PHAGE T7 LYSOZYME mRNA TRANSCRIPTION AND TRANSLATION

in vivo and in vitro

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SUMMARY: The experiments reported here show that T7 lysozyme mRNA synthesis is under the control of T7 RNA polymerase (gene 1), both in vivo and in vitro. In addition, the translation of T7 lysozyme mRNA is significantly diminished in male cells, even though potentially translatable lysozyme mRNA is present. These data support the previously reported conclusion that certain T7 mRNAs are subject to a translational control system mediated by the F-factor of the E. coli host.

The control of gene expression in E. coli infected with bacteriophage T7 has been studied at the transcriptional (1,2,3) and the translational level (4). These analyses revealed that the switch from early gene expression to late gene expression is under the control of an early gene (gene 1), which codes for a new RNA polymerase which is specific for the late genes of T7 (3,5). The T7 mRNA made in vivo is unusually stable and has a half-life of greater than 30 minutes at 30°C (6). However, analysis of phage protein synthesis suggests that these stable RNAs may be subject to translational controls (4). Further evidence for translational control of gene expression in T7 comes from the work of Malamy and his collaborators (7,8), who showed that while the full complement of T7 RNAs are found after infection of nonpermissive F^+ cells, at least some of the late class of T7 proteins are undetectable by gel electrophoretic analysis (7). They suggested that the F-factor is responsible for the inhibition of the translation of certain of the T7 mRNAs.

In our study of the messenger properties of T7 RNA, we have analyzed the ability of T7 RNA samples to make T7 lysozyme, a late protein. Simultaneous analyses for the T7 lysozyme and lysozyme messenger activity show that lysozyme mRNA is present in F' cells, but lysozyme is not. Mixing experiments showed that these results do not reflect the presence of a lysozyme inhibitor in F' cells. These studies suggest the existence of an untranslated, but poten-

tially functional late mRNA in F' cells infected with phage T7.

Initial experiments showed that the messenger for T7 lysozyme is a late RNA, that is, it is under the control of gene 1, the T7 RNA polymerase. RNA was isolated by the diethyl pyrocarbonate method (9), and used to program an in vitro protein synthesis system as described by Schweiger and Gold (10). Lysozyme was assayed by excorporation of radioactivity from filter discs to which had been bound ^{3}H-diamino pimelic acid-labeled E. coli cell walls (10).

It was first determined that the amount of lysozyme synthesis was dependent on the mRNA content of the protein synthesis reaction mixture. Total RNA from E. coli SY106 (1) infected with $T7^{+}$ was used to program lysozyme synthesis. Over a concentration range of 0 to 75μg/ml, the amount of lysozyme made was a reasonably proportional to the amount of mRNA added to the system (Fig. 1). This relationship established that lysozyme synthesis could be used to estimate the lysozyme mRNA content of an unknown RNA sample.

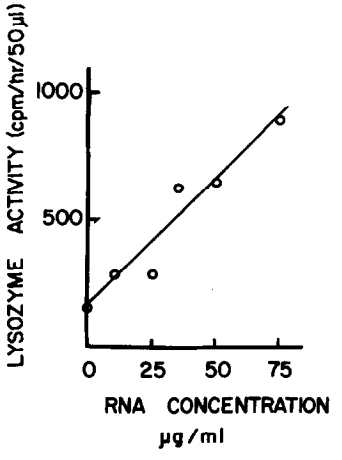

FIGURE 1. Relationship between lysozyme activity synthesized under conditions described in Table I to the amount of RNA added to the incubation mixture. RNA was extracted from E. coli SY106 twelve minutes after infection with $T7^{+}$ at 30°C and at a multiplicity of 15 phage per bacterium.

Table I shows the lysozyme mRNA activity of RNA isolated after infection of E. coli SY106 with several T7 amber mutants or with $T7^{+}$ in the presence of 200μg/ml of chloramphenicol (CM). Very little lysozyme mRNA is made when phage-specific protein synthesis is blocked with chloramphenicol, or, more specifically, if gene 1 (T7 RNA polymerase) is rendered inactive by an amber mutation.

TABLE I

RNA Sample	Lysozyme Activity Synthesized (cpm/hr/0.05 ml)
Uninfected *E. coli* SY106	2
$T7^+$	630
$T7^+$ (+ CM)	64
T7 am 23 (gene 1)	12
T7 am 28 (gene 5)	482
in vitro RNA (*E. coli* polymerase)	49
in vitro RNA (T7 polymerase)	226

In vivo RNA samples were prepared from 20 ml cultures (5×10^8/ml) of *E. coli* SY106 (su^-) (1), ten minutes after infection at 30° C, at a multiplicity of 8-12 phage per bacterium. The T7 amber mutants were from the collection of F. W. Studier (13). *E. coli* RNA polymerase was prepared by the method of Burgess (14) through the two zones sedimentation steps. The synthesis conditions were: 0.1 M KCl, 0.04 M Tris-Cl, pH 7.8, 0.02 M $MgCl_2$, 0.01 M mercaptoethanol, 0.4 mM each of ATP, GTP, CTP, UTP, 75 μg/ml T7 DNA and RNA polymerase. Incubation was for 20 min. at 37 C. T7 RNA polymerase was prepared and the synthesis run as previously described (3, 5). The *in vitro* protein synthesizing system (10) was prepared from *E. coli* RV (F^-, su^-) (15). Each reaction (0.050 ml) contained 50 mM Tris-acetate pH 8, 1 mM dithiothritol, 0.1 M NH_4Cl, 50 mM K-acetate, 20 mM phosphoenol pyruvate, 2 mM ATP, 0.5 mM each GTP, CTP, and UTP, 0.2 mM of each of 20 amino acids, 0.2 mM folinic acid, 50 μg tRNA (Schwarz/Mann), 500 μg ribosomes, 300 μg of S100 protein which eluted from a DEAE column between 0.05 M NH_4Cl and 0.26 M NH_4Cl, 3 μg RNA (except 0.5 μg of the *in vitro* RNAs were used), 8 mM Mg-acetate, which was optimal for the RNA-directed synthesis of lysozyme, and 5 μg/ml of actinomycin D to inhibit any DNA-directed, coupled synthesis (10). Protein synthesis was carried out for 40 min. at 35° C and then a 0.030 ml aliquot of the reaction mixture was assayed for the lysozyme (10). Release of radioactivity from ^3H-cell wall material bound to 2.5 cm Whatman 3 mm filters was linear for the first two hours and this data was used to estimate lysozyme activity. A background (no RNA) of 80 cpm/hr/0.05 ml was subtracted.

The barely detectable levels of lysozyme mRNA made in the presence of chloramphenicol, or by the *E. coli* RNA polymerase *in vitro*, probably result from 'read through' of the normal termination signal between the early and late region

of the T7 genome (11). The fact that no lysozyme mRNA is seen in cells infected with the T7 amber mutants in gene1, suggests that the host shut-off function (12) can minimize the 'read through' seen in the presence of chloramphenicol. This hypothesis is compatible with the idea that the host shut-off function is inhibited by chloramphenicol and acts in some way to block the E. coli RNA polymerase activity. Amber mutations in gene 5 (DNA polymerase) are without effect on T7 lysozyme mRNA synthesis. RNAs made in vitro from T7 DNA by E. coli RNA polymerase and T7 polymerase were also tested. The results confirm the conclusion obtained from the in vivo RNA analysis: that is, lysozyme is a late T7 function, its message being transcribed by the T7 polymerase.

To test the possibility that the late RNA which codes for T7 lysozyme is translated in female (F^-) cells but not in male (F') cells, RNA and protein extracts were prepared from the same cultures of infected male and female cells.

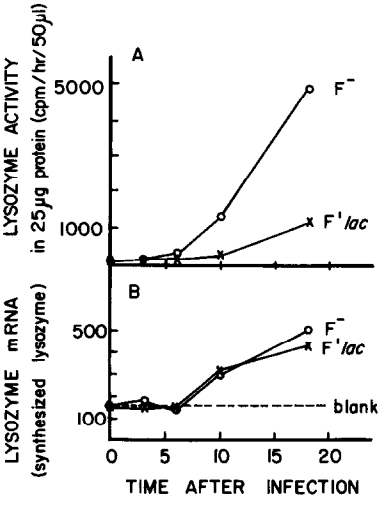

FIGURE 2. Time course of lysozyme synthesis (A) after T7-infection of E. coli X5119 (F^-) (15) (-O-) or E. coli X5119 (F'lac) (-X-) at 30° at a multiplicity of 10 phage per bacterium. Sonic extracts were prepared and 25 µg of protein (by the Lowry method) was assayed as described in Table I. Total RNA was extracted from aliquots of the same infected cultures and the lysozyme mRNA content estimated (B) by its ability to program the in vitro synthesis of lysozyme which was then assayed as described in Table I. The uninfected control samples were taken just prior to infection and are shown at the zero time points. The assay blank was 150 cpm/hr/ 0.05 ml and is shown (----) in (B). The F^- culture lysed at 32 minutes while the F'lac culture was still unlysed at 180 minutes.

The lysozyme activity in the protein extracts was determined directly to measure the in vivo levels of T7 lysozyme. The RNA samples were then assayed for lysozyme mRNA by in vitro protein synthesis. Figure 2A shows the time course of T7 lysozyme synthesis in F^- and F' cells. Parallel assays for lysozyme mRNA are shown in Figure 2B. It is clear that T7 lysozyme synthesis is significantly depressed in the host carrying the F' lac episome when compared to lysozyme synthesis in F^- cells. The restriction of T7 in male cells results in a decrease in the average burst size from about 60 to about 5 phage per cell (7). In light of this, it is not surprising that a small amount of lysozyme is found in T7-infected male cells. In contrast to these results, are the data for the lysozyme mRNA content of T7-infected male and female cells. Virtually the same amount of translatable lysozyme mRNA is found in male and female cells infected with T7. The time course (Fig. 2) of synthesis of both the enzyme and its mRNA show that this protein is temporally an 'intermediate protein' (14). At the same time, the results with the amber mutants in gene 1 and infection in the

TABLE II

Sample	Protein (μg)	Lysozyme Activity (cpm/hr/0.05 ml
$T7^+$ in F^- Host	25	389
Uninfected F' Host	50	11
$T7^+$ in F' Host	50	172
T7 in F^- Host plus	25	365 (400)
Uninfected F' Host	50	
T7 in F^- Host plus	25	553 (561)
T7 in F' Host	50	

Test for inhibition of T7 lysozyme by extracts of uninfected E. coli X5119 F'lac cells or $T7^+$ -infected F' lac host cells. The indicated amounts of protein extracts were assayed separately or in combination. The activity values in parentheses are the sums of the activities of individual components of the mixed assays. Infected cell extracts were prepared ten min. after infection at 30° C. The ^3H substrate was a different preparation from that used in Table I and Figures 1 and 2. No assay blank has been subtracted.

presence of chloramphenicol (Table I) clearly establish that lysozyme is a class 2 (7), or 'late' (1), function with respect to transcriptional control.

To exclude the possibility that an inhibitor of lysozyme activity is present in F' cells, the appropriate mixing experiments were done. Extracts from T7-infected F^- cells (10 min. sample) were assayed with and without addition of extracts from either uninfected or T7-infected F' cells. The results (Table II) show that the extracts from F' cells do not inhibit the lysozyme activity present in the T7-infected F^- cells since the resultant activities are exactly additive.

The results of these experiments demonstrate that translatable lysozyme mRNA existes in T7-infected F' lac cells, but that in vivo this mRNA is not translated. The mechanism of this translational control by the F-factor is not understood at present, but several testable explanations might be suggested. In addition to hypotheses directly related to the mechanisms of protein synthesis (7), other models are not yet excluded, such as the selective sequestration, or 'packaging', of certain mRNAs to make them unavailable for translation until extracted from the cell.

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