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A LARGE-MOLECULAR-WEIGHT REGULATING FACTOR FOR CHROMATIN-DEPENDENT RNA POLYMERASE II REACTIONS IN RAT LIVER CYTOPLASM

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Summary : The author found a sugar-containing 70 KDa factor in rat liver nuclei as described in the previous report (1), which repressed RNA chain initiation by all classes of rat liver RNA polymerases and specifically stimulated chromatin-dependent RNA chain elongation catalyzed by RNA polymerase II. In this communication, the localization of this factor in rat hepatic cells was studied. In the cytoplasmic fraction, a large-molecular-weight regulating activity for chromatin-dependent RNA polymerase II reactions was observed and it was characterized to be a 250 KDa glycoprotein. This factor showed the same biological activity for chromatin-dependent RNA polymerase II reactions as that of the factor reported previously (1). Furthermore, this factor was converted to the 70 KDa factor by the action of exogenous or unknown endogenous protease(s). These results suggest that a conversion mechanism from cytoplasmic factor to nuclear factor for RNA polymerase II-dependent transcription exists.

Heparin has been known as an artificial modulator for chromatin-dependent RNA polymerase II reactions in vitro (2). This molecule shows an inhibition on RNA chain initiation and a stimulation on the elongation reaction. On the other hand, previously, Kinoshita suggested that a heparin-like substance is a possible initiator of RNA synthesis in sea urchin embryo and such a substance migrates from cytoplasm to nuclei at the gastrulation stage (3,4). However, the details of the material has not been elucidated completely.

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Recently, the author found a novel factor in rat liver nuclei, which shows the same actions for chromatin-dependent RNA polymerase II reactions as that of heparin (1). This factor is not heparin as judged by the physicochemical analysis and it is characterized to be a 70 KDa sugar-containing material (1).

In this paper, the author further analyzed the localization of the factor mentioned above. A large-molecular-weight regulating activity for chromatin-dependent RNA polymerase II reactions was found in the cytoplasmic fraction as that of heparin. This factor was characterized to be a 250 KDa glycoprotein, which can be converted to the 70 KDa sugar-containing factor by the exogenous or endogenous protease(s). The significance of such conversion between cytoplasmic factor and nuclear factor is discussed from the standpoint of RNA polymerase II-dependent transcription.

MATERIALS AND METHODS

Preparation of rat liver cytosol and isolation of 250 KDa factor

Rat liver cytosol was prepared by the following method. Liver of male Sprague-Dawley rats (about 200 g body weight) was minced with scissors in two volumes of cold 2.1 M sucrose and 3 mM $MgCl_2$ and homogenized with 10 strokes in a Potter-type homogenizer. The homogenate was filtrated through 4 layers of gauze, mixed with 8 volumes of the same medium and centrifuged at 28 K rpm for 1 hr in a Hitachi RP-30 rotor. Nuclear pellet was used for the preparation of RNA polymerase II and nuclear factor. The cytoplasmic supernatant was dialyzed against TGMED buffer (50 mM Tris-HCl pH 7.9, 20 % grycerol, 5 mM $MgCl_2$, 0.1 mM EDTA and 0.1 mM dithiothreitol), applied on a DEAE Sephadex A-25 column (1.8 x 12 cm) and eluted with TGMED buffer containing 30 mM ammonium sulphate. The eluates were concentrated by salting out with two volumes of saturated ammonium sulphate (pH 7.9). After centrifugation at 16 K rpm for 15 min in a Beckman JA-20 rotor, the precipitate was dissolved with TGED buffer (10 mM Tris-HCl pH 7.5, 12.5 % glycerol, 0.5 mM EDTA and 0.2 mM dithiothreitol), applied on a Bio gel A-1.5m column (0.6 x 94 cm) and eluted with TGED buffer containing 0.5 M NaCl at 1.5 ml per fraction. Each fraction was concentrated by salting out with two

volumes of saturated ammonium sulphate (pH 7.9) and centrifuged. The precipitate was dissolved with 0.5 ml of TGMED buffer and 50 μl was used for the assay. If necessary, after the trypsin digestion (bovine pancreas, Sigma, 50 μg/ml) was performed at 37°C for 1 hr, the rechromatography of Bio gel A-1.5m was carried out.

Preparation of 70 KDa factor from nuclei

70 KDa factor of nuclei was prepared by the previous method (1).

Assay for RNA polymerase II activity

DNA or chromatin-dependent RNA polymerase II activity was assayed by the previous method (1).

Analysis of the physicochemical properties of the factor

Sugar analysis was performed as described in the previous paper (1). Heparin and factors were hydrolyzed at 110°C for 5 hr in 6 N HCl. The hydrolyzate was applied on a silica gel-coated plate (Merck, 20 x 20 cm). Enzyme treatments with trypsin, DNase I, RNase A , lipase C (Sigma) and Turbo cornutus glycosidases (Seikagaku Kogyo, Japan) were carried out at 50 μg/ml for 1 hr at 37°C.

RESULTS AND DISCUSSION

As described in the previous report (1), a novel factor for chromatin-dependent RNA polymerase II reactions exists in rat liver nuclei. The factor shows the repressing effect on RNA chain initiation, whereas it exhibits the stimulatory effect on chramatin-dependent engaged RNA polymerase II reaction at a high ionic strength.

Furthermore, the author studied the localization of the factor in the cytoplasmic farction. In order to proetct the leakage of the cell nuclear materials, the cytoplasmic fraction was prepared by the use of magnesium-containing hypertonic sucrose. When the RNA polymerase I and II activities were taken as marker enzymes of the nuclear fraction, these activities were not detected (not shown). This result suggests that the nuclear substance are not leaked out into the cytoplasmic fraction. In the cytoplasmic fraction, although the author could not detect the 70 KDa factor reported previously (1), a much larger inhibitory activity for exogenous DNA-dependent

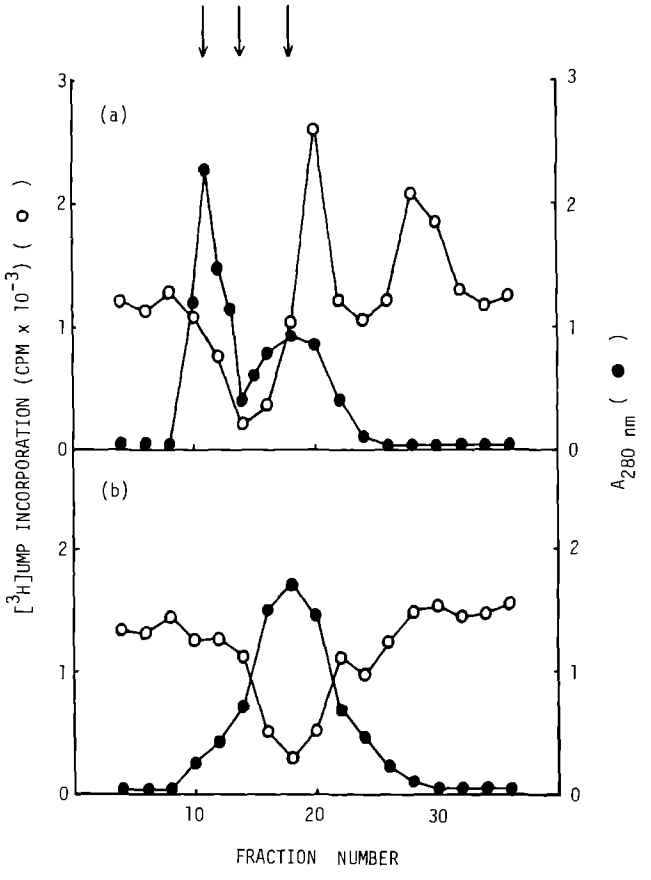


Fig.1 Bio gel A-1.5m column chromatography of the rat liver cytosol fraction. (a) The preparation of the cytosol fraction and Bio gel A-1.5m chromatography were performed as described in methods. (b) After the same crude cytosol fraction was incubated at 37 C for 1 hr, the same chromatography was carried out. Upper arrows in the figure show the markers ; Jack bean urease (480 KDa), bovine catalase (250 KDa) and bovine serum albumin (67 KDa) from the left to the right.

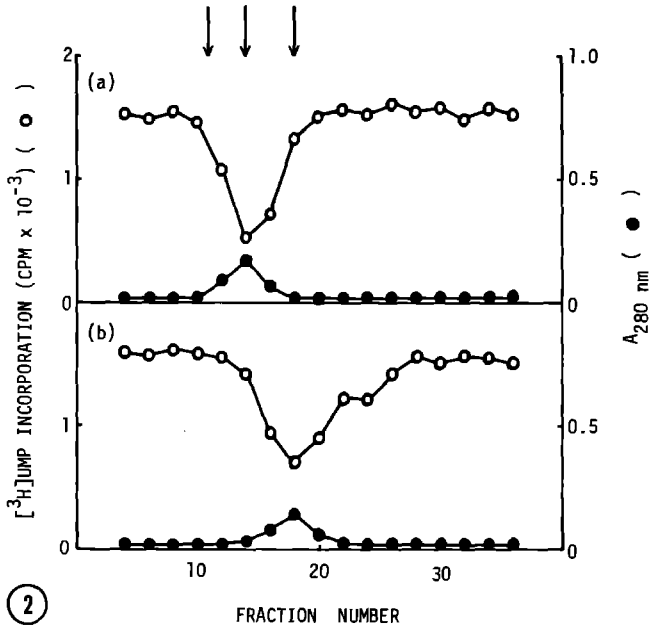
RNA polymerase II reaction was observed. As shown in Fig.1a, this large-molecular-weight factor was estimated to be about 250 KDa as judged by some markers in a Bio gel A-1.5 m chromatography. This factor showed the similar properties as that of the 70 KDa factor (1). The factor exibited the inhibitory actions for chromatin-dependent RNA polymearse reactions at a low ionic strength (50 mM ammonium sulphate) and specific stimulation for RNA polymerase II reaction at a high ionic strength (250 mM ammonium sulphate) (Table1).

Table 1 Effects of 250 KDa factor on RNA polymerase reactions in rat liver chromatin

Concentration of $(NH_4)_2SO_4$	$[^3H]$UMP Incorporation (CPM)		Treated / Control (%)
	Control	Treated	
α-amanitin sensitive activity			
50 mM	3498	854	24.4
250 mM	7154	24798	346.6
α-amanitin resistant activity			
50 mM	990	406	41.0
250 mM	980	1090	111.2

Chromatin-dependent RNA polymerase activity was assayed in the absence or presence of α-amanintin (1 μg/ml). The radioactivity is the average of triplicate assays.

Furthermore, an interesting phenomenon was observed. This large-molecular-weight factor was converted to the 70 KDa factor by an incubation of the crude fraction before the application to the gel filtration (Fig.1b). The same conversion of the 250 KDa factor was also observed by a treatment with bovine pancreas trypsin (Fig.2). This phenomenon also occurred by other proteases, but not by DNase I, RNase A and lipase C (not shown). This activity was lost by the treatment with glycosidases as shown in the analysis of the 70 KDa factor in the previous paper (1) (not shown). In addition, sugar analysis of the converted 70 KDa factor treated with trypsin showed the same pattern of sugar components as that of the 70 KDa factor from nuclei (Fig.3). In addiiton, as shown in Fig.3, these factors do not contain heparin components such as glucosamine and xylose, which were elucidated in the previous report (5). These results indicate that 250 KDa factor contains the same 70 KDa factor from nuclei and that it is not heparin or heparin complex. Possibly, 250 Kd factor seems to be a complex of sugar and protein, glycoprotein.



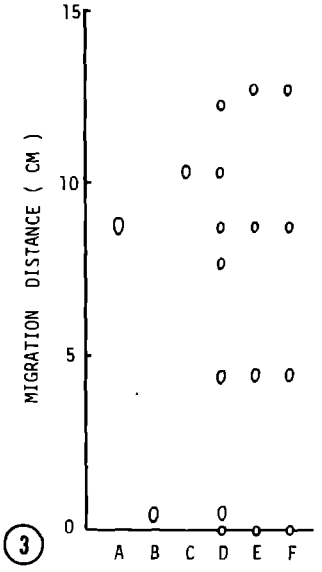


Fig.2 Bio gel A-1.5m column chromatography of the trypsin digest of 250 KDa factor. 250 KDa factor in Fig.1a was incubated at 37 °C for 1 hr in the (a) absence and (b) presence of bovine pancreas trypsin and each preparation was applied on a Bio gel A-1.5m column. Upper arrows in the figure show the same markers in Fig.1.

Fig.3 Analysis of sugar components of the factors by silica gel thin layer chromatography. A, B and C in the figure show the marker sugars ; glucose, glucosamine and xylose, respectively. D, E and F represent heparin, 70 Kd factor in Fig.2b and 70 KDa factor from nuclei, respectively. After development of thin layer chromatography with a solvent system of butanol / pyridine / water, it was burnned and darkening spots are indicated.

Next, the author analyzed the relative percent of these factors in the cytoplasmic and nuclear fractions. As shown in Table 2, the inhibitory activity of the 70 KDa factor in nuclei was estimated to be 8-15 % of the total cellular activity and the activity in the cytoplasmic fraction was 85-92 %. Amongst nuclear activities, the very small portion (1-2 % of the total activity) was tightly bound to chromatin fraction (not shown). RNA polymerase II in the tightly-bound chromatin fraction is complexed with RNA synthesis stimulatory factors and transcribes efficiently towards chromatin-template (6)

Table 2 Relative activity of the factor in the cytoplasmic and nuclear fractions

	Relative activity of total recovered activity	
	Cytoplasm	Nuclei
Experiment 1	92.2 %	7.8 %
Experiment 2	85.4 %	14.6 %

One unit of the factor was assumed as 50 % inhibition activity on the assay system of DNA-dependent RNA polymerase II reaction in this paper. The relative activity of the factor in the cytoplasm and nuclei was calculated from the units of inhibition and the sample volume of cytoplasm and nuclear fractions.

and reflects nuclear mRNA synthesis activity during physiological changes (7). The relationship between the 70 KDa factor and tightly bound RNA polymerase II in the transcriptional regulation is being studied.

Anyhow, the existence of a conversion mechanism from the cytoplasmic and nuclear factors for RNA polymearse II seems to be an interesting problem in the field of the transcriptional regulation.

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