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Control of Early Gene Expression of Bacteriophage T4: Involvement of the Host *rho* Factor and the *mot* Gene of the Bacteriophage

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Many early mRNA species of bacteriophage T4 are not synthesized after infection of *Escherichia coli* in the presence of chloramphenicol. This has been interpreted as a need for T4 protein(s) to be synthesized to allow expresssion of some early genes, e.g., those for deoxycytidinetriphosphatase, deoxynucleosidemonophosphate kinase and UDP-glucose-DNA β-glucosyltransferase. In the experiments described here, early mRNA of bacteriophage T4 was allowed to accumulate during chloramphenicol treatment. After the addition of rifampin to inhibit further RNA synthesis, and subsequent removal of chloramphenicol, the accumulated mRNA was permitted to express itself into measured enzyme activities. It was shown that the early mRNA species coding for deoxycytidinetriphosphatase and UDP-glucose-DNA β-glucosyltransferase could be formed in the presence of chloramphenicol if the *E. coli* host cell carried a mutation in the structural gene for the RNA chain termination factor *rho*. This was interpreted to mean that T4 protein(s) with anti-*rho* activity is normally required for the expression of these two early genes. An altered *rho*-factor could not, however, relieve the need of phage protein synthesis for the formation of another early mRNA, that coding for deoxynucleosidemonophosphate kinase. In this case the *mot* gene of T4 seemed to be involved, since the primary infection of *E. coli* cells with the *mot* gene mutant *ts*G1 did not allow subsequent deoxynucleoside monophosphate kinase mRNA synthesis after wild-type phage infection in the presence of chloramphenicol. In control experiments, deoxynucleoside monophosphate kinase mRNA synthesis induced by wild-type phage superinfecting in the presence of chloramphenicol was facilitated by the primary infection with T4 phage containing an unmutated *mot* gene.

During the development of bacteriophage T4 in an *Escherichia coli* cell, several different classes of T4 phage mRNA can be discerned. Different requirements can also be defined for the expression of many early T4 genes (2). If protein synthesis is inhibited by the addition of chloramphenicol before T4 infection, only a few early mRNA species, among them that coding for dCMP hydroxymethylase (EC 2.1.2.8) are synthesized (15). An explanation for this would be that phage protein(s) may be necessary for the transcription of many early genes or that chloramphenicol, by blocking translation, leaves the promoter-distal transcripts exposed to nucleolytic degradation, which would reduce the expression of promoter-distal genes (2).

In previous work we have measured the transcription of early T4 genes by an enzyme-forming capacity method (11). Early messengers were allowed to accumulate during infection in the presence of chloramphenicol. Rifampin was then added and chloramphenicol was removed by filtration to permit the messengers formed in the absence of protein synthesis to express themselves into enzyme activity. By this technique it was observed that dCTPase (EC 3.6.1.12) mRNA and dTMP kinase (EC 2.7.4.13) mRNA could not be formed in the presence of chloramphenicol. This was interpreted as a need for phage-specific protein synthesis to allow transcription of several early genes. The alleged phage protein(s) was shown to have a *trans* effect on a superinfecting phage genome, allowing the transcription of the mentioned early enzyme genes in the presence of chloramphenicol. The transcribed RNA was detected in a double infection system where the primary infection took place with a phage mutationally deficient in the measured enzyme. Superinfection was then performed with wild-type phage in the presence of chloramphenicol. By the enzyme-forming capacity method as described above, it was then demonstrated that the primary infection had created conditions for the

formation of dTMP kinase mRNA and dCTPase mRNA in the absence of protein synthesis.

In the experiments reported here, a similar approach was used to investigate the relation of the *rho* gene of the host to the early transcriptional regulation of phage T4. It could be seen that a mutation in the *rho* gene (17) of the *E. coli* host permitted the synthesis of T4 dCTPase mRNA in the presence of chloramphenicol, i.e., the rho_{ts15} mutation (3) in the host seemed to obviate the need for phage-specific protein synthesis to permit dCTPase mRNA formation. Similar observations were made with the polarity suppressor mutants of Korn and Yanofsky (9, 10) for dCTPase mRNA and for UDP-glucose-DNA β-glucosyltransferase (β-glucosyltransferase, EC 2.4.1.27) mRNA. These results were interpreted to mean that normally one or several phage proteins interact with the *rho*-protein of *E. coli* to control the formation of dCTPase and β-glucosyltransferase mRNA, respectively.

A new regulatory gene (*mot*), modifier of transcription, mapping between genes 52 and *t*, was defined by the regulatory mutant *ts*G1 isolated by Mattson et al. (13). By complementation tests, the *far*P85 mutation, isolated by Johnson and Hall (7), was also found to be located in the *mot* gene. The *mot* gene mutations cause reduced synthesis of several early proteins, e.g., those of genes 1, 32, 43, 45, 46, and rIIB (14).

In the experiments reported here, a delay in the initiation of dTMP kinase synthesis after *mot* mutant infection was observed. It could also be seen that, under nonpermissive conditions, primary infection with *ts*G1 was unable to create conditions for the formation of dTMP kinase mRNA at superinfection in the presence of chloramphenicol. This was interpreted as a direct role of the *mot* gene product in the control of dTMP kinase mRNA synthesis.

MATERIALS AND METHODS

Chemicals. The radioactive substrates [*U*-^{14}C]-dCTP (440 mCi/mmol), [2-^{14}C]dTMP (58 mCi/mmol), and [glucose-^{14}C]UDP-glucose (300 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, Great Britain. Unlabeled dCTP, dTMP, and UDP-glucose were obtained from Sigma Chemical Co., St. Louis, Mo. Rifampin and chloramphenicol were gifts from Gruppo Lepetit, Milano, Italy, and Parke, Davis & Co., Hounslow, Great Britain, respectively.

Bacteria. The rifampin-sensitive *E. coli* B strain AA446 (12) was provided by E. Lund; it requires leucine for growth and is a derivative of AS19 (20). The *rho*-deficient *E. coli* K-12 strain AD1704 (F^- *his*$^-$ *sup*^{o} *str*^{r} gal_{am57} *val*^{r} rho_{ts15}) and its *rho*$^+$, but otherwise isogenic, counterpart AD1766 were provided by A. Das. Since the rho_{ts15} mutation (3) is very unstable, it was necessary to test frequently for its presence. In such tests, the following pleiotropic properties of the mutation were examined: temperature sensitivity, valine resistance, and galactose sensitivity (3). The *rho* mutant *E. coli* K-12 derivatives W3110*rho*102, W3110*rho*103, and W3110*rho*104 and their parental strain W3110 (*rho*$^+$ *trpR*$^-$ *trpE*$^-$ *trpA*$^-$ *lacZ* U118 *val*^{r} *azi*^{r}) (9, 10) were provided by C. Yanofsky. Phage amber mutants were plated on the *supD*-containing *E. coli* K-12 strain CR63 (21).

Bacteriophage. Wild-type T4D (*am*$^+$) and mutant *am*N122 (gene 42$^-$) were obtained from J. Wiberg. Mutant *imm*2 was from J. D. Childs, and double mutant *amαgt*8*amβgt*8 was from H. Revel. The bacteriophage double and triple mutants *am*E114*imm*2 (gene 56$^-$ [dCTPase$^-$] *imm*$^-$ [23]), *am*C42*imm*2 (gene 1$^-$ [dTMP kinase$^-$] *imm*$^-$) and *αgt*$^-$*βgt*$^-$*imm*2 (*amαgt*8*amβgt*8 [α- and β-glucosyltransferase$^-$] *imm*$^-$) were constructed in the laboratory by phage crossing. The T4 mutants *far*P85 (*mot*$^-$) (7) and *ts*G1 (*mot*$^-$) (13) were obtained from D. Hall and D. Rabussay, respectively.

Media. Experiments with *E. coli* B were performed in the glycerol Casamino Acids medium described by Fraser and Jerrel (6). For the experiments with AA446, the mineral salts medium M9 (5), supplemented with glucose (5 mg/ml) and $FeCl_3$ (10 μM) was used. Required leucine was added to 50 μg/ml. The *E. coli* strains AD1704 and AD1766 were grown in LB medium (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, and 1 g of glucose per liter).

Infected cells. For most experiments bacteria were grown in bubbler tubes to about 2×10^8 cells/ml at 37°C. At infection (time zero), four phages per bacterium were added. Phage adsorption was usually better than 95%, and bacterial survival was usually less than 1% at 3 min after infection.

Enzyme assays. Extracts were prepared by sonic disruption of the cells as described earlier (11).

dHMP kinase or dTMP kinase. dHMP kinase or dTMP kinase (4) was determined by the method of Wiberg et al. (24). As a substrate, [^{14}C]dTMP was used. The background activity in extracts from uninfected bacteria was subtracted. Each assay contained 0.025 μCi (85 nmol) of [^{14}C]dTMP.

dCTPase. dCTPase was determined as described earlier (11). Each assay contained 0.025 μCi (43 nmol) of [^{14}C]dCTP.

UDP-glucose-DNA β-glucosyltransferase. UDP-glucose-DNA β-glucosyltransferase was determined by a modification of the method of Baros and Witmer (1). The reaction mixture contained (in 0.5 ml): extract; $MgCl_2$, 3.8 μmol; K_2HPO_4, 15 μmol; dithiothreitol, 15.0 μmol; T2 DNA, 60 μg; and [glucose-^{14}C]UDP-glucose, 4.8 nmol (0.075 μCi). After 60 min at 37°C, the amount of label incorporated into acid-precipitable material was measured. (Activity was expressed as nanomoles of glucose transferred to DNA per hour and 1.5×10^8 cells at 37°C.)

RESULTS

Deficiency of *rho* function in the host relieves the need for phage-specific protein synthesis during the formation of dCTPase and β-glucosyltransferase mRNA. The *rho*

protein of *E. coli* is involved in the normal termination of bacterial transcription. The *E. coli* strain XA7007 carries a mutation (*su*A) in the *rho* gene (17). In experiments by Young (25), it was shown that the presence of this mutation in the host did not increase either the amount or the number of species of mRNA formed after T4-infection in the presence of chloramphenicol. In agreement with this, we observed (11) that dTMP kinase mRNA was not synthesized in XA7007 (*su*A$^-$) infected with T4 in the presence of chloramphenicol.

A conditional lethal mutation in the *rho* gene, *rho*$_{ts15}$, was later found and characterized by Das et al. (3). The *E. coli* K-12 strain AD1704 carries this mutation. Cells of this *rho*$^-$ mutant strain were infected with *am*N122 (this phage mutant was used to prevent lysis of the cells late in infection) in the presence of chloramphenicol. Early mRNA was allowed to accumulate for various amounts of time, rifampin was then added to block further RNA synthesis, and chloramphenicol was finally removed to allow the mRNA synthesized during the chloramphenicol treatment to express itself into enzyme activity. It is shown (Fig. 1A) that under these conditions dCTPase mRNA was formed to some extent in the absence of protein synthesis. Similar results were obtained for β-glucosyltransferase mRNA (data not shown). With dTMP kinase mRNA, however, no synthesis could be observed in the absence of protein synthesis as could be seen in Fig. 1B. When strain AD1766 (*rho*$^+$) was used instead as host in a control experiment, no dCTPase mRNA was formed under otherwise identical conditions (Fig. 2). Similar results were obtained for β-glucosyltransferase mRNA (data not shown). The strain AD1766 is isogenic with AD1704, except that it has the wild-type allele of *rho*.

Mutants W3110*rho*101 through 104 of *E. coli* were isolated by Korn and Yanofsky (9, 10). Their mutations induce suppression of polarity and give a defective transcription attenuation at the tryptophan operon. These phenomena were explained by a deficiency in transcription termination due to an altered *rho* factor (9, 10).

When experiments identical to those described above were performed with these mutants as hosts, it was observed that, in both W3110*rho*102 and W3110*rho*103, dCTPase mRNA and β-glucosyltransferase mRNA, respectively, could be formed in the absence of protein synthesis. With W3110*rho*$^+$ or W3110*rho*104, however, no synthesis of these mRNA types could be detected in the presence of chloramphenicol (Fig. 3 and Table 1). The formation of dTMP kinase mRNA under chlor-

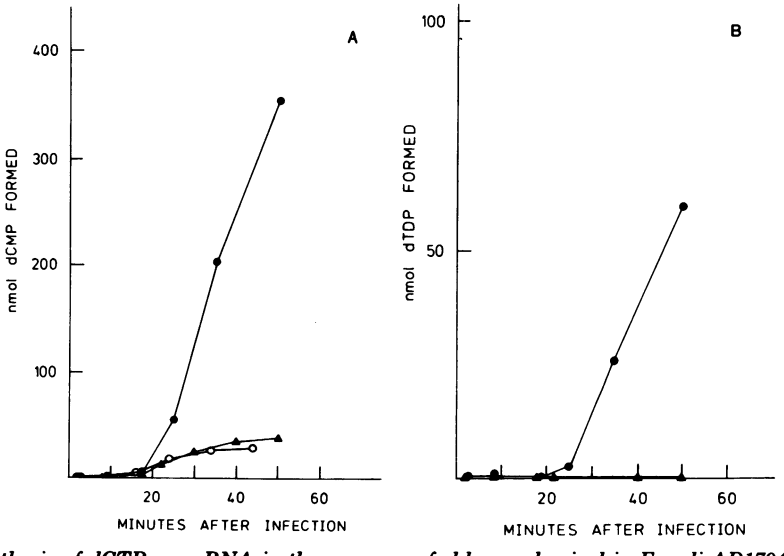

FIG. 1. *Synthesis of dCTPase mRNA in the presence of chloramphenicol in E. coli AD1704 (rho*$_{ts15}$*) after infection with amN122. (A) Cells of E. coli AD1704 were grown at 30°C and infected (time zero) at 36°C in LB medium. At the indicated times, 2-ml samples were withdrawn and extracts were prepared and assayed for dCTPase activity as described in the text. Ordinate: nanomoles of dCMP formed per hour per 1.5 × 10*^{8} *cells at 37°C. (B) The same extracts as in A were assayed for dTMP kinase activity as described above. Ordinate: nanomoles of dTDP formed per hour per 1.5 × 10*^{8} *cells at 37°C. Symbols: ▲, chloramphenicol (200 μg/ml) added at 10 min before infection, rifampin (200 μg/ml) added at 9 min after infection, and chloramphenicol removed by filtration at 10 min; ●, same procedure but without rifampin; ○, chloramphenicol added at 10 min before infection, rifampin added at 3 min after infection, and chloramphenicol removed by filtration at 4 min.*

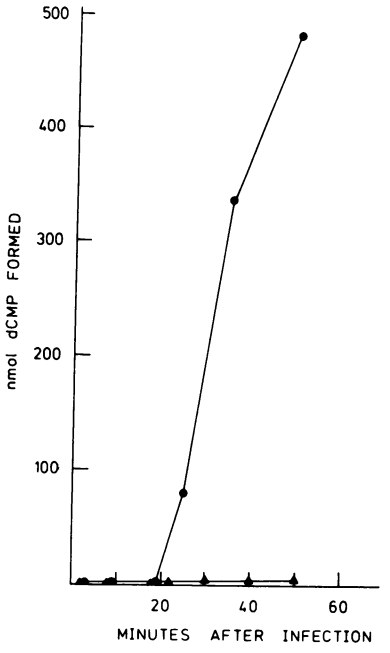

FIG. 2. *Inhibition of dCTPase mRNA synthesis in the presence of chloramphenicol in E. coli AD1766 (rho⁺) after infection with amN122. Cells of E. coli AD1766 were grown at 30°C and infected (time zero) at 36°C in LB medium. At indicated times, sample extracts were prepared and assayed for dCTPase activity as described in the text. Ordinate: nanomoles of dCMP formed per hour per 1.5 × 10⁸ cells at 37°C. Symbols: ▲, chloramphenicol (200 μg/ml) added at 10 min before infection, rifampin (200 μg/ml) added at 9 min after infection, and chloramphenicol removed by filtration at 10 min; ●, same procedure but without rifampin.*

amphenicol treatment could not be seen with any of these mutants as hosts (data not shown).

A mutationally altered *rho* factor in the host thus seems to allow synthesis of dCTPase mRNA and β-glucosyltransferase mRNA but not of dTMP kinase mRNA in the absence of protein synthesis. The difference observed here between mutants W3110*rho*102 and W3110*rho*103 on the one hand and W3110*rho*104 on the other could be explained by a relatively smaller mutational change in the *rho* function of W3110*rho*104 as reflected in the lower degree of polarity suppression seen with this mutant (10).

Effect of mutations in the *mot* gene of phage T4 on the formation of dTMP kinase mRNA. The T4 phage mutant *ts*G1, isolated by Mattson et al. (13) and defining the regulatory gene *mot*, was shown by Sauerbier et al. to be isogenic with the mutant *far*P85 (19) originally studied by Johnson and Hall (7). These authors, by enzyme determinations, also showed that *far*P85 underproduces dTMP synthetase after infection of *E. coli* (8).

When *E. coli* cells were infected at 37°C with the mutant *ts*G1 or the mutant *far*P85 (*ts*G1 is phenotypically *mot*⁻ at 37°C [19]) (Fig. 4), the initiation of dTMP kinase synthesis was delayed and the final enzyme level was seen to reach only about 50% of that in the wild-type infection. The synthesis of dCTPase (Fig. 4B), however, started at the normal time and rose to final enzyme levels that were higher than those after wild-type infection.

When the same experiment was performed at 25°C (*ts*G1 phenotypically *mot*⁺ at 25°C [19]), another pattern was obtained. The mutant *ts*G1 induced about the same amount of dTMP kinase as wild-type phage, whereas *far*P85 hardly induced any dTMP kinase synthesis at all (Fig. 4C). This deficient kinase formation with *far*P85 at low temperature was obtained repeatedly and is unexplained. The initiation of dCTPase (Fig. 4D) and β-glucosyltransferase formation (data not shown) occurred at the same time as after wild-type infection, and the final enzyme levels were similar to those after wild-type infection.

Involvement of the *mot* gene product in the control of dTMP kinase mRNA formation. To investigate the function of the *mot* gene in the synthesis of dTMP kinase mRNA, the double infection system described above was used. It was demonstrated earlier that the formation of dTMP kinase mRNA was inhibited by chloramphenicol and that one or several phage-specific proteins seemed to be involved in this synthesis regulation (11). This is also shown here in the experiment in Fig. 5A, where primary infection took place with a mutant defective in the structural gene for dTMP kinase and also containing the *imm*⁻ mutation (23) to diminish the superinfection exclusion effect. After superinfection with wild-type phage in the presence of chloramphenicol, synthesis of dTMP kinase mRNA could then be demonstrated by the enzyme-forming capacity determination. Without primary infection there was no synthesis of dTMP kinase mRNA (Fig. 5A). Very similar results were obtained for dCTPase mRNA formation (Fig. 5B) and also for that of β-glucosyltransferase mRNA (data not shown).

When the *mot* mutant *ts*G1 was used in the primary infection, however (Fig. 6A), it was observed that at 37°C no dTMP kinase mRNA was formed after wild-type superinfection in the presence of chloramphenicol. When, however, dCTPase activity is measured in the same experiment, it can be seen (Fig. 6B) that the mRNA for this enzyme was synthesized. The latter observation was also made for β-glucosyltransferase mRNA (data not shown). As a con-

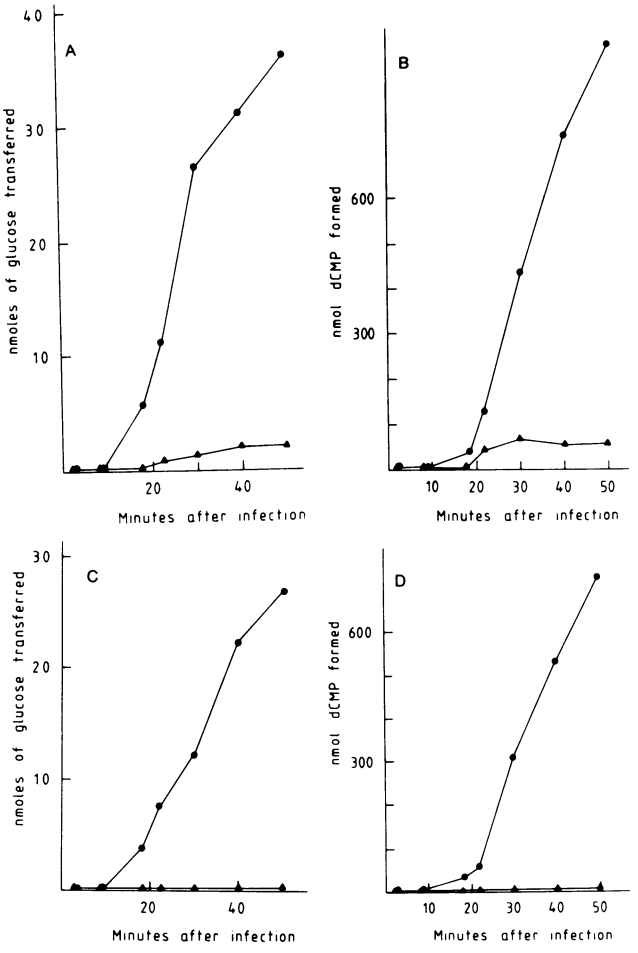

FIG. 3. *Synthesis of β-glucosyltransferase mRNA and dCTPase mRNA in the presence of chloramphenicol in E. coli W3110rho102 and E. coli W3110rho104 after infection with amN122. Cells of E. coli W3110rho102 (A and B) or E. coli W3110rho104 (C and D) were grown in LB medium at 37°C and infected with amN122 (time zero). At indicated times, samples were withdrawn, and extracts were prepared and assayed for enzyme activities as described in the text. Symbols: ▲, chloramphenicol (200 μg/ml) added at 1 min before infection, rifampin (200 μg/ml) added at 9 min after infection, and chloramphenicol removed by filtration at 10 min after infection; ●, same procedure but without rifampin. (A and C) β-Glucosyltransferase activity; values along the ordinates denote nanomoles of glucose transferred to T2 DNA per hour per 1.5 × 10^8 cells at 37°C. (B and D) dCTPase activity; ordinates are nanomoles of dCMP formed per hour per 1.5 × 10^8 cells at 37°C.*

TABLE 1. *Enzyme activities in strains W3110rho⁺ and W3110rho103 at 40 min after infection with amN122*[a]

Strain	dCTPase[b]		βgt[c]	
	−rif	+rif	−rif	+rif
W3110*rho*⁺	323	11	2.92	0.06
W3110*rho*103	742	41	3.37	0.22

[a] Experiments were performed as described in the legend to Fig. 3.

[b] Each value represents nanomoles of dCMP formed per hour per 1.5 × 10^8 cells at 37°C.

[c] Each value represents nanomoles of glucose transferred to T2 DNA per hour per 1.5 × 10^8 cells at 37°C.

trol, the same double-infection experiments were performed at 25°C, which is a permissive temperature for *ts*G1. It can be seen (Fig. 7) that in this case both dTMP kinase and dCTPase mRNA was formed after superinfection in the presence of chloramphenicol. The accomplishment of dTMP kinase mRNA synthesis thus seems to be dependent on the allelic state of the *mot* gene.

DISCUSSION

The temporal program of prereplicative transcription after bacteriophage T4 infection seems

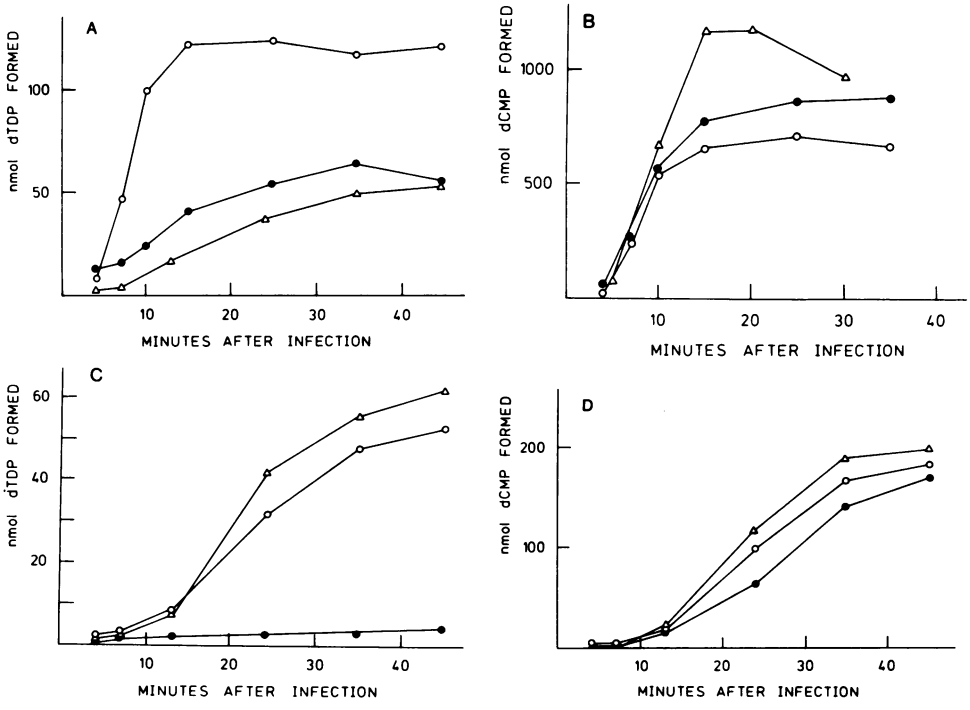

FIG. 4. *Effect of mot mutations on the formation of dTMP kinase and dCTPase. Cells of E. coli B were grown at 37°C in glycerol-Casamino Acids medium to 2 × 10⁸ cells/ml and infected with phage at time zero and again at 2 min (to obtain lysis inhibition). At different times, sample extracts were prepared and assayed for dTMP kinase and dCTPase, as described in the text. (A) dTMP kinase formation at 37°C. Ordinate: nanomoles of dTDP formed per hour per 1.5 × 10⁸ cells at 37°C. (B) dCTPase synthesis at 37°C. Ordinate: nanomoles of dCMP formed per hour per 1.5 × 10⁸ cells at 37°C. (C) dTMP kinase formation at 25°C. Ordinate as in A. (D) dCTPase synthesis at 25°C. Ordinate as in B. Symbols: ○, infection with am⁺; ●, infection with farP85; △, infection with tsG1.*

to comprise several different parts. The enzyme-forming capacity method used in the experiments presented here sheds light on the transcriptional regulation of specific cistrons in these different parts.

The chloramphenicol sensitivity of prereplicative transcription could be regarded as a polarity effect restricting transcription to promoter-proximal DNA segments due to nucleolytic sensitivity of newly formed mRNA in the absence of protein synthesis (2). In earlier work we presented some evidence for an interpretation of the chloramphenicol effect as a reflection of an active, regulatory process, i.e., that positive regulatory elements transcribed and translated from early phage genes are necessary for the expression of other early phage genes (11). Such an active regulatory process could conceivably be explained in two alternative ways. Either an early gene could produce an antiterminator protein which counteracts the *rho* factor of the host to permit continued transcription into promoter-distal genes (18) or a product of a promoter-proximal gene could stimulate the transcription of other early genes (22).

The three genes, the transcriptional pattern of which was studied in this investigation, all code for enzyme proteins involved in the biosynthesis of modified cytosine in T4 DNA. The results, demonstrating a prevention of the chloramphenicol effect in the presence of a *rho* mutation in the host, indicated that transcription of the genes for dCTPase (gene 56) and β-glucosyltransferase (gene *βgt*), respectively, were under the control of an anti-*rho* mechanism, but that of dTMP kinase (gene 1) was not. It could be noted that genes 56 and *βgt* are relatively close on the genetic map of T4 (18.5 and 23 kilobases, respectively), whereas gene 1 is situated in a different area at 74 kilobases (16). The mutation rho_{ts15} in the host counteracted the chloramphenicol effect both at the nonpermissive temperature of 30°C (Fig. 1) and at the allegedly permissive temperature of 25°C (data not shown). This observation is consistent with the original characterization of the mutant by

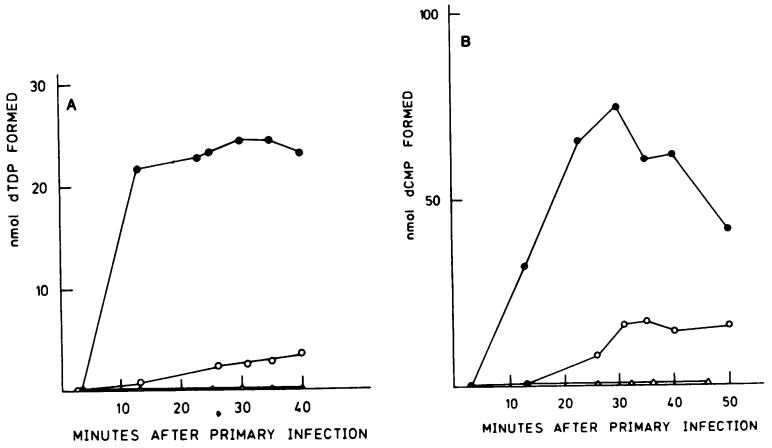

FIG. 5. *Formation of dTMP kinase and dCTPase mRNA by wild-type phage in the absence of protein synthesis after primary infection with amC42imm2 and amE114imm2, respectively. Cells of E. coli AA446 were infected with either amC42imm2 (dTMP kinase$^-$, imm$^-$) or amE114imm2 (dCTPase$^-$, imm$^-$). Superinfection with wild type took place at 5 min. At indicated times, sample extracts were prepared and assayed for dTMP kinase or dCTPase activity as described in the text. (A) Ordinate: dTMP kinase activity, nanomoles of dTDP formed per hour per 1.5 × 10^8 cells at 37°C. (B) Ordinate: dCTPase activity, nanomoles of dCMP formed per hour per 1.5 × 10^8 cells at 37°C. Symbols: ○, infection with amC42imm2 (A) or amE114imm2 (B) (time zero), chloramphenicol (20 μg/ml) added at 4 min, superinfection with am$^+$ at 5 min, rifampin (20 μg/ml) added at 14 min, chloramphenicol removed by filtration at 15 min; ●, same procedure but without chloramphenicol; △, same procedure but without primary infection.*

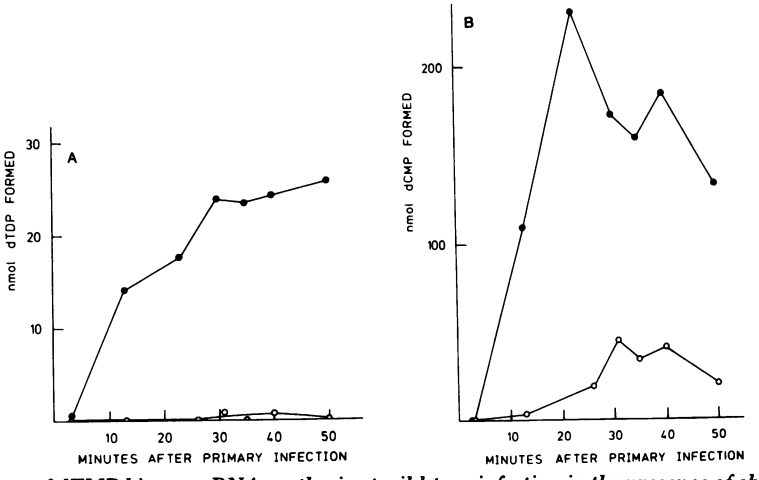

FIG. 6. *Absence of dTMP kinase mRNA synthesis at wild-type infection in the presence of chloramphenicol after primary infection with tsG1 at 37°C. Cells of E. coli AA446 were infected at 37°C with tsG1 (mot$^-$). Superinfection with wild-type phage took place at 5 min. At indicated times, sample extracts were prepared and assayed for dTMP kinase or dCTPase activity as described in the text. (A) Ordinate: dTMP kinase activity, nanomoles of dTDP formed per hour per 1.5 × 10^8 cells at 37°C. (B) Ordinate: dCTPase activity, nanomoles of dCMP formed per hour per 1.5 × 10^8 cells at 37°C. Symbols: ○, infection with tsG1 (time zero), chloramphenicol (20 μg/ml) added at 4 min, superinfection at 5 min, rifampin (20 μg/ml) added at 14 min, chloramphenicol removed by filtration at 15 min; ●, same procedure but without chloramphenicol.*

Das et al. (3), who observed that the specific *rho* function of the *rho*$_{ts15}$ mutant was defective also at the low temperature of 25°C, where the pleiotropic effects of the mutation, however, were mild enough to allow bacterial growth. A further support for the interpretation that chloramphenicol inhibits an anti-*rho* mechanism in the early transcription process is that the drug effect was counteracted also by the polarity suppression mutants of Korn and Yanofsky (9, 10), and

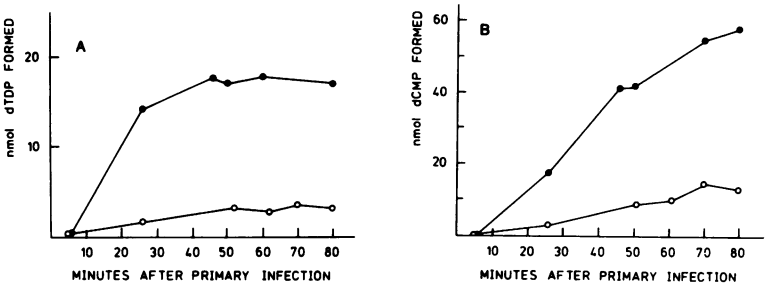

FIG. 7. *Formation of dTMP kinase and dCTPase mRNA by wild-type phage in the presence of chloramphenicol after primary infection with tsG1 at 25°C. Cells of E. coli AA446 were grown at 37°C and infected with tsG1 at 25°C. Superinfection with wild-type phage at 10 min. At indicated times sample extracts were prepared and assayed for dTMP kinase and dCTPase activity as described in the text. (A) dTMP kinase activity, nanomoles of dTDP formed per hour per 1.5 × 10^8 cells at 37°C. (B) dCTPase activity, nanomoles of dCMP formed per hour per 1.5 × 10^8 cells at 37°C. Symbols: ○, infection with tsG1 (time zero), chloramphenicol (20 μg/ml) added at 9 min, rifampin (20 μg/ml) added at 29 min, chloramphenicol removed by filtration at 30 min; ●, same procedure but without chloramphenicol.*

in such a way that a rough correlation was seen between rescue of transcription and severity of mutational change in *rho* function (10).

The idea that positive regulatory elements coded for by early genes are needed for the normal expression of other early genes is strengthened by the finding and characterization of the *mot* gene (13, 14). The effect of mutations in this gene is to decrease the expression of many early genes, among them gene 1 (dTMP kinase). This mutational effect is, however, much milder than that of chloramphenicol, since gene 1 expression is only delayed and diminished (Fig. 4), and many other early genes, the functions of which are abolished by chloramphenicol, are expressed normally in *mot* mutant infections (14). An amber mutation was isolated in the *mot* gene, indicating that it codes for a protein (14). In the superinfection experiments reported here, the *mot* protein seemed to have an effect in *trans* on the superinfecting phage. When *mot* was nonfunctional by mutation the *trans* effect, relieving the chloramphenicol inhibition of transcription, was abolished for gene 1 but not for gene 56 (dCTPase). This could be interpreted as the *mot*-protein functioning at the initiation of gene 1 transcription at a promoter recognized only some time after infection and needed for reaching the normal level of message production.

In conclusion, the results show that there is a phage-induced change in transcriptional termination which regulates the expression of the genes for dCTPase and β-glucosyltransferase, respectively. The regulatory element could be an anti-*rho* protein synthesized some time after infection, and the chloramphenicol effect would then be explained by the inhibition of that synthesis. The expression of the dTMP kinase gene, however, seemed to be unrelated to the *rho* function, but instead related to the function of the *mot* protein. By inference, the *mot* protein is thus unlikely to be involved in an attenuation mechanism.

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