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RNA Polymerase from Eukaryotic Cells

Isolation and Purification of Enzymes and Factors from Chromatin of Coconut Nuclei

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This paper describes the isolation and purification of two RNA polymerases (CI and CII) and several protein fractions (A, B and C) from the chromosomal acidic proteins of coconut endosperm nuclei. The method involves disruption of isolated nuclei in dilute salt medium, centrifugation to sediment the crude chromatin, solubilization of chromatin in concentrated salt, dialysis of soluble chromatin to dilute salt to precipitate DNA-histone complex and centrifugation to get the acidic proteins in the supernatant. Further purification involves ammonium sulphate fractionation and chromatography on DEAE-cellulose and QAE-Sephadex.

RNA polymerase CI purified through the QAE-Sephadex step gives a single band on polyacrylamide gel electrophoresis. In gels containing dodecylsulfate, this enzyme shows multiple bands indicating its subunit nature. The pH optima for both RNA polymerases is 8.0. RNA polymerase CI is maximally activated by Mn^{2+}; while CII, by Mg^{2+}. The activities of both the enzymes are stimulated by fraction B. All the fractions except A are substantially free from nucleases. Both RNA polymerases require the addition of DNA for activity. Fraction B is ineffective either with denatured coconut DNA or native λ DNA.

The existence of ribonucleic acid polymerase was recognised first by Weiss from mammalian tissue [1]. The enzyme either in soluble or aggregate form has been reported since from bacteria [2–4], plants [5] and animals systems [6]. It was thought previously that only the aggregate form was prevalent in eukaryotic organisms, but the soluble form has also been isolated from the higher organisms [7,8]. It seems that both the soluble and aggregate forms exist in the eukaryotes, their concentration varies with the physiological condition of the cell [9]. Specific RNA polymerase have been demonstrated in chloroplasts [10,11] as well as in mitochondria [12]. RNA polymerase from these organelles are sensitive to rifampicin while that from the nucleus are insensitive. There are two or three types of RNA polymerase in eukaryotic cells [13–17]. They differ in metal ion requirement for their activity [13–16] and also in sensitivity to antibiotics [17,18]. From our laboratory RNA polymerase from the chromatin of coconut differing in metal ion requirement and in the susceptibility to rifampicin have recently been reported [18]. With the discovery of initiation factor (σ factor) by Burgess *et al.* [19] and termination factor (ρ factor) by Roberts [20], a new facet of research on the transcriptional processes has been stimulated. Before these discoveries, Hara and Mitsui [21], Davidson *et al.* [22] and Khesin *et al.* [23] also isolated factors that stimulate RNA synthesis. However, it is not known whether this is similar to the σ-factor, later discovered by Burgess *et al.* [19]. A factor that stimulates RNA polymerase from coconut nuclei has been found to have a definite role in initiation [18]. In the present paper detailed procedures for isolation and purification of coconut enzymes and factors influencing transcription are described.

Unusual Abbreviation. QAE-Sephadex: Quarternary aminoethyl Sephadex.

Enzyme. RNA polymerase (EC 2.7.7.6).

Definition. A_{260} unit, the quantity of material contained in 1 ml of a solution which has an absorbance of 1 at 260 nm, when measured in a 1-cm path-length cell.

MATERIALS AND METHODS

Green coconuts (*Cocos nucifera*) 4–5 months old were obtained from the local market. ATP, GTP, UTP and ion-exchange resins were purchased from Sigma Chemical Company (St. Louis, Missouri, U.S.A). Highly polymerized calf thymus DNA was obtained from Worthington Biochemical Corporation; rifampicin (Pitman-Moore, Division of the Dow Chemical Company, Indianapolis, U.S.A.) and λ DNA were obtained as a gift from Dr. S. Adhya (Bose Institute, Calcutta); [^{14}C]ATP (160 mCi/mmol), [^{14}C]GTP (17 mCi/mmol) and [^{3}H]UTP (2 Ci/mmol) were obtained from Radiochemical Centre (Amersham, Buckinghamshire, England). Aquacide was purchased from Calbiochem (Löwengraben, Swit-

zerland); [^{32}P]orthophosphoric acid was supplied by the Department of Atomic Energy (Trombay, India).

Preparation of [^{32}P]Pyrophosphate

[^{32}P]Pyrophosphate was prepared from [^{32}P]orthophosphoric acid by the method of Bergmann *et al.* [24]. The labelled pyrophosphate was dissolved in distilled water and 5 μmol unlabelled pyrophosphate was added to it to make the final concentration 5 μmol/ml (30 counts × min^{-1} × pmol^{-1}).

Isolation of Chromatin from Nuclei

Nuclei were isolated from coconut endosperm by the method of Mondal *et al.* [25] in the presence of 0.25 M sucrose buffer (0.25 M sucrose; 0.01 M Tris-HCl pH 7.0; 1 mM 2-mercaptoethanol, 0.1 mM EDTA). The crude chromatin was isolated from the nuclei by the method of Bonner and Huang [26]. The nuclei were homogenized with a glass homogenizer fitted with a tight pestle, in the presence of 0.14 M NaCl buffer (0.14 M NaCl, 1 mM 2-mercaptoethanol, 0.1 mM EDTA and 10 mM Tris-HCl pH 7.0) and centrifuged. The pellet was washed with the same buffer until the supernatant fluid was clear. The non-histone protein was extracted by the method of Wang [27]. The crude chromatin was extracted overnight with 2 M NaCl. The supernatant obtained after centrifugation of this chromatin solution at 20000 × *g* for 1 h was dialyzed against 12 volumes of Tris-glycerol buffer (10 mM Tris-HCl pH 8.0, 1 mM 2-mercaptoethanol, 0.1 mM EDTA, 5% glycerol) for 10 h and centrifuged again at 20000 × *g* for 1 h. The clear supernatant thus obtained contained mainly acidic proteins. This constituted the source of RNA polymerase and the factors.

Polyacrylamide-Gel Electrophoresis at pH 8.0

Polyacrylamide gel electrophoresis at pH 8.0 followed the general method of Davis [28]. These gels routinely contained 5% acrylamide. Gels with added 8 M urea as described by Jovin *et al.* [29], and with 0.1% sodium dodecylsulfate as described by Weber and Osborn [30], were prepared wherever necessary. Gels were stained for at least 2 h in a 0.2% solution of coomassie brilliant blue in methanol—acetic acid—water (5:1:5, v/v/v). They were then soaked in 7.5% acetic acid—5% methanol for 30 min, destained electrophoretically, and stored in the same solvent.

Assay Procedure

Pyrophosphate-Exchange Method. Although the incorporation of pyrophosphate into nucleotides during RNA synthesis is not a true indication of total RNA synthesis, it is a very sensitive method for detected enzyme activity [31]. The reaction mixture (0.5 ml) containing 40 mM Tris HCl pH 8.0, 0.2 mM EDTA, 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 0.4 mM K_2HPO_4, 0.16 mM KCl, 0.05 mM ATP, GTP, UTP and CTP, 0.1 mM [^{32}P]PP_i (30 counts × min^{-1} × pmol^{-1}) 20 μg DNA and enzymes and factors as indicated in the legends of the figures, was incubated for 15 min at 37 °C. The reaction was terminated by adding 0.2 ml of 0.5 M EDTA (pH 6.0) followed by 0.1 ml of saturated sodium PP_i (adjusted to approximately pH 6.0 with KH_2PO_4) in ice. 0.5 ml of 10% suspension of activated charcoal (acid-washed, suspended in 10 mM sodium pyrophosohate brought to pH 6.0 with KH_2PO_4) was added to the incubation mixture followed by 3 ml of 10 mM sodium pyrophosphate (pH 6.0). After 30 min the charcoal was washed successively (at least ten times with 10 ml sodium pyrophosphate). The charcoal was transferred to aluminium planchets, dried and the radioactivity measured.

RNA-Synthesis Method. The reaction mixture (either 0.5 ml of 0.25 ml) containing 40 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$ or 2 mM $MnCl_2$, 0.4 mM K_2HPO_4, 0.16 mM KCl, 0.1 mM of each of the four triphosphates of which one was labelled ([^{14}C]ATP, specific activity 10 counts × min^{-1} × pmol^{-1} or [^{3}H]UTP, specific activity 4 counts × min^{-1} × pmol^{-1}), 40 μg/ml coconut endosperm DNA and enzymes and others as indicated in the legends of figures, was incubated at 37 °C for 15 min. The reaction was stopped by adding 10% trichloroacetic acid followed by the addition of 200 μg bovine serum albumin as a carrier. The resultant mixture was kept in ice for 30 min for complete precipitation. The pellet obtained after centrifugation was washed five times with 5% trichloroacetic acid, twice with ethanol containing 1% potassium acetate once with ethanol and finally with ether. The pellet was dissolved in 0.2 ml liquid ammonia and diluted to 1 ml with distilled water. The entire material was dissolved in 10 ml of a dioxan-base fluor and the radioactivity counted.

Alternatively an aliquot of the incubation mixture was applied on 3-cm discs of Whatman 3-MM paper, dried and then treated with 10% trichloroacetic acid. After 30 min, the discs were rinsed 10 times with 5% trichloroacetic acid and subsequently with ethanol (containing 1% potassium acetate) and ether. The discs were dried and counted with 10 ml of toluene liquifluor in a liquid scintillation counter.

RESULTS

The composition of chromatin isolated from nuclei was checked at several steps. The pertinent data are recorded in Table 1. In the crude chromatin DNA, RNA, histone and non-histone proteins were estimated. The ratio of DNA to histone has been found to be 1:1.1 (w/w). The content of non-histone protein of

ble 1. *Composition of the chromatin at different purification stages*

e chromatin and reprecipitated chromatin obtained by the hod described in Material and Methods, was defatted with anol—ether (3:1, v/v) and then extracted with 0.3 N HCl r 5 h. The extracted basic proteins were precipitated by chloroacetic acid (final concentration 20%). The precipi- ed protein was washed with acetone-HCl and finally with etone, dried at room temperature and estimated for histone. e pellet after HCl extraction was washed with 20% tri- loroacetic acid, then with ethanolic potassium acetate, and ally with ether. The washed pellet was digested with 0.3 M OH for 17 h at 37 °C and was made acidic with (0.25 M rchloric acid. After centrifugation at 10000 × *g* for 15 min, e supernatant was collected to measure RNA and the pellet e extracted with 5% perchloric acid for 15 min at 95 °C. e extracted material was estimated for DNA and the pellet s dissolved in 0.5 M KOH and was estimated for residual otein. In the case of one extract with 2 M NaCl, the chro- atin was removed by precipitation and exactly equal liquots of the supernatant were dried by dessication in the ld. One aliquot was extracted with 0.3 M HCl for basic otein and the residue after extraction was estimated for sidual protein before it was freed from nucleic acids by e perchloric acid treatment. The other aliquot was esti- mated for RNA and DNA by the usual method

action	DNA	RNA	Histone	Nonhistone protein
	% of the total weight			
rude chromatin	33.3	15.1	36.0	15.7
M NaCl extract	33.1	13.1	39.4	14.4
eprecipitated chromatin	42.2	7.0	42.2	8.6
upernatant after chromatin removal	2.8	6.2	1.0	90.0

Table 2. *Summary of the purification of RNA polymerase* Enzyme activity was assayed by the incorporation of [^{14}C]-ATP into acid-insoluble products. One unit is defined as hat amount of enzyme which can incorporate 1 pmol [^{14}C]ATP in 15 min under the assay conditions

ep	Total protein	Specific activity	Total activity
	mg	units/mg	units
. 2 M NaCl-extracted soluble chromatin	502	14	7028
. Acidic protein	143	44	6292
. CM-cellulose chromatography at pH 6.4	140	43	6020
. 30—55% $(NH_4)_2SO_4$ fractionation	27.5	256	7040
. DEAE-cellulose[a]			
(A) First activity peak (RCI)	2.0	740	1480
(B) Second activity peak (RCII)	6.0	972	5832
. QAE-Sephadex (RCI)	0.213	2340	514

[a] After this step of purification, factor B (the protein fraction in ...) was added in the assay system.

RNA is less than 50% of that of DNA. After solubilization in 2 M NaCl and centrifugation, the composition of chromatin shows little difference from that of crude chromatin. Distinct differences in the composition are noted, however, in the case of reprecipitated chromatin. The supernatant after removal of chromatin contains almost 90% non-histone protein. From this nonhistone protein, RNA polymerases were purified through several steps as outlined in Table 2.

Purification of RNA Polymerase from the Non-Histone Proteins

The clear supernatant after removal of precipitated nucleo-histone was adjusted to pH 6.4 by 0.1 M acetic acid and passed through a CM-cellulose column (20 × 1 cm) to remove any remaining histone. The resultant fluid was subjected to 30—55% saturated ammonium sulphate fractionation. The precipitate was dialysed against Tris-glycerol buffer and loaded onto a DEAE-cellulose column equilibrated previously with this buffer. After washing with the same buffer the column was eluted with a linear gradient of KCl in the above buffer. Absorbance at 280 nm and the activity for RNA polymerase were measured (Fig. 1). Two activity peaks eluted at 0.1 M and 0.2 M KCl were collected separately and concentrated by aquacide and dialysed against Tris-glycerol buffer and rechromatographed separately in a DEAE-cellulose column (Fig. 2). The activity peaks eluted at 0.1 M and 0.2 M KCl were designated as RCI and RCII, respectively. RNA polymerase CI (RCI) was rechromatographed on a QAE-Sephadex column which was previously equilibrated with Tris-glycerol buffer containing 0.05 M KCl and eluted with a linear gradient of KCl in the same buffer. Each 1-ml fraction was scanned at 280 nm and assayed for RNA polymerase (Fig. 3). RNA polymerase activity was detected in the first peak. A summary of the purification of the enzymes is given in Table 2. RNA polymerase CI and CII are purified 170- and 70-fold respectively starting from the soluble chromatin with a recovery of 7% in the former case and 83% in the later case.

Subunits of RNA Polymerase CI

Polymerase CI after purification with QAE-Sephadex (A_{50}) on polyacrylamide gel electrophoresis exhibits a single polypeptide band (Fig. 4A) nearer to the cathode end. When electrophoresed in gels with added 8 M urea it tends to be dissociated into more than one band (Fig. 4C) and in 0.1% sodium dodecylsulfate gels the subunit structure is most clear. The enzyme was resolved into at least four-peptide bands (Fig. 4B). The characterization of the subunits and their molecular weight have yet to be

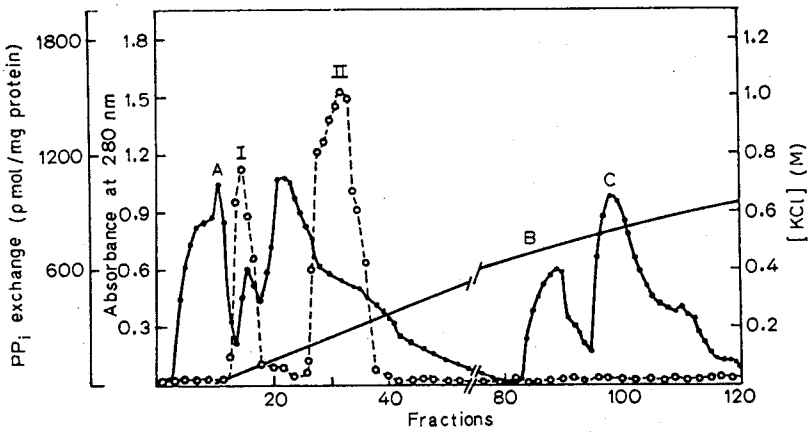

Fig. 1. *Elution profile of RNA polymerase sand factors from the DEAE-cellulose column.* The 30—55% ammonium sulphate fraction (27.5 mg) was charged on a DEAE-cellulose column (25×0.9 cm). 1-ml fractions of the KCl eluate (flow rate 1 ml/5 min) were collected and absorbance at 280 nm measured (●——●). The polymerase activity was assayed with each fraction (O----O) by the pyrophosphate exchange method as described in Materials and Methods. ——, KCl concentration

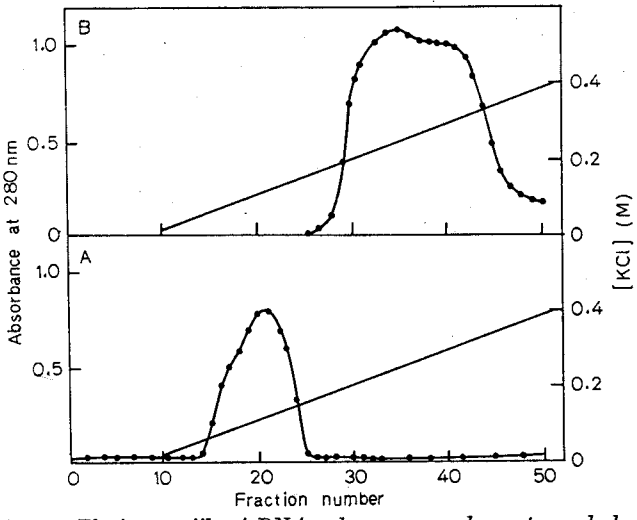

Fig. 2. *Elution profile of RNA polymerases rechromatographed on a DEAE-cellulose column.* (A) 5 ml containing 3.4 mg protein from first activity peak (RCI) and (B) 5 ml containing 10 mg protein from the second activity peak (RCII) as shown in Fig. 1 was charged separately on DEAE-cellulose column (25×0.9 cm) and eluted in KCl. 1-ml fractions (flow rate 1 ml/5 min) were collected and absorbance at 280 nm (●——●) was measured. ——, KCl concentration

assessed. Polymerase CII at the purification thus far achieved has been found to be inhomogeneous.

Other Protein Peaks from the DEAE-Cellulose Column

From Fig. 1 it is apparent that there are peaks other than RNA polymerases. The protein from the first, fourth and fifth peaks when added to RNA polymerases can influence the activity of both the enzymes and these are designated as A, B and C, respectively. Fraction A and C inhibit while fraction B stimulates the reaction. When assayed for nucleases it is found that fraction A has high RNAase activity while other fractions have practically none (Table 3). DNAase could not be detected in any of the fractions.

pH Optimum for RNA Polymerase CI and CII

It is seen from Fig. 5 that the activity of both the polymerases is maximum at pH 8.0. There is a rapid fall in the activity beyond pH 8.0. Even at pH 8.4 the activity is reduced to half of that

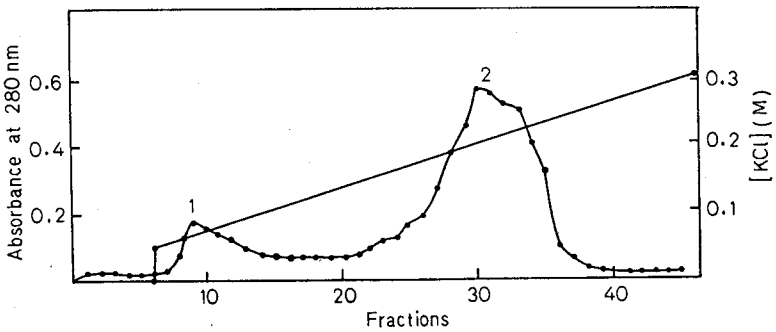

Fig. 3. *Elution profile of RNA polymerase CI from QAE-Sephadex (A_{50}).* 2 ml containing 2 mg RNA polymerase CI obtained from cellulose column chromatography was loaded onto a QAE-Sephadex column (25×0.9 cm). 1-ml fractions were collected at the rate of 1 ml/5 min and absorbance at 280 nm (●——●) was measured. ——, KCl concentration

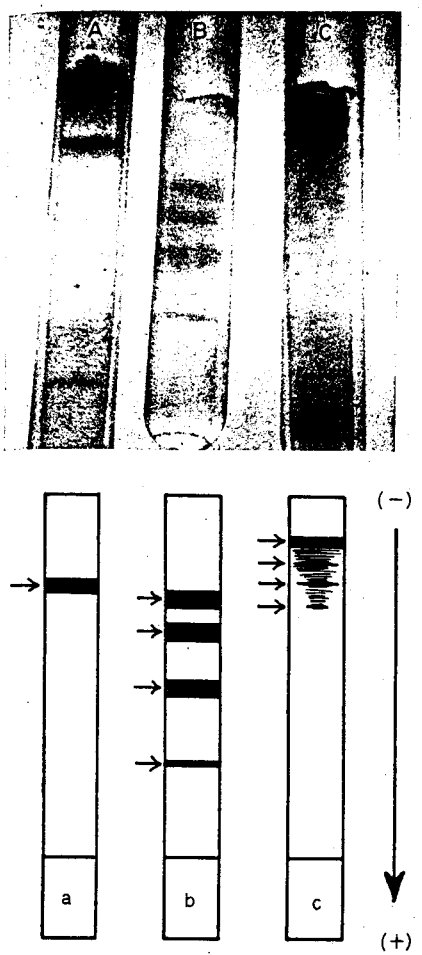

Fig. 4. *Polyacrylamide-gel electrophoresis of RNA polymerase I.* (A) Polyacrylamide gels, pH 8.3, containing 5% acrylamide, and 0.133% methylene bisacrylamide, were electrophoresed at 4 mA per tube for 2 h. RNA polymerase CI (QAE-Sephadex fraction), 10 μg in 50 μl was applied. (B) Dodecylsulfate gels, containing 0.1% sodium dodecylsulfate, 0.1 M sodium phosphate pH 7.2, 5% acrylamide and 0.135% methylene bisacrylamide, were electrophoresed at 8 mA per tube for 4 h. Polymerase CI (QAE-cellulose fraction), 20 μg in 0.1 ml of application buffer containing 0.1% sodium dodecylsulfate, 1% 2-mercaptoethanol, 0.01 M sodium phosphate, pH 7.2, 10% glycerol, and 0.002% bromophenol blue, and applied directly to the gels. (C) 8 M urea gels, pH 4.3, containing 5% acrylamide in the running gel and 2.5% acrylamide in stacking gel, were electrophoresed at 4 mA per tube for 2 h. Polymerase CI (QAE-cellulose fraction), 30 μg in 50 μl of 8 M urea containing 1% mercaptoethanol and 0.0002% bromophenol blue was applied

recorded at pH 8.0. At neutral pH, 60% of the optimal activity is exhibited.

Divalent-Cation Requirements

Fig. 6 and 7 show the requirement for divalent cations of RNA polymerase CI and CII, respectively. RNA polymerase CI displays maximum activity with Mn^{2+} at a concentration of 2 mM. Partial activity with Mg^{2+} is maintained at a broader concentration range (5—10 mM). At 8 mM Mg^{2+} only two thirds of the activity of that obtained with Mn^{2+} has been noted. However, the contrary is found with RNA polymerase CII. Maximum activity is recorded at a concentration of 10 mM Mg^{2+} while partial activity is obtained at a concentration of 2 mM Mn^{2+}, but this activity presents only 40% of that with Mg^{2+}. This pattern is maintained even in presence of different amounts of enzymes and nucleotides in both the cases. Apparently, RNA polymerase CI prefers Mn^{2+} while polymerase CII prefers Mg^{2+}.

Table 3. *RNAase assay with DEAE-cellulose fractions*
1 ml incubation mixture containing 0.04 M Tris-HCl pH 8.0; 0.2 mM EDTA; 5 mM 2-mercaptoethanol; 2 mM $MnCl_2$; 0.01 M $MgCl_2$; 0.4 mM K_2HPO_4; 0.08 mM KCl; 1.52 A_{260} units RNA (coconut endosperm) and 50 μg protein from each peak was incubated at 37 °C for 30 min; the reaction was stopped by perchloric acid and centrifuged at 10000 × *g* for 15 min. The supernatant was collected and the absorbance was measured at 260 nm. The results were corrected for the blank at which the reaction was stopped at zero time. 1 unit of activity corresponds to 0.001 A_{260} unit increase/min under the assay condition

Materials	Activity
	units/mg
1st DEAE-cellulose peak (factor A)	800
2nd DEAE-cellulose peak (RCI)	11
Purified RCI (QAE-Sephadex)	0
3rd DEAE-cellulose peak (RCII)	15
4th DEAE-cellulose (factor B)	10
Purified factor B (QAE-Sephadex)	0
5th DEAE-cellulose (Factor C)	9
Purified factor C (electrophoresis)	0

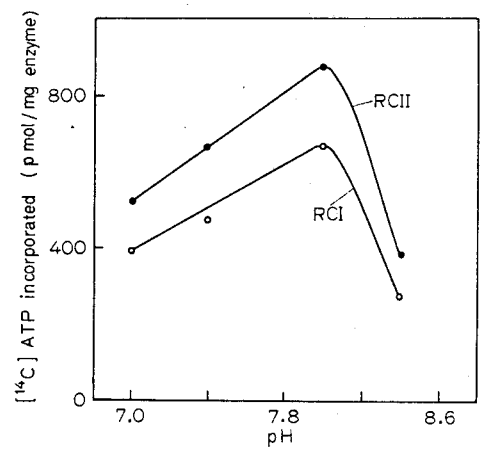

Fig. 5. *pH optimum for the enzyme activity.* The incubation system was the same as described under Materials and Methods except the 0.01 M Tris-HCl was of different pH. 50 μg RNA polymerase CI from DEAE-cellulose fractions (○——○) or 50 μg RNA polymerase CII from DEAE-cellulose fractions (●——●) and 5 μg of factor B were used in each system

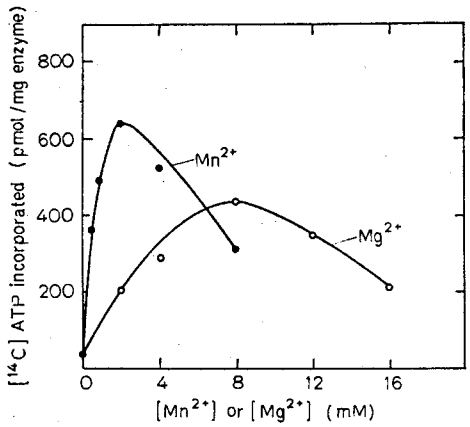

Fig. 6. *Divalent-cation requirement for RNA polymerase CI.* The incubation systems were the same as described in Materials and Methods except for divalent cations. $MnCl_2$ or $MgCl_2$ was used in different concentrations. 50 µg RNA polymerase CI (RCI) from DEAE-cellulose fractions. and 5 µg factor B were used in these experiments

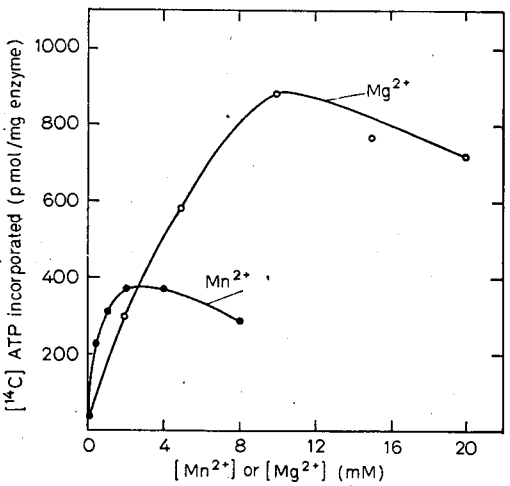

Fig. 7. *Divalent-cation requirement for RNA polymerase CII.* The conditions of the experiments were as used in Fig. 6. 100 µg RNA polymerase CII (RCII) and 10 µg factor B were used in these experiments

Requirements for RNA Polymerase CI and CII

RNA polymerase CI and CII after purification through a DEAE-cellulose column exhibit an absolute requirement for DNA (Table 4). Denatured DNA is very slightly effective in case of both polymerase CI and CII. If fraction B is omitted, the activity decreases to 7—10% of that with fraction B. However, with denatured DNA or λ DNA this decrease is not noted suggesting that the fraction B acts only with double-stranded DNA and that as well is very specific. When fraction C is added at zero time, an inhibition in RNA synthesis is exhibited. RNA polymerase CI requires more DNA (30 µg/50 µg protein) to yield maximum activity whereas 10 µg DNA can satisfy the requirement in case of polymerase CII (Fig. 8). However, this figure may not be comparable since polymerase CII

Table 4. *Requirements for RNA polymerase*
The incubation system was the same as described under Materials and Methods; 250 µg RNA polymerase CI, 250 µg RNA polymerase CII and 30 µg each of factor B and C obtained from the DEAE-cellulose (Fig. 1) were used in appropriate cases

Conditions of the experiment	[14C]ATP incorporated	
	CI	CII
	pmol/mg enzyme	
Complete system[a]	740	972
— Coconut DNA	0	0
— Factor B	80	132
— Coconut DNA, — Factor B, — denatured coconut DNA	51	90
— Coconut DNA, + denatured coconut DNA	50	137
— Coconut DNA, + λ DNA, — Factor B	145	—
— Coconut DNA, + λ DNA,	180	—
— Coconut DNA, + calf thymus DNA — Factor C	632 213	901 300
— Coconut DNA, + denatured DNA, — factor C	110	130
— Mn^{2+}, + Mg^{2+}	382	—
— Mg^{2+}, + Mn^{2+}	—	448
— 0.2 M KCl	1072	1436

[a] Complete system contains factor B also.

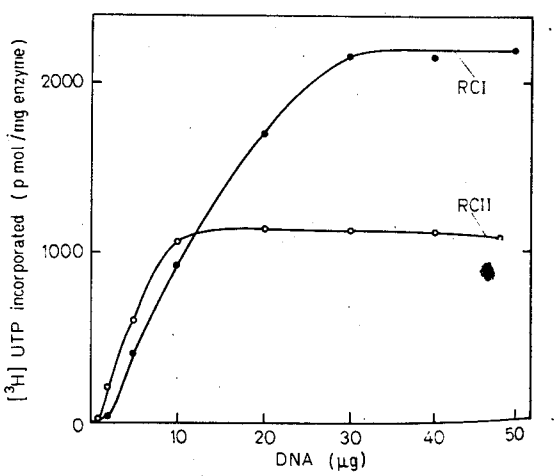

Fig. 8. *Optimal concentration of DNA for the enzyme activity.* The incubation system was the same as described in Materials and Methods except with different concentration of DNA (coconut endosperm). 50 µg RNA polymerase CI (QAE-Sephadex fraction) or 50 µg RNA polymerase CII (DEAE-cellulose fraction) and 5 µg factor B were used in each incubation system

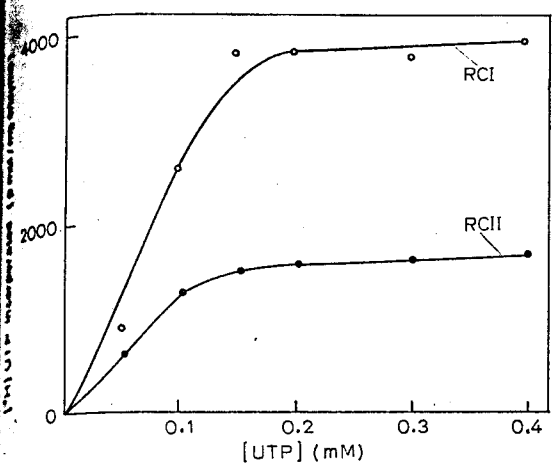

Fig. 9. *Optimal concentration of UTP for the enzyme activity.* The incubation system was the same as described in Materials and Methods except with different concentration of nucleotides of which UTP was labelled. The concentration of other nucleotides was same as that of UTP. 50 μg RNA polymerase CI (QAE-Sephadex fraction) (○) or 50 μg RNA polymerase CII (DEAE-cellulose fraction) (●) and 5 μg of factor B were used in each incubation system

less pure. Optimal activity has been recorded with 0.15 mM UTP in the case of both the enzymes (Fig. 9). The enzyme activities are influenced by the ionic strength as is usually observed with other RNA polymersaes. In presence of 0.2 M KCl an increase of 40—50% in enzyme activity is recorded. Since RNA polymerase CI is highly purified most of the work has been done with this polymerase only.

DISCUSSION

RNA polymerase from higher organisms has been reported earlier but due to some inherent difficulties with the system this enzyme could not be purified [6]. In contrast, RNA polymerase from bacterial systems has been purified extensively and a homogeneous protein with its subunits has also been characterized [2]. In the case of higher organisms, RNA polymerase has been resolved into two or three species and the requirements of these species were shown to be different [33, 34]. The earlier reports, however, suggest that one is associated with the nucleolus and the other with the nucleoplasm [15]. With the present system two forms are isolated from the chromatin that includes the nucleochromosomal apparatus. Not only RNA polymerase but also two factors which can modulate the activity are also detected in the chromatin. Comparison of the two forms from the chromatin with the forms I and II of others may be fortuitous as in these other cases sonication or other drastic procedures are commonly used for solubilization of polymerases. In the present case RNA polymerase CI has been purified to homogeneity and contains at least four-subunits as evidenced from gel electrophoretic pattern (Fig. 4). Its specificity for native DNA from an homologous system is established. DNA from the calf thymus is less effective than cocconut DNA when the factor B is used. With λ DNA there is no increase in RNA synthesis on addition of factor B. The relationship between the present two forms of RNA polymerase and the RNA polymerases described by others [16, 33, 34] from nucleolar (I) and nucleoplasm (II) has yet to be established.

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