FACTORS AND RIFAMPICIN INFLUENCING RNA POLYMERASE ISOLATED FROM CHROMATIN OF EUKARYOTIC CELL

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Summary : Two RNA-polymerases (I and II) and several protein fractions (A, B and C) have been isolated from chromosomal acidic proteins of coconut nuclei. Fraction B stimulates the activity of both the RNA-polymerases and appears to be an initiation factor. RNA-polymerase I is sensitive to rifampicin when the drug is added to the enzyme first before the addition of factor B, but insensitive when the order of addition is reversed. Fraction C has an inhibitory effect on both the RNA-polymerases.

While working with the chromatin material from coconut nuclei, RNA-polymerase activity was detected with the acidic protein fraction of the complex. RNA-polymerase activity was resolved into two distinct peaks after passing through DEAE cellulose column with a purification of 70 - 80 fold (manuscript in preparation). When the present work was in progress multiple forms of RNA-polymerases were reported from rat liver and embryonic sea urchin nuclei (1). These forms were found to be distinct and different from each other in the requirement of divalent and monovalent cations. That one RNA-polymerase from thymus is sensitive to α-amanitin and the other is not has also been reported very recently (2). However, there is no report that RNA-polymerases from eukaryotes require any other factor for initiation or termination analogous to that reported in case of *E. coli* RNA-polymerase (3, 4). The purpose of this paper is to report such factors influencing RNA-polymerase activity in case of eukaryotic cells as well.

The nuclei were isolated from the thin layers of endosperm of coconuts (Cocos nucifera) by the method of Mondal et al (5). The nuclear preparation has been found to be free from mitochondria. The crude chromatin was prepared according to Huang and Bonner (6). The acidic proteins were extracted by the method

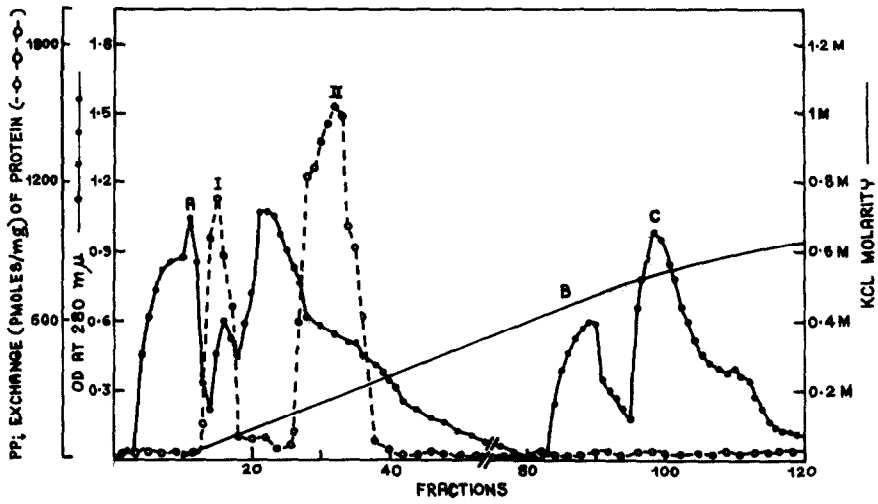

Fig. 1. Elution profile of RNA-polymerases and factors from DEAE cellulose column. Chromatinic acidic protein (143 mg) was passed through CM cellulose (10 x 0.9 cm) to remove contaminating histone, and 27.5 mg of 30-55% ammonium sulphate saturated protein fraction from the effluent was charged on a DEAE cellulose column (25 x 0.9 cm). Fractions of 1 ml were collected at a flow rate of 1 ml/5 min and O.D. at 280 mμ measured. The RNA-polymerase assay system (0.5 ml) containing Tris HCl pH 8.0, 0.04 M; EDTA, 0.2 mM; mercaptoethanol, 5 mM; $MgCl_2$, 0.01 M; $MnCl_2$, 2 mM; KCl, 0.08 M; K_2HPO_4, 0.4 mM; ATP, GTP, CTP and UTP, 0.1 mM each; $^{32}PP_i$ (3 x 10^7 cpm/μmole), 0.2 mM; DNA, 20 μg; 50 μg of enzyme and 5 μg of fraction B was incubated at 37°C for 15 min. The reaction was terminated by chilling and adding 0.2 ml of 0.5 M EDTA (pH 6.0) followed by 0.1 ml of saturated sodium pyrophosphate (adjusted to pH 6.0 by adding KH_2PO_4). 0.5 ml of 10% suspension of activated charcoal was then added. The charcoal was washed throughly with 0.01 M sodium pyrophosphate (pH 6.0), plated and counted in End window counter. All the counts were corrected for the blank in which the reaction was stopped at zero min.

of Wang (7) and this was passed through CM cellulose and the effluent was saturated with 30-55% ammonium sulphate. The precipitate was dissolved and dialyzed against TME (Tris buffer 0.01 M, pH 8.0, mercaptoethanol, 0.001 M and EDTA, 0.0001 M). This was charged on DEAE cellulose column equilibrated with the same buffer TME and eluted with linear gradient of KCl in the TME buffer. RNA-polymerase activities were assayed by PP_i exchange (8). The enzymic activities eluted at 0.1 M KCl and 0.2 M KCl were designated as RNA-polymerase I and II respectively. Along with the polymerases three other fractions of proteins (A, B, C) were eluted at 0.05 M, 0.55 M and 0.62 M KCl respectively (Fig. 1). The protein corresponding to the enzymic activity was concentrated with aquacide and passed through DEAE cellulose column again and eluted and assayed under same conditions as described. RNA-polymerase I displayed maximum activity in presence of Mn^{2+} (2 μmole/ml), while polymerase II in presence of Mg^{2+} (10 μmole/ml). Both of them required an addition of DNA for the activity. Fraction B enhanced the activity of both the RNA-polymerases. Fraction B alone increased the activity of RNA-polymerase I by about 9-10 fold. This increase was manifested only when native DNA from coconut or thymus was used but not with λ DNA. Fraction C had an inhibitory effect on the activity of both the polymerases (Table 1). However, no RNase or DNase activity has been detected with the fractions B and C.

The mode of action of fraction B on RNA-polymerase was further elucidated. When assayed with $\beta\gamma$-^{32}P-ATP as well as ^{14}C-ATP it was apparent that in presence of fraction B incorporation of ^{32}P into acid insoluble fraction was linear at the beginning and then a plateau was obtained in 6 min whereas ^{14}C-incorporation was negligible at the begining

Table 1. Requirements for RNA-polymerase

Conditions of the experiment	^{14}C-ATP incorporated μμmoles/mg of RNA-polymerase I	^{14}C-ATP incorporated μμmoles/mg RNA-polymerase II
1. Complete system	740	972
2. 1 - DNA	0	0
3. 1 - B	80	132
4. 2 + denatured DNA - B	51	-
5. 4 + B	50	-
6. 2 + λ DNA - B	145	-
7. 6 + B	180	-
8. 1 + C	213	300
9. 5 + C	110	130
10. 1 - Mn^{2+} + Mg^{2+}	382	-
11. 1 - Mg^{2+} + Mn^{2+}	-	448

The complete incubation mixture (0.5 ml) contained all the components as described under Fig. 1 except pyrophosphate, and ^{14}C-ATP (Sp. Ac. 1.8 x 10^7 cpm/μmole) was added in place of cold ATP. Mn^{2+} was used for the assay of RNA-polymerase I and Mg^{2+} for RNA-polymerase II. 250 μg each of polymerase I and II (purified by rechromatography on DEAE cellulose) and 30 μg each of protein fraction B and C (obtained as in Fig. 1) were used. After incubation for 15 min at 37°C, the reaction was stopped by the addition of 10% TCA and 50 μg of yeast RNA as carrier. The acid insoluble ppt. was washed in the centrifuge thrice with 10% TCA, twice with ethanol and once with ether. Finally, the residue was dissolved in dil. NaOH, plated and counted in gas flow counter.

which increased linearly from 5 - 10 min under the experimental conditions used (Fig. 2). It appears therefore that fraction B might act as an initiation factor. Other evidences for this conclusion are that fraction B binds with RNA-polymerase I itself but not with DNA (unpublished results) as well as its effectiveness with native DNA instead of single stranded DNA.

It has been known that RNA-polymerase from nuclei of higher organisms are insensitive to the antibiotic rifampicin

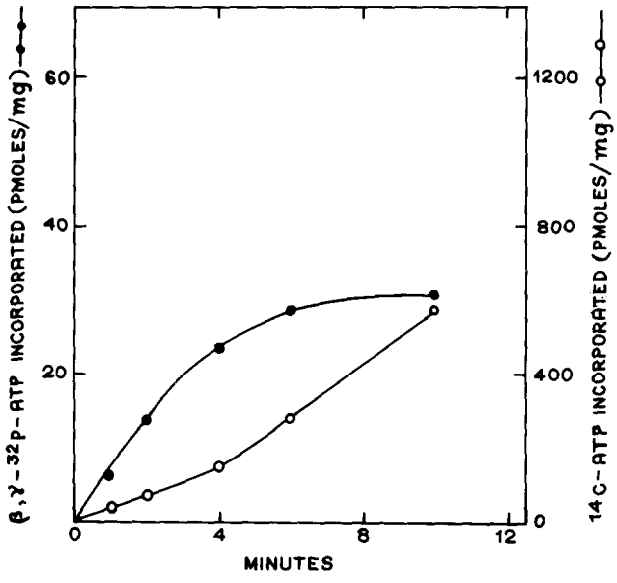

Fig. 2. Kinetics of initiation and total synthesis of RNA by polymerase I. The components of the incubation mixture were the same as in Table 1. $\beta\gamma$-^{32}P-ATP (2.3×10^7 cpm/μmole) was used for detection of initiation and ^{14}C-ATP for total synthesis. The temp. of incubation was lowered to 20°C to slow down reaction rate so that initiation could be followed better. 100 μg of RNA-polymerase I and 10 μg of fraction B were used. $\beta\gamma$-^{32}P-ATP was prepared by aminoacid-dependent PP_i exchange in presence of Azotobacter vinelandii extract (11), and when analysed, was found to contain no counts in the α-position.

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in contrast to E. coli RNA-polymerase (9). This inhibition by rifampicin in the latter case is attributed to the interaction of the drug with minimal component (PC enzyme) of the polymerase (10). In the present case when RNA-polymerase I was incubated with rifampicin followed by fraction B there was inhibition in ^{14}C-ATP incorporation and 2 μg of rifampicin/50 μg enzyme could knock off all the activity. If the fraction B was added first with the enzyme followed by rifampicin, no inhibition was recorded (Table 2). That the inhibition manifested in the former case could not be encountered by

Table 2. The effect of Rifampicin on polymerase I

Order of addition of components	^{14}C-ATP (μμmoles)/ mg of enzyme
1. NTP, DNA, Enz I, Rifam, B.	32
2. NTP, DNA, Enz I, B, Rifam.	790
3. NTP, Enz I, B, Rifam, DNA.	750
4. NTP, DNA, B, Rifam, Enz I.	52
5. DNA, NTP, Enz I, B, Rifam.	800
6. DNA, Enz I, B, NTP, Rifam.	740

The condition of the incubation was same as indicated in Table 1. Enzyme (50 μg), B (5 μg) and Rifampicin (4 μg) were used in these experiments. NTP means 4 nucleoside triphosphates including labelled ATP.

changing the order of additions of other components in the reaction mixture is shown in Table 2. The block may thus lie in the formation of factor-enzyme-system itself. However, RNA-polymerase II was found to be resistant to rifampicin. Whether the fraction C acts as a termination factor or as a general inhibitor of RNA-polymerase I is not clear at present. The details of the work are now in progress.

Since chromatin contains these factors as well as RNA polymerases it is expected that the structure of chromatin itself during different stages of growth may regulate RNA synthesis in eukaryotic cells.

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