Effect of DNA-Negative and Maturation-Defective Conditions on Accumulation of Functional Messengers for T4 Bacteriophage-Specific Dihydrofolate Reductase and Deoxynucleoside Monophosphate Kinase

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Messengers for T4 phage-specific deoxynucleoside monophosphate kinase overaccumulated in nonpermissive hosts infected with amber-defective viruses that displayed either the DNA-negative or maturation-defective phenotype. Under both conditions, however, transcription of functional messengers for dihydrofolate reductase followed essentially normal kinetics.

When nonpermissive Escherichia coli are infected with amber-defective T4 phage displaying either the DNA-negative (DO) or maturation-defective (MD) phenotypes, none of the late genes is expressed (1–3), and the shutoff of early messenger accumulation is delayed (8, 14, 15). In a general study, Wilhelm and Haselkorn (14) demonstrated that early messengers could be divided into three groups, relative to the effects of DO and MD conditions on their transcription. Transcription of group I messengers was normal under both conditions. Group II messengers were transcribed normally under MD conditions but not under DO conditions. Group III messengers, the majority, were not subjected to normal turnoff under both DO and MD conditions. Subsequent reports, from other laboratories, suggested that messengers for T4 phage-specific α-glucosyl-transferase (15) and deoxynucleoside monophosphate kinase (8) belong to groups II and III, respectively. In this

FIG. 1. Kinetics of functional messenger transcription in Escherichia coli B51 infected with DNA-positive and DNA-negative phages. Three (1 liter) cultures of bacteria were grown in basal salts-glycerol medium (5) supplemented with 50 μg of L-tryptophan per ml and 0.5% Casamino Acids (vitamin free). The temperature was 30 C. When the cells reached 4 × 10⁴/ml, they were harvested by centrifugation and resuspended into 900 ml of fresh, previously warmed (30 C) medium. Five minutes later, the cultures were infected by adding 100 ml of warm medium that contained the viruses. The multiplicity of infection was always 10 plaque-forming units/cell. At the times indicated, 100-ml aliquots were removed, poured over ice, and concentrated 50-fold by centrifugation. The cells were lysed, and RNA was isolated by the methods described by Sakiyama and Buchanan (7). RNA-directed cell-free synthesis of enzymes was carried out in 500-μl reaction mixtures as described by Sakiyama and Buchanan (7). After protein synthesis was complete, 200-μl aliquots were assayed for dihydrofolate reductase and deoxynucleoside monophosphate kinase as described by Trimble et al. (10). Messenger activity is reported in terms of milliunits of enzyme synthesized per milligram of S-30 protein (10). Symbols: ○, T4DamH26; ●, T4DamN82, no rifampin; ▼, T4DamN82, rifampin (300 μg/ml) present from 15½ min postinfection.
communication, we confirm the assignment of kinase messengers to group III and show that messengers for dihydrofolate reductase belong to group I.

When *E. coli* B<sub>S-1</sub> was infected with T4DameH26 (endolysinless), functional reductase messengers were present by the 3rd min (Fig. 1 and 2). These messengers reached their maximum level by the 6th min and, thereafter, rapidly decayed to insignificant amounts. Kinase messengers first appeared between 3 and 6 min postinfection and were present at maximum levels by the 12th min (Fig. 1 and 2).

Upon infection of *E. coli* B<sub>S-1</sub> with either T4DamN82 (gene 44, DO phenotype) or T4DamBL292 (gene 55, MD phenotype), kinase messengers subsequently accumulated to levels 30 to 50% higher than normal (Fig. 1 and 2). Beginning at the 15th min, the amount of kinase messenger present in DO- and MD-infected cells decreased but at a rate that was two or three times slower than normal.

The slower apparent rate of kinase messenger decay could reflect one of two situations. (i) It is possible that this species is more stable, at late times, under DO and MD conditions. (ii) It is possible that the apparently slower rate of decay is generated by residual kinase messenger transcription. To differentiate between these alternatives, bacteria were infected with either DO or MD viruses, and rifampin, a potent inhibitor of RNA synthesis (9, 12, 13), was added at 15½ min. The presence of rifampin more than doubled the apparent rate of kinase messenger decay (Fig. 1 and 2), implying that many of the kinase messengers present at later times (in the absence of drug) were synthesized de novo.

In contrast to the results obtained for kinase messengers, essentially normal kinetics of reductase messenger transcription were observed under both DO and MD conditions (Fig. 1 and 2). This supports the earlier data of Mathews (6) and Warner and Lewis (11) who showed that, unlike other early enzymes they studied, reductase failed to overaccumulate in DO-infected bacteria.

The results presented in this paper are consistent with the interpretation that transcription of reductase and kinase messengers is not subject to unit control. In this context, it is relevant to mention that mutants defective in the turn-off of reductase synthesis have recently been described (4). However, it is not clear if these mutants control reductase synthesis at the level of transcription or translation.

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**LITERATURE CITED**


