Control of Gene Function in Bacteriophage T4

III. Preventing the Shutoff of Early Enzyme Synthesis

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Synthesis of early T4 protein, which is normally shut off at 10 min after infection, continues until lysis when host cells have been preinfected with T3 sam⁺. In host cells preinfected with T3 sam⁻, synthesis of early enzymes is shut off as normal. Thus, S-adenosylmethionine is required for the turnoff of early T4 functions (at least when host cells have been preinfected with T3).

Synthesis of early T4 proteins starts shortly after infection of permissive host bacteria and stops at about the middle of the latent period (2, 4, 14, 19), whereas messenger ribonucleic acid (mRNA) homologous to early genes of T4 is found throughout the lytic cycle (1, 5, 8). It remains questionable, however, whether all early T4 genes are represented in the class of T4 mRNA which persists throughout the latent period (13, 21). If mRNA of all T4 genes is present during the whole latent period, then it is likely that the termination of early protein synthesis is mediated by a translational control. We hypothesize that changes in the available transfer RNA (tRNA) (18), alone or in concert with other factors involved in translation, could be instrumental in turning off the translation of early T4 mRNA. We further assume that a methylating capacity is required to bring about the necessary changes in tRNA species. These assumptions lead to the prediction that host bacteria with a deficient methylating capacity would fail to shut off synthesis of early T4 proteins.

Here we report the continuous synthesis of two early T4 enzymes, β -glucosyltransferase and deoxycytidine monophosphate deaminase, in bacteria with impaired methylating capacities.

MATERIALS AND METHODS

Bacteriophages. T4B, originally obtained from S. Benzer, and T4B h2⁺, constructed by R. Hertel by a cross with T2 and four backcrosses against T4B, are from our collection. The lysozyme deficient T4D am H26 was kindly provided by G. Streisinger. T3 wild was from our stock. T3 H222 sam⁺ (9) carrying an amber mutation in the RNA polymerase gene 1 (3; R. Hausmann, *personal communication*), T3 sam⁻ (9), and the double mutant T3 A19 sam⁻ with an amber deficiency in gene 1 and incapable of synthesizing S-adenosylmethionine (SAM)-cleaving enzyme were

generously made available by R. Hausmann. Lysates of T3 were regularly tested for their ability or inability to induce synthesis of SAM-cleaving enzyme.

Bacteria. *Escherichia coli* B₈₋₁ (12) was used throughout this study except for growing and assaying of the various amber phage for which *E. coli* BBW/1 (10) was used.

Growth and infection of bacteria. Nutrient broth consisting of Difco nutrient broth (8 g) NaCl (5 g), water (1,000 ml), pH 6.9, and tryptone broth consisting of Difco tryptone (10 g), NaCl (5 g), water (1,000 ml), pH 6.8, were used for growth of bacteria, adsorption of phage, and incubation of phage-infected bacteria. Bacteria were infected at either 5×10^8 cells/ml or 10^9 cells/ml with 10 T3 particles per cell or 5 T4 particles per cell or both. Infection was controlled by assaying for colony-forming bacteria at appropriate times after phage addition. In the experiments presented in this report, at least 95% of the bacteria had lost the colony-forming ability in cases of T3 infections and more than 98% in cases of T4 infections.

Assay for β -glucosyltransferase. Five- or ten-microliter amounts of cell-free extract were incubated with 20 μ liters of assay mixture for 15 min at 37 C and then chilled. Two milliliters of cold 10% trichloroacetic acid was added, and the precipitate was collected and washed on nitrocellulose filters. The assay mixture (7) contained: uridine-diphospho-¹⁴C-glucose (16 μ Ci/ μ mole), T4 α gt⁻, β gt⁻ deoxyribonucleic acid (DNA) (40 μ g/ml) as acceptor, and magnesium acetate (0.06 M). Radioactivity was determined in Packard Tri-Carb scintillation counters.

Assay for deoxycytidinemonophosphate deaminase (dCMP deaminase). The assay was the one described by Schweiger and Gold (16).

Assay for lysozyme. The method of Leutgeb as described by Schweiger and Gold (16) was used. Filters loaded with bacteria containing 10^6 counts/min incorporated from ³H-labeled diaminopimelic acid were incubated with 20 μ liters of cell extract, and portions (0.2 ml) were withdrawn for measuring the radioactivity released by lysozyme action.

UV irradiations. T3 sam^+ and T3 sam^- were ultraviolet (UV)-inactivated to 10^{-7} to 10^{-8} survivors (as assayed on B_{s-1}) by the light of a low pressure mercury lamp (254 nm). Experiments utilizing UV irradiated phage were carried out in dim yellow light.

Preparation of cell-free extracts from phage-infected bacteria. Portions (10 ml) were withdrawn from the infected cultures at various times after infection, chilled on crushed ice, centrifuged at 0°C, and suspended in 1 ml of cold buffer [0.01 M tris(hydroxymethyl)aminomethane, 0.01 M MgCl_2 , 0.022 M NH_4Cl , and 0.001 M dithiothreitol]. The cell suspensions were then quickly frozen in dry ice-acetone mixtures and stored in a freezer. After thawing, cells were mixed with 2 volumes of dry glass beads and crushed at 1°C in a Sorvall Omnimixer. Thereafter, glass beads and cell debris were removed by centrifugation, and portions of the supernatant fluids were assayed for enzyme activities.

RESULTS

β -Glucosyltransferase activity in *E. coli* B_{s-1} after infection with T4 H26. The experiment was performed to verify that β -glucosyltransferase is a typical early T4 enzyme, synthesis of which stops at 10 min after infection (Fig. 1, solid line). Use of the lysozyme-deficient T4 H26 allowed an extension of the observation period from 20 to 30 min at 37°C without lysis of cells (Fig. 1, broken line) or any known interference with T4 regulatory processes.

β -Glucosyltransferase activity in *E. coli* B_{s-1} preinfected with UV-irradiated T3 sam^+ and superinfected with T4B h2^+ . Gefer et al. (6) have shown that UV irradiation of T3 does not efficiently reduce the T3-directed synthesis of SAM-cleaving enzyme, which is due to the proximity of the structural gene for the enzyme to the early T3 promoter (*unpublished data*; Hausmann, *personal communication*). Furthermore, it has been shown that infection with UV-irradiated T3 sam^+ can sufficiently reduce the intracellular level of *S*-adenosylmethionine to impair the methylation of nucleic acids (15). For these reasons, UV-irradiated T3 sam^+ (10^{-7} to 10^{-8} survivors) was used to block, or reduce, the methylating capacity of bacterial cells.

Six to eight minutes after addition of T3 to bacteria, when the SAM-cleaving enzyme had reached maximum concentrations (9), T4B h2^+ was added and the activity of β -glucosyltransferase was measured in cell-free extracts of samples taken at various times after T4 addition (Fig. 2). In contrast to the results presented in Fig. 1, synthesis of β -glucosyltransferase continued at a constant but reduced (60% of normal) rate until lysis of cells.

β -Glucosyltransferase activity in *E. coli* B_{s-1} preinfected with UV-irradiated T3 sam^- and super-

FIG. 1. Activity of β -glucosyltransferase in T4 H26-infected *E. coli* B_{s-1} (left ordinate, open circles, solid line). Turbidity of the infected culture (right ordinate, crosses, broken line). Cell-free extracts (1 ml) were made from 5.5×10^9 infected cells. A 10- μ liter amount thereof was incubated for 15 min at 37°C with 20 μ liters of the β -glucosyltransferase assay mixture.

infected with T4B h2^+ or T4 H26. To show that the SAM-cleaving enzyme of T3 prevents the turnoff of early T4 protein synthesis, the experiment in Fig. 2 was duplicated except UV-irradiated T3 sam^- was used as the preinfecting phage. The same bacterial culture was used for both experiments, divided into two equal batches shortly before infection with either T3. For superinfection, T4B h2^+ was used. In contrast to the continuous synthesis shown in Fig. 2, β -glucosyltransferase stopped being synthesized at 12 min after T4 infection (Fig. 3, curve A). The same experiment was repeated using the lysozyme-deficient T4 H26 to extend the observation period from 20 to 30 min. The results (Fig. 3, curve B) show that synthesis of β -glucosyltransferase is shut off at 10 or 12 min after T4 infection, as was the case without T3 preinfection (Fig. 1).

Activities of β -glucosyltransferase and dCMP-deaminase in *E. coli* B_{s-1} preinfected with T3 H222 sam^+ and superinfected with T4 H26. To reduce the number of active T3 genes and to avoid UV irradiations, T3 H222 sam^+ , deficient in the synthesis of T3 RNA polymerase, was introduced. The gene for the SAM-cleaving enzyme, together with three or four other genes, is transcribed by bacterial RNA polymerase (11); the remaining T3 genes require T3 RNA polymerase for transcription (3), and are thus silent.

E. coli B_{s-1} , preinfected with T3 H222 sam^+ , was superinfected with T4 H26 at 7 min after T3 H222 addition. Samples were withdrawn at various times after T4 H26 infection, and the activity of β -glucosyltransferase was assayed in cell-free

FIG. 2. Activity of β -glucosyltransferase in *E. coli* B_{s-1} , ultraviolet T3 sa^{+} -preinfected for 8 min and superinfected with T4B $h2^{+}$ at time 0. Sample size and assay conditions were the same as in Fig. 1.

extracts thereof. Figure 4 shows that again synthesis continues at a nearly constant rate until lysis of cells.

To show that the failure to shut off synthesis of β -glucosyltransferase is not limited to one early T4 gene, the activity of dCMP-deaminase was assayed in cell-free extracts from the preceding (Fig. 4) experiment. The data in Fig. 5 show a continuous increase in dCMP-deaminase activity throughout the lytic cycle, whereas synthesis of dCMP-deaminase (14) normally stops at 10 min after infection (20).

Effects of T3 H222 sa^{+} preinfection on eclipse and latent periods, burst size, and lysozyme synthesis of T4B. As was shown in the preceding experiments, preinfecting T3 sa^{+} reduced the rates of early enzyme synthesis by about 40%. In this and subsequent experiments, the question was raised whether T3 sa^{+} affects the synthesis of late T4 proteins and of T4 DNA. If so, this could lead to (i) exclusion of superinfecting T4, (ii) extension of the latent period, (iii) reduction of the burst size, or both ii and iii.

B_{s-1} and, in parallel, B_{s-1} infected with T3 H222 sa^{+} , were infected with T4B at multiplicities of five, and the number of plaque-forming units was measured at various times thereafter. The results (Fig. 6 and 7, solid lines) show that only about 15% of the preinfected bacteria T3 H222 excluded the superinfecting T4B. The T4 latent period appeared to be only slightly extended. The burst size, however, was reduced to about 35% of normal, suggesting that synthesis of late functions is reduced by the preinfecting T3 about as much as synthesis of early functions. T3 does not introduce a strong heterogeneity between the latent periods of individual T4B-infected complexes. Following T4B infection, cells lysed between 20 and 28 min, with T3-preinfected

FIG. 3. Curve A, activity of β -glucosyltransferase in *E. coli* B_{s-1} , ultraviolet T3 sa^{+} -preinfected for 8 min and superinfected with T4B $h2^{+}$ at time 0. Sample size and assay conditions were the same as in Fig. 1. Curve B, activity of β -glucosyltransferase in *E. coli* B_{s-1} , preinfected with ultraviolet T3 sa^{+} for 6 min and superinfected with T4 H26 at time 0 min (left ordinate, open circles, solid line). Turbidity of the infected culture of curve B (right ordinate, crosses, broken line). Cell-free extracts (1 ml) were made from 1.2×10^{10} infected cells. A 10- μ liter amount thereof was incubated for 15 min at 37 C for the β -glucosyltransferase assay with 20 μ liters of the β -glucosyltransferase assay mixture described in Materials and Methods.

bacteria lysis occurring between 21 and 36 min (Fig. 6 and 7, solid lines). With and without T3 preinfection, the first mature T4 phage appeared at 12 min after T4 addition, and the ratio of mature phage to plaque-forming units reached one at 15 min (with T3 preinfection) and 17 min (without T3 preinfection). The value of 17 min is taken from the dotted line in Fig. 7, which gives the actual number of mature T4 phage corrected for the reduction in burst size (i.e., multiplied by a factor of 3).

Since a delayed onset of T4 development in about two-thirds of the T3 sa^{+} -preinfected bacteria could have simulated the failure to shut off early functions, this possibility was tested by two additional experiments. In the case of delayed onset, the period of late enzyme synthesis would be equivalently extended (to at least 40 to 42 min), and lysis of cells would be protracted sim-

FIG. 4. Activity of β -glucosyltransferase in *E. coli* B_{8-1} , 7 min preinfected with T3 H222 (gene-1⁻ sam⁺) and superinfected with T4 H26 at time 0 (left ordinate, open circles, solid line). Turbidity of infected culture (right ordinate, crosses, broken line). Same bacterial culture as in Fig. 5. Cell-free extract (1 ml) was made from 5.0×10^8 infected cells. A 5- μ liter amount thereof was incubated with 20 μ liters of β -glucosyl-transferase assay mixture for 15 min at 37 C.

FIG. 5. Activity of dCMP-deaminase in *E. coli* B_{8-1} , 7 min preinfected with T3 H222 (gene-1⁻, sam⁺) and superinfected with T4 H26 at time 0 (left ordinate, open circles, solid line). Turbidity of the infected culture (right ordinate, crosses, broken line). Assays were made with the same cell-free extracts as in Fig. 4. A 10- μ liter amount of extract was incubated for 45 min at 37 C with 10 μ liters of water, 2 μ liters of dCMP solution, and 1 μ liter of dCTP solution.

ilarly. However, Fig. 8 shows that lysozyme synthesis proceeds at a linear (not continuously increasing, as predicted by the delayed onset model) rate between 10 and 30 min and stops

FIG. 6. Eclipse period, latent period and burst size of T4B in *E. coli* B_{8-1} at 37 C. Lawn bacteria, *E. coli* B_{8-1} . Infective centers at various times after T4 addition (solid line). Plaque-forming units after CHCl_3 treatment of infected cells, mature phage (broken line).

thereafter. Furthermore, comparison of the latent periods and burst sizes of T4 in T3 gene-1 sam⁺- and T3 gene-1 sam⁻-preinfected bacteria shows no, or very minor, differences in the eclipse and latent periods and in the burst sizes of T4 (Fig. 9 and 10). Comparison with Fig. 6 reveals that the end of the latent period possibly is delayed by 4 to 6 min by preinfection with either T3, which agrees with the data in Fig. 7. Latent periods and burst sizes were also measured with T4B h2⁺-infected cells which had been preinfected with UV-irradiated T3 sam⁺. The results (data not shown) were identical to those observed with T3 H222 preinfection (Fig. 9).

DISCUSSION

Our experiments show that in bacteria in which the methylating capacity has been eliminated or reduced by T3 sam⁺ the early T4 enzymes β -glucosyltransferase and dCMP-deaminase continue to be synthesized at a constant rate until lysis of cells. Controls with T3 sam⁻-preinfected bacteria display the normal turnoff of early T4 protein synthesis at 10 min after infection.

The question remains whether continuous synthesis is caused by a defect in the shutoff mecha-

FIG. 7. Eclipse period, latent period, and burst size of T4B in *E. coli* B₈₋₁, 7 min preinfected with T3 H222 sam⁺ at 37°C. Bacteria are from the same culture as in Fig. 6. Lawn bacteria, *E. coli* B₈₋₁. Infective centers at various times after T4 addition (solid line). Plaque-forming units after CHCl₃ treatment of infected cells, mature phage (broken line). The dotted line represents the broken line multiplied by 3 (the reduction in burst size as compared with Fig. 6) to facilitate comparison of the eclipse times in Fig. 7 and 6.

FIG. 8. Lysozyme activity in 7 min T3 H222 sam⁺-preinfected *E. coli* B₈₋₁ after superinfection with T4B at time 0 (left ordinate, open circles, solid line). Turbidity of the infected culture (right ordinate, crosses, broken line).

nism in a synchronously developing population of T4-infected cells (model A), or by introduction of a strong heterogeneity in the onset of T4 development in which the subpopulations turn off early T4 proteins as usual (model B). To account

FIG. 9. Eclipse period, latent period, and burst size of T4B in *E. coli* B₈₋₁, 7 min preinfected with T3 H222 sam⁺ at 37°C. Lawn bacteria, *E. coli* B₈₋₁. Infective centers at various times after T4 addition (solid line). Plaque-forming units after CHCl₃ treatment of infected cells, mature phage (broken line).

FIG. 10. Same experiment as in Fig. 8, except T3 gene-I⁻ sam⁻ was used as the preinfecting phage.

for the linear rate in β -glucosyltransferase synthesis up to 30 min after T4 infection, model B must postulate that the onset of T4 development is delayed by 20 min in one-third of the population. Model B thus predicts a reduction in the rate of β -glucosyltransferase synthesis to 33% of normal, a continuous synthesis of lysozyme until 40 min after T4 infection, and a protracted period of cell lysis beginning at 20 min and ending at 48 min after T4 infection. Model A predicts a normal rate of β -glucosyltransferase synthesis, a termination of lysozyme synthesis by 28 to 30 min, and no protraction of the lysis period. Synthesis of β -glucosyltransferase (Fig. 2 and 4), lysozyme

synthesis (Fig. 8), and the lengths of the lysis periods (Fig. 6, 7, 9, and 10) show that there is little heterogeneity introduced by the preinfecting T3 sam⁺, thus ruling out model B.

We have shown that the mechanism which turns off early T4 protein synthesis depends upon the availability of S-adenosylmethionine (at least in T3-preinfected bacteria). This finding is in agreement, but does not prove, our working hypothesis that methylation of tRNA is instrumental in the apparent translational control of early T4 enzymes. Experiments are being conducted to probe this hypothesis and alternative hypotheses.

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