the physical and genetic maps has been derived from various sources (4, 10, 21). At the bottom, the transcription products of the *E. coli* RNA polymerase (those of the early region) and of the T7 RNA polymerase are located with respect to the physical map (10). Transcripts A, B, and C are minor products that arise from failure of the T7 RNA polymerase to recognize the termination signal at 61 units (10). The arrangement of *Hpa* I fragments within the T7 DNA molecule is from McDonell *et al.* (18).

regions of the T7 genome are transcribed, RNA that had been labeled with ^{32}P at intervals after infection was isolated from infected cells and hybridized to restriction fragments of T7 DNA. The results of this analysis demonstrate that transcription of T7 DNA is temporally regulated (Fig. 2*A*). The early region (which includes *Hpa* I fragments F, H, G, and Q) is transcribed strongly during the interval 0–4 min after infection; transcription of this region ceases at some time during the 4 to 8-min labeling period. [T7 RNAs hybridizing to *Hpa* I fragments smaller than *Hpa* I·O are not presented in Fig. 2*A*; they have been analyzed in separate experiments (not shown)]. Transcription of the class II genes (*Hpa* I fragments G, Q, C, D, I, and O) begins during the interval 4–8 min after infection but starts to drop off at 8–12 min after infection; very little class II RNA is detectable after 16 min. The majority of the class III genes (those located to the right of 61 T7 map units) are not transcribed until 8–12 min after infection, and these transcripts remain the predominant newly synthesized T7 RNAs thereafter (see Fig. 2*A*, *Hpa* I fragments B, E, J, K, L, M, and N).

Transcription of T7 genes 8, 9, and 10 (*Hpa* I fragment A) is initiated at the same time as that of the class II genes but continues, along with that of the other class III genes, after class II RNA synthesis has been terminated. A previous study (10) had demonstrated that there is an overlapping mode of transcription of the *Hpa* I·A region in which transcription products initiated at both class II and class III promotors share a common termination signal at 61 map units (see Fig. 1). The kinetics of RNA synthesis *in vivo* reflect this overlapping mode of transcription.

The region of the genome corresponding to *Hpa* I·K appears to be transcribed weakly during the interval of 4–8 min but strongly from 8–12 min and thereafter. Weak transcription of this region during the former interval may arise from inefficient termination of class II RNAs at the termination signal at 61 T7 map units (see legend to Fig. 1).

Transcription of T7 DNA *In Vitro*. Previous reports have noted that the T7 RNA polymerase synthesizes class II RNA poorly *in vitro* (8–10). Because the class II genes are transcribed strongly by this enzyme *in vivo* during certain time intervals, we wished to determine whether the conditions used for RNA synthesis *in vitro* affect the regions of the template that are transcribed. The maximum rate of RNA synthesis *in vitro* is observed at a Mg^{2+} concentration of 20 mM and a monovalent cation concentration <50 mM (22). When conditions in the *in vitro* assay system are altered, it is found that the regions of T7 DNA that are transcribed by the phage enzyme vary according to the ionic conditions (Fig. 3). Whereas class III RNAs are synthesized over a broad range of Mg^{2+} concentrations, class II RNAs are synthesized best in the range 5–10 mM $MgCl_2$. A similar dependence of the types of RNA synthesized upon the concentration of monovalent cations is also observed: at a Mg^{2+} concentration of 10 mM, class II RNAs are synthesized only at KCl concentrations <0.1 M (not shown). As yet, we have not found conditions that will permit the synthesis of class II RNAs but which will prevent the synthesis of class III RNAs. (*In vitro* RNAs hybridizing to restriction fragments smaller than *Hpa* I·J are not clearly visible in Fig. 3; they have been analyzed in separate experiments.)

Effects of Lysozyme on T7 Late Transcription. In view of the dependence of transcription *in vitro* upon ionic conditions, we reasoned that alterations in the intracellular ionic conditions during T7 infection might play a role in the shift from class II to class III RNA synthesis *in vivo*. Condit (23) has shown that T7-infected cells undergo a marked permeability change at about 10–15 min after infection and has identified the T7 gene product responsible for this effect as lysozyme (the product of gene 3.5). The permeability change is manifested by failure of the cells to exclude o-nitrophenyl β-galactoside (a substrate for the bacterial enzyme β-galactosidase). When the program of transcription in cells infected with phage defective in lysozyme (3.5^- phage) is compared to that of cells infected with wild-type phage, it is clear that transcription of the class II region (*Hpa* I fragments G,Q, C, D, I, and O) is not shut off normally (cf. Fig. 2 *A* and *B*). Synthesis of class III RNAs, on the other hand, appears to be normal in 3.5^--infected cells.

Because the amount of labeled RNA present in the hybrid-

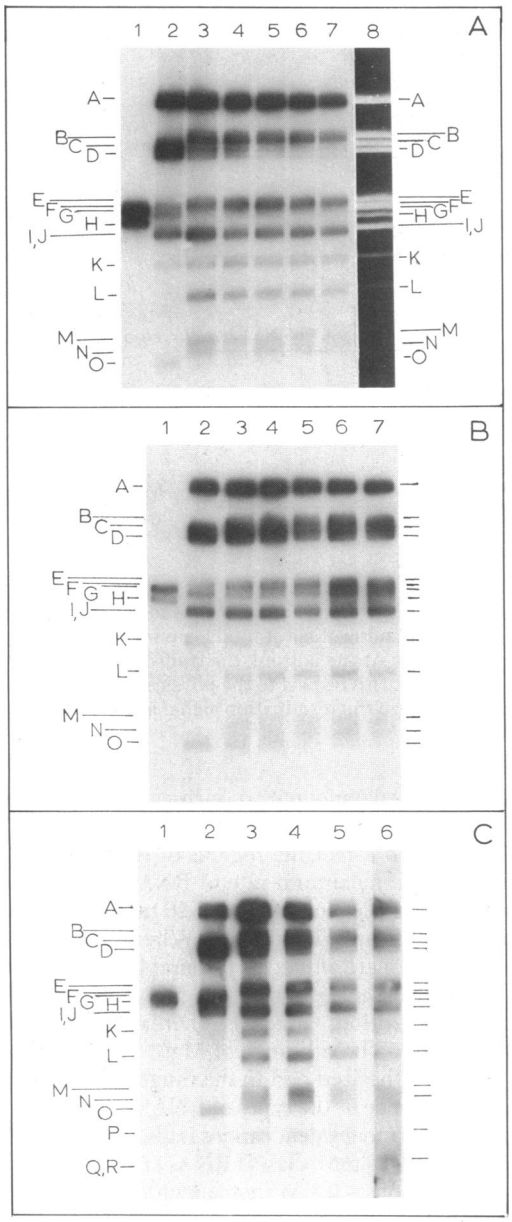

FIG. 2. Regulation of transcription in T7-infected cells. *Hpa* I cleavage products of T7 DNA were resolved by electrophoresis in 0.7% agarose gels, denatured, and transferred to strips of nitrocellulose filter (19). Each filter strip was incubated with 10^5 cpm of ^{32}P-labeled RNA that had been isolated from T7-infected cells. After hybridization, the filter strips were washed, treated with RNase, and exposed to x-ray film. Each lane in the figure is the autoradiogram of one filter strip. A photograph of the ethidium bromide-stained DNA fragments, as observed in the gel prior to transfer, is presented in lane 8 of panel *A*; the fragments are identified by letter (see Fig. 1). The intervals during which incorporation of label took place in the infected cells were as follows: lane 1, 0–4 min after infection; lane 2, 4–8 min; lane 3, 8–12 min; lane 4, 12–16 min; lane 5, 16–20 min; lane 6, 20–24 min; lane 7, 24–28 min. Cells were infected as follows: (*A*) wild-type bacteriophage T7; (*B*) T7 *am* lys 13a (gene 3.5$^-$); (*C*) T7 *am* 29, 28 (gene 3$^-$,5$^-$).

ization reactions presented in Fig. 2 was adjusted to a constant level (10^5 cpm) in all cases, the autoradiograms presented in this figure do not reflect the changing rates of RNA synthesis that occur in T7-infected cells. Summers (24) has shown that the rate of uridine incorporation in T7-infected cells drops off rapidly at about 10 min after infection. It is not clear whether this decrease in uridine incorporation reflects a decline in the rate of

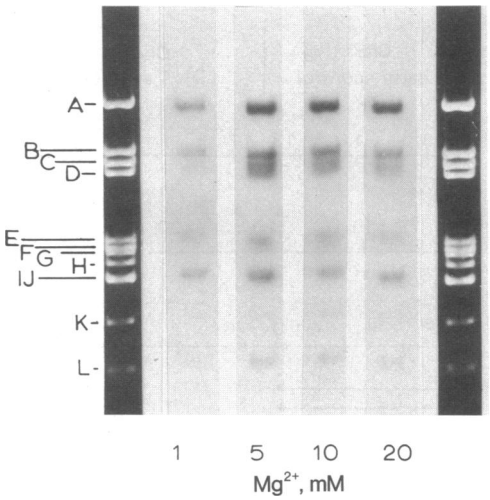

FIG. 3. Effect of Mg^{2+} concentration on the synthesis of T7 RNA *in vitro*. ^{32}P-Labeled RNAs synthesized by T7 RNA polymerase *in vitro* in the presence of varying concentrations of Mg^{2+} (as indicated) were hybridized to *Hpa* I restriction fragments of T7 DNA as described in Fig. 2. Autoradiograms of the filter strips are flanked by photographs of the ethidium bromide-stained restriction fragments of T7 DNA as observed in agarose gels.

RNA synthesis or merely a change in the utilization of exogenous precursors. Hopper *et al.* (5) have found that the accumulation of functional T7 mRNA in the infected cell reaches a maximum at about the same time, suggesting that uridine incorporation can serve as an indicator of RNA synthesis. As shown in Fig. 4*A*, the sharp drop in uridine incorporation observed after infection with wild-type phage is not observed in cells infected with 3.5$^-$ phage. We conclude that T7 lysozyme affects not only the types of T7 RNA made but also the rate of RNA synthesis as well. Because host RNA synthesis is shut off by 5 min after infection under all conditions tested (25), these differences reflect the rates of T7 RNA synthesis in the infected cells.

To examine the possibility that lysozyme might affect transcription in T7-infected cells by disrupting the barriers to passage of ions from or to the medium, we measured the rates of uridine incorporation in cells infected with wild-type phage under various ionic conditions (Fig. 4*B*). Although increasing the concentration of Mg^{2+} in the medium from 1 mM to 8 mM resulted in a slight increase in the rate of uridine incorporation between 3 and 5 min after infection, the rate soon dropped off in a fashion identical to that observed under the usual conditions. No differences in the regulation of transcription were apparent when RNAs synthesized under these conditions were hybridized to restriction fragments of T7 DNA (not shown). At higher concentrations of monovalent cations (0.15 M KCl) the rate of incorporation was diminished between 3 and 5 min after infection but appeared to be normal thereafter.

Role of DNA Synthesis in T7 Late Gene Transcription. The role of phage lysozyme during T7 infection is complex. Unlike the lysozymes of bacteriophages T4 and λ, that of T7 is synthesized midway during infection and is not required for lysis of the cell (2). It has recently been found that this enzyme participates in the release of host and progeny DNA from a membrane-bound complex and that, in the absence of this function, phage DNA synthesis proceeds at only about 45% of the rate observed in wild-type infected cells (14).

To determine whether the effects of lysozyme on T7 late RNA synthesis reflect the role of this enzyme in DNA synthesis, we examined the pattern of transcription in cells infected with

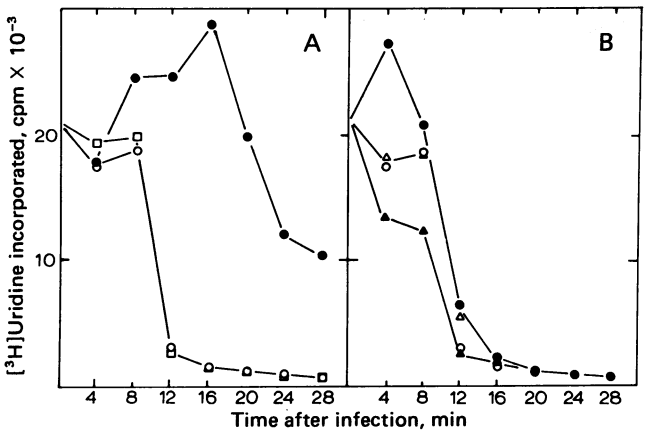

FIG. 4. Rates of RNA synthesis in T7-infected cells. (*A*) Cultures of *E. coli* growing in B2 medium supplemented with 0.1 mM phosphate and 0.1% (wt/vol) casamino acids were infected with bacteriophage T7 as indicated. At intervals after infection, portions of the culture were withdrawn and incubated with [^{3}H]uridine for 2 min. The amount of label incorporated into acid-insoluble material was determined as described (25) and plotted at the midpoint of the labeling interval. ○, Wild-type T7; □, T7 *am* 29, 28 (gene 3$^-$,5$^-$); ●, T7 *am* lys 13a (gene 3.5$^-$). (*B*) Bacteria grown in the media described below were infected with wild-type T7. Rates of RNA synthesis in the infected cells were determined as described above. ○, B2 medium supplemented as above (B2 medium is 20 mM in buffer, 80 mM in monovalent cations, and 1 mM in $MgCl_2$); △, 0.5 × B2 medium supplemented as above; ●, 0.5 × B2 medium supplemented as above and containing 8 mM $MgCl_2$; ▲, 0.5 × B2 medium supplemented as above and containing 8 mM $MgCl_2$ and 0.15 M KCl.

phage defective in gene 3 (endonuclease) and gene 5 (DNA polymerase). Unlike the lesion in gene 3.5, the defects in genes 3 and 5 lead to a complete block in DNA synthesis (11). As shown in Fig. 4*A*, the sharp drop in the rate of uridine incorporation observed after infection with wild-type phage is also observed in cells infected with (3$^-$,5$^-$) phage. This feature of T7 transcription is therefore independent of DNA replication.

Although the shutoff of class II RNA synthesis in (3$^-$,5$^-$)-infected cells is not as clean as that observed during wild-type infection, the failure to shut off class II RNA synthesis is not as dramatic as that observed in cells infected with 3.5$^-$ phage (Fig. 2 *B* and *C*). The effect of phage lysozyme on class II RNA synthesis may therefore reflect the role of this enzyme in DNA metabolism. It is unlikely, however, that failure to shut off class II RNA synthesis during 3.5$^-$-infection is due merely to the decreased rate of DNA synthesis in 3.5$^-$-infected cells.

DISCUSSION

In this report we have demonstrated that the synthesis of the late T7 RNAs is temporally regulated. Comparison of the results presented here with those of previous investigators (1, 2) reveals that there is a close correlation between the times during which the T7 late genes are transcribed and the appearance of the corresponding proteins in the infected cell. Although the time-dependent synthesis of the T7 RNAs can account for the observed pattern of protein synthesis, we cannot exclude the possibility that post-transcriptional events might also be involved in the regulation of late gene expression. In view of the rapid drop in the rate of T7 RNA synthesis that occurs at about 10 min after infection, the preferential synthesis of class III proteins at later times might be attributed to a selective inactivation of the class II mRNAs as well as to the continued (low level) synthesis of class III mRNAs that we have observed.

We have found that the regulation of transcription of the T7 late genes requires the product of gene 3.5, the phage lysozyme; neither the rapid decrease in the rate of T7 RNA synthesis nor the shutoff of class II RNA synthesis was observed in cells infected with phage defective in this gene. F. W. Studier and R. C. Condit (personal communications) have observed that synthesis of class II proteins is prolonged in 3.5$^-$-infected cells; no other late T7 gene has been found to affect the synthesis of this class of proteins.

The mechanism by which lysozyme affects transcription of the T7 late genes is not clear. Because we have demonstrated that the transcriptional specificity of the T7 RNA polymerase *in vitro* is dependent upon ionic conditions, it would be tempting to speculate that lysozyme alters the intracellular ionic conditions after infection. However, we were unable to demonstrate an effect of lysozyme upon leakage of rubidium (a potassium analog) from infected cells (unpublished data), and attempts to alter the pattern of T7 transcription by adjusting the ionic balance of the growth medium have thus far been unsuccessful (Fig. 4*B*). Although these experiments do not rule out the possibility that lysozyme might alter the intracellular ionic balance in a specific manner, other mechanisms by which lysozyme might affect the transcriptional specificity of the T7 RNA polymerase *in vivo* should also be considered. For example, lysozyme-induced alterations in membrane integrity may cause the loss or inactivation of host (or phage) factors required for efficient transcription of the class II genes. It has been found that parental phage DNA is associated with the cell membrane in a fashion distinct from that of progeny DNA (26); the change in the nature of this association may be intimately concerned with both DNA replication and the shutoff of class II RNA synthesis.

Ponta *et al.* (27) have reported that T7-infected cells become permeable to monovalent cations shortly after infection and have found that this permeability change requires the product of T7 gene 0.3. This early loss of monovalent cations does not appear to be involved in the regulation of T7 late transcription; we have observed that the class II and class III RNAs are synthesized with normal kinetics in cells infected with a mutant of T7 (D104,LG3) that is defective in the early permeability change (unpublished data).

It should be noted that, whereas the changes induced by lysozyme result in shutoff of class II RNA synthesis, they are not required for the "turning on" of class III RNA synthesis: synthesis of class III RNAs occurs with normal kinetics in cells infected with lysozyme defective phage (Fig. 2*B*). Because all late T7 genes (both class II and class III) are transcribed by the phage RNA polymerase *in vitro*, the question arises as to why transcription of the class III genes is blocked *in vivo* until 8 min after infection. As yet, we have no information concerning this point.

To determine the role of T7 lysozyme in late RNA synthesis, we used a mutant of T7, *am* lys 13a, that contains an *amber* mutation in gene 3.5 (2, 14). It is possible that a secondary mutation elsewhere in the genome could account for the observations made here and by others (14) concerning the multiple functions of this enzyme in phage development. The isolation of independent lysozyme mutants should help to answer this question.

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