A NEW EUKARYOTIC RNA POLYMERASE FACTOR: A FACTOR FROM CHICKEN MYELOBLASTOSIS CELLS WHICH STIMULATES TRANSCRIPTION OF DENATURED DNA

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Summary. A heat-stable protein factor, capable of stimulating RNA synthesis by nuclear RNA polymerase II, was found in isolated nuclei of chicken myeloblastosis cells. It is adsorbed to a DEAE-Sephadex column used for RNA polymerase purification and then is eluted with 0.1 M ammonium sulfate. This factor appears to differ from previously reported eukaryotic RNA polymerase factors in its property of stimulating the activity of denatured (or single-stranded) DNA template. When heated, this factor contains no detectable endonuclease or exonuclease activity. The degree of stimulation is greater with chicken myeloblastosis RNA polymerase IIb than IIa and is most efficient when homologous DNA is used as template. This factor causes no stimulation of E. coli RNA polymerase.

Studies on RNA polymerase of bacteria and other microorganisms indicate that protein factors may play a major role in the positive regulation of gene expression (1). The sigma factor (2,3) is described as a constituent of the E. coli complete enzyme and is responsible for the accurate and efficient initiation of RNA chains at specific sites on the DNA template. There are known accessory factors for RNA synthesis which either provide for an increased frequency of initiation (4) or which determine the specific termination of RNA chains (5).

Reports have appeared concerning the existence of stimulating factors of RNA polymerase in eukaryotes (6-12). These factors have specificities directed toward either polymerase I or polymerase II, but stimulate only when double-stranded (native) DNA is used as template. Enzyme activities measured with denatured or single-stranded DNA are either unaffected (7,10) or, in some cases, completely inhibited (6,11,12) by the addition of these factors. The mechanism of the action of these factors is not understood. Since RNA synthesis by isolated polymerase uses denatured DNA as a preferential template (polymerase II) or uses it as well as native DNA (polymerase I), factors which modify enzyme activity on denatured DNA template would be significant in

understanding the mechanism of eukaryotic gene transcription.

In the present communication we report the isolation of a factor which stimulates nucleoplasmic RNA polymerase (polymerase II) of chicken myeloblastosis cells when denatured or single-stranded DNA is used as template.

MATERIALS AND METHODS

Purification of nuclear RNA polymerase II from chicken myeloblastosis cells (13): DNA-dependent RNA polymerase was isolated from nuclei of 6 grams of chicken myeloblastosis cells (14) and purified through DEAE-Sephadex column according to a procedure reported by Roeder and Rutter (15). RNA polymerase I, IIa and IIb were resolved from the column and IIa and IIb were further purified by glycerol gradient centrifugation (10-40%). The activities of polymerases IIa and IIb require the presence of all four nucleoside triphosphates, depend entirely upon added DNA template, are sensitive to DNAse and yield RNase-sensitive products. Both enzymes are stimulated by ammonium sulfate with the maximum stimulation being at 0.02 M. According to kinetics of UMP incorporation at high enzyme concentrations, the enzymes appear to be devoid of nuclease activity (13).

RNA polymerase assay: The standard assay system contained (in a 0.1 ml reaction mixture): 50 mM Tris-HCl (pH 7.9), 1 mM $MnCl_2$, 10 mM KCl, 2 µg pyruvate kinase, 4 mM phosphenol pyruvate, 1 mM dithiothreitol, 0.2 mM each of ATP, GTP and CTP, 0.04 mM UTP and ^{3}H-UTP (600-700 cpm/p mole) 40 µg denatured (heated at 100°C for 4 min followed by quick cooling) calf thymus DNA and enzyme. Ammonium sulfate was adjusted to a final concentration of 0.02 M. After incubation for 45 min at 37°C, the reactions were stopped by the addition of CCl_3COOH, samples filtered, and radioactivity counted in a scintillation counter.

RESULTS

DEAE-Sephadex chromatography of nuclear RNA polymerase and the existence of a stimulation factor: DEAE-Sephadex chromatography of soluble nuclear RNA polymerase of chicken myeloblastosis cells resolves a minor peak and two major peaks of enzyme activity (Fig. 1). They were designated peak I, IIa and IIb, respectively, with regard to their sensitivity to alpha-amanitin (Calbiochem) inhibition. Peak fractions of enzyme IIa and IIb were pooled separately and further purified by glycerol gradient centrifugation. These partially purified enzymes were assayed in the presence of 5 µl aliquots of each wash fraction from the DEAE-Sephadex column and a stimulation factor was found (Fig. 2). The activity of enzyme IIb was stimulated more than IIa and its profile was sharper. Peak activity of the stimulation factor (Fig. 2) was close to the peak activity of polymerase I (Fig. 1) but they were not superimposed. However, in the following experiments where factor is included, the activity

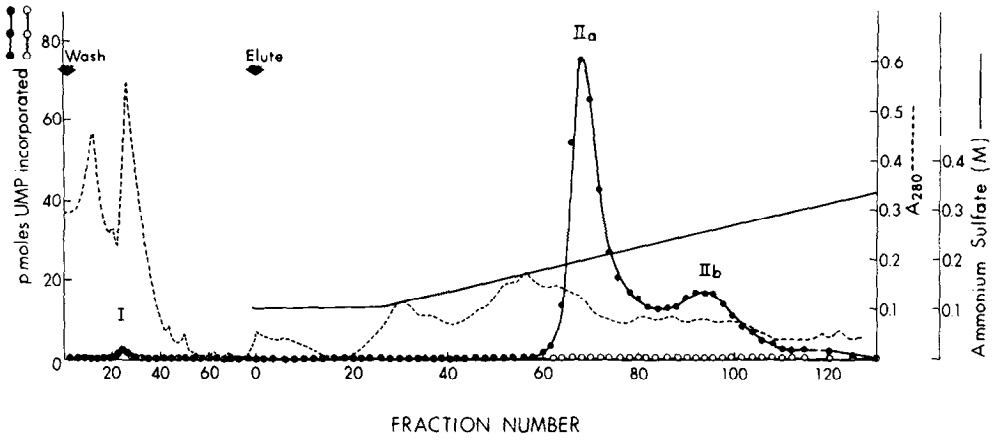

Figure 1

DEAE-Sephadex chromatography of nuclear RNA polymerase from chicken myeloblastosis cells: Soluble nuclear RNA polymerase was chromatographed on a 1.5 x 16 cm DEAE-Sephadex A-25 column, which was pre-equilibrated with 0.05 M ammonium sulfate in TGMED (15). After sample loading, the column was washed with 40 ml of 0.1 M ammonium sulfate in TGMED, and eluted with a linear gradient of TGMED buffer from 0.1 to 0.4 M ammonium sulfate. Wash fractions were collected in 0.5 ml aliquots and the gradient was collected at 1 ml per fraction at flow rate of 0.5 ml/min. 0.025 ml of each fraction was assayed for RNA polymerase activity. The assay conditions were as described as in Materials and Methods, except 5 mM Mg^{++} was included and $(NH_4)_2SO_4$ was omitted. The UMP incorporation represents p moles UMP incorporation in 30 min. per 0.025 ml of each fraction assayed in 0.1 ml reaction mixture. (●-●-●), Activity in the absence of ∝-amanitin. (o-o-o), Activity in the presence of 2 µg/ml ∝-amanitin.

due to RNA polymerase I was subtracted (usually extremely low due to low quantity of factor added) and the stimulation effect of the factor on the enzymes is not a result of an additive effect of two polymerases. Kinetic study shows that the factor only stimulates the activity of the myeloblastosis enzymes but does not effect E. coli RNA polymerase (Fig. 3).

Characterization of the stimulation factor: Heating for 10 min at 100°C or prolonged dialysis of the factor did not destroy its stimulatory activity, whereas protease treatment resulted in loss of activity (Table I). Exonuclease activity was found in the stimulation factor, but it is lost upon heating at 100°C for 15 min (Table II). The heated factor had no detectable endonuclease activity under the conditions of the RNA polymerase assay (Fig. 4).

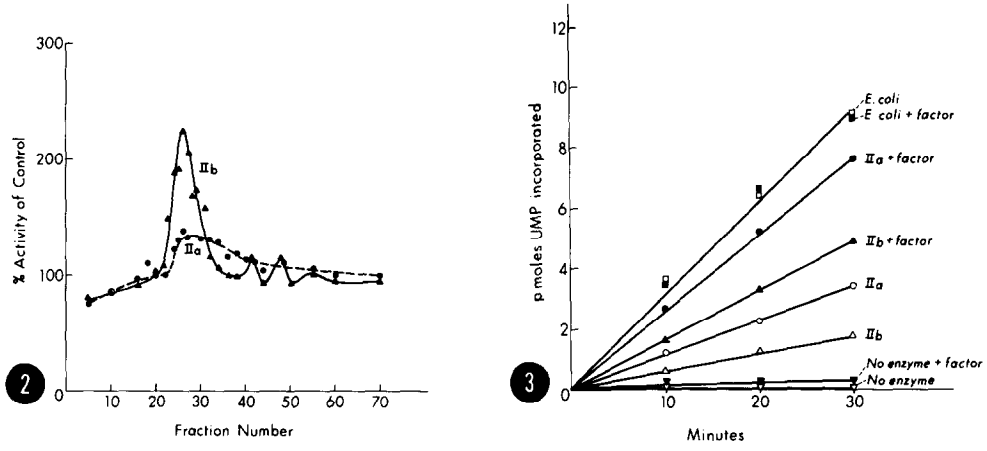

Figure 2

Existence of a stimulation factor in the DEAE-Sephadex wash fractions and its influence on RNA synthesis by enzymes IIa and IIb: A 5 µl aliquot of each 0.1 M ammonium sulfate wash fraction from DEAE-Sephadex chromatography (Fig. 1) was added to a 0.1 ml assay mixture containing the glycerol gradient prepared enzyme IIa or IIb. The amounts of enzyme IIa or enzyme IIb added were adjusted so that the control (no factor added) counts of both enzymes were about the same (in the range of 6-7 pmoles). The activity due to the factor alone in each fraction (less than 10%) was subtracted to calculate the % activity. The other assay conditions were as described in Materials and Methods.

Figure 3

The effect of the factor on the rate of RNA synthesis: Peak fractions of the stimulation factor were combined and 0.02 ml of the combined solution was added to each assay (assay volume 0.4 ml) containing the glycerol gradient enzyme IIa or enzyme IIb, or E. coli RNA polymerase (Biopolymers, General Biochemicals). Aliquots (75 µl) were removed from the incubation mixture at the time indicated and the pmoles of UMP incorporated determined. The other assay conditions were as described in Materials and Methods.

Stimulation of RNA polymerase IIa and IIb on denatured or single-stranded DNA template activities by the factor: Enzymes IIa and IIb have a strong preference for denatured over native DNA template. However, there is no template specificity for the stimulatory effect of the factor (Table III): it stimulates RNA synthesis with native DNA, denatured DNA or synthetic polymer poly-(dA-dT)·(dA-dT) template. The effect is more pronounced with homologous DNA, stimulating enzyme IIa and IIb 1.4 and 2.0-fold respectively with denatured calf thymus DNA and 2.5 and 3.0-fold with denatured chicken DNA. The factor serves no template function (Table III); UMP incorporation by both enzymes saturates at

TABLE I

Effect of Heat, Dialysis or Protease Treatment on the Stimulation Factor

Assay Conditions	Activity (pmoles UMP incorporated per 30 minutes) IIa	IIb
Exp. 1		
Enzyme alone	12.2	6.4
Enzyme + factor	18.4	11.8
Enzyme + factor (heated)	16.4	11.2
Enzyme + factor (dialyzed)	16.7	12.4
Exp. 2		
Enzyme alone	9.4	4.1
Enzyme + factor	15.4	10.4
Enzyme + factor treated with protease	10.6	6.7
Enzyme + buffer treated with protease	9.3	5.2

Peak fractions of the stimulation factor were combined and 5 µl aliquots were added to each assay (0.1 ml reaction mixture) containing glycerol gradient enzyme IIa or enzyme IIb. Activity due to the factor alone was subtracted from the results of enzyme plus factor. The assay conditions were as described in Materials and Methods. Heating was for 10 minutes at 100°C; dialysis was for 18 hours against 0.1 M ammonium sulfate in TGMED. Protease treated factor or buffer (0.1 M ammonium sulfate in TGMED) was treated with protease (200 µg/ml pronase VI, Sigma) at 37°C for 20 minutes followed by heat treatment at 100°C for 10 minutes.

5µg calf thymus DNA, an amount far less than that used in each assay. To exclude selective stimulation of reannealed denatured DNA, we assayed in the presence of single-stranded ϕX 174 DNA (untreated or heated) template. Both enzymes were stimulated (Table III) and again stimulation is also observed at saturated concentrations of DNA (Fig. 5).

DISCUSSION

The existence of protein factors which stimulate eukaryotic RNA polymerase activities has been reported in several biological systems (6-12). These factors do not adsorb to DEAE-Sephadex or DEAE-cellulose columns and stimulation is observed only with double-stranded DNA template. Since RNA polymerase II prefers denatured DNA as template, it has been suggested that these factors

TABLE II

DNA Exonuclease Activity of the Stimulating Factor: Sensitivity to Heat Treatment

Experimental Conditions			^{3}H-DNA left
Factor (µl)	Heated Factor (µl)	Time of Incubation (minutes)	CPM
40	-	0	7391 (control)
10	-	45	7372
40	-	90	4119
-	10	45	7403
-	40	90	7220

DNA exonuclease activity was determined by measuring the amount of TCA-precipitable E. coli ^{3}H-DNA remaining after incubation with factor at 37°C. The reaction mixture contained (in a 0.2 ml reaction mixture): 50mM Tris-HCl (pH 7.9), 1 mM $MnCl_2$, 10 mM KCl, 1 mM dithiothreitol, E. coli ^{3}H-DNA and factor. $(NH_4)_2SO_4$ was adjusted to a final concentration of 0.02 M. Heated factor refers to heating at 100°C for 15 min.; the heated factor retained RNA polymerase stimulation activity.

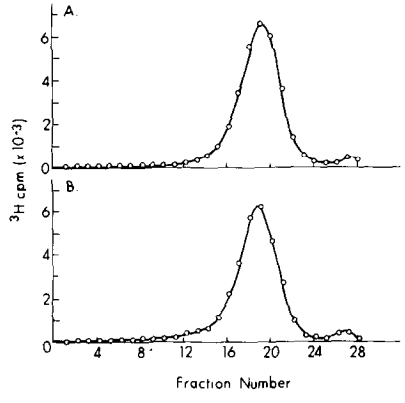

Figure 4.
Alkaline sucrose gradient centrifugation analysis of E. coli ^{3}H-DNA after incubation with the heated factor preparation: E. coli ^{3}H-DNA was incubated (A) without the factor (B) with 10 µl heated (100°C, 15 min.) factor in a standard assay mixture minus calf thymus DNA, nucleoside triphosphates, pyruvate kinase and phosphoenol pyruvate and enzyme. After incubation at 37°C for 45 min., the reaction was terminated by the addition of phenol-cresol. After mixing, shaking, centrifugation and back extraction of the phenol phase, the combined aqueous phase was loaded on a 5-20% linear sucrose gradient containing 0.3 M NaOH, 0.8 M NaCl, 0.001 M EDTA. The gradient was then centrifuged at 50,000 rpm in a SW 50.1 rotor for 3.5 hr. Fractions were collected from the bottom of the tube and TCA-insoluble material was collected and radioactivity counted.

TABLE III

Effects of Different Templates on Enzyme Stimulation by the Factor

Template	p mole UMP incorporation per 30 min			
	Enzyme IIa		Enzyme IIb	
	- Factor	+ Factor	- Factor	+ Factor
Calf thymus DNA				
denatured	3.86	5.42	4.21	8.40
native	0.041	0.052	0.118	0.194
Chicken blood DNA				
denatured	1.43	3.56	2.46	7.49
native	0.115	0.265	0.161	0.706
Poly (dA-dT)·poly (dA-dT)	0.302	0.89	0.49	1.17
ϕX 174 Single-stranded DNA	6.9	9.8	6.3	12.2
ϕX 174 Single-stranded DNA, heat treated	2.8	4.6	5.9	11.7
No template	0	0	0	0

5 µl of the peak fraction (no. 26 of Fig. 2) of stimulation factor was added to a 0.1 ml assay mixture containing glycerol gradient enzyme IIa or IIb in the presence of different templates. Calf thymus DNA or chicken blood DNA was used at 40 µg per assay; double-stranded poly(dA-dT)·poly(dA-dT) and ϕX 174 single-stranded DNA used per assay were 20 µg and 3.6 µg respectively. Heat-treated ϕX 174 single-stranded DNA was heated at 100°C for 4 minutes followed by quick cooling. The activity due to the factor alone (less than 10%) was subtracted to calculate the enzyme activity in the presence of the factor. The other assay conditions were as described in Materials and Methods.

unwind the DNA and enable the enzyme to transcribe helical DNA more efficiently (7,8,10). It has also been suggested that the inhibition of RNA polymerase by factors added in the presence of denatured DNA template may be due to contaminating amounts of RNase H or to exonuclease (6,11,12).

This report describes a stimulatory factor isolated from the nuclei of chicken myeloblastosis cells; it adsorbs to DEAE-Sephadex and is eluted with 0.1 M ammonium sulfate. This factor is a macromolecule having a protease-sensitive moiety required for activity. The property of its marked heat-stability is similar to that of the calf thymus factor of Stein and Hausen (6)

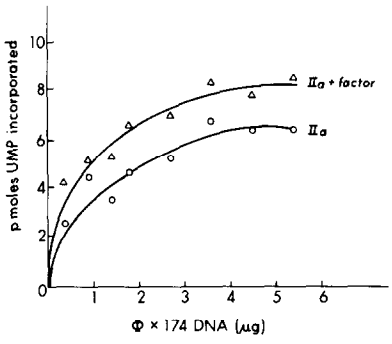

Figure 5.
The effect of the factor on ϕX 174 single-stranded DNA template activity. Glycerol gradient prepared enzyme IIa was assayed at various concentrations of ϕX 174 single-stranded DNA template in the presence or absence of the factor. The other assay conditions were as described as in Materials and Methods.

and the ascites HSF factor of Lee and Dahmus (10). We found exonuclease activity in this factor but it is lost upon heating: endonuclease activity is not detected in the heated factor. Therefore the stimulatory activity of the factor is not ascribed to DNase activity. The differential responses of polymerases IIa and IIb to the factor and of homologous vs heterologous DNA, as well as the lack of response of E. coli RNA polymerase, again indicate that stimulation is not due to a non-specific effect of nuclease, and rather suggest a possible regulatory function.

The unique property of this factor is its stimulatory activity on denatured DNA template, and particularly with single-stranded ϕX 174 DNA. Double-stranded DNA template was stimulated but did not serve as a better template for factor stimulation. Therefore this factor may have the capacity of controlling RNA synthesis at the enzyme level as opposed to merely changing template specificities. Chicken myeloblastosis leukemia is induced by the avian myeloblastosis virus (14). Following RNA virus infection RNA-dependent DNA polymerase is involved in converting viral RNA to DNA (16,17). However, subsequent steps of DNA expression in the cell remain unclear. It is tempting to speculate that the transcription of a single-stranded DNA product from RNA-dependent DNA polymer-

ase is enhanced due to a specific factor such as the one reported here. It is possible that this factor is a sigma-like protein and that polymerase IIa and IIb behave like E. coli complete and core enzymes. However, we cannot exclude the possibility that the preparation contains more than one factor. Work is in progress to further purify this factor from chicken leukemia and other tissues and to study its mechanism of action.

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