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Transcription of Bacteriophage T4 Genome *in Vitro*. Heterogeneity of RNA Polymerase in Crude Extracts of Normal and T4-Infected *Escherichia coli* B†

Madhusudan P. Pitale and Ramamirtha Jayaraman*,‡

ABSTRACT: In order to obtain RNA polymerase preparations carrying the necessary specificity determinants to transcribe the delayed-early genes of bacteriophage T4, crude extracts of uninfected and T4-infected *Escherichia coli* were fractionated in glycerol gradients of low ionic strength. In contrast to the reported sedimentation behavior of the purified enzyme, the RNA polymerase activity in crude extracts of normal and infected cells sedimented heterogeneously over a wide range of sedimentation coefficients. When the "heavy" (24–33 S) and "light" (14–20 S) regions of the gradient were precipitated with ammonium sulfate and recentrifuged, the former split into two subfractions, one again sedimenting heavy and the other sedimenting light. The latter did not split under the same conditions. The resulting subfractions from uninfected cell extracts had different thermal stabilities at 50° (half-lives ranging from 2–3 to 25 min) while those from T4-infected cell extracts were very thermolabile (half-life of 1–2 min). All the subfractions were more active on T4 DNA than on calf-thymus DNA. They also formed rifampicin-resistant, RNA chain initiation complexes with T4 DNA. Based on the kinetics of heat inactivation with T4 and calf thymus DNAs as templates and preferential transcription of T4 DNA, it is proposed that the T4-infected cell enzymes prepared as described here harbor heat-labile initiation factor(s). During infection the heavy sedimenting RNA polymerase activity disappears after 2.5 min at 37°. This appears to require phage-specific protein synthesis because (a) it does not happen in the presence of chloramphenicol and (b) it does not happen in T4 ghost-infected cells.

Four broad classes of RNA have been described in bacteriophage T4-infected cells of *Escherichia coli* called immediate-early, delayed-early, quasi-late, and late species of RNA depending upon the respective times of appearance and relative abundance at a given time in the infected cell. The immediate-early and delayed-early species could be distinguished by the fact that phage-specific protein synthesis is needed for the synthesis of delayed-early RNA *in vivo* (Grasso and Buchanan, 1969; Salser *et al.*, 1970) but not *in vitro*, using T4 DNA and purified *E. coli* RNA polymerase (Milanesi *et al.*, 1969). The apparent requirement for phage-specific protein synthesis to turn on delayed-early transcription *in vivo* has given rise to two models. The one proposed by Travers (1969, 1970) postulates specific initiation factor(s), analogous to the σ factor of *E. coli*. The other model postulates a putative anti-termination factor (Schmidt *et al.*, 1970; Brody *et al.*, 1970; Black and Gold,

† From the Molecular Biology Unit, Tata Institute of Fundamental Research, Colaba, Bombay-400005, India. *Received July 9, 1974.*
‡ Present address: Department of Biological Sciences, Madurai University, Madurai-625021, India.

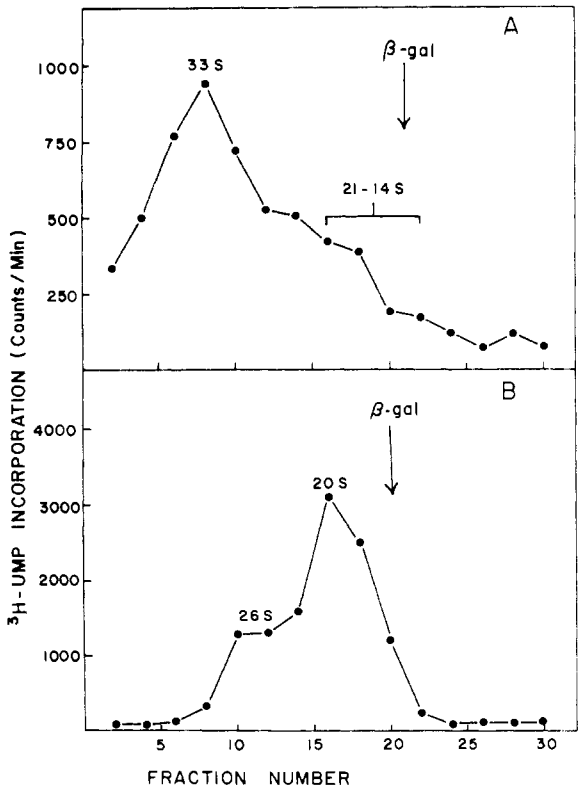

FIGURE 1: Sedimentation of RNA polymerase activity in crude extracts of uninfected and T4-infected *E. coli* B. (A) Ribosome-free DNase treated extracts were prepared from 6.0 g wet weight of *E. coli* B grown to a density of 2×10^8 cells/ml. Three milliliters of the extract (64 mg of protein) was centrifuged in a 10–30% v/v glycerol gradient as described under Materials and Methods. RNA polymerase activity was assayed using 5 μg of T4 DNA as the template. (B) *E. coli* B was grown to a density of 4×10^8 cells/ml and infected with phage T4 $D_{ac\,41}$, at a multiplicity of 7. The survivors after 2.5 min were less than 0.2%. Cells were collected 5 min after infection. Extracts were prepared from 7.5 g of infected cells and 3 ml (41 mg of protein) was layered on gradients and processed as described above. All gradients had 5 μg of β-galactosidase as marker protein.

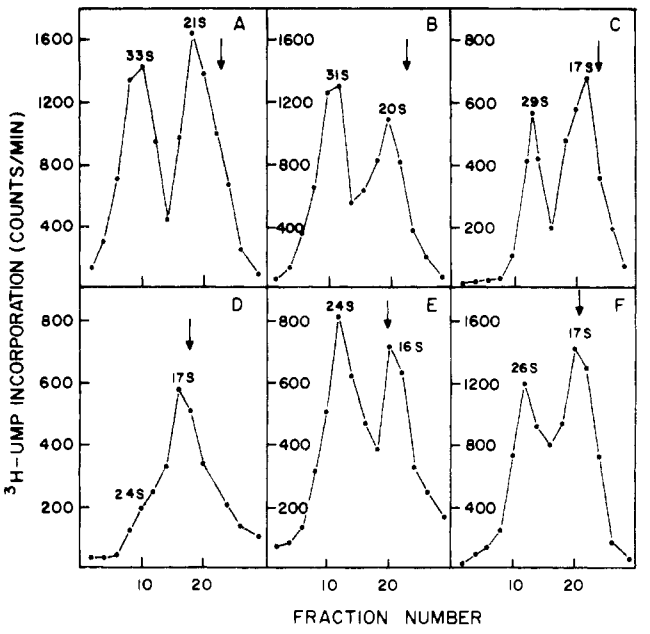

FIGURE 2: Sedimentation of RNA polymerase activity in crude extracts of *E. coli* B at different times after infection. *E. coli* B was grown to a density of $3\text{-}4 \times 10^8$ cells/ml and infected with phage T4 $D_{ac\,41}$ at a multiplicity of 10. At the indicated times after infection the culture was poured onto ice and collected by centrifugation and crude extracts were prepared as described under Materials and Methods. Approximately 30 mg of protein was layered on each gradient: (A) uninfected cells; (B) 1 min after infection; (C) 2.5 min after infection; (D) 5 min after infection; (E) 5 min after infection in the presence of 100 μg/ml of chloramphenicol added at −2 min; (F) 5 min after infection with T4 ghosts. The arrows indicate the position of marker β-galactosidase. The preparation of ghosts had less than 1% viable phage. Cell survival in all cases was less than 1%.

1971; Schachner *et al.*, 1971). The two models are not mutually exclusive (O'Farrel and Gold, 1973).

In an earlier paper one of us (Jayaraman, 1972) presented evidence suggesting the involvement of specific initiation factors in the transcription of at least some of the delayed-early genes. In an attempt to isolate such factor(s) ribosome free, DNase-treated lysates of T4-infected *E. coli* were subjected to glycerol gradient centrifugation yielding an RNA polymerase preparation which is 3–4 times more active on T4 DNA than on calf-thymus DNA. A similar procedure with lysates of uninfected cells revealed certain interesting properties of the enzyme. These data will be presented and discussed below. While this work was in progress Snyder (1973) and Travers and Buckland (1973) reported similar data on sedimentation profiles of RNA polymerase in crude lysates of *E. coli* and T4-infected *E. coli*.

Materials and Methods

Strains. Escherichia coli B and bacteriophage $T4D_{ac\,41}$ (both from Dr. Edward Goldberg's collection) were used throughout.

Media. The cells were grown to a density of $3\text{-}5 \times 10^8$ cells/ml at 37° with vigorous shaking, in a rich medium containing per liter: nutrient broth (Oxoid), 24 g; Bactopeptone, 15 g; NaCl, 15 g; and glucose, 3 g. Phage infection was carried out at 37° in the same medium with vigorous aeration. The cells were plated for survivors 2.5 min after infection.

Preparation of Extracts. At the appropriate time after growth or after phage infection the cells were poured onto slightly more than an equal volume of crushed ice, collected by centrifugation at 10,000 rpm for 7 min, washed once with the buffer containing 0.01 M Tris-HCl (pH 7.9), 0.005 M $MgCl_2$, 0.05 M KCl, 0.1 mM EDTA, 5% v/v glycerol, and 1 mM β-mercaptoethanol, and stored frozen at −20°. The frozen cells were ground in a chilled mortar with three times the weight of acid-washed sand (E. Merck, washed three times with water and twice with the above buffer to remove traces of acid and chilled to 4°). The paste was transferred to centrifuge tubes with small volumes of the above buffer. Sand, unbroken cells, and debri were removed by centrifugation at 10,000 rpm for 10 min. The residue was washed two or three times with small amounts of cold buffer; the supernatants were combined with the extract and made up to the desired volume. Pancreatic DNase (Sigma) was added to a final concentration of 20–40 μg/ml and left in ice for 1 hr. Solid KCl was added to a final concentration of 0.5 M. DNA and ribosomes were removed by centrifugation at 38,000 rpm for 2 hr in the type 50 rotor of the Beckman Model L ultracentrifuge. The clear, yellow supernatant was dialyzed overnight against the above buffer to remove KCl, layered on a 10–30% v/v glycerol gradient in the above buffer, and centrifuged at 23,000 rpm for 23–24 hr at 4° in the SW 25.1 rotor of the Model L ultracentrifuge. Fractions (25 drops, approximately 1 ml) were col-

lected from the bottom. The polymerase activity was assayed using 0.1 ml of the fractions.

Assay. RNA polymerase was assayed according to Burgess (1969) as described elsewhere (Jayaraman, 1972) except that [^{3}H]UTP (Radiochemical Center, Amersham, England) was used at a specific activity of 2 μCi/μmol.

Ammonium Sulfate Precipitation. Pooled fractions were precipitated at 66% saturation of ammonium sulfate at 4° by dropwise addition of 2 vol of saturated ammonium sulfate (neutralized to pH 7.5 with liquor ammonia) with constant, gentle stirring. Dithiothreitol was added prior to ammonium sulfate such that the final concentration after the addition would be 0.1 mM. Stirring was continued for another 30 min after the addition, and the precipitate was collected at 10,000 rpm for 10 min, dissolved in 3 ml of the above buffer but containing 0.02 M KCl, and dialyzed overnight against the same buffer. The entire amount was layered on a 10–30% v/v glycerol gradient in the same buffer (0.02 M KCl) and centrifuged as before. Fractions were collected and assayed as described above.

Rifampicin-resistant initiation complex was assayed as described earlier (Jayaraman, 1972).

Heat Inactivation. The sample (0.1–0.2 ml) was distributed into several tubes of approximately uniform wall thickness; bovine serum albumin was added to 200 μg/ml and kept in a water bath maintained at 50 ± 0.5°. At the appropriate time the tubes were removed, chilled in an ice-water slurry, and transferred to crushed ice. Residual activity was assayed as described above. Inclusion of bovine serum albumin gave reproducible inactivation kinetics, especially when the protein concentration was low.

Protein was assayed according to Lowry *et al.* (1951).

Results

Sedimentation of RNA Polymerase Activity from Crude Lysates of Uninfected and T4-Infected E. coli in Low Salt Glycerol Gradients. Figure 1A shows the sedimentation of RNA polymerase activity from a DNase treated, ribosome-free extract of uninfected *E. coli* in a 10–30% glycerol gradient containing 0.05 M KCl (ionic strength = 0.075). The enzyme sediments heterogeneously as a broad peak over nearly two-thirds of the gradient covering a range of sedimentation values from about 33 to 14 S. Often we have observed two distinct peaks of activity at the heavy and light regions of the gradient (see Figure 2A). The enzyme from T4-infected cells also sedimented as a broad band (Figure 1B); the 33S enzyme activity was absent and the bulk of the activity sedimented around 17–20 S with a distinct shoulder at 24–26 S. To decide whether these changes are physiological we compared the sedimentation profiles of RNA polymerase activity in crude extracts of T4-infected *E. coli* at various intervals after infection. The results are shown in Figures 2A–F. Both the heavy and light sedimenting activities were observed up to 2.5 min after infection (Figures 2A–C). By 5 min the activity in the heavy region disappeared and a shoulder appeared at approximately 24 S (Figure 2D). This did not occur in the presence of chloramphenicol and in cells infected with T4 ghosts (Figures 2E,F). The data suggest that two events occur side by side in T4-infected cells. On the one hand the heavy and light peaks of activity appear to become progressively lighter after infection. This occurs even in the presence of chloramphenicol and in ghost-infected cells and might reflect the effect of changes in the intracellular environment due to infection. On the other hand the disappearance of the activity under the heavy region appears to be related to phage development since it could be prevented by chloramphenicol.

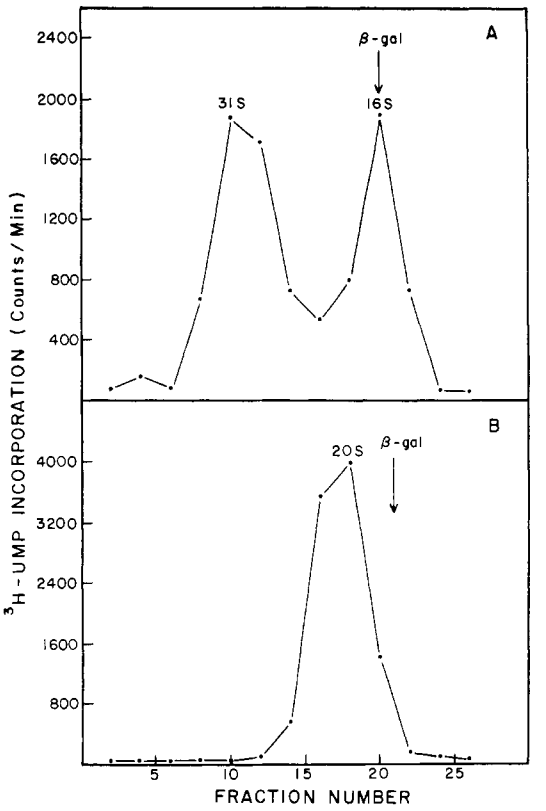

FIGURE 3: Effect of ammonium sulfate precipitation and recentrifugation in a glycerol gradient containing 0.02 M KCl (uninfected cell fractions). (A) The 33S regions of the gradient (Figure 1A, fractions 1–12) were pooled, precipitated at 66% saturation of ammonium sulfate, dissolved in 3 ml of low salt (0.02 M KCl) buffer, and dialyzed overnight against the same buffer. The entire amount (4 mg of protein) was layered on a 10–30% glycerol gradient containing 0.02 M KCl, centrifuged, and assayed on T4 DNA (5 μg) as described under Figure 1. (B) The 14–21S regions of the gradient (Figure 1A, fractions 16–22) were pooled and precipitated as described above. Both the gradients had 5 μg of β-galactosidase as marker protein.

Splitting of the 33S Enzyme on Ammonium Sulfate Precipitation. In the beginning we felt that the heterogeneous sedimentation reflected an equilibrium mixture of different aggregates of the enzyme. We pooled the material sedimenting at 33 and 14–21 S separately, precipitated the proteins at 66% saturation of ammonium sulfate, and centrifuged in another glycerol gradient which contained 0.02 M KCl (ionic strength 0.045) hoping to recover the activity as the dimer. Under these conditions the 33S material from uninfected cells split into two distinct peaks of activity, one sedimenting at ~30 S and other sedimenting at 16 S (Figure 3A), whereas the material from the 14–21S region gave a single peak at 19 S (Figure 3B). This observation argues against a simple equilibrium. Moreover, when the 33S material was precipitated and centrifuged in another glycerol gradient containing 0.05 M KCl, splitting was still observed (data not shown) suggesting that the change in ionic strength was not causing an artifact.

When the 24–26S material from a 0.05 M KCl glycerol gradient of T4-infected cell lysate was precipitated, recentrifuged as above, and assayed on T4 DNA the bulk of the activity appeared in the 16S region (Figure 4A). However, with calf-thymus DNA as template, the 24–26S region also showed some activity (Figure 4A). The light sedimenting

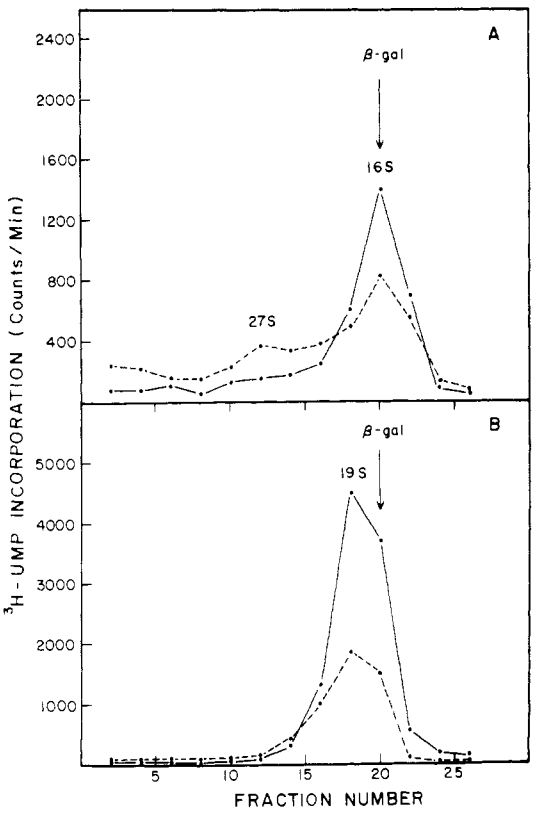

FIGURE 4: Effect of ammonium sulfate precipitation and recentrifugation in a glycerol gradient containing 0.02 M KCl (T4-infected cell fractions). (A) The 26S regions of the gradient (Figure 1B, fractions 8–12) were pooled and precipitated as described under Figure 3. Protein (4.2 mg) was layered on the gradient and centrifuged. The fractions were assayed using 5 μg of T4 DNA (solid line) and 10 μg of calf-thymus DNA (broken line) as the templates. (B) The 20S regions (Figure 1B, fractions 14–21) were pooled and precipitated as above. Protein (22.5 mg) was layered on gradient and centrifuged. The fractions were assayed on T4 DNA (solid line) and calf-thymus DNA (broken line). Both the gradients had 5 μg of β-galactosidase as marker protein.

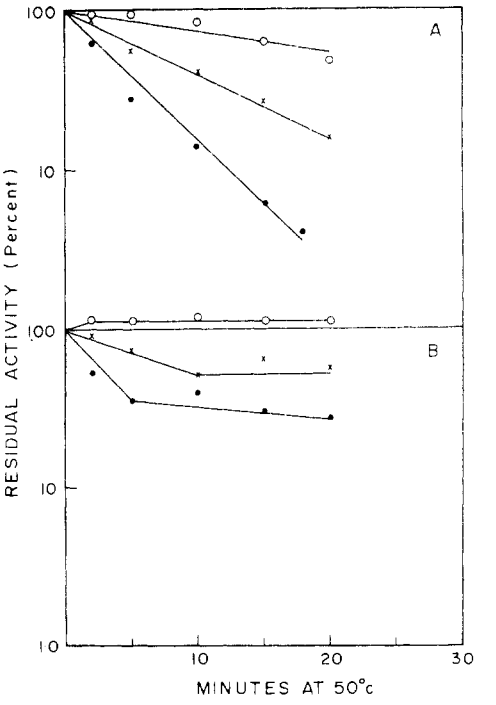

FIGURE 5: Kinetics of heat inactivation of pooled fractions from uninfected *E. coli* B after ammonium sulfate precipitation. The peak fractions of Figures 3A and 3B were pooled separately. Aliquots of the pooled fractions were heated in the presence of bovine serum albumin at 50° for various times as described under Materials and Methods and assayed (A) on 7.5 μg of T4 DNA and (B) 10 μg of calf-thymus DNA: (●) H-H_{EC}; (X) L-H_{EC}; (○) L-L_{EC}. One-hundred per cent activity corresponds to 869, 1114, and 3562 cpm on T4 DNA and 542, 378, and 964 cpm on calf-thymus DNA, respectively. See text for explanation of abbreviations.

fractions from a 0.05 M KCl glycerol gradient of infected cell lysate continued to sediment light after precipitation and recentrifugation (Figure 4B). No activity was observed in the heavier regions of the gradient either with T4 or calf-thymus DNA as the template.

Thermal Sensitivity of Heavy and Light RNA Polymerases. The above experiments suggested structural and/or functional heterogeneity in *E. coli* RNA polymerase. A simple way to test this notion would be to examine the thermal sensitivity of the species. When the active fractions from the second glycerol gradient containing 0.02 M KCl were pooled and examined for thermal stability, a very reproducible pattern was observed (Figure 5A). For convenience these fractions will be abbreviated as follows: H-H_{EC} (heavy fraction split from original heavy fraction of *E. coli* enzyme), L-H_{EC} (light fraction split from original heavy fraction of *E. coli* enzyme), and L-L_{EC} (light fraction split from the original light fraction of *E. coli* enzyme). The half-lives of these fractions at 50° were 2–3, 8–10, and 22–25 min, respectively (Figure 5A). The heat inactivation curves were monophasic when T4 DNA was used as the template. However, when calf-thymus DNA was used as template biphasic heat inactivation curves were obtained (Figure 5B). The half-life of the fast decaying component of a given species with calf-thymus DNA was approximately the same as that with T4 DNA. The slow decaying component of each species had a half-life of more than 30 min at 50°.

The kinetics of heat inactivation of the fractions pooled from the second (0.02 M KCl) glycerol gradient of phage-infected cell extracts gave a different picture. By analogy with the uninfected cell counterparts these will be referred to as H-H_{T4}, L-H_{T4}, and L-L_{T4}. Of these, the H-H_{T4} was not studied since it was inactive on T4 DNA (see Figure 4A). Using T4 DNA as the template both L-H_{T4} and L-L_{T4} showed biphasic heat inactivation kinetics, the half-life of the fast decaying component being 1–2 min at 50° (Figure 6A). It could be seen from Figure 6A that the slow decaying species in L-H_{T4} and L-L_{T4} constitute about 60 and 15%, respectively.

With calf-thymus DNA as the template the phage-infected enzymes were almost heat resistant (Figure 6B). There was, however, an initial decay which was slower on this template than on T4 DNA. These observations will be discussed at length later on. Table I summarizes the data on heat inactivation of normal and T4-infected enzymes.

Formation of Rifampicin-Resistant Initiation Complexes with Unsplit and Split Fractions from Normal and T4-Infected Cell Enzymes. Purified RNA polymerase holoenzyme from *E. coli* is known to form rifampicin-resistant initiation complexes when incubated with DNA in the absence of substrates (Hinkle and Chamberlin, 1970; Zillig *et al.*, 1970; Sippel and Hartmann, 1970; Jayaraman, 1972). The ability to form this complex could be taken as a stringent test for the functional integrity of the enzyme. It would also provide a means to test possible changes in initiation

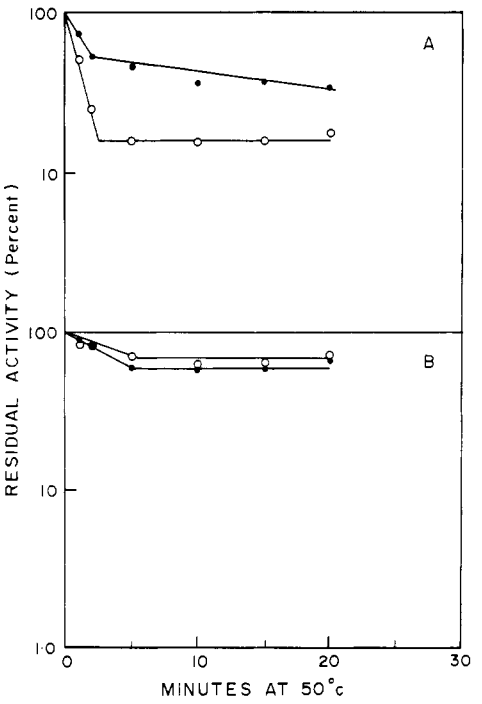

FIGURE 6: Kinetics of heat inactivation of pooled fractions from T4-infected *E. coli* B after ammonium sulfate precipitation. The active fractions of Figures 4A and 4B were pooled, heated at 50°, and assayed as described under Figure 5. The activity was monitored on (A) 7.5 μg of T4 DNA and (B) on 10 μg of calf-thymus DNA: (●) L-H_{T4}; (○) L-L_{T4}. One-hundred per cent activity corresponds to 885 and 18,385 cpm on T4 DNA and 1210 and 3972 cpm on calf-thymus DNA, respectively. See text for an explanation of abbreviations.

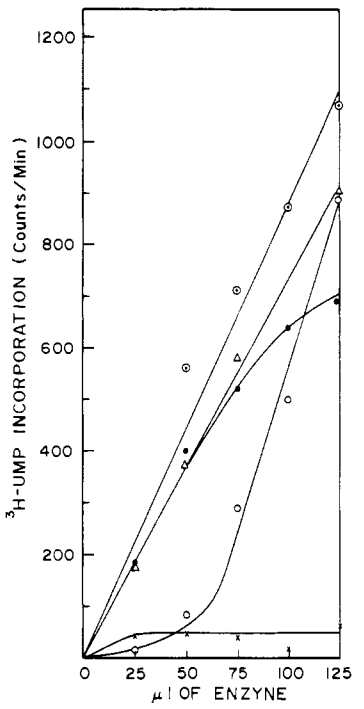

FIGURE 7: Formation of rifampicin-resistant initiation complex with enzymes from uninfected *E. coli* B before and after ammonium sulfate precipitation. The enzymes were prepared as described under Figures 1A and 3. Rifampicin-resistant initiation complexes were assayed as described earlier (Jayaraman, 1972): (●) H_{EC}; (X) L_{EC}; (△) H-H_{EC}; (○) L-H_{EC}; (⊙) L-L_{EC}. See text for an explanation of abbreviations.

Table I: Summary of Heat Inactivation Data with Normal and T4-Infected Cell Enzymes.

	T4 DNA as Template		Calf-Thymus DNA as Template	
Enzyme	Half-Life at 50° (min)	Slow Decaying Fraction (%)	Half-Life at 50° (min)	Slow Decaying Fraction (%)
H-H_{EC}	2–3	0	2–3; >30	40
L-H_{EC}	8–10	0	10; >30	50
L-L_{EC}	22–25	0	—; >30	100
L-H_{T4}	1–2; 22	55	7–8; >30	60
L-L_{T4}	1–2; >30	15	8–10; >30	70

specificity. We therefore studied the formation of rifampicin-resistant initiation complexes with the enzyme fractions described above.

The data obtained with uninfected cell fractions are presented in Figure 7. It could be seen that both the unsplit and split fractions from the heavy species formed rifampicin-resistant initiation complexes efficiently. The light species before ammonium sulfate precipitation formed very little, if any, complex although it was quite active on T4 DNA. After precipitation and recentrifugation, it too formed the rifampicin-resistant complex. None of these fractions formed the complex at 0.3 M KCl, in agreement with the reported instability of the complex at high ionic strength (Bautz and Bautz, 1970).

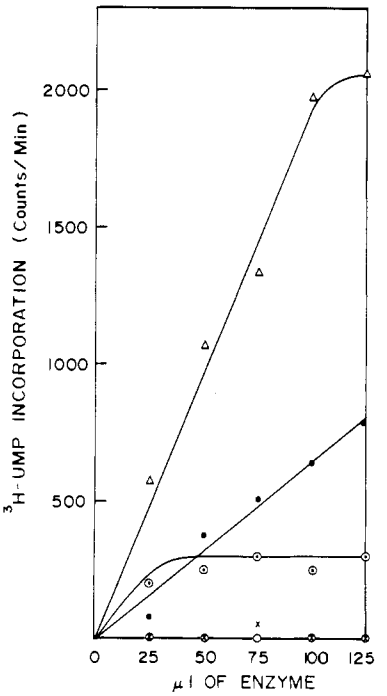

FIGURE 8: Formation of rifampicin-resistant initiation complexes with enzymes from T4-infected *E. coli* B. The enzymes were prepared from T4-infected *E. coli* B as described under Figures 1B and 4. The rifampicin-resistant initiation complexes were assayed as described by Jayaraman (1972): (●) H_{T4}; (⊙) L_{T4}; (X) H-H_{T4}; (○) L-H_{T4}; (△) L-L_{T4}. See text for an explanation of abbreviations.

The formation of the complex with the phage-infected enzyme is presented in Figure 8. Before ammonium sulfate precipitation, the heavy species formed the complex very well. The light species also formed the complex, but it satu-

Table II: Template Specificity of Unsplit and Split Fractions from Normal and T4-Infected *E. coli* B.[a]

Enzyme	[^{3}H]UMP Incorp. (cpm) T4 DNA (7.5 μg)	CT DNA (7.5 μg)	*E. coli* DNA (6.0 μg)	T4/CT
H_{EC}	1564	456	200	3.5
L_{EC}	2941	369	130	8.0
$H–H_{EC}$	988	315	120	3.1
$L–H_{EC}$	1243	373	154	3.3
$L–L_{EC}$	2044	732	479	2.8
H_{T4}	1060	495	420	2.1
L_{T4}	3300	750	400	4.4
$L–H_{T4}$	1350	636		2.1
$L–L_{T4}$	4600	1470		3.1

[a] One-tenth milliliter of the indicated fractions was assayed with T4 DNA, calf-thymus DNA, and *E. coli* DNA as described earlier (Jayaraman, 1972).

rated at low enzyme concentration. But after precipitation both the $H–H_{T4}$ and $L–H_{T4}$ were virtually ineffective in forming the complex. The reason for this discrepancy is not clear at the moment. (It could be recalled that $H–H_{T4}$ was inactive with T4 DNA as template (Figure 4A).) It is surprising that $L–H_{T4}$ which was fairly active on T4 DNA failed to form the complex. On the other hand, the ability of the light species to form rifampicin-resistant initiation complex increased markedly after precipitation and centrifugation in a second gradient. It appears that the ability of the light fractions from the first (0.05 M KCl) gradient of *E. coli* and T4-infected *E. coli* extracts to form the complex is somehow masked and precipitation and recentrifugation overcome this effect.

Template Specificity of Unsplit and Split Fractions from Normal and T4-Infected Cell Extracts. Originally the object of the present investigation was to obtain an RNA polymerase from T4-infected *E. coli*, able to transcribe the delayed-early region of phage T4 preferentially. Therefore, the activities of the different fractions from normal and T4-infected cells were studied on T4 DNA, calf-thymus DNA, and *E. coli* DNA. The data are presented in Table II. It could be seen that all the fractions have preferential activity on T4 DNA over calf-thymus (CT) DNA, the T4/CT ratio being greater than 2.0. The data obtained with the fractions from T4-infected cells are especially significant since it is known that in infected cells the σ factor does not co-purify with RNA polymerase (Bautz and Dunn, 1969; Schachner *et al.*, 1971) although it is shown to be present in such cells (Stevens, 1972). Although the T4/CT ratios of $L–L_{EC}$ and $L–L_{T4}$ are almost identical (Table II) they differ markedly in their thermal sensitivities (Figures 5 and 6). Whether the normal and T4-infected cell enzymes transcribe different genetic loci has not been determined yet.

Discussion

Sedimentation Behavior of Crude RNA Polymerase from Normal and T4-Infected E. coli. Purified RNA polymerase sediments homogeneously at 23–25S at low ionic strength and at 15S at high ionic strength corresponding to dimeric and monomeric forms of the enzyme, respectively (Berg *et al.*, 1971). Our observations presented here and those of Snyder (1973) and Travers and Buckland (1973) reported elsewhere have shown that in the crude state the enzyme activity sediments heterogeneously. The facts that the 33S material transcribes T4 DNA and could form rifampicin-resistant initiation complex argue against the notion that it could be core polymerase which is known to form multimeric aggregates (Berg *et al.*, 1971). A more plausible explanation seems to be that in the crude state the enzyme sediments in association with various factors which control its activity *in vivo*, such as the Tu–Ts factors (Travers *et al.*, 1970; Blumenthal *et al.*, 1972) and the M factor (Davison *et al.*, 1969) and possibly other, yet unidentified, factors. Does this physical heterogeneity indeed reflect the functional heterogeneity of the enzyme? Although we have not tried to answer this question, Snyder (1973) and Travers and Buckland (1973) have shown that different regions of the gradient transcribed different templates, natural and synthetic, with varying efficiency. This suggests that functional heterogeneity could exist.

The heterogeneous sedimentation of RNA polymerase activity is also observed in extracts of T4-infected cells. In this case the significant observation is the disappearance of the activity under the heavy peak by 5 min post-infection. This appears to require phage-specific protein synthesis and is at variance with the observation of Snyder (1973) who reported that the disappearance of the heavy enzyme was independent of phage-specific protein synthesis. The interesting point is that the near total disappearance of the activity in the heavy region is not accompanied by a corresponding increase of that in the light region. This rules out the conversion of the heavy enzyme activity to light species provided there are no differences in the specific activity of the enzymes. We do not yet know the mechanism of disappearance of the heavy polymerase after infection. Whatever be the mechanism the significant point to note is that it involves only the heavy enzyme. As suggested by Snyder (1973) this could be involved in the shut-off of host transcription.

Ammonium Sulfate Precipitation of the Crude Fractions and Differential Thermal Sensitivity of Resultant Products. The splitting of the 33S material into two fractions was unexpected. Coincident with the splitting there were also changes in the thermal sensitivity after precipitation. As far as we can tell, both the heavy and light fractions have equal thermal sensitivity using T4 DNA as the template (half-life approximately 10 min at 50°) before precipitation. After precipitation marked heterogeneity in thermal sensitivity was observed, the half-lives ranging from 2–3 to more than 20 min. The fact that the ammonium sulfate precipitated light enzyme ($L–L_{EC}$) has altered thermal sensitivity although it has approximately the same sedimentation coefficient as the parent suggests that it too has undergone some change after precipitation.

The ammonium sulfate precipitated enzymes showed monophasic heat inactivation curves with T4 DNA and biphasic curves with calf-thymus DNA as templates (Figure 5). It is known that σ is heat-labile and is required for the initiation of transcription of T4 DNA (Burgess *et al.*, 1969). The different half-lives obtained with T4 DNA as the template could possibly reflect heterogeneity among the population of σ. If heating converts the holoenzyme to the core (or some form functionally analogous to the core) the heat inactivation curve with T4 DNA template would be monophasic since the core is poorly active with this template. With calf-thymus DNA, on the other hand, the

curves would be biphasic since the core has considerable activity on this template. Extrapolation of the slower decay curve would then give the ratio of activity of the holoenzyme and core enzyme on calf-thymus DNA (per cent slow decaying fraction in Table I). The observed value of 40–50% is in good agreement with the data of Burgess *et al.* (1969). The fraction L–L_{EC} behaves differently giving monophasic heat inactivation curves on both T4 and calf-thymus DNA templates. Its preferential activity on T4 DNA (Table II) and its ability to form a rifampicin-resistant initiation complex (Figure 7) argue against the notion that it could be core polymerase which is poor in both respects. This fraction could represent a form of the enzyme in which the σ subunit is fairly heat stable; heating does not seem to alter its ability to use calf-thymus DNA as the template. It thus appears that there could be enormous heterogeneity among the RNA polymerase molecules of *E. coli*. The molecular basis and physiological significance, if any, of such heterogeneity are obscure at the moment.

What Is the Nature of the Enzyme from T4-Infected Cells? Although Travers (1969, 1970) described a σ-like factor in T4-infected cells, it has not been purified so far. We started this work to obtain an enzyme from T4-infected cells able to transcribe T4 DNA efficiently. Many earlier reports (Bautz and Dunn, 1969; Schachner *et al.*, 1971; Stevens, 1972) suggested that the best way to obtain such an enzyme is to look for it in relatively crude preparations. We have reported here the isolation of an enzyme activity from T4-infected cells transcribing T4 DNA preferentially. The ratio of activity on T4/CT DNA of the T4-infected cell enzymes very closely resembles that of the uninfected cell enzymes. Although this is *not* conclusive evidence for changes in initiation specificity, we feel that they are different from the normal cell enzymes. First of all, the infected cell enzymes are more heat-labile than the *E. coli* enzymes. The difference is very marked in the case of L–L_{EC} and L–L_{T4} and somewhat less in the case of L–H_{EC} and L–H_{T4} (see Table I). Secondly the infected cell enzymes decay more slowly on calf-thymus DNA than on T4 DNA (Table I). Thirdly, a certain fraction, ranging from 15 to 55%, of the T4-infected cell enzymes seems to have acquired moderate heat resistance even on T4 DNA. These observations are difficult to explain without invoking certain assumptions. Nevertheless, they do suggest that following T4 infection RNA polymerase has become more heat labile than the *E. coli* enzyme and this heat lability is apparent only when T4 DNA is used as the template. We feel the enzyme activity isolated by our procedure is associated with a heat-labile specificity determinant, probably an initiation factor. At the moment this is only a speculation; conclusive evidence needs further experimentation.

The formation of rifampicin-resistant initiation complexes could be attributed to the presence of σ in the case of uninfected cell enzymes but it raises some interesting questions in the case of T4-infected cell enzymes. Does the putative T4 factor function like *E. coli* σ in forming rifampicin-resistant complexes and, if so, does it act at the same or different loci where the *E. coli* σ functions? Is the factor a new entity or a modified form of σ? Does T4 transcription need both σ and the T4 factor during the delayed-early transcriptive phase? These are some of the questions which we are trying to answer.

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