**In vitro** system for induction of delayed early RNA of bacteriophage T4

(regulation of RNA synthesis/chloramphenicol RNA)

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ABSTRACT Concentrated lysates of *Escherichia coli* that had been infected with bacteriophage T4 in the presence of chloramphenicol (CAM), only a fraction of the total early RNA is found 5 min after infection (1). This RNA, called immediate early (IE), corresponds to the promoter-proximal portions of polycistronic transcription units (2, 3). The promoter-distal parts of these transcription units code for delayed early (DE) RNA. Superimposed on this transcription pattern is another CAM-sensitive mode of prereplicative transcription, called quasi-late (QL) or middle. Middle transcripts are not derived from IE promoters (5--7). A number of proteins are known to be synthesized from messenger RNA molecules which start either at IE or middle promoters (5).

In vitro transcription of T4 DNA with purified RNA polymerase and T4-DNA-dependent coupled transcription-translation show IE promoter recognition and the extension of RNA chains from IE into DE regions (8, 9). Middle promoters have not yet been recognized in vitro, nor has the CAM effect on T4 transcription been reproduced in vitro (10).

Recent experiments in vivo show that T4 infection can modify the cell to allow production of rIIA and rIIB mRNA in the presence of CAM (11). When the rIIA and rIIB genes are introduced into a cell only after that cell has become insensitive to the CAM effect, these RNA species appear to be expressed as middle species (11). We have interpreted this to mean that overcoming the CAM effect and middle mode expression depend on a change in the state of the infecting DNA. We report here on an in vitro system which supports this conclusion.

MATERIAL AND METHODS

**Bacteria, Bacteriophage, and T4-Infected Cells.** *E. coli* B° is the standard host for T4 D infection. B°-rif 4 was selected as a spontaneous mutant resistant to 150 μg/ml of rifampicin in minimal medium agar plates. The strain grows normally in liquid culture with 200 μg/ml of rifampicin.

T4 em 292 is a derivative of T4 D which contains a mutation in gene 55; it has a normal development during the early period. T4 NB 2226 is a derivative of T4 B; it is the largest known deletion in the rII region, covering all of rIIA, rIIB, D1, D9a, and D2b (see ref. 12 for full details). This phage and the other smaller deletions used to prepare competitor RNA for rIIA and rIIB messenger analysis are described in refs. 11 and 12.

Bacteria were grown to 5 × 10⁹ cells per ml at 30° in M9 medium with 1% casamino acids (13). When restricted lysates were to be prepared, CAM was added to 200 μg/ml 5 min before infection. L-tryptophan was added to 20 μg/ml and then the cells were infected at a multiplicity of 5 phage per bacterium. Infections were usually stopped 5 min after infection by chilling rapidly to 1–3°. In preparation of “exhausted” unrestricted lysates, rifampicin was added to 200 μg/ml 5 min after infection, and the infection was allowed to continue another 10 min, after which the cells were chilled. Infected cells were centrifuged for 10 min at 4000 × g, then resuspended in ½ the original volume of 10 mM Tris-HCl at pH 7.5, 10 mM MgCl₂, 100 mM NaCl, and recentrifuged. The cell pellet was then resuspended in ½ the original volume of 0.5% Brij 58, 10 mM Tris-HCl at pH 7.5, 10 mM NaCl, after which cells were frozen at −20°. Frozen cells were stored at −20° until they were ready to be used for lysate preparation and in vitro synthesis.

**Lysate Preparation and In Vitro RNA Synthesis.** Frozen infected cells at 5 × 10¹⁰/ml were thawed and mixed with 0.8 volumes of lysozyme at 1 mg/ml. They were then incubated 5 min at 37°, after which the lysates were kept at 0° until they were used for RNA synthesis. Between 100 and 300 μl of these lysates (or combinations of various lysates) were then added per ml of incubation mixture for RNA synthesis. The standard incubation mixture contained 20 mM 3-(N-morpholino)propanesulfonic acid (Mops) at pH 7.5, 100 mM KCl, 10 mM MgCl₂, 1 mM ATP, 0.2 mM GTP, 0.2 mM CTP, and 0.02 mM [³H]UTP at a specific activity of 300–600 μCi/μmol. Incubation was at 37° for 30 min. The in vitro reaction mixtures were then made 1% sodium dodecyl sulfate, and it was at this time that samples were taken for measurements of RNA synthesis. Sodium acetate, pH 5.2, was added to 100 mM, then phenol was added, and phenol extractions were carried out at 65° for purification of the RNA (13). When infected cells were labeled with [¹⁴C]uridine in vitro, and lysates prepared from such cells were used for [³H]UTP incorporation in vitro, the percentage of label recovered after purification was the same for ¹⁴C and ³⁵S (55–70%).

**Purification of RNA Polymerase.** *E. coli* RNA polymerase was purified as described previously (14). Purified RNA polymerase from strain B°-rif 4 was 50–80% resistant to 50 μg/ml of rifampicin when T4 DNA was used as a template.

**Hybridization-Competition Analyses of RNA.** All methods have been published previously. T4 DNA strand separations with poly(U,G) were carried out as in ref. 15. Hybridization-competition techniques have been described (15, 16). Analyses of rIIA, rIIB, and D₂ mRNA follow techniques given in refs. 11 and 12, except that all hybridization-competition analyses were done using the purified λ strand of T4 DNA. The cpm annealed to T4 λ strand DNA in the absence of competitor RNA...
RESULTS AND DISCUSSION

In Vitro RNA Synthesis Limited to IE Regions. We labeled cells infected with T4 with [14C]uridine 1–5 min after infection; parallel infections were done in the presence and absence of 200 μg/ml of CAM. In the presence of CAM, infected lysates were therefore used to prepare concentrated lysates and RNA was synthesized with [3H]UTP in the absence of CAM. We incubated 30 min at 37°C, since this is about five times longer than required for RNA polymerase to transcribe the longest early transcription units in vitro (2, 3, 10). Even with this long incubation time, lysates from cells that were restricted in vivo show similar restriction for in vitro RNA synthesis (Fig. 1). Simultaneous competition of in vivo and in vitro RNA with unlabeled RNA from T4-infected, CAM-pretreated cells shows that the lysates maintain the specificity they had in vivo during the in vitro incubation.* There is a small difference in the competition curves between in vivo IE RNA and RNA synthesized from lysates of cells producing this RNA, but this specific difference is found for the in vitro RNA and the in vivo RNA from the corresponding unrestricted lysate (Fig. 1). The specificity of these lysates does not depend on the presence of CAM in the in vitro reaction mixture; in addition, the specificity is the same in vitro whether or not new initiation of chains is permitted, since in vitro RNA made from unrestricted lysates in the presence or absence of rifampicin shows the same properties (Fig. 1). Since rifampicin reduces [3H]UTP incorporation only by about 1/5 in these lysates, most RNA is made by chain extension of RNA molecules initiated in vivo. The RNA synthesized in vitro with these lysates is specific for the l strand of T4 DNA (data not shown).

We have added 50 μg of purified E. coli RNA polymerase to a restricted lysate (200 μl, which is the equivalent of 10^10 cells) in 1 ml of reaction mixture; this stimulated 3-fold the amount of RNA synthesized, but did not affect the specificity of synthesis (to be published). Thus, the restriction acts efficiently on chains that are completely synthesized in vitro.

Complementation of Restricted Lysates by Unrestricted Lysates. About 2 min after T4 infection, an event occurs which overcomes the capacity of CAM to restrict RNA synthesis to IE species (11). We therefore tried to complement restricted lysates with various lysates prepared from unrestricted T4 infected cells to effect an in vitro induction of DE RNA. Since unrestricted cells are usually in the process of transcribing DE RNA when they are concentrated for lysate formation, it is essential to show that a product from the unrestricted lysate diffuses to the restricted lysate to induce synthesis of DE RNA. To do this, we have used a rifampicin-resistant derivative of our normal host as a source of restricted lysates. The unrestricted lysates were made from T4-infected rifampicin-sensitive cells. Half of these cells were isolated 5 min after infection; to the other half, we added 200 μg/ml of rifampicin 5 min after infection and continued incubation until 15 min to allow RNA polymerase to run off to the end of early transcription units and be inactivated by the antibiotic. RNA synthesis, as measured by uridine incorporation, was measured before addition of rifampicin and just before isolation (data not shown). The in vivo RNA synthetic capacity at 15 min is 1–3% of what it was at 5 min. These cells were isolated and lysates were then prepared. The lysates were tested for RNA synthetic capacity in vitro in the presence of rifampicin to assure that RNA polymerase had been inactivated. Lysates which had been isolated after 10 min of incubation in the presence of rifampicin incorporate only about 6% as much [3H]UTP into RNA in 30 min at 37°C as does a normal 5 min lysate (data not shown). Detailed kinetics under a variety of conditions confirm that this treatment in vivo has "exhausted" RNA synthetic capacity in vitro (to be published).

Various quantities of 5 min and “exhausted” lysates were mixed with restricted lysates from rifampicin-resistant cells, and in vitro RNA synthesis was carried out in the presence of rifampicin (and CAM, although this is irrelevant to the result). We find DE RNA synthesized in the two complementation systems, and the quantity of DE RNA increases with increasing quantities of either normal or “exhausted” unrestricted lysate (Fig. 2). Since total RNA synthesis is about the same in all mixtures of these lysates, the percentages of RNA can be interpreted as amounts of RNA of each class. The amount of DE RNA in the mixed system using “exhausted” lysates far exceeds the RNA synthetic capacity of these lysates; thus, the DE RNA seen in this complementation system is derived from a component of the unrestricted lysate and a component from the restricted lysate. Other experiments show that uninfected E. coli or E. coli infected by T4 after the cells had been in 200

* One difference between transcription-restricted and unrestricted lysates is that the former have a higher viscosity, since CAM treatment of host cells prevents T4-induced degradation of host DNA (17). We showed restricted lysates through a syringe with a 21-gauge needle until they had the same viscosity (as measured by flow in a 1 ml pipette) as unrestricted lysates. This had no effect on IE RNA synthesis from these lysates (to be published). We also tested whether supercoiling of the DNA in such lysates might affect their restrictive synthesis, since any structure analogous to the host "chromoid" would be unaffected by our lysing procedure (18). We calculate that 100 μg of ethidium bromide should relax all supercoils in a "chromoid-like" structure, and that twice this concentration would put positive supercoils into the DNA. Incubation with these concentrations of ethidium bromide does not affect the restriction to IE species made in vitro (to be published). Ethidium bromide does decrease the total amount of RNA synthesized, as would be expected from the experiments of Richardson (19).
they don't RIIA Thus, when deletion inletion in DNA destroyed. The concentration for analyzed amount for DE RNA was made after T4 am 292 infection. “Exhausted” lysates were made after rifampicin treatment in vivo (see Materials and Methods). In vitro RNA synthesis using these lysates was carried out (1 ml reaction mixtures) in the presence of 34 μg/ml of rifampicin and 200 μg/ml of CAM. In vitro RNA preparations were analyzed for DE RNA by plotting the percent RNA hybridizing to T4 strand DNA as a function of the reciprocal of the CAM RNA competitor concentration and extrapolating these curves to infinite competitor concentration. The ordinate represents the intercepts of such curves. The abscissa is the number of μl of complementing lysate used to complement restricted lysates. The concentration of restricted lysate was not constant; it was always added so that the total lysate volume was 300 μl. The control experiments (•) made from 100 and 300 μl restricted lysate are shown. (●), 5 min unrestricted lysate; (○), “exhausted” unrestricted lysate; (△) uninfected E. coli B#2 lysate; (△), lysate made from B#2 infected by am 292 where 200 μg/ml of rifampicin was added with the phage (isolation was 5 min after infection); (●), lysate made from B#2 infected by am 292, where 200 μg/ml of rifampicin was added 5 min before infection (isolation was 5 min after infection). The arrows indicate complementing lysates which had been heated 15 min at 85°C before being used for complementation with restricted lysates.

µg/ml of rifampicin for 5 min yield lysates which have no capacity to complement restricted lysates (Fig. 2). Finally, when normal 5 min lysates or “exhausted” lysates are heated at 85°C for 15 min, their complementing capacity is completely destroyed. DE RNA made by in vitro complementation of lysates shows the same hybridization specificity for the I strand as does in vivo early RNA (data not shown). We have also verified that unlabeled 5 min in vitro RNA competes with material that unlabeled CAM RNA does not compete with.

To find out which lysate is providing the RNA polymerase and which lysate the DNA in this mixture, we have taken advantage of the many known deletions in the rII region of T4. We prepared restricted lysates from rifampicin-resistant, CAM-pretreated, T4 am 292-infected cells. These express only IE message in the region defined by NB 2226, the largest deletion in the rII region. In particular, no rIIA or rIIB RNA is expressed when cells are infected in the presence of CAM (20). We prepared unrestricted lysates, both normal and “exhausted,” from rifampicin-sensitive cells infected with T4 NB 2226. Thus, in vitro neither lysate is able to express normal amounts of rIIA or rIIB RNA, the restricted lysate because it is derived from CAM-treated cells, and the unrestricted lysates because they don’t contain the genes for these species. When the lysates are mixed, we find that DE RNA is synthesized in vitro by complementation (Table 1, lines 3 and 4), but no more rIIA and B RNA is found than is found in the RNA made from the restricted lysate alone (Table 1, line 2). This suggests that the DNA from the restricted lysate is not used for DE RNA synthesis during lysate complementation, but that the rifampicin-resistant RNA polymerase diffuses to the DNA in the “exhausted” lysate to effect DE RNA synthesis. Further proof that the enzyme in the complementing system comes from the restricted lysate is obtained when the restricted lysate is made from CAM-pretreated rifampicin-sensitive cells. When such a system is used to complement an “exhausted” unrestricted lysate in the presence of rifampicin, no new chain initiation can take place. All RNA is derived from chain extension of in vitro initiated RNA. This system synthesizes only IE RNA despite the presence of DNA competent for DE RNA synthesis in the mixture (data not shown). We can, in fact, replace the restricted lysate by purified RNA polymerase holoenzyme from the rifampicin-resistant strain used in these experiments (Table 2, lines 2 and 5). The approximately 3-fold increase in the amount of DE RNA synthesized when the purified enzyme replaces the restricted lysate as the source of RNA polymerase suggests to us that DE RNA synthesized in our complementing lysate does not come from a preformed RNA primer.

The DNA in Unrestricted Lysates Is Not Equivalent to Mature T4 DNA. We next followed the kinetics of appearance of DE and rII-specific RNA to see whether the initiation of DE RNA we obtain in our complementing lysates is initiated at IE or middle promoters. The unrestricted “exhausted” lysate derived from am 292 infection was mixed with a restricted lysate derived from rifampicin-resistant, CAM-pretreated cells that had been infected with T4 carrying the deletion NB 2226. Now, the only rIIA, rIIB, and D2 genes available are in the template competent for DE RNA synthesis. We measure the amount of DE RNA and rIIA-, rIIB-, and D2-specific RNA 2, 5, and 30 min after mixing the lysates in the presence of rifampicin (and CAM) (Table 1, lines 7, 8, and 9). As would be expected, we find rII-specific RNA, since these genes are now in a competent template for their expression.

The appearance of total DE RNA and 2226-specific RNA follows the same kinetic pattern. At 2 min there is no DE RNA and almost no 2226-specific RNA, even from the IE D2 region under NB 2226. It must take at least 2 min for the RNA polymerase to diffuse to and initiate on the unrestricted template. By 5 min after mixing there is a small but significant increase in DE- and 2226-specific RNA (Table 1, line 8). The increase is all due to rIIA and rIIB RNA synthesis; in contrast, when RNA polymerase transcribes purified T4 DNA, 3 min of synthesis at 37°C gives only D2 but no rII-specific transcripts (Brody, unpublished results). This suggests that the first rIIA and rIIB transcripts synthesized in our complementing lysates are being made in the middle mode rather than as extensions of IE transcripts. We point out that the data in Table 1 show only the end-points of mixed hybridization-competition curves and underestimate the precision of this technique. It depends, in fact, on the total area between the curves generated by the mixed competitors. The relative proportions of rIIA and rIIB RNA seen after a 30 min incubation support our interpretation. The amount of rIIB RNA could exceed the amount of rIIA RNA (Table 1, line 9) in these mixed competitor experiments (see ref. 20 for a full exposition of this point) only if rIIB RNA is being made in a middle mode if rIIA RNA is being specifically degraded.

Another indication that the DNA template in the “exhausted” unrestricted lysates is not the equivalent of mature phage DNA is that exogenous T4 DNA does not overcome the restriction seen in lysates from CAM-treated cells.
Table 1. Global DE and rIIA-, rIIB-, and D2-specific RNA after in vitro synthesis with complementing lysates

<table>
<thead>
<tr>
<th>Cells</th>
<th>Phage</th>
<th>Unrestricted lysateb</th>
<th>In vitro synthesis (min)</th>
<th>% RNA</th>
<th>DEc</th>
<th>NB 2226f</th>
<th>D2g</th>
<th>rIIAh</th>
<th>rIIBi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. In vivo</strong></td>
<td>T4+ RNA (12–13 min)</td>
<td>None</td>
<td></td>
<td>13.7</td>
<td>5.0</td>
<td>6.0</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. rif 4</td>
<td>am 292</td>
<td>NB 2226 DNA</td>
<td>5 min</td>
<td>41.0</td>
<td>2.1</td>
<td>1.9</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. rif 4</td>
<td>am 292</td>
<td>NB 2226 DNA</td>
<td>Exh.</td>
<td>25.0</td>
<td>6.1</td>
<td>5.2</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. BE</td>
<td>NB 2226</td>
<td>DNA</td>
<td></td>
<td>6.0</td>
<td>2.0</td>
<td>2.0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. BE</td>
<td>NB 2226</td>
<td>DNA</td>
<td></td>
<td>10.9</td>
<td>2.6</td>
<td>2.6</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. rif 4</td>
<td>NB 2226</td>
<td>am 292 Exh.</td>
<td></td>
<td>4.8</td>
<td>0.7</td>
<td>0.7</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. rif 4</td>
<td>NB 2226</td>
<td>am 292 Exh.</td>
<td></td>
<td>8.6</td>
<td>1.3</td>
<td>0.6</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. rif 4</td>
<td>NB 2226</td>
<td>am 292 Exh.</td>
<td></td>
<td>29.3</td>
<td>3.9</td>
<td>1.4</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Cells were treated with 200 µg/ml of CAM 5 min before infection. Infection was carried out at a multiplicity of 5 phage per bacterium, and infected cells were isolated 5 min after infection. rif 4 is B+rif 4.

*b* Cells were B(rifampicin-sensitive) infected with 5 phage per bacterium. “Exhausted” lysates were made from cells treated with 200 µg/ml of rifampicin 5 min after infection and isolated 15 min after infection. Normal 5 min lysates; Exh., “exhausted” lysates.

*c* The in vitro reaction mixtures were incubated at 37°C as indicated in Materials and Methods. We use 150 µl of restricted lysate per ml of reaction mixture, and when a complementing unrestricted lysate was used, it was also present at 150 µl per ml of reaction mixture. We find approximately 10–20 pmol of [3H]UTP incorporated into RNA per 10⁸ cell equivalents in 30 min in these lysates, complemented or not. For the experiments presented in lines 2–4 and 7–9, the in vitro reaction was carried out in the presence of 34 µg/ml of rifampicin and 200 µg/ml of CAM.

*d* These are cpm hybridizing to l strand T4 DNA (at 4 µg/ml) in the presence of 1930 µg/ml of unlabeled CAM RNA (cf. ref. 4) compared to hybridization without competitor RNA.

*e, h, i* RNA specific to these regions is determined as end-points of mixed competitor curves using RNA isolated after infection from various deletions in this region (see refs. 11 and 20 for details). The only change we have made is that hybridization-competitions are carried out using 4 µg/ml of purified l strand isolated from T4+ DNA. Lines 7–9 were determined by complete competition curves using two different concentrations of [3H]RNA and of the l strand of T4 DNA. The [3H] cpm annealed to the l strand of T4 DNA in the absence of competitor were: 1: 10,600; 2: 2535; 3: 6502; 4: 2449; 5: 2180; 6: 2300; 7: 2100; 8: 2088; and 9: 2387.

*f* The unrestricted lysate was replaced by 34 µg/ml of purified T4+ DNA.

We have made restricted lysates from rifampicin-sensitive cells infected with NB 2226 phage, and tried complementing these lysates with an excess of T4+ DNA extracted from purified phage particles. If we assume that the five NB 2226 phage used to infect the CAM-treated host have all injected their DNA, then the amount of NB 2226 DNA in this in vitro preparation is 4 µg. We added 34 µg of exogenous DNA. Even after 30 min of incubation, this 8.5-fold excess of exogenous DNA, ordinarily transcribed well by RNA polymerase, was poorly transcribed. Only 2.6% of the RNA made in this system was 2226-specific (Table 1, line 6), whereas a control restricted lysate made from rifampicin-sensitive cells infected with am 292 showed 11.4% NB 2226-specific RNA (data not shown). Thus, the exogenous DNA added to the restricted lysate was transcribed with βf, the probability of the endogenous DNA. Even this small amount of transcription yielded only IE (D2) transcripts from the region defined by NB 2226 on the exogenous DNA. The DNA in the unrestricted lysate has some property not found in mature DNA that permits it to act as an efficient template for rIIA and rIIB RNA synthesis in our complementation system.

**Effect of tsG1 Mutation on “Exhausted” Lysates.** If the complementing lysates used in our experiments do synthesize DE RNA in a middle mode, we should expect some effect of the tsG1 mutation on the in vitro capacity to synthesize DE RNA in these lysates. The tsG1 mutation is known to affect middle mode transcription in vitro (7) and to have essentially no effect on transcription from early transcription units. We therefore prepared “exhausted” lysates from tsG1 infected cells at 42°C (nonpermissive conditions) and 30°C (permissive conditions) and as a control we used our standard am 292-infected cells at 42°C. We complemented these lysates with either restricted lysates as described previously or with purified rifampicin resistant RNA polymerase (Table 2). Having the tsG1 mutation under nonpermissive conditions during the infection drastically reduces the capacity of the corresponding “exhausted” lysate to synthesize DE RNA, either with a restricted lysate or with purified RNA polymerase. The properties of the lysates made when tsG1 phage had infected under permissive conditions are more complex. When mixed with a restricted lysate, such an “exhausted” lysate gives scarcely more DE RNA than does an “exhausted” lysate derived from cells infected with tsG1 phage at 42°C. On the contrary, when incubated with 5 µg of purified E. coli rif 4 RNA polymerase, this “exhausted” lysate gives normal levels of DE RNA. We conclude that most, but not all, of the DE RNA is being made in a middle mode in our complementing lysate system. Moreover, our result with purified RNA polymerase suggests that the tsG1 mutation in vitro acts by blocking the capacity of the T4 DNA template to support middle mode RNA synthesis.

The effect of CAM on early T4 transcription resembles its effect on early λ transcription and on transcription from the trp operon of E. coli. In λ infections N+ phage show the transcription pattern found after λ+ infections in the presence of CAM. In the absence of the N anti-terminator, ρ factor acts at specific sites in early transcription, and one finds, as a result, only promoter-proximal early RNA (21). In T4, the CAM effect has been more difficult to interpret, because the polarity-suppressing strains examined up to now do not reverse the CAM effect (22, 23), because DE RNA expression seems not to depend on an N-like T4 gene (ref. 10 and our own unpublished results), and because a second mode of expressing many early genes is superimposed on the expression of polycistronic early transcription units (5). Despite these difficulties, it is still possible
that the CAM T4 effect is simply due to induced termination at IE-DE junctions (4) and that the proper suppressor strain will eliminate this CAM-induced polarity.

Our results show that this termination event can be reproduced in vitro. Purified DNA allows DE synthesis in vitro (3, 8, 9); after DNA injection in the presence of CAM, a host terminator, perhaps ρ, must block propagation of RNA polymerase into DE regions. We have previously proposed that CAM indirectly activates this terminator by blocking the translation of nascent RNA (11). This part of our model corresponds to the mode of action proposed for ρ (24).

We think that the "unblocking" event for T4 DNA is related to the capacity to express RNA in a middle mode. We have previously suggested that this capacity, like the capacity to inactivate the CAM-induced polarity in T4-infected cells, depends on interactions between T4 DNA and host membrane (11). It is not clear why so much of the DE RNA synthesized in "exhausted" lysates appears to be made in the middle mode. We know that IE promoter recognition is capable of yielding, via chain elongation, large amounts of DE RNA (3, 9). It is reasonable to imagine that the rifampicin-inactivated RNA polymerase in the "exhausted" lysates binds to IE promoters and prevents access of the complementing rifampicin-resistant RNA polymerase to these promoters (25). This does not, however, explain why so low a percentage of DE RNA is found in "exhausted" lysates derived from tsGl-infected cells. This mutation affects only middle mode expression in vitro, and expression of DE RNA via chain elongation after IE promoter recognition is normal in tsGl-infected cells (ref. 7 and our unpublished results). Some interrelationship between antitermination and middle mode expression has apparently been revealed in these in vitro experiments, but we do not yet understand its nature.

Finally, our lysate system and the models they have generated provide an alternative explanation for the putative T4 σ factor (26, 27). Travers reported that initiation of RNA in DE regions could be found using T4 DNA, core RNA polymerase, and either a crude ribosomal "wash" or a fraction from such a "wash" partially purified by (NH₄)₂SO₄ fractionation. We propose that these crude fractions supplied not a T4 σ factor, but rather the membrane components necessary to allow DNA to be transcribed in a middle mode (11). Whether E. coli σ factor is also necessary for such recognition is not yet known.

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