Translation Initiation Factor 2 Alters Transcriptional Selectivity of *Escherichia coli* Ribonucleic Acid Polymerase†

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ABSTRACT: Preparations of translation initiation factor IF-2 strongly stimulate the production of rRNA by *Escherichia coli* RNA polymerase but have little effect on the synthesis of other RNA species. The factor alters the sedimentation characteristics of RNA polymerase holoenzyme. We suggest a mechanism by which IF-2 alters the pattern of transcriptional selectivity of RNA polymerase.

During rapid exponential growth the bacterium *Escherichia coli* produces no more ribosomes than are required for protein synthesis (Maaløe, 1969). The molecular mechanisms by which this balance is maintained are, however, at present poorly understood (Travers, 1976a; Maaløe, 1980; Nierlich, 1978). One hypothesis proposes that transcription and translation are coupled by the cycling factors involved in protein synthesis (Travers, 1976a). On this model the factors directly interact with RNA polymerase, thereby altering the spectrum of promoters to which the enzyme can productively bind. Two such components of the translation machinery, EF-TuTs (Travers et al., 1970) and fMet-tRNA$_f^{Met}$ (Pongs & Ulbrich, 1976), alter transcriptional selectivity in vitro, preferentially stimulating and inhibiting, respectively, stable RNA, the synthesis of stable RNA species. In addition, fMet-tRNA$_f^{Met}$ binds in a highly specific fashion to polymerase holoenzyme (Pongs & Ulbrich, 1976). In this paper we report that a third component of the translation machinery, IF-2, affects transcription in vitro, specifically increasing the production of rRNA. An interaction of this factor with RNA polymerase is indicated by an alteration of the sedimentation characteristics of the enzyme. We suggest that in vivo the accumulation of this protein in a free form may serve as a signal for ribosome production.

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 0.75 mol of σ subunit and ~2 mol of ω subunit. Core RNA polymerase was prepared by the method of Burgess & Jendrisak (1975). Initiation factors 2a, 2b, and 3 were purified to homogeneity by the method of Hershey et al. (1977). IF-2b [prepared by the method of Dondon et al. (1974)] was also the generous gift of Dr. A. Danchin. Electrophoretically homogeneous EF-Tu was the gift of Dr. D. Miller.

λ d$_5$ *ilv* DNA (Jorgensen & Fiil, 1976), λ *plac* 5 DNA, λ DNA, and T2 DNA were prepared by gentle phenol extraction of purified phage particles. A ColE1–rDNA chimera, pER 24, including the EcoRI restriction fragment from λ *rif* d18 DNA containing the rRNA promoter region and the proximal portion of the 16S tRNA cistron (Brosius et al., 1978) was purified by the method of Clewell & Helinski (1970). The Cla restriction fragment from $\phi 80$ psu$^+_{III}$ DNA (Landy et al., Ross, 1974) and the *lac* 205 restriction fragment from pOP I (Backman et al., 1976) were purified as previously described (Debenham, 1978).

In Vitro Transcription. The reaction mixture (200 μL) for RNA synthesis contained, unless otherwise stated, 0.04 M Tris-HCl, pH 7.9, 10 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 25 μM each of ATP, CTP, and GTP, 4 μM [^{3}H]UTP (sp act. 23 Ci/mmol), and KCl and DNA as indicated. The reaction mixture was preincubated for 5 min at 30 °C, and RNA synthesis was started by the addition of RNA polymerase holoenzyme to a final concentration of 20–40 μg/mL and allowed to proceed for 15–30 min at 30 °C. Where appropriate, IF-2 was preincubated in equimolar proportion with RNA polymerase at 2 mg/mL (4 μM) for 5 min prior to starting the reaction.

To determine preinitiation complex formation, we preincubated reaction mixtures lacking nucleotide triphosphates and containing 12 μg/mL pER 24 DNA and 0.01 M KCl for 5 min at 30 °C, then added RNA polymerase to 100 μg/mL with or without IF-2a at a final concentration of 25 μg/mL, and continued the incubation for a further 10 min at 30 °C. Heparin was then added to a final concentration of 400 μg/mL together with the nucleoside triphosphates including 0.018 mM [^{3}H]UTP (4.5 Ci/mmol), and RNA synthesis was allowed to proceed for 20 min at 30 °C.

Analysis of in Vitro Transcript. rRNA synthesis was analyzed as previously described (Travers, 1976b). Transcription from the DNA restriction fragments containing the *lac* UV5 and *su*$^+_{III}$ tRNA promoters was analyzed by electrophoresis on an 11% acrylamide gel in Tris–glycine buffer. The gel was dried and fluorographed (Bonner & Laskey, 1974), and the individual RNA bands on the fluorograph were quantitated by densitometry on a Joyce Loebel Mark III densitometer.

Other Assays. IF-2a activity was assayed as described by Hershey et al. (1977).

Zone Sedimentation. RNA polymerase with or without IF-2 was layered on a 4.6-mL 15–30% glycerol gradient containing 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. The gradients were centrifuged at 5 °C for 20 h at 39 000 rpm in a Beckman SW 50.1 rotor or for 24 h at 39 000 rpm in a Beckman SW 40 rotor. One- or two-drop fractions were collected, and 15-μL aliquots were assayed for activity with

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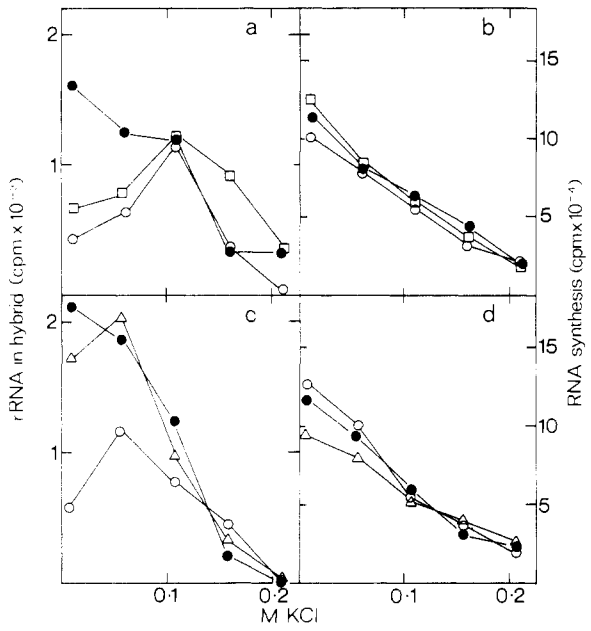

FIGURE 1: Effect of IF-2a on rRNA synthesis in vitro. (a and b) rRNA (a) and total RNA (b) synthesis from pER 24 DNA with RNA polymerase alone (○), RNA polymerase plus IF-2a (●), and RNA polymerase plus IF-3 (□); (c and d) rRNA (c) and total RNA (d) synthesis from pER 24 DNA with RNA polymerase alone (○), RNA polymerase plus IF-2a (●), and RNA polymerase plus EF-Tu (△). Final concentrations of RNA polymerase, IF-2a, IF-3, EF-Tu, and DNA were 13, 3, 1, 1.8, and 31 μg/mL, respectively. Hybridization efficiency for rRNA was 23%. Data points are for 50-μL aliquots of reaction mixtures.

calf thymus DNA as the template (Travers, 1976b). Catalase and β-galactosidase were run both as internal and external markers.

Results

IF-2 Stimulates rRNA Production. IF-2 mediates the binding of fMet-tRNA to ribosomes for initiating mRNA translation (Salas et al., 1967; Eisenstadt & Brawerman, 1967; Revel et al., 1968; Kolakofsky et al., 1969; Rudland et al., 1971). The factor is normally isolated as a mixture of two forms (Kolakofsky et al., 1969; Lelong et al., 1970; Mazumder, 1971; Fakunding et al., 1972; Miller & Wahba, 1973), IF-2a and IF-2b, with molecular weights of 118 000 and 90 000, respectively (Hershey et al., 1977). IF-2b is believed to be a degradation product of IF-2a (Hershey et al., 1977).

To test the possibility that IF-2 might act as a transcription factor in vitro, we measured the effect of IF-2a on rRNA synthesis in vitro as a function of KCl concentration, a parameter known to affect both the nonspecific binding of RNA polymerase to DNA (Pettijohn & Kamiya, 1967) and its capacity to transcribe different DNA templates (Matsukage, 1972). We used as the template the relaxed form of pER 24 DNA, a circular ColE1–rDNA chimera containing both the promoter region and the proximal portion of the 16S rRNA cistron of the *rrnB* (Brosius et al., 1978). Figure 1a shows that when IF-2a was mixed with RNA polymerase in a 1:1 molar ratio of factor to enzyme, rRNA production was preferentially stimulated at low ionic strength, the extent of stimulation decreasing from ~200% at 0.01 M KCl to zero at KCl concentrations >0.01 M. This enhancement of rRNA synthesis was not accompanied by any significant change in the extent of total transcription from the DNA template. To check whether this change in transcriptional selectivity was a characteristic of IF-2, we tested the effect of IF-3 on pER 24 transcription in a parallel reaction. In this case the factor, again in 1:1 molar ratio, had little effect on total RNA synthesis (Figure 1b). The extent of rRNA synthesis was also not greatly changed except at high ionic strength where a reproducible preferential increase in the transcription of rRNA sequences was observed. Thus, the effect of IF-2a on transcriptional selectivity is distinguishable from that of another macromolecule required for the initiation of protein synthesis. In other experiments we tested the effect of EF-Tu, a protein previously reported to influence transcriptional selectivity (Travers, 1973). In this case the protein, again present in approximately equimolar proportion with RNA polymerase, elicited a qualitatively similar response to IF-2a (Figure 1c).

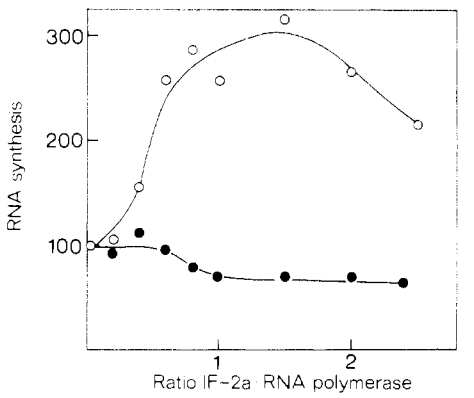

FIGURE 2: Stoichiometry of IF-2a-dependent stimulation of rRNA synthesis. RNA polymerase (final concentration 2 mg/mL) was mixed with varying amounts of IF-2a and compensating amounts of IF-2a storage buffer (0.01 M Tris, pH 7.9, 0.2 M KCl, 0.0001 M dithiothreitol, and 10% glycerol), and samples were assayed for their capacity to synthesize rRNA from pER 24 DNA at 0.01 M KCl with a concentration of polymerase in the reaction mixture of 12 μg/mL. Data are normalized. For total RNA synthesis (●) 100 represents 94 000 cpm/50-μL aliquot from the reaction mixture; for rRNA synthesis (○) 100 represents 890 cpm of rRNA in hybrid per 50-μL aliquot from the reaction mixture.

To determine the stoichiometry of IF-2a-dependent stimulation of rRNA synthesis, we mixed IF-2a with RNA polymerase in varying proportions and incubated the two components together for 10 min at 0 °C. These mixtures were then assayed for their capacity to synthesize rRNA by addition to prewarmed reaction mixtures containing pER 24 DNA and lacking KCl. Under these conditions the maximum stimulation of rRNA synthesis was attained at a molar ratio of IF-2a to polymerase of ~1.5 (Figure 2).

To establish that the observed effects on transcriptional selectivity were indeed dependent on the presence of active IF-2, we determined the relative rates of heat inactivation of the stimulation of rRNA synthesis and also of the IF-2a-dependent binding of fMet-tRNA$_f^{Met}$ to ribosomes. Figure 3 shows that when an IF-2a preparation was heated at 48 °C half the capacity for rRNA synthesis stimulation was lost by 3 min. This loss was paralleled by a similar loss in activity as a translation factor. At 10 min at 48 °C the two curves diverge slightly, the stimulation of rRNA synthesis being affected to a greater extent than the IF-2-dependent binding of fMet-tRNA. This divergence could reflect differences in the linearity of the response of these properties to increasing concentrations of IF-2a (cf. Figure 2). The temperature lability of the translational activity of IF-2 is similar to that previously observed. Since essentially no stimulation of rRNA synthesis is observed with heat-denatured IF-2, the possibility that the effect of IF-2a on transcription is merely a nonspecific consequence of protein addition is excluded.

At what stage of the transcription process does IF-2 act? To approach this question we measured the capacity of RNA

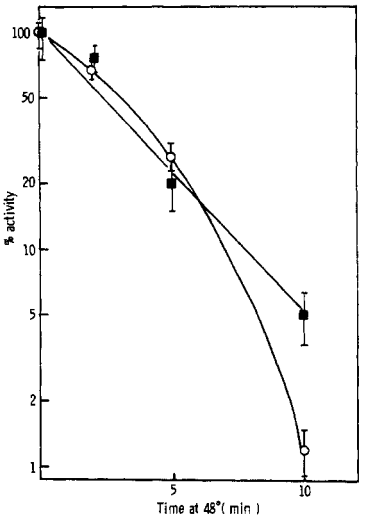

FIGURE 3: Heat inactivation of translational and transcriptional activities of IF-2a. IF-2a at 300 μg/mL was heated at 48 °C for the indicated times and cooled, and 2-μg aliquots were assayed for their ability both to stimulate rRNA synthesis from pER 24 DNA at 0.01 M KCl and to mediate AUG-directed [^{3}H]fMet-tRNA binding to ribosomes. Values plotted are the difference between rRNA synthesis with and without unheated IF-2 (○) and fMet-tRNA bound to ribosomes (■). 100% values are respectively 2032 cpm of rRNA in hybrid and 5693 cpm of [^{3}H]fMet-tRNA bound. All data points were calculated by subtraction of 937 cpm of rRNA and 542 cpm [^{3}H]-fMet-tRNA, these being the figures observed in the absence of added IF-2a. Data presented are the mean of two experiments. The specific activity of [^{3}H]fMet-tRNA was 1670 cpm/pmol.

Table I: Influence of IF-2a Preparation on the Formation of Polymerase-Promoter Preinitiation Complexes on pER 24

IF-2a present		cpm of [^{3}H]/50-μL aliquot	
during pre-incubation	during synthesis	total RNA	rRNA in hybrid
−	−	49 272	3047
+	+	45 538	5799
−	+	48 164	3425

polymerase to form preinitiation complexes at the promoters on pER 24 DNA in the presence and absence of IF-2a. The enzyme was first preincubated with the template in the absence of the nucleoside triphosphate substrates. Then preinitiation complex formation was assayed by the simultaneous addition of the nucleoside triphosphates and heparin, a polyanion that sequesters polymerase molecules which are free or weakly bound to DNA (Schafer et al., 1973; Mangel & Chamberlin, 1974). When IF-2a was present during the preincubation and subsequent RNA synthesis, rRNA production was preferentially enhanced (Table I). By contrast, IF-2a did not significantly affect either total or rRNA synthesis when it was added immediately after heparin. We conclude that to alter the pattern of rRNA synthesis IF-2a must be present prior to the initiation of transcription. The simplest interpretation of this result is that the factor influences the formation of preinitiation complexes.

IF-2a Alters the Sedimentation Coefficient of RNA Polymerase. In principle, IF-2 could affect transcription by interacting with either RNA polymerase or the DNA template. An indication that the factor interacts directly with the enzyme was obtained from studies on the sedimentation characteristics of polymerase holoenzyme in the presence of IF-2. Figure 4 shows that when IF-2a was mixed with RNA polymerase in a 1:1 molar ratio, the apparent sedimentation coefficient of

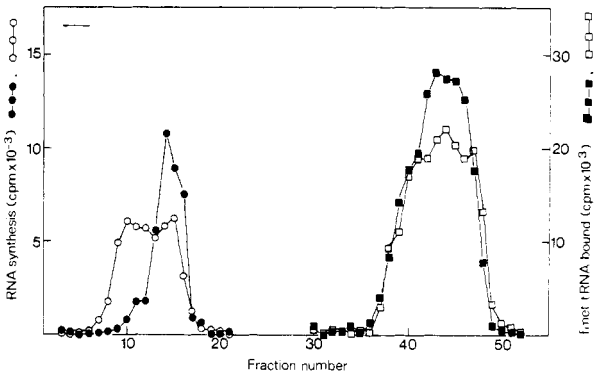

FIGURE 4: Effect of IF-2a on the sedimentation of RNA polymerase holoenzyme. Three parallel 15–30% glycerol gradients loaded with, respectively, RNA polymerase (150 μg) plus IF-2a heated for 15 min at 60 °C (45 μg), RNA polymerase (150 μg) plus IF-2a (45 μg), and IF-2a (45 μg) alone were sedimented for 20 h at 39 000 rpm in a Beckman SW 50.1 rotor. One-drop fractions were collected, yielding 57, 55, and 54 fractions, respectively. 15-μL aliquots were assayed as indicated for RNA polymerase activity on calf thymus DNA and IF-2a-dependent binding of [^{35}S]fMet-tRNA (sp act. 18 800 cpm/pmol) to ribosomes. RNA polymerase activity when sedimented with active (●) or heated (○) IF-2a; IF-2a activity when sedimented alone (□) or with RNA polymerase (■). Backgrounds of 230 and 1100 cpm have been subtracted for the polymerase and IF-2a assays, respectively. In a parallel gradient β-galactosidase and catalase activities peaked at fractions 7 and 19, respectively. The arrow indicates the direction of sedimentation.

the enzyme was reduced from ~14 to ~13–13.5 S. This effect was not significantly affected by variations of the molar ratio of polymerase to factor over the range 2:1 to 1:2. By contrast, heat-denatured IF-2a did not alter the characteristic broad sedimentation profile of RNA polymerase under these ionic conditions (Travers et al., 1980). No cosedimentation of IF-2a with RNA polymerase could be detected by polyacrylamide gel analysis (Laemmli, 1970) of the gradient fractions although such procedures would have detected IF-2a at the level of 0.1 mol/mol of polymerase. Correspondingly, assay for IF-2a activity revealed as much factor sedimenting close to the top of gradient as that in a control gradient which contained only IF-2a. Since RNA polymerase itself not only binds fMet-tRNA (Pongs & Ulbrich, 1976) but also is heterogeneous with respect to its capacity to bind this tRNA (Travers et al., 1980), it was not possible to assay for IF-2a-dependent binding of fMet-tRNA to ribosomes within the peak of polymerase activity. Nevertheless, the data suggest that at least 90% of the added IF-2a fails to cosediment with RNA polymerase.

The altered sedimentation behavior of RNA polymerase could simply result from an initial retardation of the enzyme consequent upon nonspecific interactions with IF-2. If this were the case, the properties of RNA polymerase sedimented with active and inactive IF-2a should be the same. To test this, we pooled all fractions containing polymerase activity from each gradient and assayed them immediately for their capacity to transcribe rRNA from λ d_5 *ilv* DNA. Figure 5a shows that the polymerase which had been previously mixed with active IF-2a differed in this respect from the control enzyme by synthesizing more rRNA at low ionic strength. That is, the difference between the two enzymes was similar to that normally elicited by the presence of an equimolar amount of IF-2 relative to polymerase. This difference was only apparent when the enzymes were assayed immediately after pooling. After incubation of the two polymerase samples for 30 min at 30 °C, they became functionally equivalent with respect to rRNA synthesis (Figure 5b), showing that the difference initially observed was not a consequence of unequal

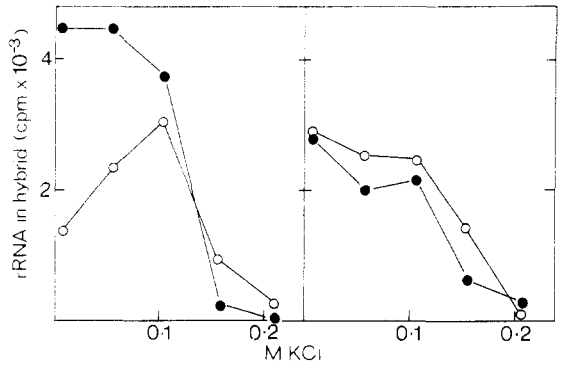

FIGURE 5: Properties of RNA polymerase sedimented with active IF-2a. rRNA synthesized from λ d_5 *ilv* DNA by RNA polymerase sedimented with heated (○) and untreated (●) IF-2a assayed immediately after pooling fractions from a similar gradient to that in Figure 4 (left-hand panel) or after incubating the pooled enzyme for 30 min at 30 °C (right-hand panel).

Table II: Effect of IF-2a on rRNA Synthesis from λ d_5 *ilv* DNA by Core RNA Polymerase[a]

[KCl] (M)		cpm/50-μL aliquot: total RNA	cpm/50-μL aliquot: rRNA in hybrid
0.01	core polymerase	8 994	337
	core polymerase plus IF-2a	9 568	266
0.11	core polymerase	5 248	215
	core polymerase plus IF-2a	5 911	292
0.01	polymerase holoenzyme	40 947	3501
	polymerase holoenzyme plus IF-2a	49 216	8214
0.11	polymerase holoenzyme	52 434	4685
	polymerase holoenzyme plus IF-2a	47 053	5520

[a] Final concentrations of core polymerase, polymerase holoenzyme, IF-2a, and DNA were 15, 19, 5, and 40 μg/mL, respectively. Data presented are the mean of two experiments.

recovery of RNA polymerase activity.

The failure to recover IF-2a cosedimenting with σ-containing RNA polymerase suggests that IF-2 does not act by displacing σ. To further substantiate this point, we tested the effect of IF-2a addition on transcription of λ d_5 *ilv* DNA by core RNA polymerase. Table II shows that IF-2a failed to enhance the low level of rRNA transcription by core enzyme. This suggests that σ is required for a transcriptional effect of IF-2 preparations, an effect paralleling that observed with EF-TuTs (Travers et al., 1970).

Although IF-2a preparation failed to stimulate the activity of core polymerase, the active factor did alter the apparent sedimentation coefficient of the enzyme, decreasing this parameter by a similar extent to that observed for holoenzyme (Figure 6). Heat-denatured IF-2a preparations were again without effect. This suggests that IF-2a may interact with core polymerase alone but that the σ polypeptide is required for an observable functional effect.

IF-2b Also Stimulates rRNA Synthesis. IF-2b is derived from IF-2a by proteolytic cleavage but retains activity as a translation factor. When tested in a transcription system with λ d_5 *ilv* DNA as the template, IF-2b, present in 1:1 molar ratio with RNA polymerase, preferentially stimulated rRNA production at low KCl concentration (Figure 7). Concomitantly, the synthesis of λ-specific RNA sequence was reduced. Thus, IF-2b preparations have a similar effect on transcriptional

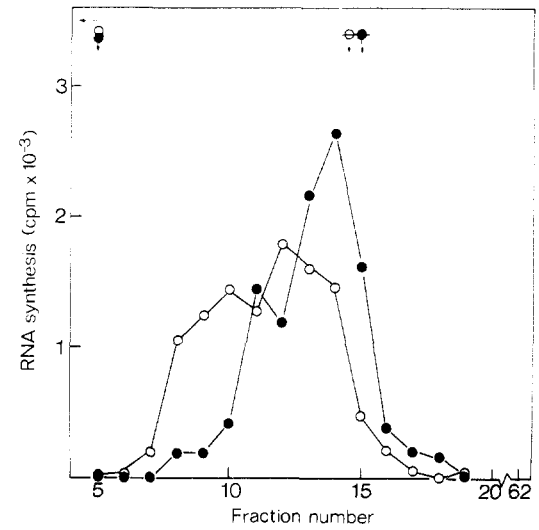

FIGURE 6: Effect of IF-2a on the sedimentation of core RNA polymerase. Parallel gradients were loaded with core RNA polymerase (110 μg) mixed with heated or untreated IF-2a (45 μg) and sedimented as described under Materials and Methods. One-drop fractions were collected, yielding 62 fractions for each gradient. 25-μL aliquots were assayed for RNA polymerase activity with calf thymus DNA as the template. (○) RNA polymerase mixed with heated IF-2a; (●) RNA polymerase mixed with untreated IF-2a. ♀ and ♀ indicate the sedimentation position of catalase and β-galactosidase sedimented as internal markers in the gradient with heated IF-2a, and ♀ and ♀ indicate the corresponding positions in the gradient with active IF-2a.

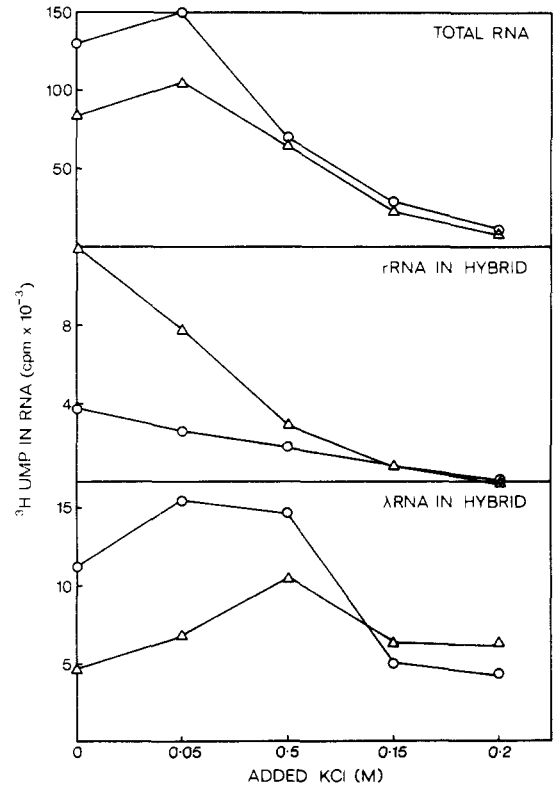

FIGURE 7: Effect of IF-2b on rRNA synthesis from λ d_5 *ilv* DNA. Final RNA polymerase, IF-2b, and DNA concentrations were 12, 2, and 31 μg/mL, respectively. Data points are for 50-μL aliquots of reaction mixtures. (○) RNA polymerase alone; (△) RNA polymerase plus IF-2b.

selectivity to IF-2a. To determine the specificity of the transcriptional effect of IF-2b, we used other DNA species as templates. With *ϕ80 psu*$^+_{III}$ DNA, the factor had little or no influence on either *ϕ80* or *su*$^+_{III}$ tRNA transcription and was similarly without effect on RNA synthesis from isolated

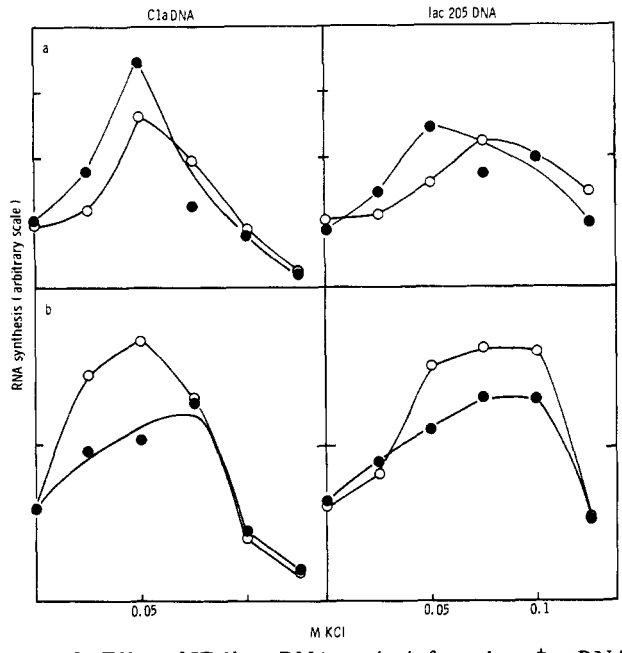

FIGURE 8: Effect of IF-2b on RNA synthesis from the su^+_{III} tRNA promoter and the *lac* UV5 promoter in a mixed transcription system. Cla DNA and *lac* 205 DNA concentrations were respectively 135 and 37 ng/mL in panel a and 270 and 106 ng/mL in panel b. RNA polymerase concentration was 16 μg/mL. RNA synthesis was for 30 min at 30 °C. The transcription products were analyzed by gel electrophoresis (see Materials and Methods). (○) and (●) indicate polymerase alone and polymerase with IF-2b, respectively.

restriction fragments containing the su^+_{III} tRNA promoter (Landy et al., 1974) and the *lac* UV5 promoter (Majors, 1975; Backman et al., 1976) at several polymerase/template ratios (data not shown). However, when these two restriction fragments were used in combination, IF-2b stimulated both su^+_{III} tRNA and *lac* transcription at low ionic strength with a high polymerase/template ratio (Figure 8). By contrast, at a lower polymerase/template ratio the factor was inhibitory to both types of RNA synthesis. In no case did the magnitude of the effect approach that observed for rRNA synthesis.

Discussion

We have shown that preparations of IF-2a and IF-2b both preferentially stimulate the in vitro synthesis of rRNA at low KCl concentrations. This effect is specific for IF-2. IF-3 has little effect on rRNA synthesis (Figure 1) while fMet-tRNA preferentially inhibits the transcription of rRNA (Debenham et al., 1980).

The possibility exists that observed stimulation by IF-2 preparations is not a property of IF-2 itself but of a contaminating protein present in sufficiently small amounts to be undetectable by gel electrophoresis. Such a contaminant would have to be inactivated at 48 °C with the same kinetics as IF-2a and would have to copurify with IF-2a, IF-2b, and possibly EF-Tu through different purification procedures. Although we cannot formally exclude this possibility, we consider it unlikely since the stoichiometric requirement for protein is consistent with IF-2a being the active component. We note that a partially purified protein of similar transcriptional effect of IF-2 has previously been characterized by Block (1976). The method of purification, approximate molecular weight, and temperature lability of this protein all bear strong resemblances to the corresponding properties of IF-2. The possibility therefore exists that the two proteins are identical.

By what mechanism does IF-2 alter transcriptional selectivity? The IF-2 preparation alters both the sedimentation and transcriptional properties of RNA polymerase (Figures 4 and 5). Yet, factor molecules do not appear to cosediment with the enzyme. This suggests that, if the factor does associate with polymerases, the dissociation constant for such a complex must be $>10^{-9}$–10^{-10} M. For comparison, the association constant of fMet-tRNA and RNA polymerase is $\sim10^{-7}$ M. It follows that, if the proposed interaction of IF-2 and RNA polymerase is of a similar magnitude, no cosedimentation would be observed. Furthermore, a much tighter association between the two proteins might be incompatible with their otherwise separate functions in vivo. We have shown elsewhere that when sedimented on a glycerol gradient, RNA polymerase exhibits functional heterogeneity such that the transcriptional selectivity of a population of enzyme molecules is dependent on its position within the overall distribution of polymerase protein (Travers et al., 1980). In particular, rRNA is synthesized most efficiently by molecules in the trailing shoulder of the peak of RNA polymerase. We may therefore explain the effects of IF-2 by assuming that the factor converts those forms of RNA polymerase which normally sediment on the leading edge to forms with a lower sedimentation coefficient corresponding to those which normally sediment at the trailing shoulder. Such a conversion would result in a stimulation of rRNA synthesis. In the absence of auxiliary factors, the distribution between the different structural forms of the enzyme is dependent on ionic strength, such that forms with a lower sedimentation coefficient are favored by KCl concentration ≥0.15 M at 30 °C (A. A. Travers, unpublished observations).

This effect would explain why IF-2 preparations only alter transcriptional selectivity at low ionic strength. A precedent for a change in polymerase function by this type of mechanism exists. Another RNA polymerase effector, ppGpp, reduces the $s_{20,w}$ of the enzyme by ~0.8 S (P. G. Debenham, P. J. G. Butler, and A. A. Travers, unpublished experiments) and concomitantly alters the transcriptional selectivity of the enzyme. The different structural forms of RNA polymerase are stable at 0 °C but equilibrate rapidly at 30 °C (Travers et al., 1980).

This phenomenon would account for the alteration by IF-2 of the sedimentation coefficient of RNA polymerase in the absence of cosedimentation of the two proteins.

The multiplicity of polymerase effectors identified does not necessarily imply a similar multiplicity of discrete binding sites on RNA polymerase. IF-2 and EF-Tu both bind to the same site on the 50S subunit of the ribosome (Heimark et al., 1976), and therefore their interaction with RNA polymerase could occur at a single site analogous to their ribosomal binding site. The similarity of the transcriptional effect of the two factor preparations (Figure 1) could be explained on this basis.

During normal bacteria growth there is considerable evidence that controlling elements other than the nucleotide ppGpp influence the pattern of transcription (Travers, 1976a; Nierlich, 1978). So far the molecular nature of these elements remains obscure. Nevertheless, the effect of IF-2 on transcription in vitro would be fully compatible with a role in which the accumulation of free factor would indicate that ribosomes were fully utilized in protein synthesis and consequently that the cell requires more ribosomes. Such a model would predict that conditional mutants of IF-2 might be defective in both rRNA synthesis and protein synthesis under the restrictive conditions.

Acknowledgments

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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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