POLY(A) SYNTHESIS BY RNA POLYMERASE II FROM RAT LIVER

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SUMMARY

Multiple forms of DNA-dependent RNA polymerase were resolved by DEAE-Sephadex chromatography. In addition to RNA polymerases, an active poly(A) polymerase was also fractionated. RNA polymerases were examined for their capacity to synthesize poly(A). None of the freshly prepared enzymes could efficiently make poly(A) in presence or absence of exogenous primers. However, "aging" of polymerase II by simple incubation at 37°C resulted in the loss of RNA polymerizing activity with a corresponding increase in poly(A) synthesizing activity. Transformation of RNA polymerase to poly(A) polymerase resulted in reduced capacity to transcribe native DNA and altered chromatographic behavior. The results suggest that subunits of polymerase II obligatory to DNA-dependent RNA synthesis were degraded by "aging" and that a stable subunit of the RNA polymerase could preferentially make poly(A).

INTRODUCTION

It is well recognized that DNA-dependent RNA polymerase (E.C.2.7.7.6) of eukaryotes exists in multiple forms with different intranuclear localizations (for a review, see ref. 1). The nucleolar enzymes (IA or IV and I) are insensitive to the mushroom toxin α-amanitin, whereas the major nucleoplasmic enzyme (form II) is completely inhibited by the toxin (2-5).

For the past few years, we have been interested in understanding a possible relationship of different forms of RNA polymerase to eukaryotic poly(A) polymerase (E.C.2.7.7.19). The need for a post-transcriptional addition of poly(A) consisting of 150-200 adenylic acid units for proper processing of most eukaryotic mRNAs (6) prompted us to investigate the role of RNA polymerase in this important cellular function. We reasoned that in analogy to the corresponding bacterial enzyme (7), poly(A) polymerase could be an integral part of eukaryotic RNA polymerase. In order to test this possibility, RNA polymerases, particularly form II due to its putative role in mRNA synthesis (1), were examined for their ability to synthesize poly(A). The results demonstrated that polymerase II had minimal poly(A) polymerase

activity but "aging" by simple incubation at 37°C resulted in its conversion to poly(A) polymerase.

METHODS

Isolation of rat liver nuclei and solubilization of nuclear RNA polymerase were essentially by the procedure described by Jacob et al (3,8). The method essentially consisted of a short incubation of isolated nuclei in an alkaline Tris-HCl buffer containing $MgCl_2$, EDTA and dithiothreitol (TMED buffer) and of subsequent centrifugation of the nuclear lysate at 40,000 x g for 40 min. The supernatant, containing all of the RNA and poly(A) polymerase activities, was precipitated with $(NH_4)_2SO_4$ (0.4 g/ml) and recentrifuged. The pellet was resuspended in 5-10 ml of 50 mM Tris-HCl (pH 7.9)-25% glycerol (v/v)-5 mM $MgCl_2$-0.1 mM EDTA-0.5 mM DTT (TGMED buffer) containing 0.03 M $(NH_4)_2SO_4$ and subjected to overnight dialysis against the same buffer. The enzyme extract was then fractionated by DEAE-Sephadex chromatography as described previously (9,10).

RESULTS

DEAE-Sephadex column chromatographic profile of RNA polymerase and poly(A) polymerase extracted from rat liver nuclei. Figure 1 represents the DEAE-Sephadex chromatographic profile of RNA polymerase and poly(A) polymerase from isolated nuclei of rat liver. As previously reported (1), RNA polymerase was resolved into four main peaks: I, II, III and IV (IA). In addition, as reported for nuclear (11) and mitochondrial (12) poly(A) polymerases, poly(A) polymerase activity was eluted in the void volume of the column. Poly(A) polymerase was assayed under optimal conditions, using synthetic poly(A) as a primer. Neither by substituting Mg^{2+} (1.5 mM) for Mn^{2+} nor by using tRNA as a primer could significant poly(A) polymerase activity be detected with the RNA polymerases. Poly(A) polymerase and RNA polymerase IV were eluted in two distinct peaks. Although these two enzymes were eluted in the wash fractions they could be resolved even further by fractional precipitation with $(NH_4)_2SO_4$. Upon further purification, poly(A) polymerase retained its characteristics such

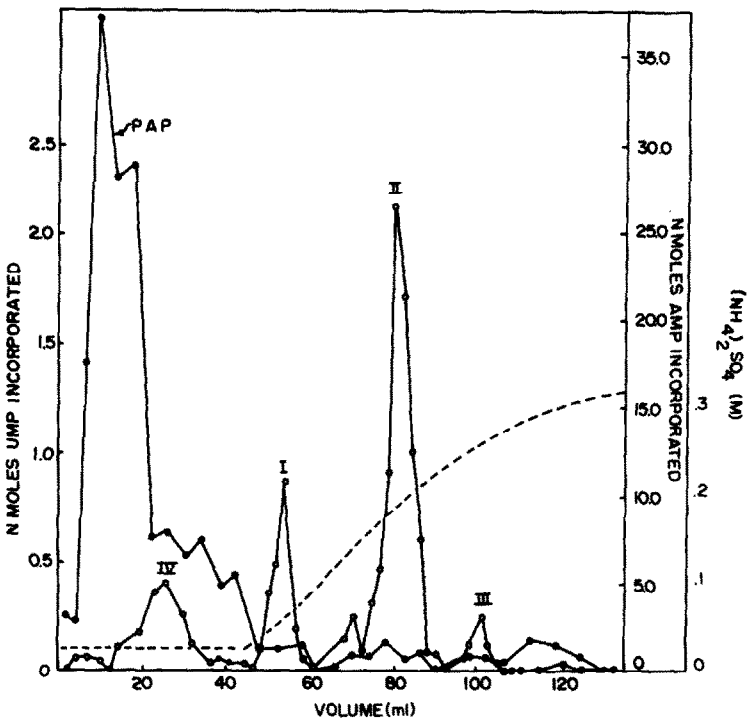

FIGURE 1: COLUMN CHROMATOGRAPHIC PROFILE OF RNA POLYMERASES AND POLY(A) POLYMERASE EXTRACTED FROM RAT LIVER NUCLEI. Dialyzed enzyme extract from 90 g liver was layered on a 12 x 1.5 cm DEAE-Sephadex A-25 column, equilibrated with TGMED buffer. The column was then washed with 20 ml of 0.03 M $(NH_4)_2SO_4$-TGMED and a linear gradient (150 ml) of 0.03-0.55 M $(NH_4)_2SO_4$ was attached. 2 ml fractions were collected and 0.1 ml aliquots were assayed for RNA polymerase as described previously (10). The reaction mixture for poly(A) polymerase activity (PAP) contained in 0.3 ml: 70 mM Tris-HCl (pH 7.8), 0.5 mM $MnCl_2$, 0.5 mM[^{3}H]ATP (2,000 cpm/nmole) and 120 µg poly(A). The reaction mixture was incubated at 37°C for 1 hr. The reaction was terminated by the addition of 100 µg ATP, 2 mg carrier protein and 1 ml 10% TCA containing 0.04 M $Na_4P_2O_7$. The nmoles AMP incorporated were determined as for the RNA polymerase. O—O RNA polymerase activity; ●—● poly(A) polymerase (PAP) activity; --- $(NH_4)_2SO_4$ concentration.

as its preference for Mn^{2+}, exclusive utilization of ATP as substrate and its requirement for a primer (manuscript in preparation). Extraction of the enzymes from isolated nuclei using the high salt procedure (13) neither increased recovery nor altered elution pattern of any of the enzymes. However, poly(A) polymerase extracted by the high salt procedure could be assayed quantitatively only after separating the enzyme from bulk of the nuclear soluble proteins by rechromatography on a phosphocellulose column. On the other hand, poly(A) polymerase extracted together with RNA polymerases by the

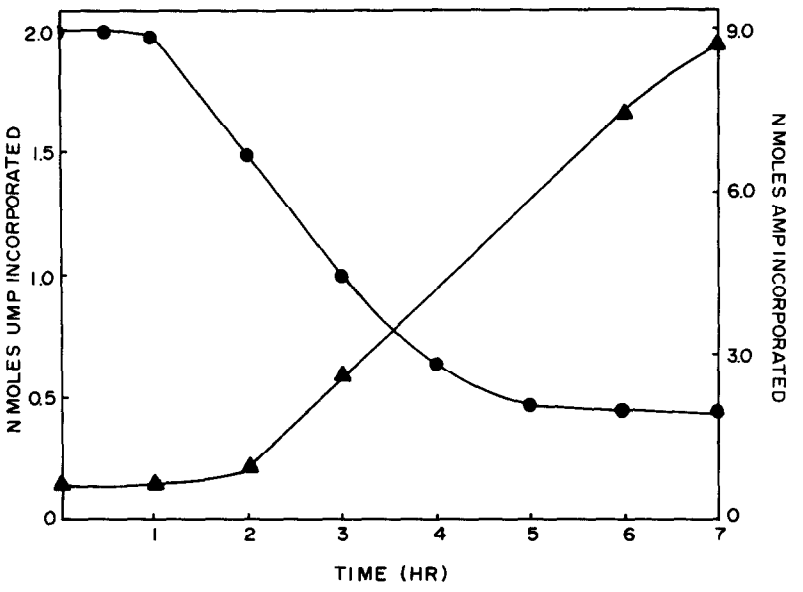

FIGURE 2: EFFECT OF INCUBATION AT 37°C ON THE RNA SYNTHESIZING CAPACITY OF RNA POLYMERASE II. RNA polymerase II, obtained from the peak fractions of a DEAE-Sephadex column, was incubated at 37°C. At the times indicated 0.1 ml aliquots were removed and assayed for RNA polymerase or poly(A) polymerase activity as described in the legend to Figure 1 except that for the poly(A) polymerase assay, the $MnCl_2$ and ATP concentrations were doubled. All assays were performed in triplicate and the results shown are the average of five experiments. ●—● RNA polymerase activity; ▲—▲ poly(A) polymerase activity.

low salt procedure could be resolved by one-step DEAE-Sephadex chromatography.

Poly(A) synthesis by RNA polymerase II after incubation at 37°C. RNA polymerase II catalyzed primarily DNA-dependent RNA synthesis (Figure 1). However, simple incubation of form II at 37°C dramatically altered the characteristics of the enzyme. With increasing time of incubation at 37°C the ability of the enzyme to incorporate UTP into RNA was progressively reduced (Figure 2) with a concurrent increase in the ability of the enzyme to function as a poly(A) polymerase. Before incubation, RNA synthesis catalyzed by enzyme II was almost completely DNA-dependent. After 7 hr at 37°C more than 80% of this enzyme was converted to poly(A) polymerase with very little capacity to incorporate UTP. Thus "aging" of the enzyme at 37°C converts polymerase II to a poly(A) polymerase. It should be pointed out that the small fraction of

"unaged" polymerase II left with bulk of the poly(A) polymerase derived from "aged" II sometimes prevents the detection of poly(A) polymerase. In such a case, poly(A) polymerase was separated from any "unaged" polymerase II by rechromatography as described in the following section.

DEAE-Sephadex chromatography of "aged" RNA polymerase II. In order to determine if the poly(A) polymerase activity of enzyme II after "aging" at 37°C was similar to the nuclear poly(A) polymerase activity present in the nuclear extract (Fig. 1) the "aged" enzyme preparation was rechromatographed on DEAE-Sephadex. Figure 3 shows the chromatographic profile of enzyme II after 7 hr of "aging" at 37°C. The bulk of the poly(A) polymerase activity was eluted

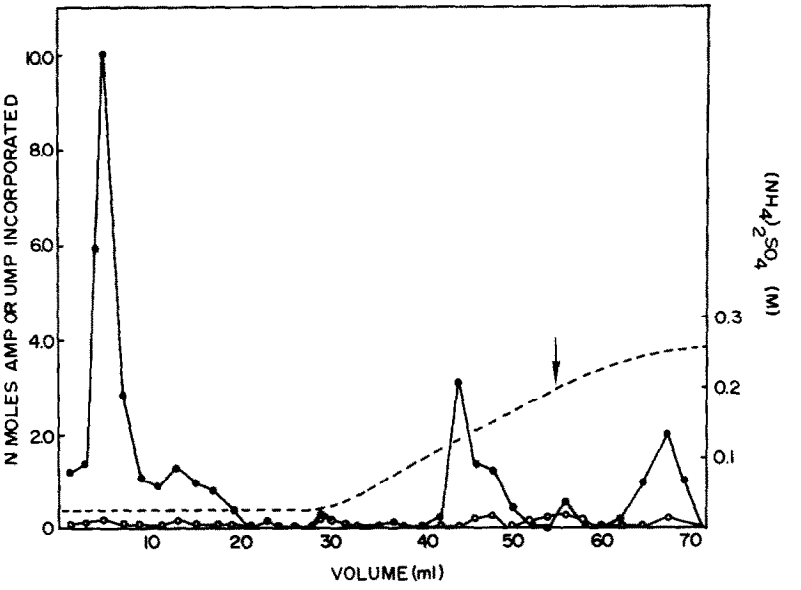

FIGURE 3: COLUMN CHROMATOGRAPHIC PROFILE OF RNA POLYMERASE II AFTER INCUBATION FOR 7 HOURS AT 37°C. The peak fractions containing RNA polymerase II were incubated at 37°C for 7 hours. The "aged" enzyme was dialyzed for 4 hours against TGMED containing 0.03 M $(NH_4)_2SO_4$ and layered on a 10 x 0.9 cm DEAE-Sephadex A-25 column that had been previously equilibrated in the same buffer. The column was then washed with 10 ml of the same buffer and a linear gradient of 0.03 M-0.5 M $(NH_4)_2SO_4$-TGMED was attached. 1 ml fractions were collected and 0.15 ml aliquots were taken to assay for RNA polymerase or poly(A) polymerase activity as described in the legend to Figure 2. ●—● poly(A) polymerase activity; O—O RNA polymerase activity. Arrow indicates position where RNA polymerase II elutes when not subjected to incubation at 37°C.

in the 0.03 M $(NH_4)_2SO_4$ wash as was observed for poly(A) polymerase extracted from whole nuclei. It should be pointed out that "unaged" polymerase II upon rechromatography on DEAE-Sephadex was eluted at 0.2 M $(NH_4)_2SO_4$ with no poly(A) polymerase activity detectable in any column fractions. Two peaks of poly(A) polymerase activity, albeit at considerably lower levels, were eluted at 0.13 M and 0.26 M $(NH_4)_2SO_4$ on rechromatography of "aged" polymerase II. These peaks appear to represent intermediates in the "aging" process. Unlike the bulk of the poly(A) polymerase eluted in the 0.03 M $(NH_4)_2SO_4$ fractions, the intermediately "aged" enzymes catalyze essentially unprimed reactions. After 3-4 hr (intermediate) of "aging" at 37°C, enzyme II could synthesize poly(A) or RNA to approximately the same extent (Fig. 2). When rechromatographed, this intermediately "aged" preparation showed a decrease in the RNA polymerase activity compared to "unaged" form II and a corresponding increase in poly(A) polymerase activity in the wash fraction.

Comparison of properties of poly(A) polymerase obtained from "aged" RNA polymerase II with those of nuclear poly(A) polymerase. Table 1 shows the characteristics of poly(A) polymerase extracted from whole nuclei and of the same enzyme derived from RNA polymerase II. They were strikingly similar in divalent metal ion (Mn^{2+} > Mg^{2+}) and primer (poly(A) > tRNA) requirements. Both enzymes utilized ATP exclusively as substrate. Both reactions were insensitive to actinomycin D or ethidium bromide. Since ethidium bromide can bind to poly(A) used as the primer (12,14), the insensitivity of the poly(A) polymerase reaction to this compound shows that poly(A) does not act as a template. Both enzymes were insensitive to α-amanitin. Finally, the bulk of the poly(A) polymerase derived from polymerase II and nuclear poly(A) polymerase had similar sedimentation constants (4.2S) as determined by glycerol density gradient centrifugation. In addition, poly(A) polymerase obtained from "aged" II could catalyze an unprimed synthesis of poly(A) to some extent.

DISCUSSION

The present study clearly demonstrates that "aging" of RNA polymerase II by incubation at the physiological temperature results in progressive loss of

TABLE I

COMPARISON OF PROPERTIES OF POLY(A) POLYMERASE CHROMATOGRAPHICALLY SEPARATED FROM NUCLEAR RNA POLYMERASES WITH POLY(A) POLYMERASE DERIVED FROM FORM II BY "AGING"

	NUCLEAR POLY(A) POLYMERASE	POLY(A) POLYMERASE FROM "AGED" FORM II
DIVALENT CATION	$Mn^{++} > Mg^{++}$	$Mn^{++} > Mg^{++}$
SUBSTRATE	ATP	ATP
PRIMER (600 µg/ml)	poly(A)> tRNA	poly(A)> tRNA
ETHIDIUM BROMIDE (150 µg/ml)	insensitive (100%)	insensitive (100%)
α-AMANITIN	insensitive (100%)	insensitive (100%)
SEDIMENTATION CONSTANT	4.2S	4.2S

Nuclear poly(A) polymerase from the 0.03 M $(NH_4)_2SO_4$ wash of the DEAE column of the nuclear extract was further purified by phosphocellulose and hydroxylapatite chromatography (manuscript in preparation). Poly(A) polymerase from "aged" RNA polymerase II was obtained from the wash fractions of the DEAE column of the "aged" enzyme. Sedimentation coefficients were estimated from glycerol gradient (10-30%) centrifugation. The gradients were centrifuged at 42,000 rpm for 15 hr in SW50L rotor. BSA (4.5S), glyceraldehyde 3-P-dehydrogenase (7S) and E.coli RNA polymerase (15S) were used as markers. Enzyme assays were carried out as described in the legend to Figure 1.

its RNA-synthesizing capacity with corresponding gain in its ability to catalyze a primer-dependent synthesis of poly(A). Poly(A) polymerase of eukaryotes may be a stable subunit of polymerase II remaining after degradation of the relatively unstable subunits required for RNA synthesis. A comparison of the subunit compositions of the two enzymes is in progress in order to elucidate their interrelationship.

Several examples of proteolytic modification of prokaryotic RNA polymerase exist in the literature. Thus, vegetative RNA polymerase from B.subtilis can be converted into the homologous spore enzyme (15,16) by a proteolytic modification of β subunit. Recently, Leighton et al (17) have unequivocally demonstrated that a basic serine protease is responsible for the modification of RNA polymerase in B.subtilis during sporulation. Whether such a specific

protease is required for the modification of eukaryotic RNA polymerase II is currently under investigation.

Since polymerase II presumably synthesizes mRNA (1), it is conceivable that the same enzyme adds poly(A) post-transcriptionally to the 3' end of mRNA. It is not yet known whether poly(A) polymerase routinely detected in the wash fractions of DEAE-Sephadex columns and RNA polymerase II are the products of two separate genes or whether the former enzyme is derived from RNA polymerase II by structural modifications.

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