TWO DISTINCT POLY(A) POLYMERASES IN YEAST NUCLEI

Lawrence A. Haff and Elizabeth B. Keller

Biochemistry, Molecular and Cell Biology, Cornell University
Ithaca, New York 14850

Received February 20,1973

SUMMARY: Yeast extracts have a poly(A) polymerase activity which catalyzes the addition of adenylate residues to the 3'-terminus of primer RNA. On purification of this activity, two poly(A) polymerases were found. Poly(A) polymerase I elutes from a DEAE-cellulose column at very low salt concentration and prefers as primer an RNA without an oligo(A) segment at the 3'-terminus. Poly(A) polymerase II elutes at a higher salt concentration from DEAE-cellulose and has a requirement for a primer with an oligo(A) segment at the 3'-terminus. The two enzymes are localized largely in the nucleus. It is suggested that these two enzymes function sequentially, first I then II, in forming the long poly(A) sequences that are found on messenger RNAs in yeast.

Poly(A) tracts have been located at the 3'-hydroxyl terminus of messenger RNA (mRNA) molecules from many eukaryotic organisms (1-4). Extracts from a number of eukaryotes have poly(A) polymerase activity which catalyzes the addition of adenylate residues to the 3'-hydroxyl terminus of RNA molecules in vitro (5-7). Since this enzyme activity has been found in yeast (7) and poly(A) tracts have been found on yeast mRNA (8,9), we decided to employ the yeast system to investigate the role of poly(A) polymerase in the synthesis of poly(A) tracts on mRNA. Upon purification of the yeast activity it was found that there are in fact two distinct poly(A) polymerases in yeast. This report describes the properties and cellular localization of these two enzymes.

MATERIALS AND METHODS

Poly(A) was obtained from Miles Laboratories. Other synthetic polynucleotides were the gift of Dr. Leon Heppel. The yeast ribosomal RNA (rRNA) preparation used was precipitated by 10% NaCl (10) from the total RNA extracted from a yeast ribosomal pellet by a phenol-sodium dodecyl sulfate treatment. Enzymes were prepared from log-phase S. cerevisiae haploid strain S288C (wild type).

The poly(A) polymerase assay contained the following in a volume of 0.25 ml: 0.5 mM [^{3}H]-ATP (4 µCi/µmole, Schwartz-Mann), 50 mM Tris-HCl (pH 8.5), 5.0 mM mercaptoethanol, 1.0 mM $MnCl_2$, 1.0 mg/ml primer RNA, and enzyme. Incubations were for 30 minutes at 30°. AMP incorporation was determined essentially as described by Twu and Bretthauer (7). A unit of enzyme is defined as that amount of enzyme which causes the incorporation of one nanomole of AMP (from ATP) into acid insoluble material in ten minutes.

The chain length of labeled oligo(A) product was determined by precipitating and washing reaction mixtures with 5% trichloroacetic acid and hydrolyzing the RNA with 0.3 M KOH at 37° for 18 hours. The hydrolyzed sample was co-chromatographed with standards of AMP and adenosine on polyethyleneimine-cellulose sheets (Brinkmann) with 0.4 M LiCl (11). The appropriate areas were cut out and counted in toluene scintillation solution. The ratio of total counts (AMP and adenosine) to counts appearing as adenosine is equal to the chain length.

RESULTS

Yeast Nuclei Contain Two Poly(A) Polymerases - Nuclei were obtained from yeast spheroplasts as described by Rozijn and Tonino (12) and further purified in a sorbitol gradient as described by Bhargya and Halvorson (13). These nuclei typically had a DNA:RNA:protein ratio of 1:5:25, and contained 90% of the total poly(A) polymerase activity of the cell. The ribosomal pellet prepared from the same spheroplasts contained most of the remaining activity. Nuclei were homogenized in 0.02 M Tris-HCl (pH 8.0), 0.5 mM EDTA, 0.1 mM dithioerythritol and centrifuged at 200,000 x g for two hours. The supernatant was chromatographed on DEAE-cellulose and was found to produce two major peaks of activity (Fig. 1). The enzymes that elute at low and high salt concentration are referred to as poly(A) polymerase I and poly(A) polymerase II, respectively. They differ in their primer RNA requirements as will be shown below.

To study the properties of these two enzymes it was more convenient to

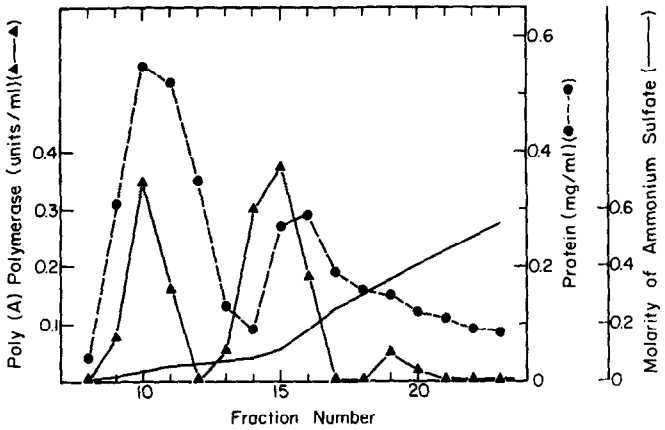

Figure 1. DEAE-cellulose Chromatography of Nuclear Extract. The column (1.2 x 10 cm) was eluted with a linear gradient from 0 to 1.0 M ammonium sulfate in 20 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 0.1 mM dithioerythritol. The total volume of the gradient was 20 ml, and each fraction was 0.75 ml. (- ▲ -) poly(A) polymerase assayed with 1.0 mg/ml poly(A) as primer, (- ● -) protein concentration, (——) molarity of ammonium sulfate from measurement of conductivity of fractions.

purify them from a homogenate produced by a Gaulin homogenizer as described in the following sections.

Poly(A) Polymerase I Preferentially Uses Messenger-Like RNA as Primer - When a yeast extract was made by a method which totally disrupted the cells, including the nuclei, about 50% of the total cell poly(A) polymerase activity was found in the soluble protein fraction (7). Upon DEAE-cellulose chromatography this activity eluted as a single peak at a low salt concentration corresponding to peak I in Fig. 1. This enzyme, which has been purified 100 fold (Haff and Keller, manuscript in preparation) has a nearly absolute requirement for primer (Table 1). The primer action of rRNA and poly(A,C,G,U) was tested because these RNAs more nearly resemble nascent mRNA than do homopolymers. They proved to be the most active primers, much more active than poly(A). Poly(G), poly(C), and to some extent poly(U), function as primers, showing that enzyme I can initiate poly(A) chains by adding an adenylate residue to a 3'-hydroxyl C, G, or U. The time course of polymerization onto rRNA shown in Fig. 2 suggested that enzyme I actually prefers non-oligo(A) termini, for as the oligo(A) chain lengthened, the rate declined. In this experiment the rate had

TABLE 1

PRIMER SPECIFICITY OF POLY(A) POLYMERASES

Primer	Initial Rate Relative to Rate with Poly(A) I	II
None	0.02	0.00
Poly(A)	1.00	1.00
Poly(C)	0.55	0.00
Poly(G)	1.70	0.00
Poly(U)	0.09	0.00
Poly(A,U)	1.70	0.00
Poly(A_4,U)	1.15	0.00
Poly(A,C,G,U)	5.78	0.00
rRNA	4.00	0.05

Each RNA was tested at a concentration of 1.0 mg/ml, which gave a maximal initial rate in each case. An exception was poly(A,C,G,U), which was tested at 0.5 mg/ml because of limited supply.

markedly declined by 120 minutes when analysis showed that an average of seven adenylate residues had been added. When a second portion of rRNA was added, the rate was nearly restored to its initial value, demonstrating that the slowing of the reaction was not primarily due to loss of enzyme activity. The extra activity resulted in a reduction in the average chain length of newly polymerized adenylates from seven before the addition to four after the addition (at 280 minutes). This combination of marked rate stimulation and decrease in average chain length further confirms the preference of enzyme I for a RNA primer without added terminal oligo(A) sequences.

Poly(A) Polymerase II Uses Only Poly(A) Sequences as Primer - When a yeast extract was made as in the preceding section by a method which disrupted the entire cell, including the nuclei, about 50% of the poly(A) polymerase activity of the cell was present in the ribosomal pellet, from which it could be extracted using 0.5 M ammonium chloride. These results confirm those of Twu and Bretthauer (7). Upon DEAE-cellulose chromatography of the ribosomal extract, two peaks of activity were found eluting at positions identical to

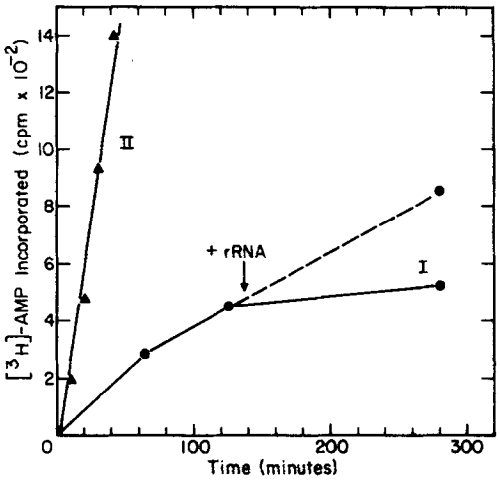

Figure 2. Time Course of Polymerization by Two Poly(A) Polymerases. Purified poly(A) polymerase I was assayed with rRNA and purified II with poly(A). The concentration of primer in each case was 1.0 mg/ml, which gave the maximal initial rate. An additional portion (1.0 mg/ml) of rRNA was added to one assay tube of I at 125 minutes as indicated by the arrow. (- ▲ -) enzyme II, (- ● -) enzyme I, (-- ● --) enzyme I with additional rRNA.

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those shown in Fig. 1. All of the cell's enzyme II and about one third of enzyme I appeared to be bound to the ribosomal pellet under these conditions in which nuclei had been disrupted. A 50-fold purified preparation of enzyme II (Haff and Keller, manuscript in preparation) required poly(A) for activity (Table 1). No other primer tested had significant activity. In contrast to enzyme I, activity did not fall off with increasing polymerization (Fig. 2). The rate was still linear when the chain length analysis showed an average of ten As per chain.

The Two Polymerases Have Many Properties in Common - The enzymes are very similar in their behavior towards nucleoside triphosphates (Table 2). Both have a strong preference for ATP, but both can utilize CTP at about 15% of the rate of ATP. GTP and UTP can be utilized at about 1-5% the rate of ATP. With both enzymes labeled AMP incorporation is inhibited strongly by GTP and inhibited less by CTP or UTP. Both enzymes function optimally in the presence of 1 mM Mn^{++}, but Mg^{++} can substitute for Mn^{++} with enzyme I, though poorly. The two enzymes chromatograph identically on Sephadex G-150 columns with an apparent molecular weight of 100,000 daltons. Neither is inhibited by actinomycin or α-amanitin. Both are completely inhibited by 1 mM p-hydroxymercuribenzoate.

TABLE 2

COMPARATIVE PROPERTIES OF POLY(A) POLYMERASES

Assay Conditions	Relative Polymerization Rate: I with rRNA primer	Relative Polymerization Rate: II with poly(A) primer
Complete system, [^{3}H]-ATP	1.00	1.00
-Mn^{++}, + 4 mM Mg^{++}	0.24	0.00 †
+ 1 mM CTP	0.45	0.78
+ 1 mM UTP	0.44	0.70
+ 1 mM GTP	0.07	0.09
Complete system, [^{3}H]-CTP ‡	0.15	0.13
Complete system, [^{3}H]-UTP ‡	0.03	0.01
Complete system, [^{3}H]-GTP ‡	0.05	0.03

‡ [^{3}H]-ATP omitted. Concentration and specific activity of substitute triphosphates were the same as those of [^{3}H]-ATP.

† Poly(A) becomes insoluble at about 4.0 mM Mg^{++}.

DISCUSSION

The poly(A) polymerase activity in yeast extracts reported by Twu and Bretthauer (7) is shown here to be due to two polymerases which differ in chromatographic behavior, primer requirement, and time course of polymerization. Poly(A) polymerase I can initiate chains on messenger-like RNA, but long chains can be formed only slowly. Poly(A) polymerase II cannot initiate on messenger-like RNA. The fact that it can use poly(A) as a primer suggests that it may be able to utilize the product of poly(A) polymerase I and elongate it. Further work, perhaps with temperature sensitive mutants for one or both of the polymerases, will be needed to establish this sequential mechanism for the synthesis of the long poly(A) tracts found on yeast mRNA (8,9).

ACKNOWLEDGEMENTS

We would like to thank Dr. Calvin McLaughlin for sending us a manuscript before publication (9). This investigation was supported by PHS Research Grant GM 10791 and Training Grant No. GM 00824.

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