# A FACTOR STIMULATING RNA POLYMERASE I HAVING ELONGATION ACTIVITY

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## 1. Introduction

Multiple RNA polymerases exist in eukaryotic cells and they have been separated into at least two, and possibly three fractions [1–3]. Of these fractions, RNA polymerase I is located in the nucleolus, and RNA polymerase II and III in the nucleoplasm. The transcriptional system of RNA polymerase I is probably much simpler than that of RNA polymerase II, since its only product in vivo is thought to be ribosomal precursor [2,4]. Therefore, it is easier to study its transcriptional system than that of RNA polymerase II as a model of general transcription in eukaryotes. The first step in studies on regulation of gene expression in eukaryotic cells seems to be to look for factors affecting transcription in vitro. This paper describes the isolation and characterization of a factor stimulating RNA polymerase I from Ehrlich ascites tumor cells. It was found that the RNA synthesized in the presence of this factor was larger in molecular size than that synthesized by RNA polymerase I alone.

## 2. Methods

### 2.1. *Assay of RNA polymerase and stimulation of RNA synthesis*

The assay mixture contained in a total volume of 0.25 ml, 6 μmol of Tris-HCl, pH 7.9, 1.15 μmol of $MgCl_2$, 0.75 μmol of $MnCl_2$, 12.5 μmol of $(NH_4)_2SO_4$, 0.017 μmol of EDTA, 1 μmol of β-mercaptoethanol, 0.0625 μmol each of ATP, GTP and CTP, 0.00625 μmol of UTP, 0.5 μCi of [$^3$H]UTP (20 Ci/mmole, New England Nuclear Co.), 5 μg of Ehrlich ascites tumor DNA and 10 to 50 μl of enzyme solution. After incubation for 60 min at 37°C, the radioactivity incorporated into the acid-insoluble fraction was counted. One unit of enzyme was defined as the amount causing incorporation of 1 pmol of UMP under the above conditions. To test the stimulatory effects of various fractions on RNA synthesis, 1 to 50 μl of these fractions were added to the assay mixture and the incorporation of UMP into the acid-insoluble fraction was compared with that in reaction mixture without the test fraction.

### 2.2. *Determination of nicking activity of the stimulatory factor*

A sample of 0.08 μg of [$^3$H]thymidine-labeled SV40 DNA was incubated with 6 μg of stimulatory factor (an amount causing maximum stimulation) in 0.2 ml of reaction mixture containing 5 μmol of Tris-HCl, pH 7.9, 0.92 μmol of $MgCl_2$, 0.6 μmol of $MnCl_2$, 10 μmol of $(NH_4)_2SO_4$, 0.014 μmol of EDTA and 0.8 μmol of β-mercaptoethanol. After incubation for 10 min at 37°C, 0.05 ml of 0.2 M EDTA was added and the mixture was layered on top of 4.8 ml of a linear 5–20% sucrose gradient containing 0.3 M NaOH, 0.5 M NaCl and 0.001 M EDTA. Gradients were centrifuged for 160 min at 40 000 rev/min in a Hitachi RPS 40T-2 rotor at 4°C and then fractions were collected from the bottom of the tubes. The DNA in each fraction was precipitated with ice-cold 5% TCA and collected on a Whatman GF-C glass fiber filter and its radioactivity was counted.

## 3. Results

RNA polymerase I was partially purified from Ehrlich ascites tumor cells according to the method of



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Roeder and Rutter [3] with minor modifications. The procedure consisted of the following steps: extraction of RNA polymerase I by sonication in solution of high salt concentration, ammonium sulfate precipitation, DEAE-cellulose chromatography and glycerol gradient centrifugation. The enzyme was insensitive to 20 μg/ml of α-amanitin under the conditions described in the Methods. No RNase or DNase activity was detected in this enzyme preparation. During purification of RNA polymerase I it was found that the material which was not adsorbed on DEAE-cellulose partially stimulated purified RNA polymerase I. Therefore, this fraction was futher purified on a column of phosphocellulose. As shown in fig.1, a peak of stimulatory activity was eluted from phosphocellulose with 0.34 M KCl. A maximum of about 2.5-fold stimulation was observed under the conditions used.

It was found that 0.05 M $(NH_4)_2SO_4$ was essential for obtaining maximum stimulation. When $(NH_4)_2SO_4$ was omitted, no stimulation of RNA synthesis by this factor was observed.

The mol. wt. of this factor was estimated by centrifugation in a glycerol gradient, using catalase, alkaline phosphatase and α-chymotrypsinogen as markers. The mol. wt. calculated in this way was about 37 000, as shown in fig.2. This factor was rapidly inactivated when heated for 10 min at 50°C, as shown in fig.3, indicating that it is a protein.

Using excess amounts of this factor, its template specificity was examined and results are summarized

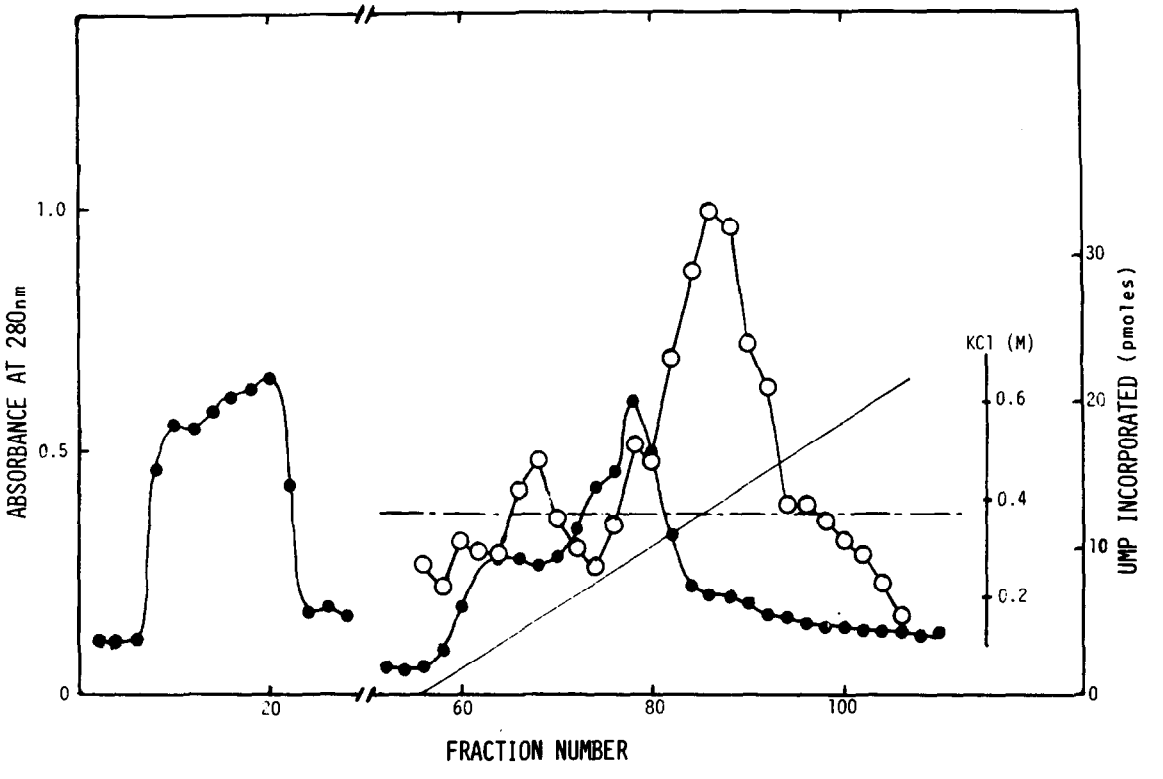


Fig.1. Phosphocellulose column chromatography of the material not adsorbed on DEAE-cellulose. The unadsorbed material in 100 ml of eluate from the DEAE-cellulose column, containing 350 mg of protein, was dialyzed against a buffer (0.05 M Tris-HCl, pH 7.9, 0.005 M β-mercaptoethanol) for 4 h at 4°C. Then it was applied to a column of phosphocellulose (2.7 × 12 cm, Whatman P-11) which had been equilibrated with the same buffer. The adsorbed material was eluted with a linear gradient of 0.1 M to 0.8 M KCl in a total of 400 ml of the buffer. Fractions of 7 ml were collected and their volume was reduced by dialysis against buffer containing 0.01 M Tris-HCl, pH 7.9, 0.005 M of β-mercaptoethanol and 50% glycerol for 2 hrs. The effect of 50 μl of each fraction on RNA synthesis was examined. (o——o) RNA polymerase activity; (•——•) optical density at 280 nm; (——) KCl concentration in elution buffer; (- - - -) base line of RNA polymerase I alone.

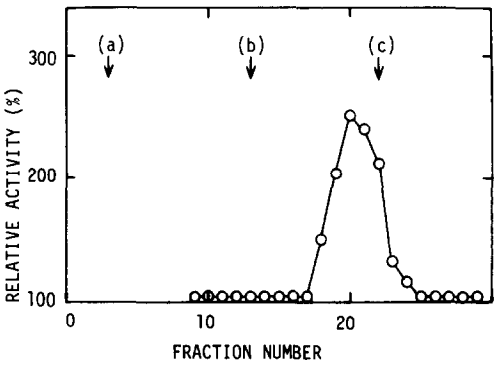


Fig.2. Estimation of the mol. wt. of the stimulatory factor. The concentrated factor was layered on a gradient of 10 to 30% glycerol in 0.01 M Tris-HCl, pH 7.9, 0.01 M $MgCl_2$, 0.2 M KCl, 0.0001 M EDTA and 0.0001 M DTT. The tube was centrifuged for 22 h at 54 000 rev/min in a Hitachi RPS-65T rotor at 0°C. The stimulation of RNA synthesis was assayed under the standard conditions with 15 units of RNA polymerase I. Arrows show the positions of marker proteins sedimented under the same conditions. (a) catalase (232 000), (b) alkaline phosphatase (83 700), (c) α-chymotrypsinogen (25 000).

in fig.4. This factor stimulated RNA synthesis on all native DNAs tested, but inhibited RNA synthesis on denatured DNA or poly dAT.

It is known that endonuclease apparently stimulates RNA synthesis in vitro by making nicks on template

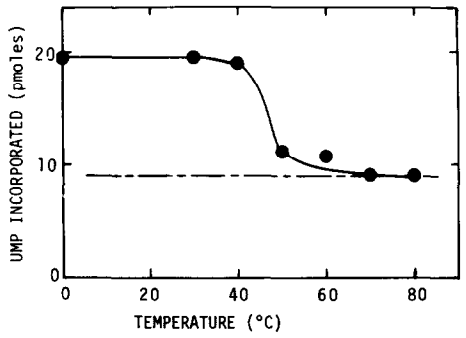


Fig.3. Heat stability of the stimulatory factor. The factor was heated at the indicated temperatures for 10 min in 0.01 M Tris-HCl buffer, pH 7.9, containing 0.005 M β-mercaptoethanol and 50% glycerol, and then its ability to stimulate RNA synthesis was tested using 30 μg of heated factor and 10 units of RNA polymerase I under the standard conditions (- - - -) Base line of RNA polymerase I alone.

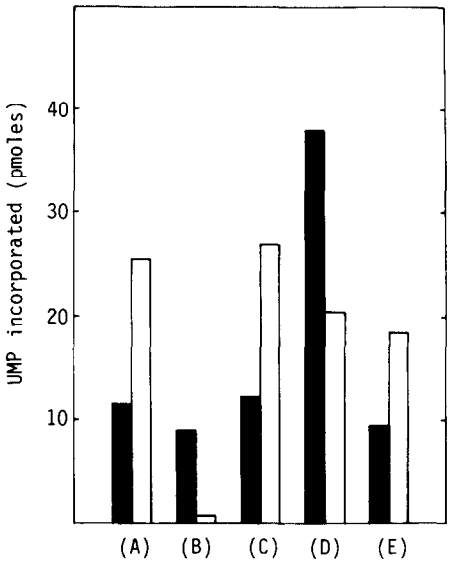


Fig.4. Effect of the factor on RNA synthesis with various templates. For assay, 10 units of RNA polymerase I and 20 μg of factor fraction were used. (A) : Ehrlich ascites tumor DNA; (B): denatured Ehrlich ascites tumor DNA; (C): *E. coli* DNA; (D): poly dAT; (E): $\Phi_{80}$ DNA, (■), RNA polymerase I alone; (□) RNA polymerase I with the factor.

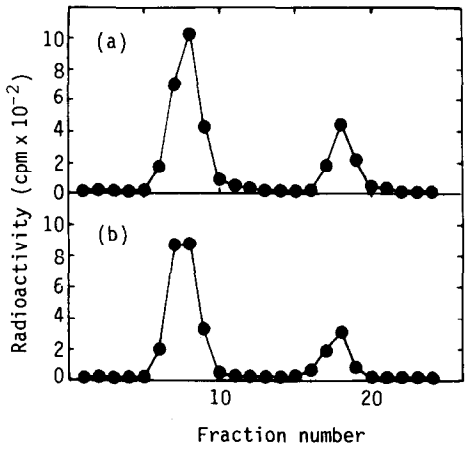


Fig.5. Alkaline sucrose density gradient centrifugation of SV40 DNA. 0.08 μg of [$^3$H]SV40 DNA was incubated with 6 μg of the factor and the mixture was analyzed by alkaline sucrose density gradient centrifugation. Sedimentation was from right to left. (a) SV40 DNA incubated with the factor; (b) control DNA incubated without the factor.

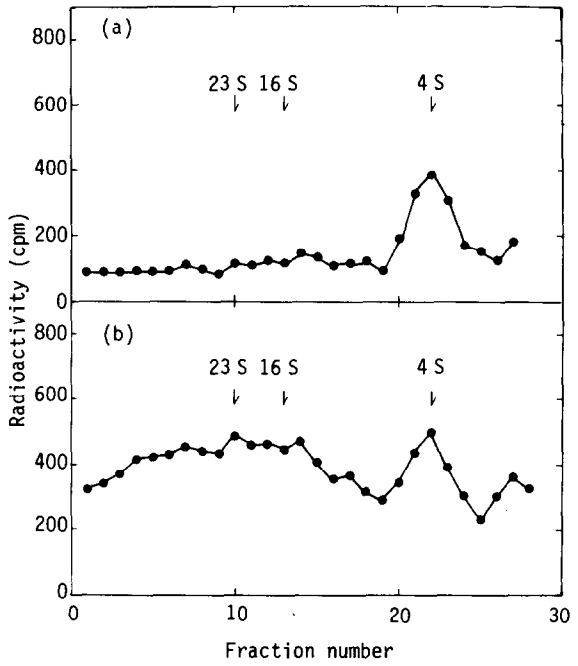


Fig.6. Sucrose gradient analysis of RNA synthesized in vitro. RNA was synthesized in 0.5 ml of reaction mixture using 40 units of partially purified RNA polymerase I. After incubation for 60 min at 37°C, RNase-free DNase was added and incubation was continued for 15 min at 37°C to digest template DNA. Then SDS was added to 0.1% and the mixture was dialyzed against 0.01 M Tris-HCl, pH 7.9, containing 0.01 M $MgCl_2$ for 90 min at room temperature. The dialyzed RNA was layered on a 12 ml linear gradient of 7–25% sucrose in solution containing 0.01 M Tris-HCl, pH 7.4, 0.01 M NaCl and 0.001 M EDTA and centrifuged for 16 h at 34 000 rev/min in a Hitachi RPS-40T rotor. Sedimentation was from right to left. Each gradient was calibrated internally with [$^{14}$C]uracil-labeled *E. coli* RNA. (a) RNA synthesized by RNA polymerase I alone, (b) RNA synthesized in the presence of the factor.

DNA [5]. Therefore, the nicking activity of this factor was examined with [$^3$H]SV40 DNA as substrate. As shown in fig.5, the sedimentation profile of SV40 DNA which had been preincubated with this factor was almost the same as that of control DNA, indicating that this factor has negligible nicking activity.

The RNA synthesized in the presence of this factor was heterogeneous and its average molecular size was much larger than that of control RNA synthesized by RNA polymerase I alone, as shown in fig.6.

Therefore, this factor seems to be effective at the elongation step of transcription.

## 4. Discussion

A protein factor stimulating the activity of RNA polymerase I was isolated from Ehrlich ascites tumor cells. This factor stimulates RNA synthesis on $\Phi_{80}$ DNA or *E. coli* DNA as well as on Ehrlich ascites tumor DNA. It showed no significant nicking activity so its effect in stimulating RNA synthesis is probably not due to modification of DNA. The possibility that this factor is an inhibitor of nuclease is unlikely, since there was no detectable nuclease activity in the preparation of RNA polymerase I used. It was found that the RNA synthesized in the presence of this factor was of larger molecular size than that synthesized by RNA polymerase I alone. It seems likely that the effect of this factor is to stabilize enzyme–DNA complex, resulting in the stimulation of RNA synthesis and production of larger RNA molecules. Similar factors stimulating RNA polymerase I have been found in rat liver [6] and Novicoff ascites tumor cells [7], though their characteristics have not been described in detail.

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