APPARENT DIFFERENCE IN THE WAY OF RNA SYNTHESIS STIMULATION BY TWO STIMULATORY FACTORS OF RNA POLYMERASE II

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1. Introduction

There are 3 species of RNA polymerase in eukaryotic cells. Among them, RNA polymerase II is known to participate in the transcription of heterogeneous nuclear RNA [1]. However, it is unknown how the enzyme transcribes various genes differently. A hypothesis was proposed [2] that structural modifications of chromatin govern the transcribability of specific genes. Since some evidence has been obtained in support of this hypothesis, it is possible that the state of the template is important in regulation of eukaryotic transcription [3–5]. In addition, specific proteins affecting the activity of RNA polymerase II may regulate eukaryotic transcription. Such proteins have been found in various eukaryotic cells, although their functions are unknown [6–11].

We have reported two protein factors that stimulate RNA polymerase II of Ehrlich ascites tumor cells [12]. One of these factors, named S-II, has been purified [13] and shown to enhance the formation of the initiation complex with homologous RNA polymerase II and DNA in the presence of nucleoside triphosphates [14]. This paper describes evidence that, like S-II, the other stimulatory factor, named S-I, specifically stimulates RNA polymerase II, but that the modes of action of the two factors are probably different.

2. Materials and methods

2.1. *RNA polymerase I, II and stimulatory factor S-II*

RNA polymerase I, II and stimulatory factor S-II were purified from Ehrlich ascites tumor cells as in [13]. One unit of RNA polymerase was defined as the amount catalyzing the incorporation of 1 pmol UMP in 60 min under the standard incubation conditions [12].

2.2. *Stimulation of RNA synthesis*

The reaction mixture contained, in total 0.25 ml, 10 μmol Tris–HCl, pH 7.9, 0.75 μmol $MnCl_2$, 1.15 μmol $MgCl_2$, 12.5 μmol $(NH_4)_2SO_4$, 0.017 μmol EDTA, 1 μmol β-mercaptoethanol, 0.0625 μmol each CTP, GTP and ATP, 0.00625 μmol UTP, 0.5 μCi [^{3}H]UTP (20 Ci/mmol), 5 μg Ehrlich ascites tumor DNA, RNA polymerase II, and 10–50 μl test fraction. The incorporation of UMP into the acid-insoluble fraction in 60 min at 37°C was compared with that in reaction mixture without the test fraction. One stimulation unit was defined as the amount which enhanced the activity of RNA polymerase II from 10–11 units under these conditions.

3. Results

We reported that Ehrlich ascites tumor cells contain at least two protein factors which enhance the activity of RNA polymerase II in vitro [12]. These two factors could be separated on a column of phosphocellulose, as shown in fig.1. The second peak, named S-II, contained less protein than the first one, so its purification was much easier. It was purified further and several of its characteristics were elucidated [13].

Unlike S-II, the first peak, named S-I, has not yet

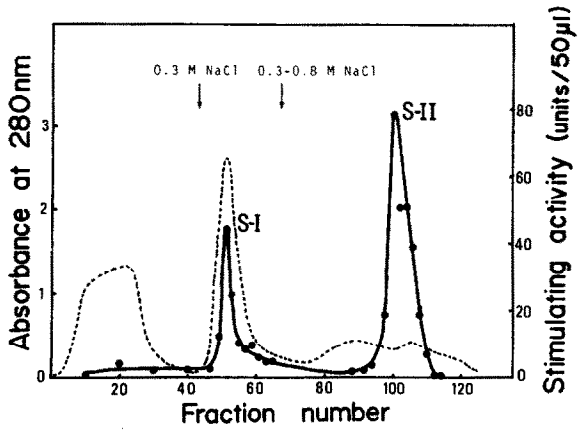

Fig.1. Phosphocellulose column chromatography of stimulatory factors of Ehrlich ascites tumor cells. The procedures for isolating the stimulatory factor are in [13]. The material not adsorbed on DEAE-cellulose was fractionated with ammonium sulfate. The protein precipitating with 50–85% saturation of ammonium sulfate was dialyzed against buffer consisting of 0.01 M Tris–HCl, pH 7.9, and 5 mM β-mercaptoethanol. About 500 mg dialysed protein was applied to a 3.2 × 7 cm column of phosphocellulose pre-equilibrated with the same buffer. The column was washed with the same buffer containing 0.3 M NaCl, and developed with a 500 ml linear gradient of 0.3–0.8 M NaCl in the same buffer. Flow rate, 30 ml/h; fraction size, 7 ml. Samples of 50 μl each fraction were used for assay of stimulatory activity. (●——●) Stimulatory activity; (– – – –), $A_{280\ nm}$.

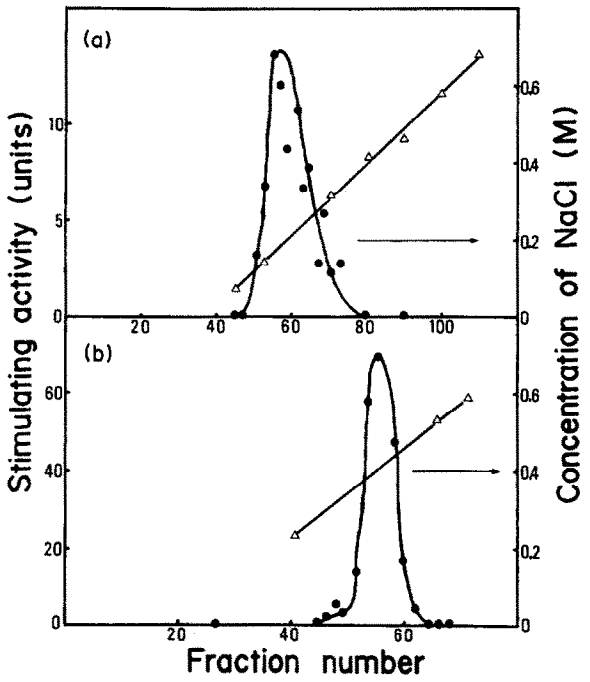

Fig.2. Rechromatography of S-I and S-II on a column of phosphocellulose. Active fractions eluted from the first phosphocellulose column were dialyzed extensively against the buffer in fig.1 legend, and then applied to a second phosphocellulose column. (a) Rechromatography of S-I. Column, 1.5 × 8.5 cm; flow rate; 25 ml/h; fraction size, 2.5 ml. The column was developed with a 200 ml linear gradient of 0.1–0.8 M NaCl. (b) Rechromatography of S-II. Column, 1 × 3 cm; flow rate, 30 ml/h; fraction size, 2 ml. The column was developed with a 100 ml linear gradient of 0.1–0.8 M NaCl. Samples of 50 μl each fraction were used for assay of stimulatory activity. Arrows indicate the NaCl concentration at which each stimulatory factor was eluted. (●——●) Stimulatory activity; (△——△) [NaCl].

been well characterized. It was found that S-I, like S-II, was a heat labile protein, being completely inactivated on heating at 60°C for 10 min (data not shown). A crucial question was whether this activity was due to contaminating S-II, or to a different protein. To investigate this problem, we examined the exact NaCl concentration at which S-I was eluted from phosphocellulose. For this, the active fractions from the phosphocellulose column were combined separately, dialyzed well, and reapplied to a column of phosphocellulose, and the column developed with a linear gradient of 0.1–0.8 M NaCl. As shown in fig.2(a), the stimulatory activity was consistently eluted from the column as a single peak at 0.2 M NaCl.

This elution position was distinctly different from that of S-II, which was eluted with 0.4 M NaCl, as shown in fig.2(b). The result suggests that S-I and S-II are different proteins. However, the specificity of S-I was the same as that of S-II, because S-I exclusively enhanced the activity of RNA polymerase II and had little effect on that of RNA polymerase I, as shown in fig.3.

The next question was whether the mechanisms of stimulation of RNA synthesis by S-I and S-II were the same. We examined this problem by testing whether the two proteins had additive stimulatory effects under conditions where the activity of S-II was maximal, and vice versa. As shown in fig.4(a) and (b), each stimulatory factor enhanced the activity of the other factor. It should be noticed that the slopes of

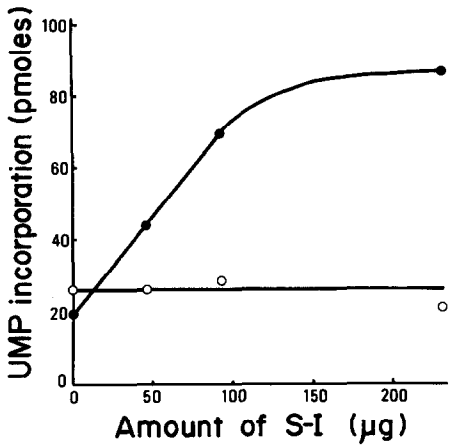

Fig.3. Enzyme specificity of S-I. RNA synthesis was examined using 25 units RNA polymerase I (○) or 20 units RNA polymerase II (●) under the standard conditions with increasing amounts of S-I. For assay of RNA polymerase I, 0.5 μg α-amanitin was added per reaction mixture.

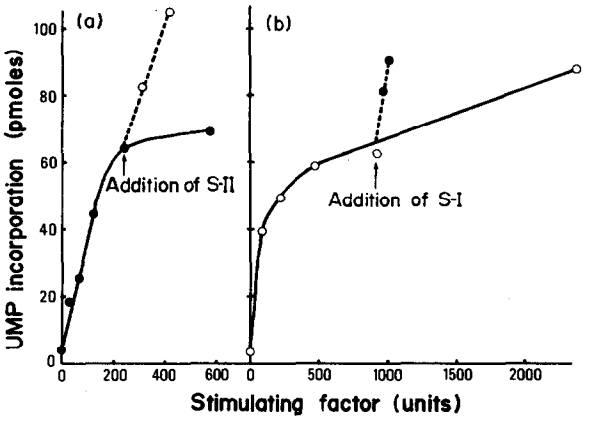

Fig.4. Additive effects of S-I and S-II. The effects of the stimulatory factors on RNA synthesis were determined using 5 units RNA polymerase II with increasing amounts of each factor. Then in the presence of nearly the saturating amount of one factor, an increasing amount of the other factor was added to see if it stimulated RNA synthesis. (a) Effect of S-II in the presence of S-I. 96 units and 240 units S-II were added to reaction mixture containing 238 units S-I. (b) Effect of S-I in the presence of S-II. 24 units and 60 units S-I were added to reaction mixture containing 960 units S-II.

the lines for incorporation of UMP into RNA versus the amount of each stimulatory factor are not influenced by the presence of the other factor, indicating that each factor functions independently. These results strongly suggest that the ways of stimulation of RNA synthesis by S-I and S-II are different.

4. Discussion

Since the first independent reports [6,7] of protein factors that stimulate eukaryotic RNA polymerase II, there have been many papers on these proteins [8–11]. However, it is difficult to evaluate the significance of these proteins at present, because their characteristics have not been fully elucidated. We have reported that Ehrlich ascites tumor cells contain two protein factors, named S-I and S-II, which stimulate the activity of homologous RNA polymerase II [12]. We showed [12] that S-I enhaced the activity of RNA polymerase II in the presence of a nearly saturating amount of S-II and vice versa. These stimulations were proportional to the amount of each stimulatory factor added. The rate of stimulation per fixed amount of one stimulatory factor was not affected by the presence of the other factor.

Thus, it is likely that the ways of stimulation of RNA synthesis by these factors are different. At least two possibilities may be considered:

(i) That S-I and S-II recognize different sequences on template DNA where RNA synthesis could be initiated.

(ii) That the molecular mechanisms of stimulation of RNA synthesis by these factors are completely different.

The former possibility is the more likely, because it is known that S-I and S-II have different template specificities [13]: namely, S-I stimulates RNA synthesis both on homologous DNA and on poly dAT, whereas S-II stimulates RNA synthesis only on homologous DNA. It is possible that the two factors recognize different initiation sequences on template DNA, and that this is why each factor can stimulate RNA synthesis in the presence of excess of the other factor.

Various protein factors seem to enhance the activity of RNA polymerase II by different molecular mechanisms. It is known that S-II enhances the formation of an initiation complex with RNA polymerase II and template DNA in the presence of

nucleoside triphosphates, whereas a factor from Novikoff ascites tumor cells has protein kinase activity and phosphorylates RNA polymerase II, resulting in apparent stimulation of the activity of RNA polymerase II [15]. The present results can also be explained by supposing that the molecular mechanisms of stimulation of RNA synthesis by these two factors are totally different. However, the mechanism of stimulation of RNA synthesis by S-I is unknown. Purification and detailed characterization of this protein should give an answer to this question.

Acknowledgement

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