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Native Deoxyribonucleic Acid Transcription by Yeast RNA Polymerase-P_{37} Complex†

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ABSTRACT: The specific activity of yeast RNA polymerases A or B, when complexed with P_{37} cofactor, compares favorably with that of *E. coli* RNA polymerase. The stimulation is observed only with double-stranded DNA but does not result from DNase action. The K_m for nucleotide substrates and the optimal conditions of transcription are not modified. P_{37} stimulates RNA synthesis by ternary transcription complexes in the presence of poly(rI) which prevents reinitiations. The RNA chain length, estimated by 5′ end labeling or sedimentation, is increased in the presence of P_{37}. On the other hand, the trinucleotide synthesis, which reflects the chain initiation reaction, is not affected. Therefore, the cofactor appears to act at the elongation step of RNA synthesis.

Purified eucaryotic RNA polymerases display a very low specific activity on double-stranded DNA templates which do not contain nicks or gaps (Gniadowski et al., 1970; Nohara et al., 1973; Flint et al., 1974; Gissinger et al., 1974; Dreyer & Hausen, 1976). Yeast RNA polymerase B in particular was shown to require single-stranded regions to transcribe native DNAs (Dezélée et al., 1974a). Several reports mention proteins which increase, to various extents, the activity of eucaryotic RNA polymerases. Most of them allow the synthesis of larger RNA transcripts on native templates (Stein & Hausen, 1970; Seifart et al., 1973; Lee & Dahmus, 1973; Sekimizu et al., 1976; Ernst & Sauer, 1977; Spindler, 1979; Revie & Dahmus, 1979). However, the functional significance of these proteins has not been clearly established.

We have recently described a yeast protein, called P_{37}, which stimulates transcription of double-stranded DNA by binding to the homologous RNA polymerases A and B, a fact that strongly suggests the in vivo involvement of P_{37} in RNA synthesis (Sawadogo et al., 1980a,b). Here, we show that the activity of the yeast RNA polymerases, in the presence of P_{37}, compares favorably with that of *E. coli* RNA polymerase even with intact DNA duplex. The mode of action of P_{37} was studied in the case of RNA polymerase B. The stimulation occurs mainly at the elongation step.

Material and Methods

RNA Polymerases and Other Enzymes. Yeast RNA polymerase B was prepared as previously described (Sawadogo et al., 1980a). Standard reaction mixtures (0.1 mL) contained

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70 mM Tris-HCl, pH 8, 2 mM $MnCl_2$, 1 mM dithiothreitol, 25 mM ammonium sulfate, 0.4 mM each of GTP, ATP, and CTP, 0.2 mM [^{3}H]UTP (10–40 cpm/pmol), and 50 μg/mL of native calf-thymus DNA. The reactions were initiated by the addition of RNA polymerase B as indicated and performed at 30 °C. Samples were processed by using the conventional acid precipitation technique to collect the RNA. Yeast RNA polymerase A was purified as previously described (Sawadogo et al., 1980b). Incubation conditions were 70 mM Tris-HCl, pH 8, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM each of GTP, ATP, and CTP, 0.5 mM [^{3}H]UTP (10–40 cpm/pmol), and 50 μg/mL of native calf-thymus DNA, *E. coli* RNA polymerase was prepared and assayed as described by Darlix et al. (1969). Specific activities are expressed in nanomoles of UMP incorporated into RNA in 1 h per milligram of RNA polymerase.

Endonuclease S_1 was from Boehringer; nicking-closing enzyme was a gift from Dr. M. Duguet (Institut Gustave Roussy).

Stimulating Factor (P_{37}). The procedure for the purification of the stimulating factor is identical with that previously described (Sawadogo et al., 1980a,b). An alternative procedure was used to obtain a P_{37} preparation free of contaminating proteins; this was required to measure the affinity of the factor for the template. After the DEAE-cellulose chromatography step of the normal procedure, the preparation (14 mL, 0.11 mg of protein/mL) was loaded onto a CM-glycophase (Corning) column (0.8 × 7 cm) previously equilibrated with a buffer containing 0.02 M Tris-HCl, pH 8, 1 mM EDTA, 10 mM 2-mercaptoethanol, 25 mM ammonium sulfate, and 5% glycerol. Elution was performed with a 80-mL linear ammonium sulfate gradient between 25 and 450 mM, at a flow rate of 15 mL/h. Fractions from 240 to 280 mM, containing the stimulating activity, were pooled and dialyzed against the same buffer containing 25 mM ammonium sulfate and 70% glycerol. This step eliminated the main contaminant (P_{49} protein) from the factor preparation which was then 90% pure; however, it also had adverse effects on factor stability and was therefore not used in further tests.

Nucleic Acids. Calf-thymus DNA was from Boehringer. It was treated by endonuclease S_1 as described by Dezélée et al. (1974a). Plasmid pJD14 (form I) containing the structural gene for yeast alcohol dehydrogenase I (Williamson et al., 1980) was prepared according to the method described by Guerry et al. (1973). Closed relaxed pJD14 DNA was obtained by treatment with nicking-closing enzyme as described by Germond et al. (1975).

Determination of DNase Activities in the Factor Preparation. Exonuclease activity was assayed by measuring the release of acid-soluble radioactivity from T_7 ^{3}H-labeled DNA (5 μg; 60 000 cpm/μg) after a 20-min incubation, in a final volume of 100 μL, with 1.2 μg of factor, under the conditions used for transcription. Endonuclease activity was monitored by the conversion of supercoiled DNA to the nicked relaxed form on agarose gel electrophoresis.

Testing for Permanent Alterations in Native DNA Template Induced by Incubation with the Factor. Endonuclease S_1 treated calf-thymus DNA (75 μg/mL) was incubated for 25 min at 30 °C with or without the factor preparation (40 μg/mL, CM-Sephadex fraction) in the standard RNA polymerase assay conditions. The reactions were stopped by the addition of 0.1% $NaDodSO_4$, and the two DNAs were extracted successively with phenol, phenol–chloroform (1:1 v/v), chloroform–isoamyl alcohol (24:1 v/v), and ether. They were then extensively dialyzed against a buffer containing 2 mM Tris-HCl, pH 7.5, 10 mM NaCl, and 0.1 mM EDTA and then concentrated against the same buffer in 60% glycerol. After a last dialysis to eliminate glycerol, these DNAs were used, at different concentrations, as template in standard conditions for RNA polymerase B in the presence or absence of P_{37}.

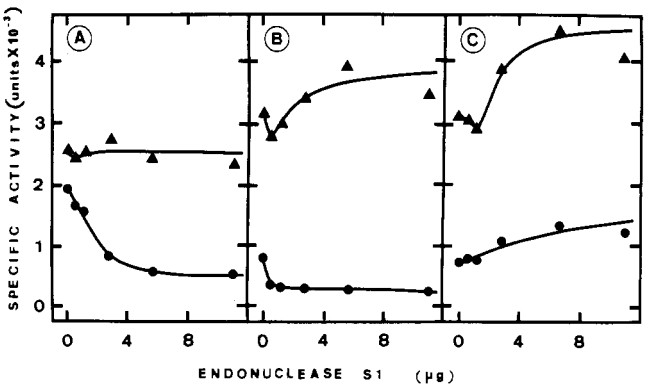

FIGURE 1: Influence of the template integrity on the specific activity of yeast RNA polymerase A and B in the presence or absence of P_{37}. Comparison with *E. coli* RNA polymerase; calf-thymus DNA (100 μg) was incubated 30 min at 37 °C with increasing amounts of endonuclease S_1. The different DNAs were then used as template under the standard conditions for each RNA polymerase. Incubations were carried out for 20 min. (A) Transcription by yeast RNA polymerase A (5.8 μg/mL) in the presence (▲) or absence (●) of P_{37} (8 μg/mL, CM-Sephadex fraction); (B) transcription by yeast RNA polymerase B (3.9 μg/mL) in the presence of (▲) or absence (●) of P_{37} (8 μg/mL, CM-Sephadex fraction); (C) transcription by *E. coli* RNA polymerase (6 μg/mL), core enzyme (●) or holoenzyme (▲).

Michaelis Constant Determination. Incubations were performed under standard conditions using 10 μg/mL of RNA polymerase B with or without factor (12 μg/mL of CM-Sephadex fraction). The nucleotide concentration was varied in each case from 4×10^{-4} M to 4×10^{-5} M, and the K_m was determined by the linear regression method.

Synthesis of Trinucleotide Diphosphates. Incubation mixtures (50 μL) contained 70 mM Tris-HCl, pH 8, 2.5 mM $MnCl_2$, 0.5 mM dithiothreitol, 20 mM ammonium sulfate, 0.2 mM UpA, 5 μM [α-^{32}P]UTP (10 cpm/pmol), 1 μg of pJD14 DNA, and 0.5 μg of RNA polymerase. After 30-min incubation at 30 °C, the reaction was stopped by the addition of 10 μL of 0.1 M EDTA. Aliquots of 5 μL were spotted on poly(ethylenimine) sheets (Macherey-Nagel CEL 300 PEI), and the chromatograms were developed and analyzed as previously described (Lescure et al., 1981).

Results

Stimulation Depends upon the Template Transcribed. The transcription by yeast RNA polymerase B of synthetic templates such as $(dT)_n$, $d(A\text{-}T)_n$, $d(I\text{-}C)_n$, or $(rC)_n$ was unaffected by the presence of P_{37}. With denatured DNA as template, only a slight stimulation was observed, while the transcription of native DNA was enhanced, depending on the DNA preparation, from 4 to 50 times by the addition of the cofactor. It has been shown that yeast RNA polymerase B requires the presence of single-stranded DNA regions to initiate transcription (Dezélée et al., 1974a). It was therefore of interest to investigate whether intact DNA could be transcribed in the presence of P_{37}. For this purpose, the DNA was treated with increasing concentrations of endonuclease S_1 and used as template for yeast RNA polymerase A or B alone or with P_{37} cofactor (Figure 1, A and B). A control reaction was run with the bacterial RNA polymerase, holoenzyme, or core enzyme (Figure 1, C). While the activity of the yeast enzymes alone decreased with the removal of the single-stranded regions from the DNA by the endonuclease S_1 treatment, the tran-

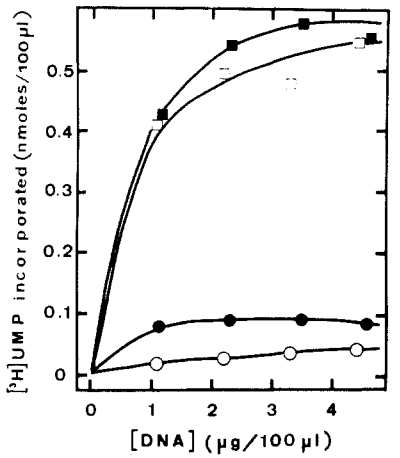

FIGURE 2: Influence of the preincubation of native DNA with the factor on its subsequent template activity, in the presence or absence of P_{37}. Standard transcription mixtures contained, at the indicated concentrations, either factor treated calf-thymus DNA (●, ■) or control DNA (○, □) (see Material and Methods). The incubations were carried out for 15 min with 5 µg/mL of RNA polymerase B, in the presence (□, ■) or absence (○, ●) of P_{37} (8 µg/mL, CM-Sephadex fraction).

scription in the presence of the cofactor remained unaffected. Rather, a slight increase in activity was observed, as with the bacterial enzyme, owing presumably to the disappearance of the nicks (Vogt, 1973), which acted as nonproductive binding sites.

The enhancement of transcription by P_{37} could be due to the presence of DNases in the factor preparation. It has been shown in the case of the yeast RNA polymerase B that the introduction of nicks in the DNA is not sufficient to increase its template activity and that single-stranded gaps have to be introduced (Dezélée et al., 1974a). The putative DNase should therefore be an exonuclease. However, there was no evidence for exonucleolytic activity in the factor preparation, since incubations of the factor with radioactive DNA, under the conditions used for transcription, did not measurably generate acid-soluble material (less than 0.5% of controls). The contaminating endonucleasic activities were equally negligible: when supercoiled DNA was transcribed in the presence of P_{37}, no more than 10% of the DNA was converted to the relaxed form as revealed by analysis of the template before and after incubation by agarose gel electrophoresis. Furthermore, as shown in Figure 2, the stimulation does not result from an irreversible modification of the template, since a DNA preparation preincubated with the factor and then phenol extracted remained poorly transcribed by RNA polymerase B alone. The factor has then to be present in the transcription mixture to allow for an efficient transcription.

Optimal Conditions for Transcription Are Unaffected by P_{37}. Many properties of the yeast RNA polymerase B are unaltered by the binding of the cofactor. Among these, optimal salt concentration, optimal concentration of divalent cations, and inhibition by α-amanitin are not modified. The affinity of the enzyme for DNA does not change appreciably either since the stimulation due to P_{37} is independent of the DNA concentration. The RNA analogue $(rI)_n$ added before the start of transcription inhibits almost to the same extent the transcription of denatured DNA by RNA polymerase B alone and the transcription of native DNA in the presence of P_{37} (results not shown).

As shown in Table I, there was no effect of the factor on the K_m for the nucleotide substrates, whichever the template transcribed. In the case of *E. coli* RNA polymerase, it has been shown that the addition of σ factor to the core enzyme stimulates transcription of $(dT)_n$ at low ATP concentration only, which reflects the effect of σ on the initiation step (Nyogi, 1972). No similar effect was obtained with P_{37} and the yeast enzyme (Table I). The mechanism by which P_{37} stimulates transcription appears in this respect different from that of σ.

Table I: Michaelis Constants for the Different Nucleoside Triphosphates

template	nucleotide	K_m[a] $-P_{37}$	K_m[a] $+P_{37}$
calf-thymus denatured DNA	GTP	42	57
	ATP	26	32
	CTP	23	13
	UTP	20	26
calf-thymus native DNA	GTP	43	35
	ATP	26	28
	CTP	14	20
$(dT)_n$	ATP	401	445

[a] Expressed in µM.

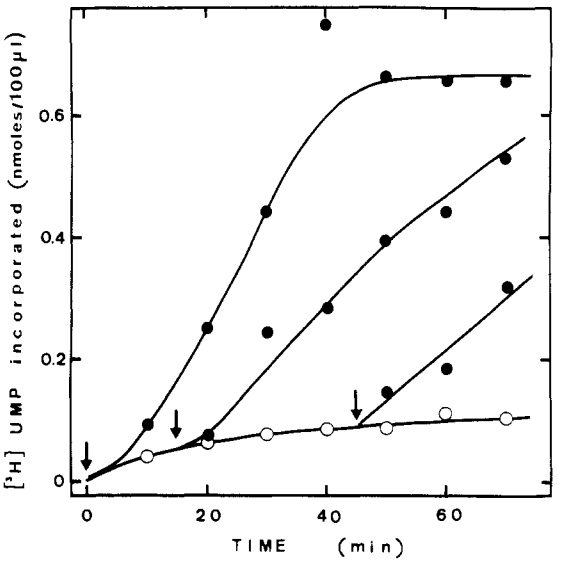

FIGURE 3: Effect of the addition of P_{37} at different times during RNA synthesis by yeast RNA polymerase B. Native calf-thymus DNA was used as template under standard conditions for RNA polymerase B (9 µg/mL). The stimulation factor (3.6 µg/mL of P_{37}, second phosphocellulose purified fraction) was either absent during the entire time course (○) or added after 0, 15, or 45 min of transcription (●).

Stimulation Affects the Elongation Step. The stimulation by P_{37} was independent of the time of factor addition (Figure 3). Often, a short lag phase was observed, before stimulation, when P_{37} was added before the start of transcription (see also Figure 5). This suggested that the factor was not required during the early steps of transcription. To investigate whether the factor acted at the initiation or elongation step of RNA synthesis, we used poly(rI) to prevent all possibilities of reinitiation. The inhibitor binds to free RNA polymerases and destabilizes the binary RNA polymerase–DNA complexes without affecting the transcribing ternary complexes (Hirschbein et al., 1967; Bautz et al., 1972; Dezélée et al., 1974b). As shown in Figure 4, the addition of P_{37} 2 min after poly(rI) brought about a strong stimulation of the residual RNA synthesis. This observation is clearly suggesting that P_{37} favored the elongation of the RNA chains made by the preexisting transcription complexes. Even after a long time had elapsed since the addition of poly(rI) (more than 1 h), it was possible to resume transcription by adding P_{37} (data not shown).

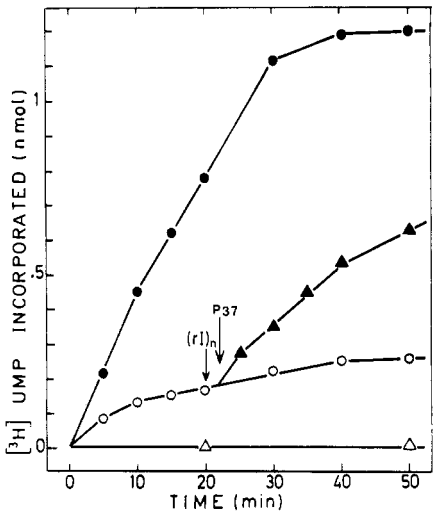

FIGURE 4: Effect of P_{37} on the residual RNA synthesis after $(rI)_n$ addition. Incubation mixtures were standard for salt and nucleotides concentrations and contained 50 μg/mL native calf-thymus DNA and 6 μg/mL RNA polymerase B. Samples of 0.1 mL were processed at different times. (O) RNA synthesis by RNA polymerase B alone; (●) RNA synthesis in the presence of P_{37} (6 μg/mL, second phosphocellulose fraction) added before incubation; (▲) RNA synthesis with P_{37} added at 22 min, 2 min after addition of $(rI)_n$ (100 μg/mL).

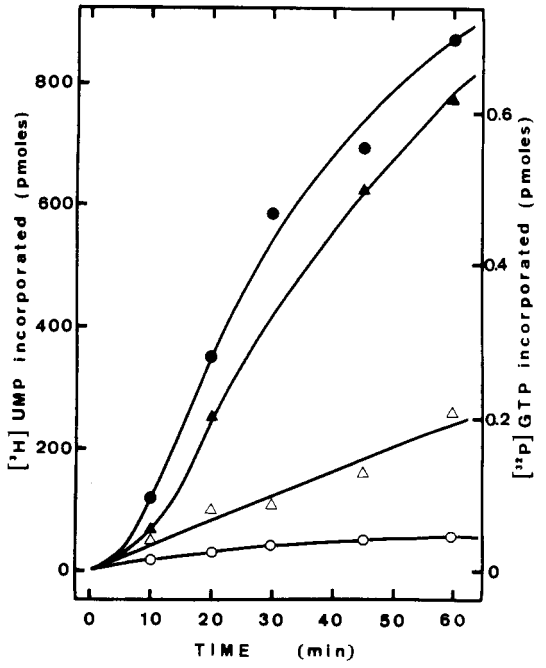

FIGURE 5: Effect of P_{37} on the kinetics of RNA synthesis and chain initiation by yeast RNA polymerase B. Transcriptions were done under standard conditions with the exception of GTP concentration which was decreased to 10^{-4} M, the other three nucleoside triphosphates being 4×10^{-4} M. Specific radioactivities were 16 cpm/pmol of [^{3}H]UTP and 1377 cpm/pmol of [γ-^{32}P]GTP. Each incubation mixture (0.1 mL) contained either 1 μg of RNA polymerase B alone (open symbols) or the same amount of enzyme and 0.4 μg of P_{37} (second phosphocellulose fraction) (filled symbols). The reactions were stopped when indicated by addition of 50 mM EDTA and 10^{-2} M cold GTP. Background incorporation of ^{32}P without DNA, with or without P_{37}, was <0.1 pmol. (O, ●) [^{3}H]UMP incorporation; (△, ▲) [γ-^{32}P]GTP incorporation.

The above interpretation of the results obtained with poly(rI) was reinforced by a double-labeling experiment where the total amount of RNA synthesized and the number of RNA chains initiated with [γ-^{32}P]GTP were measured. Disregarding the chain initiation by ATP, the apparent average chain length of 1000 to 1200 nucleotides was multiplied by 5 in the presence of P_{37} (Figure 5). The 3-fold increase in the number of chains

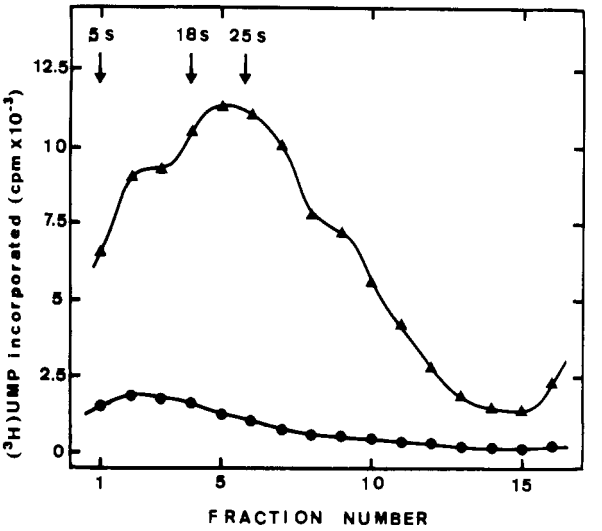

FIGURE 6: Sucrose gradient centrifugation of transcription products. Transcriptions were performed under standard conditions in the presence of RNA polymerase B (5 μg/mL) using as template a calf-thymus DNA previously treated with endonuclease S_1. The reactions, carried out for 30 min, were stopped by addition of Na-$DodSO_4$ (0.1%); 200 μL of each incubation was loaded on top of a sucrose gradient (4.2 mL, 10–30%) performed as described by Sudgen & Keller (1973). The gradients were centrifuged in a SW 56 rotor at 50000 rpm for 165 min at 17 °C. Fractions of 275 μL were collected from the top of the tubes, and the RNA was precipitated by trichloroacetic acid after addition of 5 μg of cold RNA. The specific radioactivity of the [^{3}H]UTP was 583 cpm/pmol when RNA synthesis was performed by RNA polymerase B alone (●) and 176 cpm/pmol for the transcription in the presence of P_{37} (8 μg/mL, CM-Sephadex step) (▲). The arrows indicate the sedimentation under the same conditions of ^{32}P-labeled yeast ribosomal RNA.

Table II: Effect of P_{37} on Trinucleotide Synthesis and Total RNA Synthesis

reaction	template	UMP incorporation[a] $-P_{37}$	UMP incorporation[a] $+P_{37}$[b]
trinucleotide synthesis	supercoiled	150	91
	relaxed[c]	8	10
RNA synthesis	supercoiled	210	340
	relaxed[c]	18	120

[a] Expressed in pmol/μg of RNA polymerase B. [b] 6 μg/mL of CM-Sephadex fraction. [c] Relaxed pJD14 was obtained by treatment with nicking-closing enzyme as described by Germond et al. (1975).

initiated was probably due to reinitiations occurring after completion of the RNA chains, because no stimulation of [γ-^{32}P]GTP incorporation was observed when transcription was carried out in the presence of only three nucleoside triphosphate (result not shown).

Another illustration of the effect of P_{37} on RNA chain length is provided by the experiment in Figure 6, where the RNA was sedimented in sucrose gradient. The bulk of the RNA synthesized by yeast RNA polymerase B alone sedimented around 10 S; when the factor was present in the incubation mixture, half of the transcripts were between 15 and 35 S, and many were larger.

Initiations Are Not Affected. The efficiency of RNA chain initiation can be estimated by measuring the accumulation of trinucleotides, using a dinucleotide as a primer and one nucleoside triphosphate. Yeast RNA polymerase B catalyzes a very efficient synthesis of trinucleotides on supercoiled DNA; this reaction is drastically lower on relaxed templates (Lescure et al., 1981). We compared the effect of P_{37} on RNA synthesis

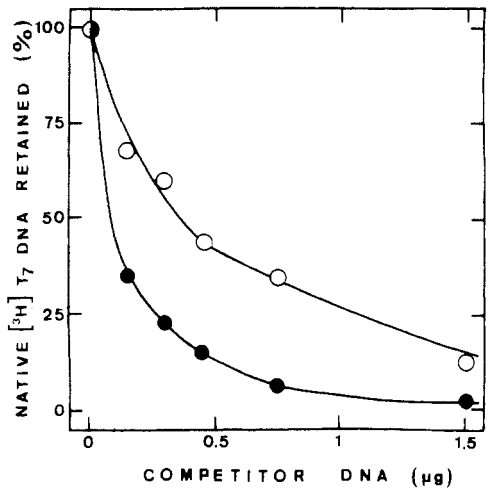

FIGURE 7: Retention of P_{37}–T_7 ^3H-labeled DNA complex on membrane filter. Effect of native or denatured competitor DNA. Binding mixtures (0.1 mL) contained 0.07 M Tris-HCl, pH 8, 1 mM dithiothreitol, 1 mM ammonium sulfate, 0.4 μg of T_7 ^3H-labeled DNA (60000 cpm/μg), and 0.17 μg of P_{37} (CM-glycophase purified; see Material and Methods). Excess unlabeled competitor was added as indicated. After 3-min incubation at 30 °C, the mixtures were diluted with 0.5 mL of the same buffer and then immediately filtered on nitrocellulose membranes. The filters were washed twice with 0.5 mL of buffer and dried, and their radioactivity was determined. (●) Denatured T_7 DNA; (○) unlabeled native DNA.

as well as on trinucleotide synthesis using as template the pJD14 plasmid, supercoiled or relaxed by the nicking-closing enzyme [pJD14 plasmid contains the structural gene for yeast alcohol dehydrogenase I cloned in pBR322: Williamson et al. (1980)]. The results are summarized in Table II. P_{37} had no stimulatory effect on the trinucleotide synthesis, whichever the structure of the template. Yet, under the same conditions, the addition of P_{37} stimulated 6 times the total RNA synthesis on the relaxed form of the DNA. Note that supercoiled DNA is a much more active template than relaxed DNA. In fact, the transcription of supercoiled DNA was only slightly enhanced by P_{37} for incubation times less than 30 min and appeared significant only for longer incubations. This experiment clearly demonstrates that the stimulation of transcription by P_{37} does not result from an effect of the factor on the initiation reaction.

The Factor Interacts with the Template. Although no permanent alteration of the template could be detected after incubation with the factor, the possibility remained that P_{37} protein, which is basic, could interact with DNA during transcription. For an investigation of the DNA binding properties of P_{37}, the factor was further purified on CM-glycophase (see Materials and Methods). The retention by P_{37} of radioactive native DNA on nitrocellulose filters is shown in Figure 7. The competition experiments showed that P_{37} bound preferentially to single-stranded DNA (Figure 7).

Discussion

We have previously established that the stimulation of native DNA transcription by yeast RNA polymerase A or B followed the binding of the factor to the enzyme (Sawadogo et al., 1980a,b). This affinity of P_{37} for the RNA polymerase is strong since the observed dissociation constant (5×10^{-8} M for RNA polymerase B) is of the same order of magnitude as that of σ factor for *E. coli* RNA polymerase (Campbell & Lowe, 1977). P_{37} must therefore be considered as a cofactor which modifies the enzymatic properties of the RNA polymerase. In the presence of P_{37}, the conditions required for optimal transcription were not modified, nor was the affinity of the RNA polymerase for DNA, RNA, or nucleoside triphosphates. No stimulation occurred when denatured DNA was transcribed. The factor is therefore required for one of the steps of RNA synthesis which are rate limiting with double-stranded DNA.

It is generally assumed that the very low specific activity of purified eucaryotic RNA polymerases on native DNA templates results from an incapacity of these enzymes to initiate (Roeder, 1976). Remarkably, the activity of the yeast RNA polymerases became, in the presence of P_{37} factor, similar to that of the bacterial RNA polymerases. Yet it appeared that the increase in RNA synthesis in the presence of P_{37} was mainly the result of an increase in RNA chain elongation and not in RNA chain initiation. A stimulation was indeed obtained under conditions where reinitiations were hindered by the addition of $(rI)_n$. The elongation inhibitor actinomycin D prevented the stimulating effect of the factor (M. Sawadogo, unpublished observations). Moreover, the trinucleotide synthesis was not affected by the addition of the factor.

Endonuclease S_1 treated templates, which are devoid of artificial initiation sites, were efficiently transcribed in the presence of P_{37}. Since P_{37} affects essentially the elongation step, yeast RNA polymerase B certainly initiates on intact double-stranded DNA. This is in accordance with our previous conclusion, based on the studies of trinucleotide synthesis, that it is the elongation, rather than the initiation, which is the limiting step of the transcription by the RNA polymerase alone (Lescure et al., 1981). Yeast RNA polymerase B synthesizes long RNA chains on denatured DNA (Dezélée & Sentenac, 1973), but the RNA chains produced with native DNA are much shorter than the in vivo transcripts. The elongation step is therefore deficient with the purified enzyme. P_{37} restores a normal elongation reaction since chains averaging 6000 nucleotides were synthesized in its presence.

One possible explanation for the effect of P_{37} on RNA chain elongation is that P_{37} prevents the inhibition of the RNA polymerase by the growing RNA chain by dissociating the DNA–RNA hybrid. However, the affinity of the enzyme for the RNA analogue $(rI)_n$ is not modified by the binding of the cofactor. An alternate possibility arises from the fact that for native DNA transcription, the DNA double helix has to be continuously melted by the translocating RNA polymerase. This has been shown to be one of the rate-limiting steps in the case of *E. coli* RNA polymerase (Solage & Cedar, 1976). P_{37}, once bound to the RNA polymerase, might favor this process by interacting with the DNA. Indeed, purified P_{37} showed some affinity for the template, with a preference for single-stranded as compared to double-stranded DNA.

Acknowledgments

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Equilibrium Studies of Ethidium–Polynucleotide Interactions†

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ABSTRACT: We report equilibrium dialysis studies of the binding of ethidium to a variety of double-helical synthetic polynucleotides containing A·U (or T) and I·C base pairs. The results are interpreted in terms of the neighbor exclusion model of drug binding, with allowance both for cooperativity of binding and for a structural switch of the helix to a different form which binds the drug more effectively. Both DNA and the alternating copolymers examined [poly[d(A-T)] and poly[d(I-C)]] showed high affinity (10^4–10^5 M^{-1}) in 1 M salt. Homopolynucleotides showed a more complicated pattern of affinities: poly(rA)·poly(rU), poly(rA)·poly(dT), and poly(dA)·poly(rU) showed high affinity, whereas poly(dA)·poly(dT), poly(rI)·poly(rC), and poly(dI)·poly(dC) showed low affinity ($\leq 10^3$ M^{-1}). The neighbor exclusion range was inferred to be two base pairs for DNA or B family helices and three for RNA or A family helices. Generally, polynucleotides showed some cooperativity in their ethidium binding. The data reveal a switch of poly[d(I-C)] to a form able to bind ethidium more effectively.

The cationic dye ethidium has found exceptionally wide use in biochemical and physical studies of nucleic acids, ranging from utilization as a fluorescent stain to crystallographic studies of its complex with oligonucleotides (Tsai et al., 1975). A problem of long-standing interest has been the extent and origin of specificity in the intercalation of ethidium into double-helical nucleic acids. Little dependence of the binding affinity on overall base composition is found (Waring, 1965; Le Pecq & Paoletti, 1967; Müller & Crothers, 1975), but the work of Krugh and his colleagues has established a definite preference for binding to pyrimidine-(3′–5′)-purine sequences compared to purine-(3′–5′)-pyrimidine in both ribo- and deoxyribooligonucleotides (Krugh et al., 1975; Krugh & Reinhardt, 1975; Kastrup et al., 1978). A similar conclusion was reached by Patel & Canuel (1976).

A general description of drug–nucleic acid binding equilibria requires not only specification of the binding constant of the drug to an isolated binding site but also a description of how the bound drug molecules interact. It has long been recognized that intercalative binding saturates well before occupation of all the spaces between the base pairs, a phenomenon which has been explained by exclusion of drug molecules from empty sites which are adjacent to a bound drug (Cairns, 1962; Crothers, 1968). Intercalative binding of ethidium to deoxyribonucleic acid (DNA)[1] saturates at one drug per two base pairs, and the complete binding isotherm fits with high accuracy to that calculated by using the neighbor exclusion model (Bauer & Vinograd, 1970; Bresloff & Crothers, 1975). The alteration of sugar pucker found in the crystal of ethidium with double-helical dinucleotides has been invoked to explain the

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[1] Abbreviations used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; EDTA, ethylenediaminetetraacetic acid; EB, ethidium bromide; Tris, tris(hydroxymethyl)aminomethane.

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