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Stimulation of transcription from accurate initiation sites by purified S-II

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The effects of transcription factors S-II and S-II′, a phosphorylated form of S-II, on accurate transcription were compared in a reconstituted transcription system greatly depleted of S-II. S-II, but not S-II′, stimulated the syntheses of run-off products of various truncated class II genes in this system, suggesting that the activity of this factor is regulated by its phosphorylation and dephosphorylation.

Transcription factor; S-II; RNA polymerase II; Accurate transcription

1. INTRODUCTION

Several transcription factors are essential for accurate transcription of class II genes [1–5]. Some of them have been purified, and their functions in formation of the initiation complex of transcription have been reported [6,7].

S-II is a protein originally purified as a stimulatory factor of RNA polymerase II from Ehrlich ascites tumor cells [8]. Subsequent studies revealed that proteins cross-reacting immunologically with Ehrlich cell S-II were present in the nucleoplasm of all eukaryotic cells examined [9]. Antibody against S-II inhibited accurate transcription of pSmaF in a HeLa cell lysate and α-amanitin-sensitive transcription in isolated nuclei of Ehrich cells [10,11]. Moreover, a stoichiometric amount of S-II was shown to form a complex with RNA polymerase II in the presence of DNA [12]. Recently, Rappaport et al. [13] reported that S-II purified from calf thymus cancels premature termination of accurate transcription of pSmaF in a HeLa cell nuclear lysate. These results suggest that S-II is a transcription factor of class II genes.

A phosphorylated form of S-II, termed S-II′, has also been purified from Ehrlich ascites tumor cells [14]. Thus, it is likely that the activity of S-II is regulated by its phosphorylation and dephosphorylation. However, no significant difference has been found between the stimulatory activities of S-II and S-II′ on RNA polymerase II in vitro [15]. Phosphorylation of S-II was shown to proceed in vivo even when RNA synthesis was blocked by actinomycin D, suggesting that there is no significant coupling of RNA synthesis and phosphorylation of S-II [16]. Thus, the physiological significance of the phosphorylation of S-II has remained unknown.

This paper reports studies on the effects of S-II and S-II′ on accurate transcription of pSmaF in a reconstituted transcription system of Ehrlich cell nuclear lysate. Results showed that S-II, but not S-II′, stimulated the synthesis of the run-off product.

2. MATERIALS AND METHODS

2.1. *Preparations of S-II and S-II′*

S-II and S-II′ were purified from Ehrlich ascites tumor cells as described previously [8]. The specific activities of S-II and S-II′, measured by stimulation of RNA polymerase II with total Ehrlich ascites tumor cell DNA as template, were 67000 and 40000 units/mg protein, respectively.

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2.2. *Preparation of nuclear extracts*

Nuclear extracts of Ehrlich ascites tumor cells were prepared as described by Dignam et al. [17]. Usually, 100 ml extracts containing 5–10 mg/ml protein were obtained from 3×10^{10} cells. The extracts were fractionated on successive columns of phosphocellulose and DEAE-cellulose as described by Dignam et al. [17]. The flow-through fraction from DEAE-cellulose was further purified on a column of single-stranded DNA agarose by the method of Sawadogo et al. [18]. The column was washed extensively with 0.1 M KCl, and then adsorbed materials were eluted with 0.6 M KCl. The fractions used for reconstruction of the transcription system are summarized in fig.1. The amount of S-II in each fraction was determined by radioimmunoassay as described before.

2.3. *Accurate transcription assay in vitro*

Transcription from promoters was assayed with truncated templates in a reconstructed system. The templates used were *Sma*I-digested pSmaF (adenovirus 2 major late promoter) [19], *Bam*HI-digested pPK44 (mouse β major globin gene) [20], and *Xho*I-digested pFb100 (*Bombyx mori* fibroin gene) [21]. Run-off transcripts labeled with [α-^{32}P]UTP were extracted with phenol, and subjected to 5% polyacrylamide gel electrophoresis under denaturing conditions. The amounts of run-off transcripts were measured quantitatively by densitometric scanning of RNA bands in autoradiograms.

3. RESULTS AND DISCUSSION

3.1. *Reconstruction of a run-off assay system greatly depleted of S-II*

To determine the effect of exogenously added S-II on accurate transcription by RNA polymerase II, we reconstructed a transcription system depleted of S-II as much as possible. For this, we fractionated nuclear extracts of Ehrlich ascites tumor cells as summarized in fig.1. The amount of S-II in each fraction was measured by radioimmunoassay (table 1). About 34% of the S-II was recovered in the 0.5 M KCl eluate from a column of phosphocellulose. When this fraction was further fractionated on a column of DEAE-cellulose, S-II was recovered in the flow-through fraction. This flow-through fraction was loaded on a column of single-stranded DNA agarose. S-II was not adsorbed to the column and was recovered in the flow-through fraction (fraction E).

A mixture of fractions A, B, C and D supported accurate transcription of pSmaF. This distribution of transcription factors is essentially the same as that obtained with a HeLa cell lysate [17]. Fraction E, which contained most of the S-II, was not required for accurate transcription, but we could not conclude from our results that S-II was not essential for transcription initiation, since the reconstituted transcription system still contained S-II, although at less than 10 ng/assay mixture, which was about 1/16 of that in the transcription system with the original nuclear extract. However, externally added S-II stimulates the synthesis of the run-off product of pSmaF in this reconstituted transcription system.

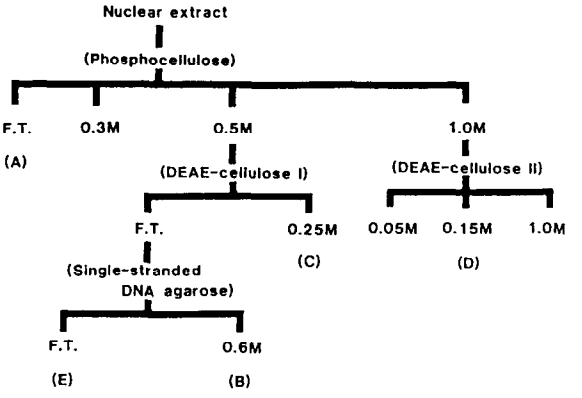

Fig.1. Procedure for fractionation of the Ehrlich nuclear extract. Values indicate concentrations of KCl for elution of each fraction. F.T., flow-through fraction. (A–D) Fractions essential for reconstitution of the accurate transcription system. (E) The fraction containing most of the S-II.

3.2. *Stimulation of accurate transcription by exogenously added S-II*

Since our reconstituted transcription system was greatly depleted of S-II, we next examined the effect of S-II on accurate transcription. As shown in fig.2, S-II caused dose-dependent stimulation of the production of run-off transcripts from adenovirus 2 major late promoter and mouse β-globin promoter, inducing up to about 1.7-fold increase in their production. Since S-II did not stimulate overall α-amanitin-sensitive RNA synthesis in this system (not shown), the stimulation was probably specific for accurate transcription from promoters.

Previously, we reported that the stimulatory activity of S-II depends on Mn^{2+} [15], which is a potent inhibitor of accurate transcription in a HeLa cell lysate [10]. This may explain why S-II did not stimulate overall α-amanitin-sensitive RNA synthesis in the reconstituted transcription system, which did not contain Mn^{2+}. Transcription of the

Table 1

Distribution of S-II during fractionation of the Ehrlich ascites tumor cell nuclear extract

Fraction	Total protein (mg)	S-II (μg)	Recovery (%)
Nuclear extract	790	1240	100
Phosphocellulose			
flow-through fraction (A)	403	40	3
0.5 M KCl eluate	66	420	34
1.0 M KCl eluate	26	19	2
DEAE-cellulose I			
flow-through fraction	25	280	23
0.25 M KCl eluate (C)	35	6	0.5
DEAE-cellulose II			
0.15 M KCl eluate (D)	4	0.6	0
DNA-agarose			
flow-through fraction (E)	8	220	18
0.6 M KCl eluate (B)	14	2	0.2

An Ehrlich nuclear extract was fractionated by the procedure shown in fig.1, and the amount of S-II in each fraction was determined by radioimmunoassay [23]. Protein was measured by the method of Bradford [24]

Bombyx mori fibroin gene (pFb100) was also stimulated by S-II in this system. Therefore, it is likely that S-II stimulates the transcription of class II genes in general in this system.

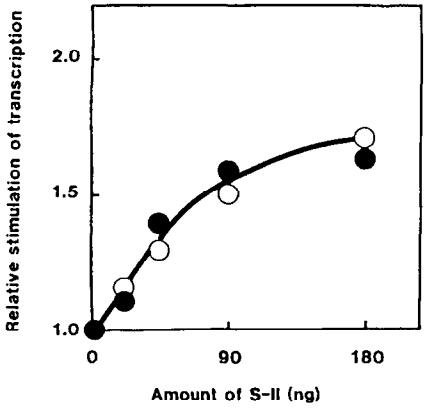

Fig.2. Effect of S-II on accurate transcription from adenovirus 2 major late promoter and mouse β-globin promoter in the reconstituted system. Increasing amounts of S-II were added to the transcription system, and the amounts of specific transcripts were measured by densitometric scanning of the bands of run-off products in autoradiograms. Then relative stimulation of transcription was calculated. The templates used were *Sma*I-digested pSmaF (○) and *Bam*HI-digested pPK44 (●).

Table 2

Selective stimulation of accurate transcription by S-II

Addition	Relative amount of specific transcript
None (control)	100
S-II	170
S-II′	100

The autoradiogram was scanned with a densitometer, and the amount of specific transcript was calculated as a percentage of that of the control

3.3. *Comparison of the effects of S-II and S-II′ on accurate transcription*

As reported before, Ehrlich ascites tumor cells also contain a phosphorylated S-II, termed S-II′, and both proteins stimulated RNA polymerase II in vitro in the same way [14,15]. Therefore, we compared the effects of these two factors on accurate transcription in the reconstituted system. As shown in table 2, S-II stimulated transcription of pSmaF, but S-II′ did not cause significant stimulation of transcription of pSmaF, although it stimulated RNA polymerase II in a regular transcription system. Similar results were obtained with β-globin and fibroin genes as templates (not shown). These results suggest that the activity of S-II needed for stimulation of accurate transcription is blocked by phosphorylation of S-II. Probably, transcription by RNA polymerase II alone and that in the reconstituted system are fundamentally different, and both S-II and S-II′ are effective in the former system, whereas only S-II is effective in the latter system. Possibly many components involved in the accurate transcription of class II genes, including RNA polymerase II [22], are regulated by phosphorylation and dephosphorylation.

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