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Functional Heterogeneity of *Escherichia coli* Ribonucleic Acid Polymerase Holoenzyme†

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ABSTRACT: On zone sedimentation *Escherichia coli* RNA polymerase holoenzyme exhibits functional heterogeneity with respect to template preference, regulation by ppGpp, and affinity for fMet-tRNA. The template preference of a subpopulation of RNA polymerase molecules correlates with both its sedimentation position and its ability to respond to effectors of polymerase selectivity. Incubation of such functionally distinct populations of enzyme molecules at physiological temperatures results in functional and structural equivalence. We suggest that RNA polymerase normally exists as a mixture of interconvertible forms and that promoter selection can be controlled by varying the number and proportions of forms present.

The pattern of promoter selection by purified *Escherichia coli* RNA polymerase can be regulated in vitro by guanosine 3′-diphosphate 5′-diphosphate (ppGpp). This nucleotide selectively inhibits RNA chain initiation from stable RNA promoters relative to λ phage promoters (van Ooyen et al., 1976; Travers, 1976a; Travers & Buckland, 1973). This differential control suggests that the enzyme can discriminate between different types of promoters. By what mechanism is this discrimination accomplished? One possibility is that RNA polymerase can exist in distinct states, each of which possesses a different promoter preference (Travers, 1976a). On this model, regulators such as ppGpp alter the pattern of

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promoter selection by changing the number and proportions of forms present. Template competition experiments with pure enzyme have suggested that the enzyme behaves functionally as a mixture of two or more forms differing in initiation specificity (Travers, 1976b). This functional heterogeneity is no longer apparent in the presence of 200 μM ppGpp.

The effect of ppGpp on promotor selection is paralleled by an average decrease of ~0.7 S in the $s_{20,w}$ of RNA polymerase (P. G. Debenham et al., unpublished experiments). Similarly, RNA polymerase containing a mutant σ subunit is changed in both sedimentation characteristics and promoter preference in a manner very similar to that elicited by ppGpp acting on wild-type enzyme (Travers et al., 1978). These results imply a correlation between the sedimentation coefficient of a population of polymerase molecules and its template specificity. Indeed, crude or partially purified preparations of RNA polymerase are demonstrably heterogeneous with respect to template specificity after zone sedimentation (Pene, 1969; Snyder, 1973; Travers & Buckland, 1973), template preference correlating with sedimentation position within a single peak of polymerase activity. This heterogeneity could simply be a consequence of different polypeptides associating with the enzyme. However, in this paper we show that heterogeneity in template preference is also characteristic of highly purified RNA polymerase holoenzyme. Furthermore, this heterogeneity correlates not only with sedimentation position but also with sensitivity to ppGpp and affinity for fMet tRNA. However, incubation of functionally distinct populations of enzyme molecules at elevated temperatures results in functional and structural equivalence. We conclude that template selection by *E. coli* RNA polymerase can be regulated by varying the number and proportions of interconvertible but functionally distinct states of the enzyme.

Materials and Methods

Materials. RNA polymerase holoenzyme was prepared by the methods of Burgess & Travers (1971) and Burgess & Jendrisak (1975) from *E. coli* MRE 600. Enzyme so prepared was >95% pure as judged by polyacrylamide gel electrophoresis and contained at least 0.7 mol of σ subunit and ~2 mol of ω subunit. Pure σ subunit was prepared by chromatography of this enzyme on Bio-Rex 70 as described by Burgess & Jendrisak (1975).

λ d_5 *ilv* DNA (Jorgensen et al., 1978) was prepared by gentle phenol extraction of purified phage particles. The Cla restriction fragment from *ϕ80* psu^+_{III} DNA (Landy et al., 1974) and the *lac* 205 restriction fragments were purified from pOPI (Backman et al., 1976) as previously described (Debenham, 1978).

ppGpp and ppApp were obtained from ICN Pharmaceuticals and checked for purity by chromatography on PEI–cellulose with 1.5 M potassium phosphate, pH 3.4. [^{35}S]-fMet-tRNA was prepared as previously described (Pongs & Ulbrich, 1976).

In Vitro Transcription. The activity of RNA polymerase fractions from a glycerol gradient was assayed with calf thymus DNA as the template as described by Burgess & Jendrisak (1975). For assay of the activity of the enzyme with individual DNA restriction fragments, reaction mixtures (100 μL) contained 0.04 M Tris-HCl, pH 7.9, 0.01 M $MgCl_2$, 0.05 M KCl, 0.006 M 2-mercaptoethanol, 0.25 mM each of ATP, CTP, and GTP, 0.01 mM [^{3}H]UTP (15 Ci/mmol), 0.5–1.0 nM DNA restriction fragment, and ~1.5 μg of RNA polymerase. In such reactions the final glycerol concentration was 5%. For rRNA synthesis from λ d_5 *ilv* DNA, reaction mixtures (200 μL) contained the above components except that KCl concentration was varied, and the concentrations of [^{3}H]UTP and λ d_5 *ilv* DNA were 0.006 mM and 0.47 nM, respectively. For ApC and ApU primed rRNA synthesis from λ d_5 *ilv* DNA, the reaction conditions were the same except that reaction mixtures contained 0.25 mM primer and all nucleoside triphosphates at 0.005 mM. In all cases RNA synthesis was for 15 min at 30 °C.

Analytical Procedures. rRNA synthesis was analyzed as previously described (Travers, 1976a). The binding of [^{35}S]fMet-tRNA to RNA polymerase was assayed in reaction mixtures (100 μL) containing 0.01 M Tris-HCl, pH 7.9, 0.05 M KCl, 0.01 M $MgCl_2$, 5% glycerol, 1.5–2.5 μg of RNA polymerase, and 10 nM [^{35}S]fMet-tRNA (sp act. 47 000–61 000 cpm/pmol). The reaction mixtures were incubated at 30 °C for the indicated times, diluted with 3 mL of cold 0.01 M Tris-HCl, pH 7.9, 0.05 M KCl, and 0.01 M $MgCl_2$ and filtered immediately through presoaked Millipore HAWP disks. The disks were washed with 3 × 3 mL of diluting buffer, counted, and dried.

Zone Sedimentation. After dialysis against 0.01 M Tris-HCl, pH 7.9, 0.01 M $MgCl_2$, 0.0001 M dithiothreitol, 0.0001 M EDTA, and 0.2 M KCl, RNA polymerase was layered on a 4.6 mL (for SW 50.1) or 11.5 mL (for SW 40) 15–30% v/v linear glycerol gradient in the above buffer. The gradients were centrifuged at 5 or 8 °C as indicated in a Beckman SW 40 or SW 50.1 rotor. Two- or four-drop fractions were collected from the gradients centrifuged in the SW 50.1 rotor.

Results

RNA Polymerase Is Functionally Heterogeneous. When RNA polymerase is centrifuged through a 15–30% glycerol gradient, the enzyme sediments as a broad peak with an average *s* value of ~14 S (Richardson, 1966). When assayed with calf thymus DNA as template, the specific activity of the enzyme across a normal sedimentation profile is approximately constant (Burgess, 1969). To test the promoter selectivity of RNA polymerase in individual fractions, we assayed equal weights of enzyme with DNA restriction fragments, each containing a single promoter. The fragments used were Cla (Landy et al., 1974) and *lac* 205 (Backman et al., 1976) containing, respectively, the su^+_{III} tRNA and *lac* UV5 promoters and no efficient terminators distal to the promoter sites. These fragments both support the transcription of a single major RNA product respectively ~150 and ~65 nucleotides long. The Cla fragment was efficiently transcribed by fractions from the trailing shoulder of the polymerase sedimentation profile while other fractions exhibited only low activity (Figure 1). A similar profile was apparent when the *lac* 205 fragment was used as the template. In this case, however, maximal transcription was observed with fractions from the trailing edge, together with a reproducible minor peak at the leading edge. These peaks of activity did not correspond with that observed with Cla DNA.

This functional heterogeneity with respect to promoter preference is also true of the promoters for rRNA synthesis. At least 5 of the rRNA cistrons found in *E. coli* have tandem promoters separated by ~100 base pairs (Glaser et al., 1977; Young & Steitz, 1979; Gilbert et al., 1979). The proximal and distal promoters of the *rrnX* cistron carried by the transducing phage λ d_5 *ilv* (Jorgensen et al., 1978) can be selectively primed by ApU and ApC, respectively, from a HaeIII restriction fragment (Young & Steitz, 1979) containing both promoters (A. A. Travers and R. Buckland, unpublished experiments). This pattern of priming is similarly observed for intact λ d_5 *ilv* DNA. When the fractions from a polymerase sedimentation profile were assayed in this way for their

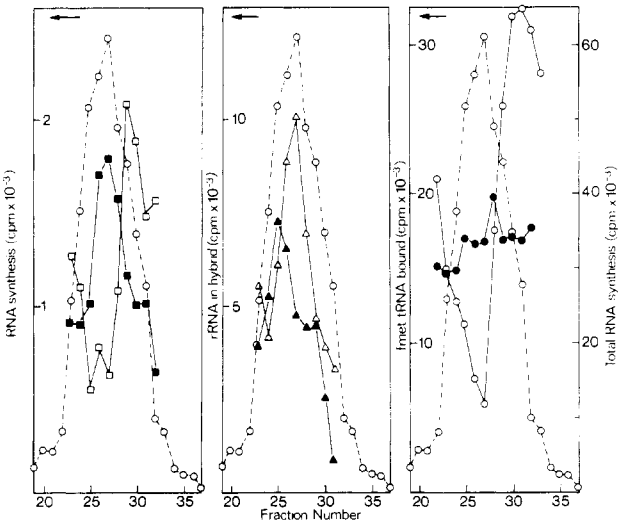

FIGURE 1: Functional heterogeneity of *E. coli* polymerase holoenzyme. 1.4 mg of polymerase holoenzyme at 2.8 mg/mL was layered on an 11.6-mL 15–30% glycerol gradient. The gradient was centrifuged at 2 °C for 30 h at 39 000 rpm in a Beckman SW 40 rotor. 75 2-drop fractions were collected and 10-μL aliquots were assayed for total RNA polymerase activity (○). *E. coli* β-galactosidase and catalase were sedimented in a parallel gradient and peaked in fractions 22 and 32, respectively. The arrow indicates direction of sedimentation. Left-hand panel: RNA synthesis from reaction mixtures (100 μL) containing 0.67 nM Cla DNA (■) or 0.9 nM *lac* 205 DNA (□) as indicated and RNA polymerase. Equal weights of RNA polymerase were used for each assay, based on 3 μL for fraction 27, containing 1.6 μg (3.2 pmol) of enzyme, corrected when necessary with an appropriate volume of gradient buffer in 25% glycerol to give a final glycerol concentration of 7.5%. Center panel: rRNA synthesis from λ d_5 *ilv* DNA primed by ApC (△) and ApU (▲). Reaction conditions were as for the left-hand panel except that reaction mixtures contained 0.25 mM primer, all nucleoside triphosphates at 0.005 mM, and 0.45 nM λ d_5 *ilv* DNA. rRNA synthesis was determined as previously described. Right-hand panel: [^{35}S]fMet-RNA bound by RNA polymerase after 1- (○) and 10-min (●) incubation at 30 °C. The specific activity of [^{35}S]fMet-tRNA was 61 000 cpm/pmol.

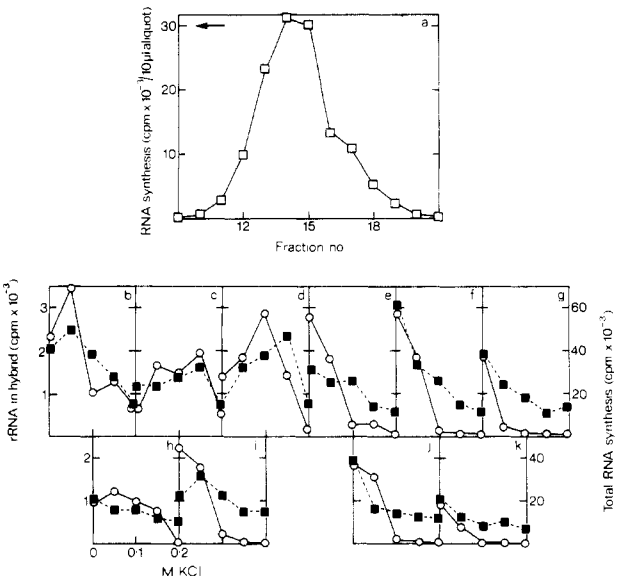

FIGURE 2: Dependence of the salt optimum for rRNA synthesis on sedimentation position. 2 mg of polymerase holoenzyme was sedimented at 5 °C for 24 h at 34 000 rpm. 44 4-drop fractions were collected. 20, 15, 10, 10, 20, and 20 μL of fractions 12–15, 17, and 18, respectively, were added to reaction mixtures containing 0.47 nM λ d_5 *ilv* DNA and 0.006 mM [^{3}H]UTP (sp act. 40 Ci/mmol). Where 10- and 15-μL aliquots were assayed, 10 and 5 μL, respectively, of gradient buffer in 25% glycerol were added to each reaction mixture. Data presented are from a single representative experiment. Similar variation of the rRNA synthesis profile has been observed in 11/11 experiments with similar sedimentation conditions. To determine the profiles of polymerase sedimented in the presence of either 10 μM ppApp or 10 μM ppGpp, we used 400 μg of holoenzyme per gradient. In these cases, in each assay the concentration of nucleoside tetraphosphate was 1 μM. (a) Polymerase sedimentation profile; (b–g) rRNA synthesis profiles for fractions 12–15, 17, and 18, respectively; (h and i) rRNA synthesis profiles for fractions from leading (h) and trailing (i) edges of RNA polymerase sedimented with 10 μM ppApp; (j and k) the same as for (h) and (i) except that 10 μM ppGpp replaces ppApp. (■) Total synthesis, from λ d_5 *ilv* DNA; (○) rRNA synthesis.

capacity to initiate at the individual rRNA promoters, the proximal promoter was utilized most efficiently by fractions from the leading shoulder while the distal promoter was favored by fractions from the trailing shoulder (Figure 1). The data suggest that the profile for the distal *rrnX* promoter parallels that of the su^+_{III} tRNA promoter. We conclude that promoter utilization by individual polymerase fractions is highly selective and differs from that of most other fractions. RNA polymerase can thus exhibit functional heterogeneity with respect to promoter preference, this heterogeneity being apparent as a correlation between the sedimentation position of the enzyme and its template preference.

Regulation of Polymerase Structure and Selectivity. Another measure of the capacity of RNA polymerase to initiate at particular promoters is the salt dependence of transcription (Fuchs et al., 1967). Analysis of the salt dependence of total rRNA transcription from λ d_5 *ilv* DNA by the individual fractions of a normal polymerase sedimentation profile (sedimented at 5 °C) revealed that rRNA synthesis became progressively less salt sensitive toward the leading edge with the possible exception of enzyme sedimenting at the extreme leading edge (Figure 2). This pattern of salt dependence correlates both in character and in position with the effects of the nucleoside tetraphosphates ppApp and ppGpp on polymerase function and structure. These nucleotides respectively increase and decrease the optimum salt concentration for rRNA synthesis (Travers, 1978). Comparison of the salt dependence of rRNA synthesis in the presence of ppApp or ppGpp acting on unfractionated holoenzyme (Travers, 1978) with that of individual fractions suggests that the characteristics of rRNA synthesis by fractions progressing from the trailing edge toward the leading edge can be approximately equated with those elicited by 200 μM ppGpp and 10 μM ppGpp and then by increasing concentrations of ppApp with the exception that the pattern shown by enzyme at the extreme leading edge has no obvious counterpart induced by nucleotide. Correspondingly, ppGpp decreases and ppApp increases the $s_{20,w}$ of polymerase holoenzyme (P. G. Debenham et al., unpublished experiments). We suggest that the changes in polymerase sedimentation and function induced by ppApp and ppGpp are mirrored in the normal sedimentation profile of RNA polymerase. A correlation between the salt profiles of rRNA synthesis and the effects of ppGpp and ppApp on polymerase function and structure would imply that the effect of the nucleotides on the capacity for rRNA synthesis should depend on the apparent s value of the enzyme. This prediction was tested by determining the effect of increasing concentrations of ppGpp on rRNA synthesis by individual fractions. Figure 3 shows that 1 mM ppGpp inhibited rRNA synthesis by fractions from the leading and trailing edges by 78 and 26%, respectively. In general, to reduce rRNA synthesis to a given absolute extent required the addition of higher concentrations of ppGpp to fractions from the leading edge than from the trailing edge except at 1 mM ppGpp when all fractions were equivalent in this respect.

A further corollary of the correlation between sedimentation

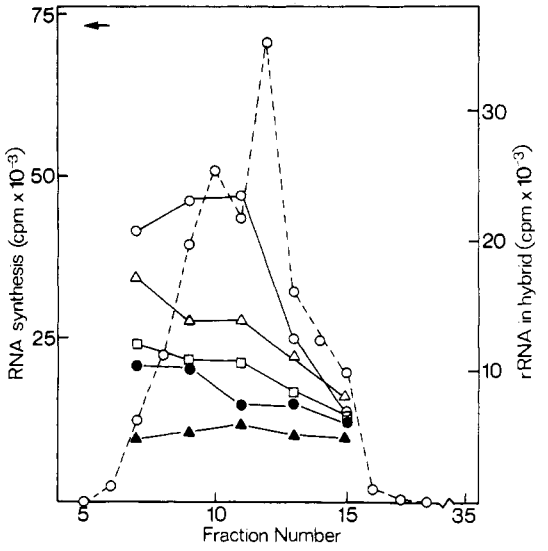

FIGURE 3: Effect of ppGpp on rRNA synthesis by equal weights of enzyme from different fractions of a polymerase sedimentation profile. Sedimentation conditions were as described in the legend to Figure 2. 35 4-drop fractions were collected. rRNA synthesis from λ d_5 *ilv* DNA in the presence of 0 (○), 10 (△), 50 (□), 200 (●), and 1000 M (▲) ppGpp. (○---○) Polymerase activity profile.

position and the functional effects of ppApp and ppGpp is that when polymerase is sedimented in the presence of these nucleotides, the range of rRNA synthesis salt profiles should be restricted in comparison with the range observed for the untreated enzyme. This is again observed. Thus, the salt profiles of rRNA synthesis by enzyme sedimented with 10 μM ppGpp most closely resemble those observed for polymerase molecules sedimenting toward the trailing edge of a normal profile (Figure 3). Similarly, the corresponding profiles for enzyme sedimented in the presence of 10 μM ppApp resemble those by polymerase molecules skewed toward the leading edge of a normal profile.

Another in vitro regulator of polymerase selectivity, $fMet\text{-}tRNA_f^{Met}$, has a functional effect very similar to that elicited by high concentrations of ppGpp; i.e., the charged tRNA preferentially inhibits the initiation of stable RNA synthesis and stimulates *lac* RNA synthesis (Debenham et al., 1980). The different polymerase fractions were assayed for their capacity to bind this tRNA by using limiting concentrations of ligand to enhance detection of any possible variations in the affinity of the enzyme for the charged tRNA. This experiment revealed a bimodal pattern of binding. The peaks of binding activity were at the leading and trailing edges and corresponded to fractions which efficiently utilized the *lac* UV5 promoter (Figure 1). Thus, those polymerase molecules which have the highest affinity for fMet-tRNA also exhibit this aspect of the promoter preference normally elicited by the tRNA.

The pattern of template preference and fMet-tRNA binding observed is reproducible provided that the conditions of sedimentation are rigorously controlled. When RNA polymerase is sedimented at a higher temperature or at a higher enzyme concentration, different, although related, patterns of template preference and fMet-tRNA binding are observed. Figure 4 shows that at a slightly higher temperature (8 °C in place of 5 °C) the pattern of template preference is duplicated such that there are two populations of polymerase molecules which utilize the *lac* 205 fragment efficiently. Similarly, there are two other distinct populations that utilize the Cla fragment. The pattern of fMet-tRNA binding is also duplicated, the greatest extent of binding again correlating with the utilization of the *lac* 205 fragment. We note that a bimodal profile of template utilization can also be observed with the *lac* 205 and Cla fragments as templates for unfractionated enzyme. In particular, there are two optimal magnesium concentrations of 4 and 10 mM for the transcription of the Cla fragment. The corresponding optima for the transcription of the *lac* 205 fragment are 6 and 12 mM, respectively (P. G. Debenham and A. A. Travers, unpublished experiments).

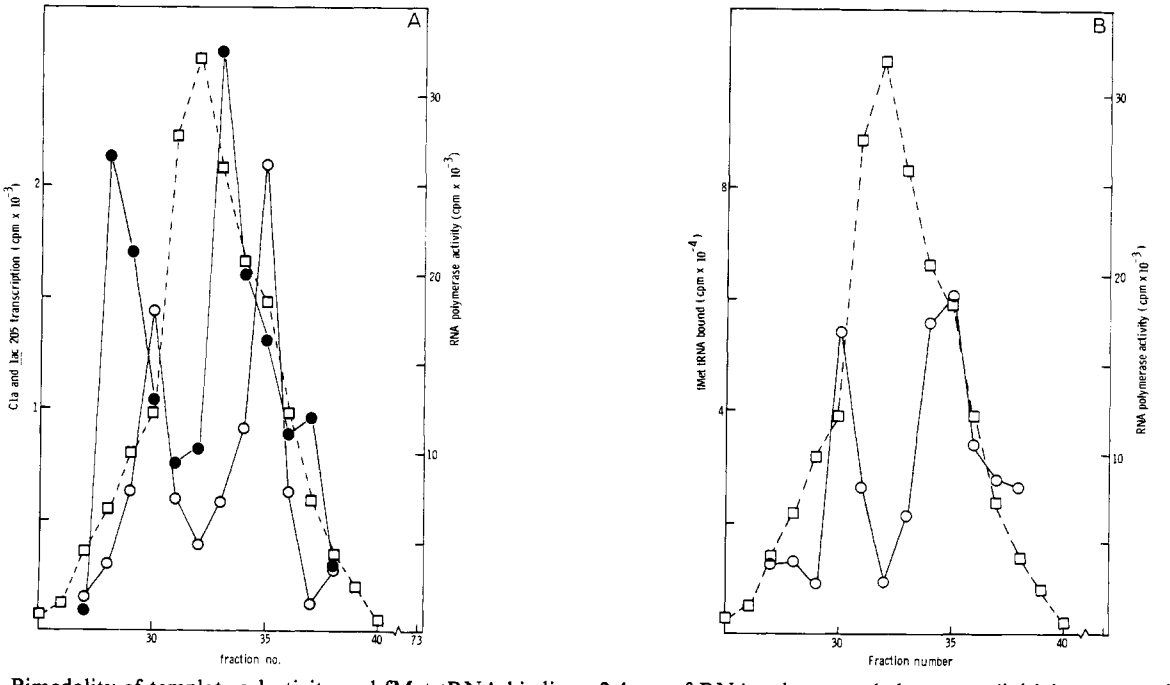

FIGURE 4: Bimodality of template selectivity and fMet-tRNA binding. 2.4 mg of RNA polymerase holoenzyme (initial concentration 4.8 mg/mL) was sedimented at 8 °C for 24 h at 39 000 rpm in a Beckman SW 40 rotor. 73 2-drop fractions were collected and assayed for RNA polymerase activity (□). Left-hand panel: activity with Cla DNA (●) and *lac* 205 DNA (○). Right-hand panel: fMet-tRNA bound by different fractions (○). Assays were performed as described in the legend to Figure 1. The specific activity of [^{35}S]fMet-tRNA was 59 000 cpm/pmol.

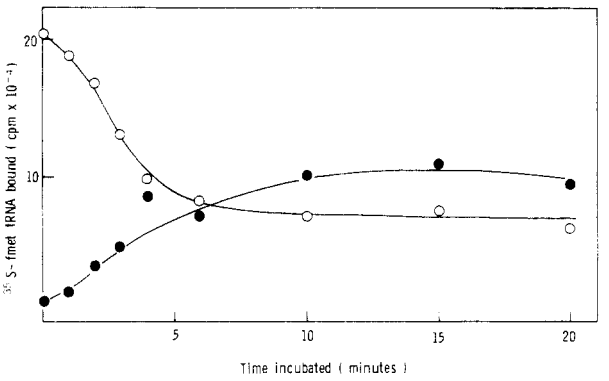

FIGURE 5: Kinetics of fMet-tRNA binding. Reaction mixtures (100 μL) containing 0.01 M Tris, pH 7.9, 0.05 M KCl, 0.01 M $MgCl_2$, 5% glycerol, and 2.4 μg of RNA polymerase were incubated at 30 °C for the indicated times. [^{35}S]fMet-tRNA (sp act. 47 000 cpm/pmol) was then added to a final concentration of 10 nM, and the incubation was continued for a further 30 s at 30 °C. The reaction mixtures were assayed as described under Materials and Methods. (○) Indicates the response of a polymerase fraction from the trailing edge (equivalent to fraction 30 in Figure 1) and (●) indicates that of a fraction from the center of a gradient profile (equivalent to fractions 25 and 26 in Figure 1).

We conclude that in addition to the observed correlation between sedimentation position and promoter preference there is an additional correlation between the former and the capacity to respond to regulators of polymerase selectivity.

Functional and Structural Equilibration of RNA Polymerase. The bimodal pattern of fMet-tRNA binding is observed only when the charged tRNA and polymerase interact for a short time, i.e., ~1 min. After 10-min incubation in the presence of limiting amounts of fMet-tRNA, there is no significant difference between polymerase fractions in their capacity to bind the charged tRNA (Figure 1). This change involves both an *increase* in binding by previously low binding fractions and a *decrease* in binding by previously high binding fractions. The average half-time for the attainment of this equivalence is 3–4 min under conditions similar to those used for in vitro transcription in the experiment shown in Figure 1 (Figure 5). This rate, which is slow relative to the initial rates of RNA chain initiation (Mangel & Chamberlin, 1974), decreases as the concentration of glycerol increases (A. A. Travers, unpublished observation). The approach to equivalance suggests that RNA polymerase molecules can equilibrate under appropriate conditions. To test whether this functional equivalence could also be attained in the pattern of transcription, we diluted fractions from the leading and trailing edges of a sedimentation peak with buffer and incubated them at equal concentrations either at 0 °C or at 30 °C for 30 min. The salt dependence of rRNA synthesis by each enzyme aliquot was then determined. Figure 6 shows that whereas the profiles of rRNA synthesis by fractions incubated at 0 °C reflected that corresponding to their sedimentation positions, those by aliquots incubated at 30 °C were very similar to each other and corresponded to profiles normally characteristic of the center of the polymerase activity peak. We conclude that incubation of RNA polymerase at elevated temperatures alters the transcriptional properties of the enzyme, allowing a functional equilibration.

If the variation in selectivity with sedimentation position reflects variation in polymerase structure, it could follow that enzyme from the individual fractions in a sedimentation profile should resediment in the same relative position. Enzyme from the leading and trailing edges behaved in this manner when kept at 0 °C (Figure 7). However, when the same enzyme fractions were incubated at 30 °C for 30 min, there was no longer any significant difference in the apparent sedimentation coefficient, enzyme from the trailing edge sedimenting faster and that from the leading edge sedimenting slower. We conclude that the equilibration of polymerase function is accompanied by equilibration of polymerase structure.

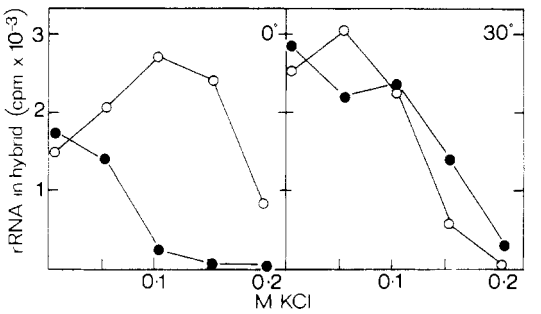

FIGURE 6: Functional equilibration of RNA polymerase. 4 mg of RNA polymerase holoenzyme (initial concentration 8 mg/mL) was sedimented at 5 °C for 24 h at 39000 rpm in a Beckman SW 40 rotor. 39 4-drop fractions were collected. A fraction from the leading edge and one from the trailing edge were diluted to give a final concentration of 450 μg/mL protein, 0.12 M KCl, and 12.5% glycerol in buffer containing 0.01 M Tris-HCl, pH 7.9, 0.01 M $MgCl_2$, and 0.0001 M dithiothreitol. Each fraction was then divided into two aliquots, one being kept at 0 °C and the other being kept at 30 °C for 30 min. All aliquots were then assayed for their capacity to synthesize rRNA from λ d_5 *ilv* DNA as a function of KCl concentration. Left-hand panel: enzyme preincubated at 0 °C. Right-hand panel: enzyme preincubated at 30 °C. (●) Polymerase from the leading edge; (○) polymerase from the trailing edge.

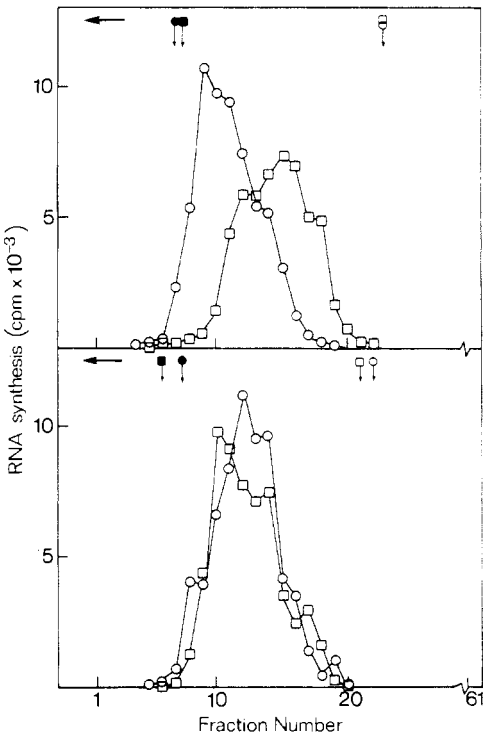

FIGURE 7: Structural equilibration of RNA polymerase. Aliquots from the leading and trailing edge of a polymerase sedimentation profile were prepared and treated as described in the legend to Figure 4. All aliquots were then sedimented at 5 °C for 24 h at 39 000 rpm in a Beckman SW 50.1 rotor. One-drop fractions were collected. In each sample β-galactosidase and catalase were included as internal markers. Upper panel: enzyme preincubated at 0 °C for 30 min. Lower panel: enzyme preincubated at 30 °C for 30 min. (○) Polymerase from the leading edge; (□) polymerase from the trailing edge. ● and ○ indicate the positions of β-galactosidase and catalase, respectively, for gradients containing enzyme from the leading edge. ■ and □ similarly indicate the markers for gradients containing enzyme from the trailing edge.

Discussion

We have shown that sedimentation of RNA polymerase holoenzyme on a glycerol gradient reveals a separation of polymerase molecules into functionally distinct populations. In particular, these populations differ in template preference, the salt dependence of rRNA synthesis, response to ppGpp, and the capacity to bind fMet-tRNA$_f^{Met}$. These observations confirm directly that the enzyme can exhibit functional heterogeneity, a conclusion which had previously been inferred from template competition (Travers, 1976b) and rifampicin binding experiments (Bahr et al., 1976).

On the basis of fMet-tRNA binding, three zones of a polymerase sedimentation profile can be distinguished, i.e., two which bind fMet-tRNA and one which does not. However, the template preferences of individual fractions (Figures 1 and 2) suggest that three is a minimum estimate and that at least four zones can be distinguished. How are these zones generated? The resedimentation in the same relative position of fractions from the leading and trailing edges of the peak of polymerase activity suggests that the observed functional heterogeneity is a consequence of structural differences, possibly stabilized by high glycerol concentrations. The variation in sedimentation position could reflect differences in subunit composition, the extent of dimerization, or protein conformation. Polypeptide analysis by polyacrylamide gel electrophoresis (Laemmli, 1970) of fractions across a sedimentation profile revealed no variation in the proportion of core polymerase subunits α, β, β', and ω, nor was there any indication of additional polypeptides that might be generated by proteolytic degradation. In some experiments there was, however, 20–30% less σ subunit at the trailing edge compared with the leading edge. Nevertheless, it is unlikely that the observed variation in function is a consequence of variable σ content since sedimentation of holoenzyme containing 1 mol of σ subunit per core per enzyme yields similar profiles of template preference and fMet-tRNA binding to those observed with polymerase containing 0.7 mol of σ subunit (data not shown). Similarly, the bimodality of template preference and fMet-tRNA binding (Figure 2) cannot be explained on the basis of variable σ content. In addition, the ability of distinct gradient fractions to attain structural and functional equivalence also argues strongly that the observed differences in sedimentation position are not a consequence of differences in σ content.

The observations reported here thus suggest that RNA polymerase is an equilibrating system. We show elsewhere (A. A. Travers et al., unpublished experiments) that variation in polymerase concentration, salt concentration, or temperature influences the sedimentation coefficient of the enzyme, its affinity for fMet-tRNA, and the salt characteristics of rRNA synthesis. This suggests that polymerase–polymerase interactions are involved in determining the position of any equilibrium. However, in the experiments described here, RNA polymerase sediments predominantly in the protomeric state rather than in the dimeric aggregate, the $s_{20,w}$ of these forms being ~15 and ~23 S, respectively (Berg & Chamberlin, 1970). It is difficult to envisage a purely aggregational mechanism for generating three to four *structural* forms of the enzyme sedimenting between ~13.5 and ~15 S. Consequently, we propose that the differences in sedimentation position may reflect, at least in part, conformational differences.

The strong correlation between the apparent sedimentation coefficient of a population of RNA polymerase molecules and its functional properties is maintained in the presence of the polymerase effectors ppGpp, ppApp, and fMet-tRNA, all of which alter the promoter preference of RNA polymerase (van Ooyen et al., 1976; Travers, 1976a; Debenham et al., 1980). The nucleotides induce opposing effects on both the pattern of rRNA synthesis (Travers, 1978) and the sedimentation characteristics of the enzyme (P. G. Debenham et al., unpublished experiments). Further, when polymerase is sedimented in the presence of either of these nucleotides, the range of salt profiles of rRNA synthesis is restricted in comparison to that observed for untreated enzyme (Figure 2). We suggest therefore that the nucleotides alter the position of an equilibrium between different structural states of the enzyme, thereby altering its promoter preference. This model is also consistent with the observed heterogeneity of fMet-tRNA binding (Figure 1). The effector-induced stimulation of *lac* transcription and inhibition of rRNA transcription may be explained by the stabilization by the charged tRNA of the *lac*-specific form of the enzyme.

The differences in promoter preference between different populations of polymerase molecules are not absolute, the extent of quantitative variation being only three- to fivefold. However, the ratio of the transcription of different RNA species, for example, *lac* and su^+_{III} tRNA, between different subpopulations, can vary by up to 10-fold. Such a change in differential rates of expression would be sufficient to account for in vivo changes in the pattern of the transcription. The data show that there is no correlation between the sequence of the −10 region (Pribnow, 1975) of a promoter and the subpopulation of polymerase molecules which expresses that promoter maximally. For example, the *lac* UV5 and proximal *rrnX* promoters have the same −10 region sequence, TATAATG, yet are utilized optimally by different populations of polymerase molecules. Similarly, the distal *rrnX* promoter and the su^+_{III} tRNA promoter have TAATATA and TATGATG, respectively, in the −10 region but are utilized optimally by similar polymerase populations. In a like manner the presence of the sequence CTTTACA centered around the −35 position of the *lac* UV5 and su^+_{III} tRNA promoters again does not correlate with maximum utilization by a particular class of polymerase molecules. We suggest, therefore, that the differences in the extent of expression which we observe is related to sequence differences in another region of the promoter. Analysis of published sequences suggests that the sequence between the −10 region and the start point of transcription is a possible candidate for this region. Thus, those promoters whose sequences in this region are GC rich are transcribed optimally by polymerase molecules which do not bind fMet-tRNA efficiently. By contrast, the *lac* UV5 promoter has an AT-rich sequence immediately before the start point and is utilized optimally by polymerase molecules which bind fMet-tRNA to the greatest extent.

To what extent does this type of regulation determine in vivo transcription patterns? The polymerase mutation *alt*-1 alters both the in vitro promoter preference and sedimentation profile of the enzyme in a manner paralleling that of 10 μM ppGpp (Travers et al., 1978). Further, the in vitro pattern of RNA synthesis by the mutant enzyme correlates with the in vivo phenotype (Silverstone et al., 1972). Thus, in this case the functional properties of RNA polymerase apparently reflect the in vivo situation. However, the observed rate of structural equlibration of RNA polymerase alone is too slow to account for the rate of change of in vivo transcription patterns. This

suggests that equilibration in vivo may be speeded up by the presence of the effectors.

In summary, we have shown that RNA polymerase can be functionally heterogeneous, different populations of polymerase molecules exhibiting distinguishable template preferences. These populations are functionally and structurally interconvertible. We suggest that regulators of polymerase specificity, such as ppGpp, alter the promoter preference of the enzyme by altering the position of an equilibrium between different forms of the enzyme.

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Preferential Stimulation of Ribosomal Protein Synthesis by Insulin and in the Absence of Ribosomal and Messenger Ribonucleic Acid Formation†

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ABSTRACT: Insulin stimulates the rate of production of cytoplasmic ribosomes in resting cultures of chick embryo fibroblasts. The effects of the hormone were studied on the synthesis of individual basic ribosomal and nonribosomal proteins and on total cell protein to gain insight into the mechanism. Insulin elevates by fourfold or more the formation of all the ribosomal proteins that were examined whereas the synthesis of individual nonribosomal proteins and of total cell protein is raised only ~1.5 times. In both control and insulin-treated cells, all or almost all newly made ribosomal proteins are used to make cytoplasmic ribosomes. The action of the hormone on ribosomal protein formation is rapid and is already more than one-half of maximum between 10 and 20 min after treatment of the resting cells. Insulin stimulates ribosomal protein synthesis in the presence of a level of actinomycin D that blocks completely the formation of rRNA and inhibits the synthesis of poly(A)-containing mRNA by 95%. Under these conditions, newly made ribosomal proteins are unstable and the majority of those that were measured decay with a half-life of 30 min or less. Rates of decay are not reduced by insulin. Inhibition of protein synthesis by cycloheximide has no effect on the rate of formation of pre-rRNA in control cells. With insulin-treated cultures, however, the antibiotic blocks completely the enhanced production of pre-rRNA that would otherwise occur. Similar results were obtained with puromycin. The observations on the synthesis of ribosomal proteins and pre-rRNA suggest that insulin acts directly to raise the production of ribosomal proteins and only secondarily to increase the formation of pre-rRNA. The effect of the hormone on ribosomal protein formation would seem to be at the level of translation.

Insulin stimulates the incorporation of radioprecursors into the cytoplasmic rRNAs[1] of cells in vivo (Wool, 1963; Steiner & King, 1966) and in organ fragment (Turkington & Riddle, 1970) and monolayer culture (Baseman et al., 1974; Baseman & Hayes, 1975). The effect of insulin on ribosome synthesis

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[1] Abbreviations used: rRNA, ribosomal ribonucleic acid; r protein, ribosomal protein; EDTA, ethylenediaminetetraacetate; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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