A FACTOR STIMULATING THE TRANSCRIPTION BY NUCLEOLAR RNA POLYMERASE IN THE NUCLEOLUS OF RAT LIVER

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SUMMARY--- The flow-through(FT) fraction of DEAE-Sephadex chromatography of RNA polymerase solubilized from isolated nucleoli of rat liver specifically stimulated transcription by nucleolar RNA polymerase. The effect was observed only with native DNA used as template but not with heat-denatured DNA. The activity was trypsin-sensitive but relatively heat stable. Although the fraction contained DNase activity as revealed by the alteration of sedimentation properties of SV40 DNA, evidence is presented that the stimulatory activity is not ascribed to this DNase activity.

A few reports have so far appeared dealing with the identification and characterization of transcription stimulating factor in eukaryotic cells(1-3). Stein and Hausen reported a factor from calf thymus which stimulated transcription by RNA polymerase II(1). Seifart discovered in rat liver cytoplasm a factor having similar properties to that of Stein and Hausen(2). Mondal et al. found in the coconut nuclei a factor stimulating the activity of both polymerase I and II of the same organism(3). In the meantime, Muramatsu et al. reported that cessation of protein synthesis in rat liver by cycloheximide *in vivo* caused a rapid and specific inhibition of nucleolar RNA synthesis while the synthesis of extranucleolar nuclear RNA was not affected(4). Since the relative activities of nucleolar and extranucleolar nuclear RNA polymerase as revealed by DEAE-Sephadex chromatography did not change by cycloheximide treatment(unpublished observations), the presence of short-lived protein factor(s) was suggested which regulates the

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transcription of nucleolar genes. During the course of study in search of such a factor, we found that the flow-through(FT) fraction of DEAE-Sephadex chromatography of crude preparation of nucleolar RNA polymerase exerted a stimulatory effect on the purified nucleolar RNA polymerase. This paper describes the identification and some chracterization of the nucleolar DEAE-Sephadex flow-through (FT) fraction mentioned above.

MATERIALS AND METHODS

Extraction and DEAE-Sephadex chromatography of RNA polymerase and preparation of nucleolar flow-through(FT) fraction Male albino rats (Donryu strain) weighing 150-200g were used throughout the experiment. Nucleoli and extranucleolar nuclear fraction were isolated with Mg^{2+}-procedure as described previously(5). Extraction of RNA polymerase from each subnuclear fraction and chromatographic purification were performed as described by Roeder and Rutter(6). The crude RNA polymerase fraction (fraction IV of Roeder and Rutter) was charged to the column of DEAE-Sephadex A-25 (1cm x 14cm). The column was washed with 10 ml of 0.1 M $(NH_4)_2SO_4$ in TGMED (0.05 M Tris-HCl(pH 7.9)-30%(v/v)glycerol-5 mM $MgCl_2$-0.1 mM EDTA-0.5 mM dithiothreitol) followed by elution with a linear gradient of 0.1-0.5 M $(NH_4)_2SO_4$ in the same buffer. Each fraction was assayed for RNA polymerase activity as described previously(7). The flow-through fractions eluted during the column was charged with the sample and washed with 0.1 M $(NH_4)_2SO_4$-TGMED were collected and concentrated to a small volume with concomitant dialysis against TGMED. The dialysate constituted the nucleolar flow-through(FT) fraction which contained transcription stimulating activity.

Alkaline sucrose gradient centrifugation analysis of SV40 DNA [^{3}H] SV40 DNA was incubated in a standard assay mixture minus calf thymus DNA and nucleoside triphosphates and with 6.8 μg of nucleolar FT fraction, heat-treated FT fraction or without FT fraction. Heat treatment was carried out at 80°C for 15 min. After incubation for 10 min at 37°C, 0.05 ml of 0.2 M EDTA was added to the reaction mixture which was placed on a 5-20%(w/w) linear sucrose gradient containing 0.3 M NaOH, 0.7 M NaCl and 0.001 M EDTA. The gradient was centrifuged

at 40,000 rpm in a Hitachi RPS 40 rotor for 2 hr at 4°C. Fractions were collected from the punctured bottom of the tube and CCl_3COOH insoluble material was collected onto a glass fibre disc(Whatman GF/C). After washing the disc with 5% CCl_3COOH and ethanol, radioactivity was determined in a liquid scitillation spectrometer using toluene scitillator.

RESULTS AND DISCUSSION

In search of the transcription regulating substance in the crude preparation of nucleolar RNA polymerase, DEAE-Sephadex flow-through(FT) fraction was tested for the ability to affect the purified RNA polymerase activity. As seen from Fig.1-b, the peak I(nucleolar) RNA polymerase exhibited a higher activity when assayed in the presence of FT fraction(prepared form fractions 1-10 of Fig.1-a). The factor having stimulatory activity is not adsorbed to DEAE-Sephadex and distributed over the early regions of chromatographic elution(Fig.2). Fig.3 shows that the nucleolar FT fraction was effective only when native DNA was used as template, a finding compatible with those reported thus far(1-3). With denatured DNA slight decrease in [^{14}C] UMP incorporation was observed as the concentration of FT was increased, presumably due to contaminating nucleases in the nucleolar FT fraction of which mention will be made later. Fig.2 also shows that the FT fraction contains certain amount of protein as detected by absorbancy at 280 nm. These protein molecules may stabilize the polymerase molecule or coprecipitate with the syhthesized RNA on the filter when exposed to CCl_3COOH, thus resulting in an apparent higher activity. Indeed, as seen from Table 1, bovine serum albumin(BSA) when added to the assay mixture exhibited an increase in the incorporation of [^{14}C] UMP into RNA. However, similar enhancement of incorporation to that caused by 320 μg of BSA could be attained by the addition of only 4.6 μg(in terms of protein) of FT fraction into the assay mixture. In addition, further stimulation was observed by the addition of nucleolar FT fraction to the assay system containing 320 μg/assay of BSA, a saturating concentration of BSA for stimulation in this experiment. Another evidence that the

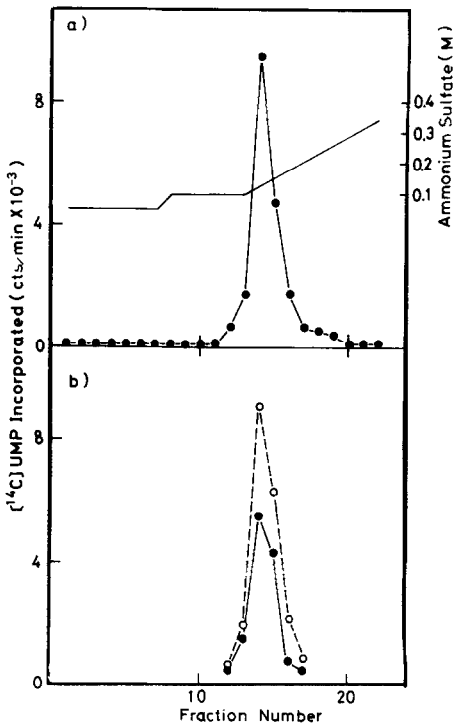

Fig. 1.

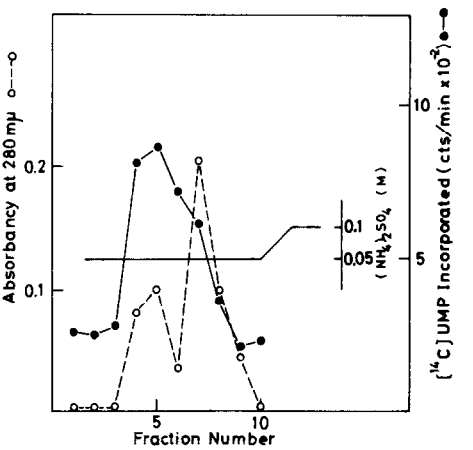

Fig. 2.

Fig.1. a) DEAE-Sephadex A-25 chromatography of nucleolar RNA polymerase. Assay of RNA polymerase activity was performed as described previously(7). Nucleolar FT fraction was prepared from fractions 1-10 as described in MATERIALS AND METHODS. b) Assay of peak fractions in the presence (o----o) or absence(●——●) of nucleolar FT fraction.

Fig.2. Distribution of stimulatory activity over the nucleolar flow-through fraction. The nucleolar RNA polymerase peak fractions (fractions 12-17 of Fig.1-a) were combined and assayed in the presence of 50 μl aliquot from each flow-through fraction.

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factor is not the BSA-like substance was provided by the specificity of the nucleolar FT fraction on the nucleolar RNA polymerase. When nucleolar FT fraction was tested for the stimulatory activity on RNA polymerase I and II at the same time(Fig.4), stimulation was noted only with nucleolar RNA polymerase and not with the extranucleolar nuclear RNA polymerase. As seen from Table 2, the activity of FT was destroyed by treatment with trypsin. However the activity is shown to be relatively stable to heat treatment(Table 3). 58% of stimulatory

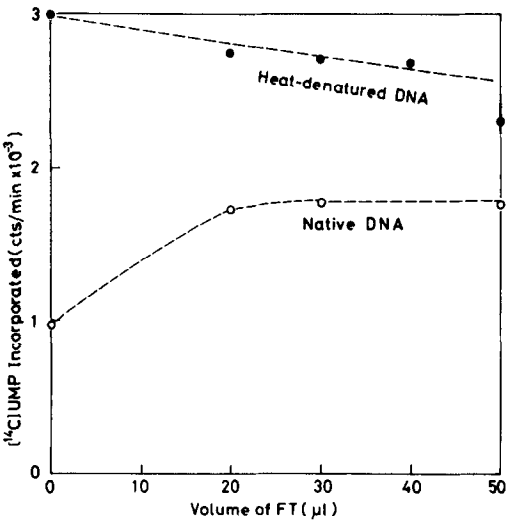

Fig. 3.

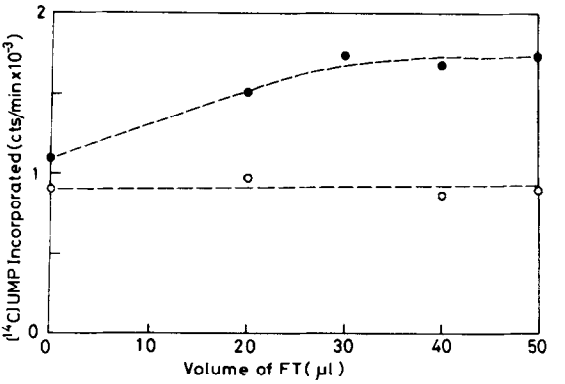

Fig. 4.

Fig.3. Dependence of stimulatory activity of nucleolar FT fraction upon the strandedness of template DNA.

Fig.4. Effect of nucleolar FT fraction on nucleolar and extranucleolar nuclear RNA polymerase. Purified nucleolar and extranucleolar nuclear RNA polymerase were assayed in the presence of various amount of nucleolar FT fraction together with 100 µg/assay of BSA. ●----● Nucleolar RNA polymerase : o----o Extranucleolar nuclear RNA polymerase.

Table 1. Efficiency of Stimulation of Nucleolar FT Fraction

	Incorporation (cpm)	Stimulation (cpm)	Stimulation ratio (%)
Control	865		
BSA (320 µg/assay)	1032	167	19
FT*	1100	235	27
BSA (320 µg/assay) + FT*	1204	339	39

* protein 4.6 µg/assay

Purified nucleolar RNA polymerase was assayed in the presence of either saturating amount of bovine serum albumin (320 µg/assay) or nucleolar FT fraction or both. Determination of protein was performed as described by Lowry et al. (10).

Table 2. Sensitivity of the Stimulatory Activity to Trypsin

Time of preincubation (min)	Stimulation (%)	
	Control	Trypsin
0	40	---
2	43	4
5	31	6
10	41	7

To 0.3 ml of nucleolar FT fraction was added 0.03 ml of 1.1 mg/ml of trypsin (Sigma, 2 x crystalized, Type III). The mixture was incubated at 37°C and at intervals during incubation, aliquot(0.03 ml) was assayed for the stimulatory activity on purified nucleolar RNA polymerase in the presence of 1 mM AMCHA-CEP (4"-(2"-carboxy)ethylphenyl trans-4-aminoethylcyclohexanecarboxylate hydrochloride), a potent trypsin inhibitor(11). For control, nucleolar FT fraction incubated with TGMED instead of trypsin was assayed in the same way. One mM AMCHA-CEP alone did not inhibit either polymerase activity or stimulatory activity of FT.

Table 3. Effect of Heat on the Stimulatory Activity of Nucleolar FT Fraction

	Incorporation (cpm)	Stimulation (cpm)	(%)	Residual stimulation after heat treatment (%)
Expt. I.				
Control	588			
FT(untreated)	1190	602	120	
FT(37°C, 20 min)	1031	443	76	63
FT(100°C, 5 min)	995	407	69	58
Expt. II.				
Control	1022			
FT(untreated)	1606	584	57	
FT(80°C, 15 min)	1472	450	44	77

Nucleolar FT fraction was treated as indicated in the Table. Purified nucleolar RNA polymerase was assayed in the presence of untreated or treated nucleolar FT fraction. In Experiment II, the same preparation of nucleolar FT fraction was used for sucrose gradient analysis of SV40 DNA (see Fig.5-b).

activity remained after heating FT at 100°C for 5 min. These data support the view that the factor is a relatively heat stable protein or a substance other than protein associated with protein molecule which is essential for the stimulatory activity.

The observed stimulation of RNA synthesis could be pertinently explained

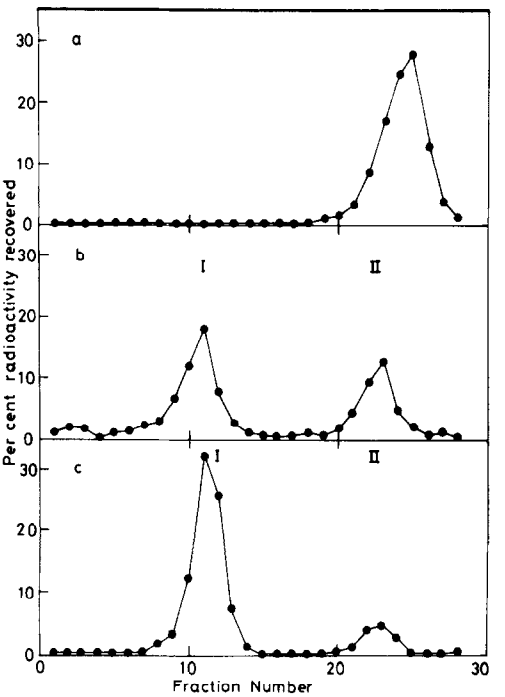

Fig.5. Alkaline sucrose gradient centrifugation analysis of SV40 DNA after incubation with nucleolar FT fraction. [^{3}H] SV40 DNA was incubated in a standard assay mixture minus DNA and nucleoside triphosphates and with 6.8 μg of nucleolar FT fraction(a), heat-treated FT fraction(b), or without FT fraction(c), and analyzed on alkaline sucrose density gradient as described in MATERIALS AND METHODS.

by the activation of DNA template due to the action of contaminating DNase(8,9). Therefore we have examined the nucleolar FT fraction as to the presence of DNase activity. As shown in Fig.5, [^{3}H] SV40 DNA incubated with nucleolar FT fraction sedimented as component II on alkaline sucrose gradient(Fig.5-a), while incubation without FT fraction did not change the sedimentation characteristics of SV40 DNA, which sedimented on the most part as component I in the same gradient(Fig.5-c). These results show that the FT fraction contained considerable DNase activity. However, at least 75% of this DNase activity was lost by heating at 80°C for 15 min as calculated from the amount of component I which survived the treatment (Fig.5-b). On the other hand, the same heat-treated FT preparation still retained about 80% of the stimulatory activity as compared with the non-heated control

(Table 3, Expt.II). The unparalleled behaviour of DNase activity and the stimulatory activity of the FT fraction in the heating experiment appears, in contrast to the finding by Keller and Goor(9), to rule out the possibility that the stimulatory activity comes exclusively from the DNase activity associated with FT fraction. Actually, assay of RNA polymerase activity in the presence of minute amount of exogenously added DNase did not show any stimulation, rather a progressive decrease in incorporation was noted in our system with increasing concentration of DNase (unpublished observations). Experiments with radioactive ribosomal RNA and pancreatic RNase showed that FT fraction did not contain either RNase or RNase inhibitor activity.

All these lines of evidence, especially the specificity of FT fraction on nucleolar RNA polymerase, strongly suggest some positive role of FT in the regulation of nucleolar gene activity, namely the regulation of ribosomal RNA synthesis in the nucleolus. Although much is yet to be known as to chemical nature and the functional role of the nucleolar FT fraction, the evidence listed above warrants further investigation of this fraction as a candidate for a new transcription stimulating factor of nucleolar origin.

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