Control of Gene Function in Bacteriophage T4

II. Synthesis of Messenger Ribonucleic Acid and Protein After Interrupting Deoxyribonucleic Acid Replication and Glucosylation¹

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Replication of T4 deoxyribonucleic acid (DNA) is known to be required for the onset of transcription of late T4 genes. Once late gene transcription has been initiated, DNA replication is no longer required for maintaining synthesis of late or early T4 messenger ribonucleic acid (mRNA). Late phage proteins (lysozyme and tail fibers) continue to be produced at constant rates after interrupting T4 DNA synthesis. The ability of the host cell to glucosylate the T4 progeny DNA has no demonstrable influence on the rates at which T4 mRNA and late proteins are synthesized after the interruption of DNA synthesis. To explain the requirement of T4 DNA replication for the onset of late gene transcription, we suggest that T4 DNA in a nascent state is mandatory for the initial late gene transcription, or perhaps for late gene transcription throughout the lytic cycle. T4 DNA in a nascent state could be segregated from the bulk of the replicating DNA, used only as template for RNA synthesis, and prevented from being modified by methylation, glucosylation, or maturation processes. The fact that no, or very little, non-glucosylated T4 DNA is extractable from T4LB3-infected CR63 after arresting DNA synthesis does not rule out this possibility.

The chromosome of bacteriophage T4 is structurally and functionally divided into a cluster of about 30 genes which are active immediately, or shortly, after infection (early genes), and a cluster of about 80 genes which function only during the latter two-thirds of the latent period (7) (late genes). Proteins which are coded for by early genes stop being synthesized once the late genes become active. The transcription of early genes, however, continues through the whole lytic cycle of T4 (11). There are two major exceptions. (i) The structural gene for thymidylate synthetase (18) and a few adjacent genes, in spite of functioning early, are located within the region of late genes. (ii) The lysozyme gene is transcribed early and late (2), but lysozyme is synthesized only after the onset of late functions (7).

A twofold correlation between T4 deoxyribonucleic acid (DNA) synthesis and the functioning of late T4 genes has been observed (7). (i) Late proteins become demonstrable shortly after the onset of T4 DNA synthesis. (ii) When T4 DNA synthesis is inhibited from the beginning, as a rule, late T4 functions are not turned on. The existence of a mutant which allows synthesis of

DNA but not of late proteins (7) and the T4 synthesis in rII-infected K(λ) without concomitant appearance of late proteins (10, 14) show that phage DNA synthesis is not a sufficient condition for transcription of late T4 genes, although it is a necessary one. If it were also a necessary condition for maintaining the synthesis of late T4 messenger (m) ribonucleic acid (RNA), interruption of DNA synthesis should arrest the synthesis of late T4 mRNA. Since DNA replication produces temporarily nonglucosylated T4 DNA (8), the state of glucosylation could be another determinant for the transcription of late T4 genes.

In this paper we report the effects of interrupting T4 DNA synthesis and glucosylation on the synthesis of T4 mRNA and late proteins.

MATERIALS AND METHODS

Bacteria. The following strains of *Escherichia coli* were used: B; B/r(λ); CR63; CR63 UDPG⁻ which we presume to be deficient in synthesizing uridine diphosphoglucose (UDPG) for reasons described in Results, and which was isolated from HNO₃-treated CR63; 15THU (17); and *Shigella dysenteriae* Sh16.

Bacteriophage. We used T4B; T4Bh2⁺; T4r102 (3); and T4LB3, a mutant synthesizing a temperature-sensitive deoxycytidine monophosphate hydroxymethylase (5).

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FIG. 1. Amount of DNA in T4 and in T4LB3-infected CR63 at various times after infection as determined by the diphenylamine reaction. The infected cells were incubated at 25 C for 30 min, and heated within 1 min to 41.5 C (indicated by the broken lines). The lower abscissa gives time in minutes at 25 C and, after the second broken line, at 41.5 C. The upper abscissa shows time in fractions of the T4 latent period to make synthesis rates at both temperatures comparable.

Growth media. Media used were: nutrient broth [8 g of Difco nutrient broth (NB), 5 g of NaCl, and 1,000 ml of water]; M-9A [11.5 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 1 g of NH_4Cl , 3 g of KH_2PO_4 , 10 ml of 40% (w/v) glucose, 4 g of Casamino Acids, 2 ml of 0.5 M MgSO_4 , 10 ml of 0.5% (w/v) L-tryptophan, and 1,000 ml of water].

Other media. We also used phosphate buffer [5.4 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 3 g of KH_2PO_4 , 4 g of NaCl, and 1,000 ml of water]; lysozyme buffer [5.2 g tris(hydroxymethyl)aminomethane (Tris) Base, 2.336 g of ethylenediaminetetraacetate (EDTA), 0.1 ml of β -mercaptoethanol, and 800 ml of water, pH 7.5]; SSC (0.15 M NaCl, 0.015 M sodium-citrate, pH 7.2); 5 and 10% trichloroacetic acid for precipitation of nucleic acids.

Inhibition of phage DNA synthesis. T4 DNA synthesis was arrested by upshifting T4LB3-infected bacteria from 25 to 41.5 C.

DNA assay. DNA was measured by the diphenylamine method of Dische, as described by Ashwell (1).

Rate of DNA synthesis. Synthesis of DNA, in T4LB3-infected *E. coli* CR63, was studied by incorporation of ^3H -thymine (10 $\mu\text{C}/\text{ml}$, 2 μg of thymine/ml) in 5-min (at 25 C) and 2-min (41.5 C) pulses. Incorporation

was terminated by chilling at -4°C . Samples (0.2 ml) were lysed in 1% sodium dodecyl sulfate (SDS) solution at 75 C, trichloroacetic acid-precipitated, and filtered onto nitrocellulose filters; the radioactivity was then measured.

Assay for glucosylation of intracellular T4 DNA. T4LB3-infected CR63 and CR63 UDPG $^-$, incubated for 29 min after infection at 25 C, were quickly heated up to 41.5 C by adding an equal volume of M-9A at 58 C, and incubation was continued to 36 min. The complexes were then lysed by 2% SDS at 70 C for 10 min and mixed with CsCl solution to give a density of 1.7 g/ml. The DNA was recovered from the gradient after a 24-hr run at 30,000 rev/min (4 C) in an SW50L rotor. The DNA preparation was dialyzed against water and subsequently adjusted to a density of 1.46 g/ml with Cs_2SO_4 . Approximately 20 μg of DNA was centrifuged in a Spinco model E rotor at 31,410 rev/min at 25 C for 30 hr to separate glucosylated and nonglucosylated T4 DNA from bacterial DNA (8).

Pulse-labeling of RNA by ^3H -5-cytosine. ^3H -labeled 5-cytosine (Schwarz BioResearch, Inc., Orangeburg, N.Y.) was added to T4-infected bacteria at 0.5 $\mu\text{C}/\text{ml}$ for various lengths of time. At the end of a pulse, the complexes were either lysed for RNA isolation, or the radioactive cytosine was chased by a 10^4 -fold excess of cold cytosine.

Rate of RNA synthesis. Rate of RNA synthesis was studied by pulse-labeling and lysing the complexes by transferring 0.5-ml samples into 1 ml of 2% SDS at 75 C. The samples were then precipitated by 2.0 ml of cold 10% trichloroacetic acid, filtered onto Schleicher

FIG. 2. Rates of DNA synthesis in T4LB3-infected CR63 as determined by ^3H -thymine pulse-labeling. $\circ$, Lower abscissa: counts per minute incorporated with 5 min pulses. $\triangle$, Upper abscissa: counts per minute observed with 2-min pulses at 41.5 C (filtration background). $\bullet$, Upper abscissa beginning at the vertical broken line: counts per minute observed with 2-min pulses after upshift from 25 to 41.5 C at 30-min of incubation.

FIG. 3. Cs_2SO_4 density gradient centrifugations of DNA from T4LB3-infected CR63 and CR63 UPDG⁻. Replottings of microdensitometer tracings of ultraviolet absorbance photographs of analytical Cs_2SO_4 gradient centrifugations. (A) DNA extracted from T4LB3-infected CR63 and (B) from CR63 UPDG⁻ infected by the same phage. The buoyant densities are: bacterial DNA, 1.428 g/ml; glucosylated T4 DNA, 1.443 g/ml; and nonglucosylated T4 DNA, 1.435 g/ml.

& Schuell B-6 membrane filters, and washed with 100 ml of 5% trichloroacetic acid.

Half-life of mRNA. To ^3H -5-cytosine pulse-labeled complexes, a 10^4 -fold excess of cytosine was added, and the loss of acid-precipitable radioactivity was determined. The apparent half-lives of mRNA thus obtained are largely influenced by precursor pool kinetics and preferential reutilization of nucleotides from degraded mRNA.

Isolation of mRNA. For isolation and partial purification of T4 mRNA, we used a prelayered gradient of CsCl (14a).

DNA-RNA hybridizations. Hybridization-competition experiments were performed following, in essence, the technique of Hall et al. (11). Total volume per hybridization was 0.30 ml of $4.2 \times \text{SSC}$, pH 7.2; total denatured T4 DNA was 1.8 μg ; input radioactive mRNA was approximately 1.0 μg containing 1,200 counts/min of ^3H ; temperature during hybridization was 67.5 C; incubation time was 5 hr. Unhybridized RNA was digested for 25 min at 37 C by 10 μg of deoxyribonuclease-free pancreatic ribonuclease (Worthington Biochemical Corp., Freehold, N.J.). The undigested material was acid precipitated, filtered onto Schleicher & Schuell B-6 membrane filters, and assayed for radioactivity. Hybridization efficiencies varied from 25 to 35% of the input RNA. To assure the specificity of hybridization-competition experiments, we attempted to inhibit the hybridization of 1 μg of early T4 mRNA with denatured T4 DNA by

simultaneously adding 200 μg of bacterial RNA. We observed no interference of the bacterial RNA with the hybridization of the early T4 mRNA. The digestion of ^3H -RNA after incubation in $4.2 \times \text{SSC}$ in the presence of 200 μg of nonradioactive RNA was complete (30 counts/min retained on the filter out of 1,200 counts/min) when performed at 37 C. With $8 \times \text{SSC}$, the digestion was less complete, resulting in approximately 100 counts/min retained on the filter. Thus, nonspecific RNA-RNA interactions resulting in poor digestibility do not exist with our conditions.

Preparation of cell extracts containing lysozyme. Bacteria were grown in 100 ml of M-9A to 10^8 cells/ml, sedimented by centrifugation, and resuspended in 2 ml of M-9A. Phage were added to give a ratio of four phage per cell. Adsorption was allowed for 2 min at 37 C. An 18-ml amount of M-9A at 25 C was added. At different times, 2-ml samples were withdrawn, poured over 0.5 ml of a frozen 0.1 M solution of EDTA at pH 7.5, and chilled to freezing. The frozen sample was then sonically treated for 30 sec, and the sonic extract was assayed for lysozyme.

Lysozyme assay. Lysozyme was assayed at room temperature with *E. coli* 15THU as substrate. Bacteria were grown to 10^8 cells/ml in NB and CHCl_3 was added for 8 min at 37 C. The bacteria were then centrifuged in the cold, washed, and finally suspended in lysozyme buffer to a final concentration of 2×10^8 /ml. Individual preparations of substrate bacteria were calibrated against a standard of egg white lysozyme

(Mann Research Laboratories, Inc., N.Y.). Lysozyme was assayed by adding 0.1 ml of a sonic extract of infected cells to 2.4 ml of substrate bacteria and observing the decrease of turbidity of the suspension of substrate bacteria at room temperature at $\lambda = 595$ nm.

Assay for tail fibers. Tail fiber synthesis (serum-blocking power) was observed by utilizing the neutralization of T4 antiserum (4, 6).

RESULTS

DNA synthesis in CR63 infected with T4B and T4LB3 at 25 and 41.5 C. *E. coli* CR63 (5×10^8 cells/ml) was infected at 25 C with either six phage

TABLE 1. Incorporation of ^3H -5-cytosine into trichloroacetic acid-precipitable material in T4B- and T4LB3-infected CR63 and CR63 UDPG⁻ before and after upshifts from 25 to 41.5 C

Phage	Host	^3H -5-cytosine incorporated after pulsing at various times after infection ^a (counts/min $\times 10^{-3}$)				
		At 25 C		At 41.5 C		
		10-20 min	20-30 min	30-33 min	34-37 min	38-41 min
T4	CR63	9.2	7.4	5.1	3.2	1.7
LB3	CR63	7.4	6.1	2.4	2.2	1.8
T4	UDPG ⁻	9.4	13.5	10.4	8.5	6.7
LB3	UDPG ⁻	9.8	8.6	8.8	6.1	6.0

^a Ten minutes at 25 C are approximately equivalent to 3 min at 41.5 C, since the T4 latent period is 65 min at 25 C and 22 min at 41.5 C.

T4LB3 per cell, or with six phage T4B per cell. At 10, 20, 25, and 30 min after infection, 10-ml samples were withdrawn, and the amount of DNA per sample was determined. At 30 min, the cultures were shifted to 41.5 C, and samples for DNA measurement were withdrawn at 1-min intervals up to 35 min, and two more samples were taken at 37.5 and 40 min. The results (Fig. 1) indicate a cessation of DNA synthesis within 2 min from the beginning of the upshift to 41.5 C in the T4LB3-infected CR63, but not in the wild-type-infected cells.

Pulse-label studies of the rate of DNA synthesis were performed with T4LB3-infected CR63 under three conditions (Fig. 2). One bacterial culture was multiply infected at 25 C, incubated until 30 min, and divided thereafter. One-half remained at 25 C; the other half was quickly heated to and incubated at 41.5 C. At the times and temperatures indicated in Fig. 2, samples were withdrawn and pulsed with ^3H -thymine. Another bacterial culture was infected with T4LB3, incubated, and pulsed with ^3H -thymine at 41.5 C. No DNA synthesis occurred when infection and incubation were carried out at 41.5 C (Fig. 2, open triangles). When infection and incubation were performed at 25 C, DNA synthesis started at 10 min and reached a constant rate at about 30 min (Fig. 2, open circles). When part of this culture was heated to 41.5 C, incorporation of ^3H -thymine ceased abruptly (fig. 2, closed circles).

Thus, after upshifting of T4LB3-infected cells to 41.5 C, no DNA synthesis was detected. The limit of detection was about 0.2 T4 equivalent of

FIG. 4. Half-life of T4 mRNA. (A) mRNA half-life in T4r102-infected *E. coli* B (solid line, half-life = 2.6 min) and in B/r(λ) (broken line, half-life = 6.1 min). (B) mRNA half-lives in T4LB3-infected CR63 (solid line, half-life = 12 min) and CR63 UDPG⁻ (broken line, half-life = 13.5 min). Incubation temperature for part A was 37 C, for part B, 41.5 C. The durations of the radioactive pulses are given by the horizontal lines. Time of chase by 10^4 excess of nonradioactive cytosine is indicated by arrows.

DNA synthesized per cell per minute (approximately 1.25% the normal rate) in Fig. 1. For the experiments in Fig. 2, this level was at about 5 to 7%.

Nature of T4 DNA synthesized in UDPG⁻. T4 released from CR63 UDPG⁻ plated with about 10^{-3} efficiency on *E. coli* B and CR63 as compared with the plating efficiency on *Shigella* Sh16. This suggests a deficient glucosylation of the T4 DNA (12, 15, 18). To demonstrate the absence of glucosylation in T4 DNA synthesized by CR63 UDPG⁻, we banded the DNA in Cs₂SO₄ gradients, which separate glucosylated and non-glucosylated T4 DNA (8). Replottings of microdensitometer readings of ultraviolet absorbance photographs are given in Fig. 3 for host CR63 and for host CR63 UDPG⁻. They show that the DNA harvested from T4LB3-infected CR63 UDPG⁻ is made up of nonglucosylated T4 DNA and bacterial DNA. The DNA harvested from infected CR63 is preferentially composed of glucosylated T4 DNA and bacterial DNA.

Effects of temperature upshifts on the rate of RNA synthesis in T4B- and in T4LB3-infected CR63 and CR63 UDPG⁻. In these experiments, we studied the effects of interrupting synthesis and glucosylation of T4 DNA on the rate of mRNA synthesis. CR63 and CR63 UDPG⁻, grown to 5×10^8 cells/ml in M-9A, were infected with T4B at a multiplicity of infection of 8 at 25 C, and pulses of ³H-5-cytosine (0.5 μ C per 0.1 μ mole per ml) were given from 10 to 20 min and from 20 to 30 min. The amount of tritium incorporated into trichloroacetic acid-insoluble material is shown in Table 1; the levels of incorporation were approximately the same. By 30 min, both cultures were upshifted to 41.5 C and pulsed with ³H-5-cytosine for periods of 3 min from 30 to 33 min, 34 to 37 min, and 38 to 41 min. As indicated in Table 1, the amount incorporated dropped to approximately one-fifth of the values obtained at 25 C; with CR63 UDPG⁻, the reduction was to one-third. With T4LB3-infected CR63 and CR63 UDPG⁻, very similar results were obtained (Table 1). With both T4 wild and T4LB3, the incorporation of ³H-5-cytosine remained on a higher level with UDPG⁻ hosts than with CR63.

mRNA half-life in T4-infected bacteria. *E. coli* B and B/r(λ) were infected at 37 C with T4r102 at a multiplicity of infection of 8 in M-9A. From 8 to 12 min, a pulse of ³H-labeled 5-cytosine (0.5 μ C/ml) was given, followed by a chase with 50 μ C of cytosine per ml, amounting to a 10^4 -fold excess over the radioactive cytosine. Samples (0.5 ml) were withdrawn and pipetted into 1 ml of 2% SDS at 78 C to accomplish immediate lysis and inhibition of ribonucleases. After 3 min at 78 C, the samples were chilled, acid-precipitated, fil-

tered onto membrane filters, and assayed for radioactivity. Figure 4A shows an mRNA half-life of 2.6 min with host B and 6.1 min with B/r(λ). The same type of experiment with CR63 and CR63 UDPG⁻ infected with T4LB3 yielded mRNA half-lives at 41.5 C of 12 and 13 min, respectively (Fig. 4B). Thus, at least with host *E. coli* B, we may expect a cessation of mRNA synthesis to lead to a reduction in protein synthesis after a delay of 2 to 4 min.

RNA synthesized after upshift to 41.5 C in T4LB3-infected CR63 and CR63 UDPG⁻. To

FIG. 5. RNA-DNA hybridization-competition experiments. (A) Tritium-labeled mRNA from T4LB3-infected CR63, isolated 10 min after upshifting from 25 to 41.5 C (upshift at 30 min after infection), was hybridized with denatured T4 DNA in the presence of increasing amounts of nonradioactive T4 mRNA which was isolated from T4B-infected *E. coli* B at 18 min after infection at 37 C (late competitor). Competitor RNA concentration: 20 μ C of mRNA/ml. (B) Tritium-labeled mRNA from T4B-infected *E. coli* B isolated 7 min after infection at 37 C competed by non-radioactive 7-min T4 mRNA (early competitor). Competitor concentration: 60 μ C of mRNA/ml. Input per hybridization was 1,200 counts/min; input DNA was 1.8 μ C. Hybridization efficiency in the absence of competitor was $30 \pm 5\%$.

study the nature of the RNA synthesized after the upshift, we infected CR63 and CR63 UDPG⁻ with T4LB3 (multiplicity of infection, 8) at 25 C. After 30 min, both cultures were quickly upshifted to 41.5 C. Five minutes later, 0.5 μ C of ³H-5-cytosine per ml was added and left for 5 min. The complexes were then lysed, and the pulse-labeled RNA was isolated.

The RNA obtained in two different experiments hybridizes with denatured T4 DNA with the same efficiency as does RNA harvested from T4 wild-type-infected *E. coli* B ($30 \pm 5\%$). This indicates that most, or all, of the pulse-labeled RNA is T4 mRNA. In Fig. 5B, we show a control hybridiza-

tion of ³H-labeled, 4-min T4 mRNA isolated from *E. coli* B. The competitor RNA is a nonradioactive, 7-min T4 mRNA. The competition was complete. When the same 7-min competitor RNA was used in competing against the T4LB3 mRNA synthesized after the arrest of DNA synthesis, the 7-min competitor chased only 70% of the radioactive RNA (Fig. 6), whereas an 18-min competitor RNA chased completely (Fig. 5A). Thus, the T4 mRNA synthesized after the arrest of T4 DNA synthesis had the same ratio of early versus late mRNA as reported for T2 (11).

Effects of arresting T4 DNA replication on lysozyme synthesis. A total of 8×10^9 cells of either host were sedimented and suspended in 2 ml of M-9A. T4LB3 (2×10^{10}) was added and adsorbed for 2 min at 37 C; the mixture was poured into 18 ml of M-9A at 25 C. Samples (2 ml) were withdrawn at 10, 20, and 28 min of incubation at 25 C, and every 2 min after the upshift to 41.5 C. The samples were pipetted onto 0.5 ml of frozen 0.1 M

FIG. 6. RNA-DNA hybridization-competition experiments. (A) Tritium-labeled mRNA from T4LB3-infected CR63 isolated 10 min after upshifting from 25 to 41.5 C (upshift 30 min after infection) was hybridized with denatured T4 DNA in the presence of increasing amounts of early competitor (7-min T4 mRNA, same as in Fig. 4B). (B) Same as A, except for the host bacteria which were CR63 UDPG⁻. mRNA input per hybridization was 1,200 counts/min; DNA input was 2 μ g. The hybridization efficiency in the absence of competitor is $30 \pm 5\%$. Broken lines: theoretical curves for a complete chase, based on the amount of radioactive versus nonradioactive RNA and the experimental value for 30% competition.

FIG. 7. Lysozyme synthesis in T4LB3-infected cells after upshift from 25 to 41.5 C. (A) Bacterial host was CR63. (B) Bacterial host was CR63 UDPG⁻. The vertical, broken lines indicate the times of upshifting to 41.5 C. The ordinates (proportional to $\Delta OD_{595}/\text{min}$) are normalized to the same lysozyme standard, resulting in identical ordinate units for A and B.

EDTA; they were frozen, sonically treated, and assayed for lysozyme. Figure 7 shows the rate of lysozyme synthesis to be constant after the upshift to 41.5 C for 15 min, after which time the cells lysed. A similar experiment with *E. coli* B as host showed a linear increase in lysozyme concentration for 8 min after the temperature shift (Fig. 8).

For reasons unknown, the burst size of T4 in CR63 UDPG⁻ was only 10 to 20% of the one observed with CR63. [The same observation was made by R. L. Russell (Ph.D. Thesis, California Institute of Technology, Pasadena, 1967) with two other UDPG-deficient strains *E. coli* B/40 and *Shigella* Sh/40.] The reduction in burst size with host CR63 UDPG⁻ is paralleled by a low rate of lysozyme synthesis both at 25 C and at 41.5 C, as shown in Fig. 9.

Tail fiber synthesis in T4LB3-infected CR63 and CR63 UDPG⁻ after arresting DNA synthesis. Synthesis of tail fibers, as evidenced by the serum-blocking power of lysates of T4-infected cells, is a late T4 function (4, 6; Fig. 10). When serum-blocking power is measured in T4LB3-infected CR63 and CR63 UDPG⁻ after the upshift from 25 to 41.5 C, it is seen that tail fibers accumulate at a linear rate until 12 to 14 min after the upshift. The synthesis then slows down and stops, owing to lysis of cells (Fig. 11).

DISCUSSION

Our results (Fig. 1-3) have shown that we (i) can turn off DNA synthesis by raising the incuba-

tion temperature to 41.5 C with T4LB3 and (ii) that the T4 DNA synthesized in CR63 UDPG⁻ is nonglucosylated. From Table 1 we see that, after the upshift to 41.5 C, the incorporation of ³H-labeled 5-cytosine into RNA gradually decreases to similar extents both with T4- and T4LB3-infected hosts. Thus, interrupting DNA synthesis

FIG. 9. Lysozyme synthesis in T4-infected CR63 and CR63 UDPG⁻. (A) Synthesis at 25 C in T4LB3-infected hosts. (B) Synthesis at 41.5 C in T4LB3-infected hosts. The ordinate (proportional to $\Delta OD_{595}/\text{min}$) is normalized by designating the highest lysozyme concentration reached in CR63 to be 1.0.

FIG. 8. Lysozyme synthesis in T4LB3-infected *E. coli* B before and after upshift to 41.5 C. Lysis of complexes starts at 43 min.

FIG. 10. Tail fiber synthesis in T4B-infected CR63 at 30°C. Neutralization of T4 antiserum by samples of T4-infected CR63 was determined at times indicated. The ordinate value 1.0 equals the total amount of T4 antiserum added to the reaction.

with T4LB3 does not lead to an additional reduction of RNA synthesis. The reduction, both with wild-type T4 and with T4LB3, is caused by the high temperature alone. CR63 and CR63 UDPG⁻ hosts differ somewhat in the decrease of RNA synthesis, but irrespective of DNA replication (Table 1). RNA production with UDPG⁻ hosts remains on a level, approximately 2.5 times the level of CR63. This could mean that nonglucosylated DNA is a better template for in vivo transcription.

mRNA synthesized in T4r102-infected *E. coli* B and B/r(λ) displayed half-lives of 2.6 and 6.1 min, respectively. The apparent half-lives of T4LB3 mRNA in CR63 and in CR63 UDPG⁻ after the upshift to 41.5°C are 12 and 13 min, respectively. We do not know whether the longer mRNA half-lives are real, or are caused by slow uptakes of chaser cytosine or other differences in the precursor pool kinetics. Should they represent the "true" mRNA half-lives in these hosts, we could not experimentally observe a turn-off of late protein synthesis by turning off late mRNA synthesis 10 min before lysis of cells. Thus, observations on the mRNA synthesis will give more reliable results.

T4 mRNA synthesized between 5 and 10 min after the upshift from 25 to 41.5°C, both in CR63 and in CR63 UDPG⁻ infected by T4LB3, is composed of 70% early mRNA and 30% late mRNA (Fig. 5 and 6). Thus, neither interrupting DNA replication nor permitting reglucosylation of T4 DNA turn off synthesis of late T4 mRNA.

As one would expect from the continuation of late mRNA synthesis, the interruption of T4 DNA replication has no, or little, effect on late protein synthesis. The temperature-shift experi-

ments with T4LB3 presented in Fig. 7A and B show continuation of lysozyme synthesis at a constant rate until lysis of cells, and irrespective of the host's ability to glucosylate the T4 DNA. The apparently reduced rate of mRNA synthesis (Table 1) after upshifting to 41.5°C is not reflected in lysozyme synthesis. This means either that the mRNA half-life is indeed about 12 min, or that there is an excess of mRNA which may drop to one-fourth without affecting the rate of protein synthesis. In *E. coli* B we had observed an mRNA half-life of 2.6 min at 37°C (Fig. 3A). This short half-life, together with the reduced rate of mRNA synthesis at 41.5°C, could lead to a gradually decreasing lysozyme production. The data presented in Fig. 8 agree with this assumption. Nonetheless, lysozyme synthesis continues until lysis.

Tail fiber synthesis, as determined by T4 antiserum neutralization, is a late function in host CR63 (Fig. 10). When T4 DNA synthesis is interrupted in T4LB3-infected host cells CR63 and CR63 UDPG⁻, tail fiber synthesis continues at a

FIG. 11. Synthesis of serum-blocking power in T4LB3-infected hosts after upshift from 25 to 41.5°C at 30 min after infection. (A) In CR63, (B) in CR63 UDPG⁻. The ordinate value 1.0 equals the total amount of T4 antiserum added to the reaction.

constant rate, independent of the host's ability to glycosylate the T4 DNA (Fig. 11A and B).

The conclusions we draw from our experiments are as follows. (i) Continuation of T4 DNA replication is not required for continuation of late mRNA and of late protein synthesis. (ii) Once the parental DNA has been replicated and DNA synthesis is arrested thereafter, late T4 mRNA continues to be synthesized, irrespective of the host's ability to glycosylate T4 DNA. (iii) Since replication of the parental DNA is a prerequisite for turning on late mRNA synthesis, and since continued DNA synthesis is not required for transcription of late genes, part of the replicated DNA is possibly put aside in the bacterial cell to serve as a template for transcription.

In a compartmentalized cell, T4 DNA replication and RNA synthesis could utilize different templates without interference. Methyl- and glucosyltransferases, and maturation factors which act upon replicated DNA, could be prevented from acting on certain chromosome copies, leaving some of the DNA in a nascent state. This nascent state could be one requirement for a normal rate of late mRNA synthesis.

Using T4-infected protoplasts of *E. coli* B and inhibiting DNA synthesis by 5-fluorodeoxyuridine after the onset of late T4 functions, Lembach et al. (13) observed a reduction in serum-blocking power and late T4 mRNA synthesis. However, they achieved a complete turn-off of serum-blocking power synthesis only when RNA synthesis was prevented by an additional rifamycin treatment. Riva and Geiduscheck (Federation Proc., p. 660, 1969) have reported a reduced rate of late mRNA synthesis after-shifts to nonpermissive temperatures with a temperature-sensitive DNA polymerase mutant (in gene no. 43) of T4. Similar results were obtained by Zograf et al. (19). Although these observations possibly differ from our results in quantitative terms, they agree on the fact that late mRNA synthesis is not (completely) turned off by arresting the replication of T4 DNA.

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