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A 5′→3′ exoribonuclease of human placental nuclei: purification and substrate specificity

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ABSTRACT

An exoribonuclease that hydrolyzes single-stranded RNA by a 5'→3' mode yielding 5'-mononucleotides has been purified from human placental nuclei. Chromatographic studies of crude placental nuclear extracts suggest that the enzyme is a relatively abundant nuclear RNase. Poly(A) is degraded by a processive mechanism while rRNA is degraded in a partially non-processive manner, possibly because of its secondary structure. The enzyme has an apparent molecular weight of 113,000, derived from determinations of the Stokes radius (43 A) and sedimentation coefficient (6.3 S). Substrates with 5'-phosphomonoester end groups are 10-20 times better than 5'-dephosphorylated substrates. The locale of the enzyme in nuclei of normal human cells as well as its mode of action suggest a role in nuclear RNA processing or turnover.

INTRODUCTION

The greater resistance of capped mRNA to hydrolysis than its decapped counterpart in Xenopus oocytes (1) and in crude extracts of mouse L cells (1) and wheat germ (1, 2) first suggested the possible presence of 5'→3' exoribonucleolytic activity in these cells. Several papers (3-5) from this laboratory have described the purification and characterization of an exoribonuclease of Saccharomyces cerevisiae; the enzyme is a processive exoribonuclease hydrolyzing substrates with 5'-phosphate end groups to 5'-mononucleotides by a 5'→3' mode of hydrolysis. Capped mRNA is quite resistant (3). The enzyme recently has been purified to near-homogeneity and its mass (160 kDa) and further features of its substrate specificity have been reported (6). Recently, Lasater and Eichler (7) also described a 5'→3' exoribonuclease isolated from nucleoli of Ehrlich ascites tumor cells. The nucleolar location suggests that the enzyme may be involved in the heretofore enzymatically uncharacterized process of rRNA precursor processing, but the authors presented no data on the activity of the enzyme in cellular fractions other than nucleoli. Green et al. (8) reported that capped globin mRNA is

stable in Xenopus oocyte nuclei while the same decapped mRNA is not, suggesting the presence of a nucleoplasmic 5'→3' exoribonuclease.

In order to analyze the possible function of 5'→3' exoribonucleolytic activity, the locale, mechanism, and substrate specificity of such an enzyme from another higher eukaryotic source have been studied. Placenta was chosen because it is a readily available human source. The data presented here suggest that the enzyme is an abundant nuclear RNase, possibly having a role in RNA processing or turnover involving substrates with 5'-phosphate termini. Its possible use in isolating and studying RNA-protein complexes is suggested by the RNA splicing studies of Noble et al. (9).

MATERIALS AND METHODS

Preparation of Human Placental Cell Fractions

Fresh term placentas (250-500 g) were obtained from local hospitals and transported in ice. The membrane was removed and the placenta was cut into small pieces which were washed several times with 20 mM Tris-HCl buffer (pH 7.4) containing 1.5 mM $MgCl_2$, 10% glycerol, 1 mM α-tosyl fluoride, and 1 mM β-mercaptoethanol. The placental pieces were then suspended in 2 volumes (on the basis of the original placental weight) of the same buffer and homogenized using a Polytron homogenizer for about 10 min at low-to-medium speed so that most of the pieces were broken. The homogenate was poured through cheesecloth, and any unbroken pieces were resuspended in one volume of the buffer described above. The homogenization and filtration were repeated, and the combined filtrates were centrifuged at 6000 x g for 10 min. The cytoplasmic fraction was poured off, and the pellet was suspended in one volume of the same buffer and the centrifugation was repeated. The nuclear pellet at this stage was used for the purification of the 5'→3' exoribonuclease and could be frozen (-30°C) before proceeding.

For preparation of nucleoli and nucleoplasm for separate assays, the procedure of Lasater and Eichler was followed (7). Aliquots of the resuspended nuclei were spun and the nuclear pellet was washed by suspension in 0.25 M sucrose containing 10 mM Tris-HCl buffer (pH 8.0), 1 mM $MgCl_2$, and 0.5 mM dithiothreitol, followed by centrifugation through 0.88 M sucrose containing the same reagents. The washed nuclear pellet was disrupted by sonication, and after centrifugation, the supernatant solution was assayed for nucleoplasmic activity. The pellet fraction was treated with potassium phosphate buffer as described (7) and, following centrifugation, the supernatant solution was assayed for nucleolar activity.

Purification of the 5'→3' Exoribonuclease

For the purification of the exoribonuclease, a nuclear supernatant fraction was prepared in the manner described by Dignam et al. (10). The nuclear fraction was suspended in one-half volume (based on the original placental weight) of 20 mM Tris-HCl buffer (pH 7.7), 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM α-tosyl fluoride, and 0.5 mM dithiothreitol. The suspension was homogenized and brought to 0.5 M NaCl by adding 5 M NaCl. After stirring for 30 min, the fraction was centrifuged for 30 min at 25,000 x g. Ammonium sulfate was then added to the supernatant solution to 80% saturation, and after 30 min of stirring, the precipitate was collected by centrifuging for 30 min at 25,000 x g. The pellet was dissolved in 40 ml of 20 mM Tris-HCl buffer (pH 7.4) containing 50 mM KCl, 50% glycerol, 0.5 mM α-tosyl fluoride, and 0.5 mM dithiothreitol and could be stored at -20°C. The ammonium sulfate fraction was applied in two portions to DEAE-cellulose (DE-23, Whatman) columns (2 × 19 cm) following dialysis overnight (16 h) against 2 L of 20 mM Tris-HCl buffer (pH 7.7), 10% glycerol, 0.5 mM dithiothreitol (buffer A), containing 50 mM KCl. The DEAE cellulose columns were eluted stepwise with the following buffers: 80 ml of buffer A containing 50 mM KCl; 90 ml of buffer A containing 100 mM KCl; 100 ml of buffer A containing 250 mM KCl. Fractions (10 ml) of the 250 mM KCl wash were collected and assayed, and the active fractions were combined and precipitated with ammonium sulfate (0-80%) as described above. The precipitate was dissolved in 8 ml of buffer A plus 50 mM KCl and dialyzed overnight against 1 L of the same buffer. The dialyzed fraction was applied to a DEAE-cellulose column (2 × 10 cm) and eluted with a 480 ml linear gradient of 50 to 500 mM KCl in buffer A. Fractions (12 ml) were collected and assayed. Two peaks of activity were usually found and the second dominant one was precipitated with ammonium sulfate (0-80%). The precipitate was dissolved in 4 ml of buffer A containing 25 mM ammonium sulfate and dialyzed overnight against 1 L of the same buffer. The dialyzed fraction was applied to a heparin-agarose (Sigma) column (0.9 × 6 cm) and eluted with an 80 ml linear gradient of 25 mM to 300 mM ammonium sulfate in buffer A. Fractions (2 ml) were collected and assayed, and the active fractions were concentrated to about 2 ml using an Amicon YM-10 filter. To completely inactivate a low level 3'→5' exoribonuclease that elutes from the Sephacryl S-200 column at the same place as the enzyme described here, the heparin-agarose concentrate was heated at 60°C for 10 min prior to loading. By prior testing of a small aliquot (0.25 ml), we selected the time required for 50% inactivation of the poly(A)-hydrolyzing

activity, and the remaining fraction was then heated in the same manner. The fraction was then loaded on a Sephacryl S-200 (Sigma) column (2 × 25 cm) and eluted with buffer A containing 0.4 M KCl. Fractions (1.3 ml) were collected and assayed. The Sephacryl fractions were frozen in liquid nitrogen and stored at -40°C for periods of up to 6 months. The enzyme could be frozen in a similar manner after each purification step.

Assay of Exoribonuclease

For assays of the exoribonuclease during purification, the reaction mixtures (150 μl) contained [^{3}H]poly(A) (1 nmol, 6.5 × 10^4 cpm), poly(A) (Miles) (125 nmol), 33 mM sodium glycinate buffer (pH 9.5), 2 mM $MgCl_2$, 50 mM NH_4Cl, 0.5 mM dithiothreitol, 100 μg of acetylated bovine serum albumin (Bethesda Research Laboratories) and enzyme (1-5 units). (pH 9.5 was used for the enzyme assays to avoid acidic and neutral contaminating RNases in the cruder fractions.) After incubation at 37°C for 15 min, the reactions were stopped by the addition of 100 μl of 7% $HClO_4$. The mixtures were allowed to stand in ice for 10 min and centrifuged for 5 min in an Eppendorf centrifuge. The amount of acid-soluble radioactivity was measured using 100 μl of the supernatant solution. A reaction mixture lacking enzyme was used as a control. One unit of enzyme activity is the amount needed to release 5 nmol of [^{3}H]AMP (calculated on the basis of the total reaction mixture). For determinations of the substrate specificity and the mechanism of the reaction, reaction mixtures (50 μl) contained the same reagents except Tris-HCl buffer (pH 7.0) replaced the pH 9.5 buffer and the poly(A) was replaced by the substrate listed in the Figure or Table legends. The mixtures were incubated for 15 min at 37°C, then 50 μl of 7% $HClO_4$ were added, and after 5 min the mixtures were centrifuged as described above. Radioactivity was determined on 50 μl of the supernatant solution.

Preparation of Substrates

The labeled synthetic polyribonucleotides, [^{3}H]poly(A)·poly(U), [^{3}H]poly(A)·poly(dT), an RNA·M13 DNA hybrid, and [^{3}H]poly(A) with a 5'-triphosphate end group were prepared as previously described (6), as were the labeled yeast RNAs (11), T4 DNA (12), and the oligonucleotides (5). [5'-^{32}P,^{3}H]poly(A), 26S [5'-^{32}P,^{3}H]rRNA, and dephosphorylated poly(A) were prepared in the manner described by Donis-Keller *et al.* (13).

Protein and Polynucleotide Determinations

Protein was determined by UV absorbance at 280 nm. The concentrations of polynucleotides were determined by UV measurements at 260 nm using the appropriate E_M and are expressed as nmol of nucleotide.

Other Materials

Unlabeled synthetic polynucleotides were obtained from Pharmacia. Marker proteins were obtained from Sigma.

RESULTS

Purification of the 5'→3' Exoribonuclease

The results of a typical preparation of the enzyme are summarized in Table I. The first step in the purification procedure is an ammonium sulfate precipitation of the nuclear supernatant fraction. To determine the distribution of both the poly(A)-hydrolyzing activity and the total RNase activity of this early fraction, it was chromatographed on a DEAE-cellulose column. The fractions from the column were assayed under four conditions: (1) poly(A) hydrolysis at pH 9.5, (2) poly(A) hydrolysis at pH 9.5 in the presence of EDTA, (3) yeast 17S rRNA hydrolysis at pH 7.0, and (4) yeast 17S rRNA hydrolysis at pH 7.0 in the presence of EDTA. The results of the chromatography of poly(A)-hydrolyzing activity (Fig. 1A) show that the main peak of activity elutes in fractions 21-22 and a smaller peak of activity elutes at fraction 17. No poly(A) hydrolysis occurs in the presence of EDTA.

TABLE I. Purification data for the exoribonuclease of placental nuclei

Fraction	Volume (ml)	Protein (mg/ml)	Total activity (units × 10^{-3})	Specific activity (units/mg × 10^{-3})
Nuclear Supernatant	162	23	149	0.04
Ammonium Sulfate Concentrate of Nuclear Supernatant	48	20	120	0.12
DEAE-cellulose 1 concentrate	8	15	171	1.4
DEAE-cellulose 2 concentrate	4	7.5	80	2.6
Heparin-Agarose concentrate	1.5	2.3	63	18.2
Heated Heparin-Agarose concentrate	1.5	2.3	31	8.9
Sephacryl S-200 peak tube	1.3	0.15	5.5	28.2 (× 700)
Sephacryl S-200 peak combined	9.1	0.14	22	17.3 (× 430)

1 unit = 5 nmol of [^{3}H]poly(A) hydrolyzed per 15 min at 37°C for the total reaction mixture.

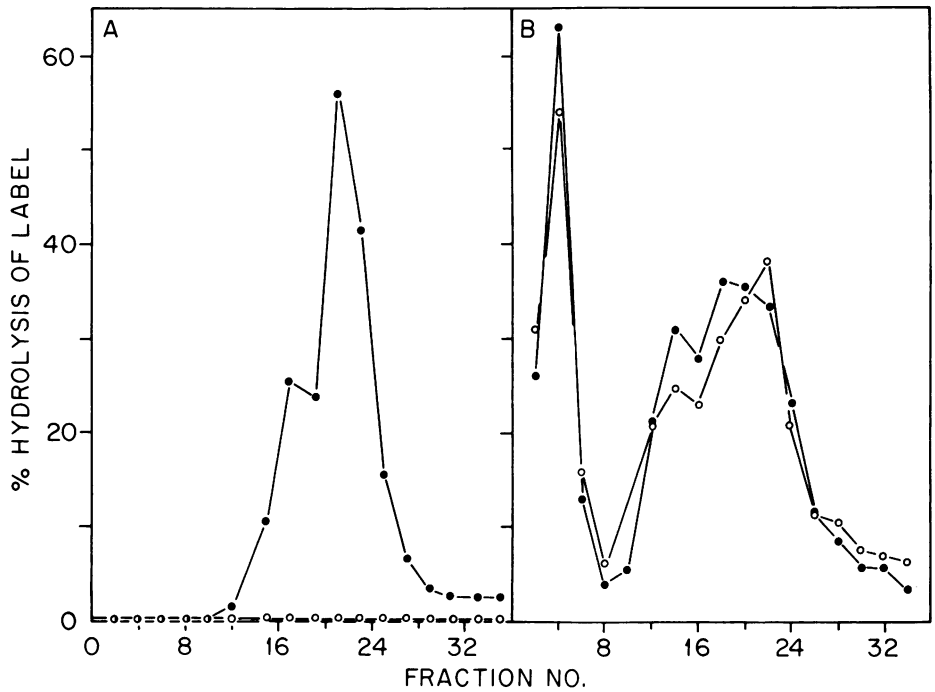

Figure 1. Analyses of poly(A)-hydrolytic and rRNA-hydrolytic activity by DEAE cellulose chromatography of an ammonium sulfate concentrate of a human placental nuclear fraction. Placenta was processed to the ammonium sulfate concentration and dialysis step of the nuclear supernatant fraction. An aliquot of the dialyzed fraction (4 ml) was applied to a 1.2 × 10 cm column of DEAE-cellulose and eluted with a 120 ml linear gradient of buffer A from 50 mM to 500 mM KCl. Fractions (3 ml) were collected and aliquots (5 µl) were assayed with poly(A) at pH 9.5 and with yeast 17S rRNA at pH 7.0 as described for analyzes of substrate specificity under Materials and Methods. One nmol of the two substrates was used and reactions were carried out with and without EDTA (20 mM). A. poly(A) minus EDTA, ●—● ; poly(A) plus EDTA, ○—○ . B. rRNA minus EDTA, ○—○ ; rRNA plus EDTA, ●—● .

The hydrolysis of RNA with the same column fractions (Fig. 1B) shows that a peak of RNA-hydrolyzing activity not retained by the column is found both in the absence and presence of EDTA. RNase activity measurable in the absence or presence of EDTA is also found in fractions 12-30. The main peak of poly(A)-hydrolytic activity shown in Fig. 1A is the fraction further purified as described in Table I and found to yield a high level of 5'→3' exoribonuclease activity. The results shown in Fig. 1 then suggest that the 5'→3' exoribonuclease is a relatively abundant RNase of nuclei since the poly(A)-hydrolytic activity accounts for about 30% of the total RNase activity found in the assays.

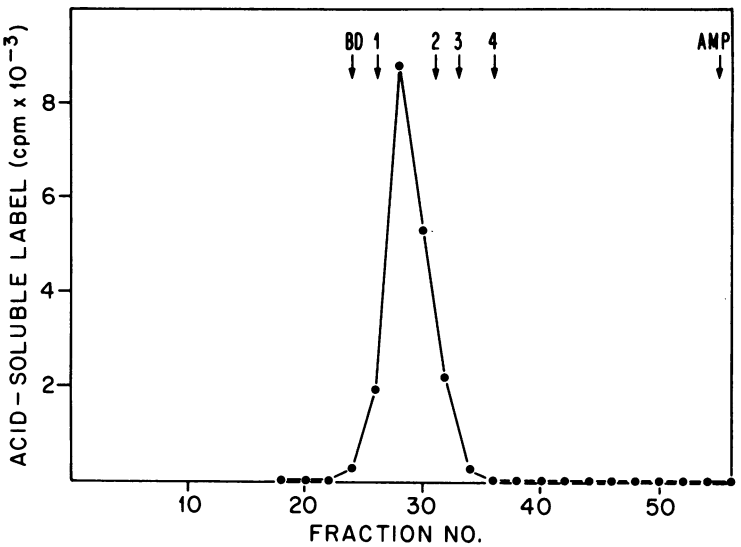

Figure 2. Gel filtration of the exoribonuclease using Sephacryl S-200. One ml of a heated heparin-agarose concentrate was chromatographed on a Sephacryl S-200 column and the eluted fractions assayed as described under Materials and Methods. Arrows denote the positions of elution of blue dextran (BD), aldolase (1), alkaline phosphatase (2), bovine serum albumin (3), ovalbumin (4), and 5'-AMP.

The overall purification data (Table I) show that the final Sephacryl peak fraction is purified 700-fold, while the combined Sephacryl peak is purified 430-fold. The recovery of activity is 14% in the Sephacryl peak. The studies of the enzyme described below show that it is free of other RNase activity, DNase activity, and phosphatase activity.

Assay of nucleoplasmic, nucleolar, and cytoplasmic fractions prepared from two different placental preparations as described under Materials and Methods was also done to determine the distribution of poly(A)-hydrolytic activity at pH 9.5. In the first preparation, 57% of the activity was found in the total nuclear fraction, of which 26% was found in the nucleolar fraction. In the second preparation, 68% of the activity was found in the total nuclear fraction, and the level in nucleoli was only 8%.

Physical Properties and Apparent Molecular Weight of the Exoribonuclease

To determine the apparent molecular weight of the exoribonuclease, the enzyme was subjected to molecular-sieve chromatography to determine the Stokes radius and to gradient centrifugation to determine the sedimentation coefficient. The enzyme was chromatographed on a Sephacryl S-200 column

TABLE II. Substrate specificity of the exoribonuclease

	Substrate	Relative activity %
A.	[^{3}H]poly(A), untreated	100
	[^{3}H]poly(A), dephoshorylated	6
	[^{3}H]poly(A), 5'-triphosphate end	55
	[^{3}H]poly(C), 5'-phosphate end	10
B.	yeast 17S [^{3}H]rRNA	18
	yeast 26S [^{3}H]rRNA	10
	yeast [^{3}H]m^{7}Gppp[^{14}C]RNA	<2
C.	[^{3}H]poly(A)•poly(U)	6
	[^{3}H]poly(A)•poly(dT)	2
	[^{3}H]RNA•M13 DNA	N.D.
	heated T4 DNA	N.D.
	native T4 DNA	N.D.
D.	[^{3}H](pA)$_4$	10
	[^{3}H](Ap)$_4$A	N.D.

The reaction mixtures (50 µl) were as described under Materials and Methods and contained 0.9 - 1.5 nmol of each substrate and 1.2 - 1.6 units of enzyme. Under these conditions, 20-30% of [^{3}H]poly(A) is hydrolyzed. Activities are expressed relative to the hydrolysis of [^{3}H]poly(A) as 100%. Reaction mixtures lacking enzyme served as controls. The hydrolysis of (pA)$_4$ and (Ap)$_4$A was measured by paper chromatography of reaction mixtures using N-propanol/NH_4OH/H_2O (60/30/10) as the solvent system. N.D. = not detectable.

which was standardized with proteins of known molecular weight (E. coli alkaline phosphatase, ovalbumin, bovine serum albumin, and aldolase). A single peak of poly(A)-hydrolyzing activity was found, eluting between the marker proteins aldolase and alkaline phosphatase (Fig. 2). A Stokes radius of 43 Å was determined from the elution volume as described by Siegel and Monty (14). A sedimentation coefficient of 6.3 S for the exoribonuclease was determined according to the procedure of Martin and Ames (15) using ovalbumin (3.6 S), bovine serum albumin (4.3 S), aldolase (7.5 S), and catalase (11.3 S) as marker proteins. Using the Svedberg equation (14) with the values for Stokes radius (43 Å) and sedimentation coefficient (6.3 S), an apparent molecular weight of 113,000 was calculated. (A partial specific volume of 0.73 was assumed.)

Properties of the Enzyme and Features of the Reaction

Effect of pH and divalent and monovalent cations - With poly(A) as the substrate, the optimum pH for activity of the enzyme is from pH 6.5 - pH 7.5. No activity is found at pH 5.3 and 60% and 40% at 8.5 and 9.5 respectively.

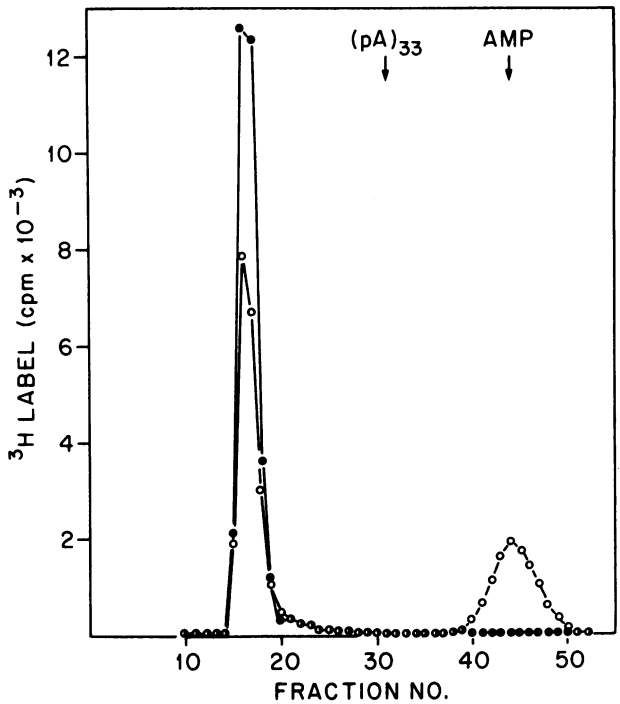

Figure 3. Bio-Gel A-5m chromatography of the products of the degradation of [^{3}H]poly(A). Reaction mixtures (150 µl) were set up as described for the assay of the exoribonuclease with no enzyme (●—●) or with 10 units of enzyme (○—○). After 15 min at 37°C, EDTA (to 10 mM) and 5'-AMP (500 nmol) were added and each reaction mixture was chromatographed on a Bio-Gel A-5m column (2.0 × 23 cm) and eluted with 100 mM Tris-HCl buffer (pH 8.0). Fractions (1.3 ml) were collected and analyzed for radioactivity.

Divalent cation is required for activity since EDTA inhibits the reaction completely. Mg^{2+} stimulates maximally at 0.5 mM and inhibits the reaction 95% at 10 mM. Mn^{2+} stimulates only 20% as well, being optimal at about 20 µM. NH_4Cl, KCl, and NaCl stimulate the reaction about 2-fold at 50 mM, but begin to inhibit at 150 mM. EDTA completely inhibits rRNA hydrolysis, thereby showing that the RNase activity depicted in Fig. 1 with a crude fraction was removed.

Nature of the product - Using poly(A) as a substrate, the only product formed is 5'-AMP, identified by paper electrophoresis at pH 3.5 and by paper chromatography using isopropanol/NH_4OH/0.1M boric acid, 70/10/30, as the solvent system. Thus, the products are 5'-mononucleotides.

Substrate specificity - The substrate specificity of the enzyme is shown in Table II. Table IIA shows that poly(A) treated with *E. coli* alkaline

phosphatase has only 6% of the activity of untreated poly(A). This preparation of untreated poly(A) apparently has a high level of 5'-phoshate end groups since no effect on its hydrolysis is observed after a 5'-phosphorylation reaction. Poly(A) with a 5'-triphosphate end group is hydrolyzed at about one-half the rate of untreated poly(A). Poly(C) is a poor substrate. As shown in Table IIB, rRNA is a substrate, but capped mRNA is a poor substrate. That [^{3}H]m^{7}Gppp[^{14}C]RNA is not decapped by the enzyme was shown using the decapping assay described previously (16) (data not shown). As shown in Table IIC, double-stranded polymers are hydrolyzed at less than 10% of the rate of poly(A), and DNA, native or denatured, is not a substrate. To test the requirement for a 5'-phosphate end group in another manner, the hydrolysis of $(pA)_4$ and $(Ap)_4A$ was measured. $(pA)_4$ is a good substrate, while hydrolysis of $(Ap)_4A$ is not detectable (less than 1% of the rate of $(pA)_4$) (Table IID). Studies of the hydrolysis of these two

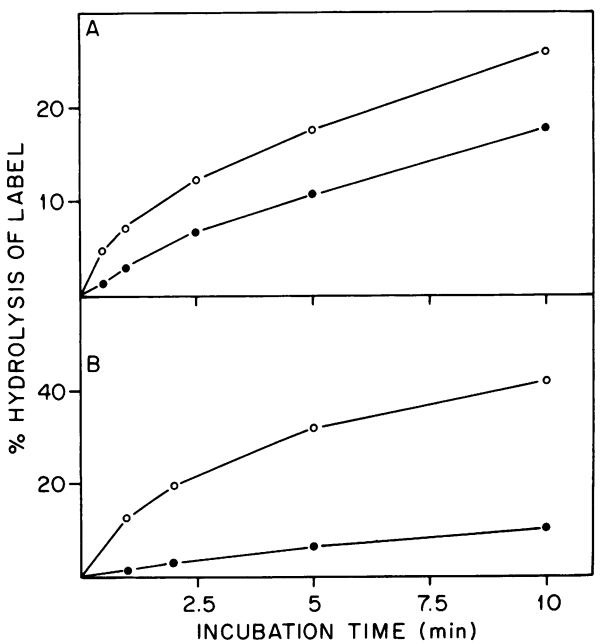

Figure 4. Hydrolysis of [^{3}H]poly(A) and 26S [^{3}H]rRNA containing 5'-terminal ^{32}P label by the exoribonuclease of placental nuclei. For A, 1 nmol of [5'-^{32}P,^{3}H]poly(A) was incubated at 0°C for the times shown in reaction mixtures (50 µl) containing 100 units of exoribonuclease. For B, 1.3 nmol of 26S [5'-^{32}P,^{3}H]rRNA were used as a substrate with 12 units of exoribonuclease and the reaction mixtures were incubated at 36°C. O—O, ^{32}P label; ●—●, ^{3}H label.

oligonucleotides with enzyme purified without the heating step showed that enzyme fractions from the Sephacryl column contained low levels of 3'→5' exoribonuclease activity. $(Ap)_4A$ was hydrolyzed to $(Ap)_3A$ and 5'-AMP at about 5-10% of the rate of hydrolysis of $(pA)_4$. This activity eluted approximately two tubes prior to the enzyme described here. The heating step destroys the contaminating activity.

Possessivity of the exoribonuclease - Bio-Gel A-5m chromatography of reaction mixtures with poly(A) and with or without enzyme (Fig. 3) shows that the hydrolysis of poly(A) follows a processive mechanism (17, 18), resulting in the degradation of one poly(A) molecule before attack on another begins. The processive mechanism is shown by the fact that the size of the poly(A) did not change and the only product detectable eluted at the position of 5'-AMP. That rRNA may be degraded by a partially non-processive mechanism is shown below.

Evidence for a 5'→3' mode of hydrolysis - Studies of the direction of hydrolysis by the enzyme were carried out using [^{3}H]poly(A) and 26S [^{3}H]rRNA with 5'-^{32}p-terminal labels as substrates. The reaction with poly(A) was

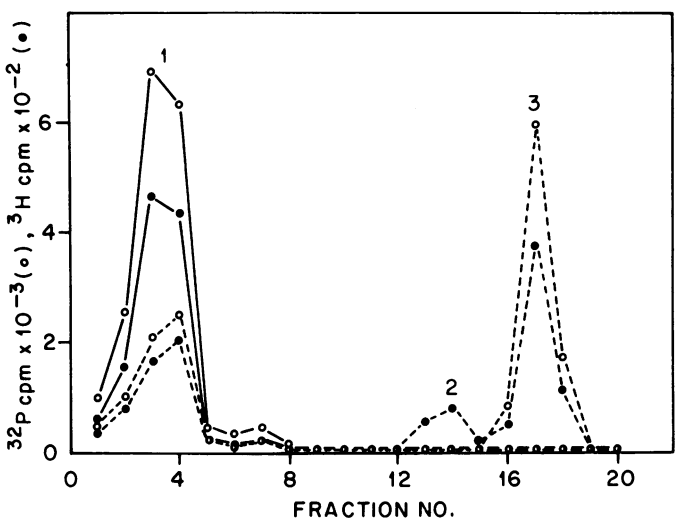

Figure 5. Paper chromatography of the products of the hydrolysis of [^{3}H]$(pA)_4$, labeled at the 5'-terminus with ^{32}P, by the exoribonuclease. [^{3}H]$(pA)_4$ (2.1 nmol) labeled at the 5'-terminus with ^{32}P was incubated at 37°C in reaction mixtures (50 µl) as described under Materials and Methods in the absence and presence of 7 units of enzyme. After 60 min at 37°, the reaction mixture was applied to paper and chromatography was carried out for 21 hours using N-propanol/NH_4OH/H_2O (60/30/10) as the solvent system. One cm segments (from the origin) of the paper were eluted with 0.5 ml of water and counted. 1=$(pA)_4$; 2=$(pA)_2$; 3=5'-AMP. ——, minus enzyme; ----, plus enzyme.

carried out at 0°C to slow the rate of hydrolysis and with a large amount of enzyme to saturate as many ends as possible. The result is shown in Fig. 4A. At the shortest time interval (30 s), three times more ^{32}P than ^{3}H is rendered acid-soluble. With 5'-[^{32}P]rRNA, the reactions were carried out at 36°C, and the results are shown in Fig. 4B. At the shortest time interval (1 min), ten times more ^{32}P than ^{3}H is rendered acid-soluble. That release of ^{32}P is considerably faster than release of ^{3}H with the 26S rRNA even at 36°C suggests that the degradation of this substrate is partially non-processive. The results with both substrates show that the enzyme hydrolyzes in a 5'→3' direction.

Evidence for a 5'→3' direction of hydrolysis was also obtained by determining label in the products of hydrolysis of [^{3}H](pA)$_4$ containing 5'-terminal ^{32}P. Chromatography of reaction mixtures (Fig. 5) shows that (pA)$_2$ is a readily detectable intermediate of the hydrolysis and that it contains no ^{32}P label. That the enzyme hydrolyzes in a 5'→3' direction is also suggested by the low reactivity of dephosphorylated poly(A) and (Ap)$_4$A as described above.

DISCUSSION

When a 5'→3' exoribonuclease activity was first suspected (1,2) and such an enzyme purified from yeast (5), it seemed that the enzyme might be involved in the degradation of mRNA *in vivo*. This was suggested as a likely function for the enzyme (4, 5), since the direction of degradation would be the same as that of translation. The finding of a 5'→3' exoribonuclease in nucleoli of Ehrlich ascites tumor cells (7) and the studies described here on the isolation of a similar enzyme from nuclei of normal human cells (human term placenta) suggest an important role in nuclear RNA processing or turnover. That a share of the hnRNA may turn over in the nucleus has long been postulated since only 5%-10% of the hnRNA synthesized reaches the cytoplasm (19). Whether introns constitute that high a percentage of hnRNA remains a moot point. The processing of rRNA precursors may very well involve such an enzyme as could the turnover of introns and 3' sequences resulting from cleavage of transcripts prior to polyadenylation.

The human placental enzyme described in this report differs in distinct ways from both the highly-purified yeast enzyme (6) and the tumor nucleolar enzyme (7). It differs from the yeast 5'→3' exoribonuclease in having very low activity with double-stranded polymers, namely poly(A)·poly(U) and poly(A)·poly(dT). It also hydrolyzes rRNA at a lower rate; moreover, the

hydrolysis of this substrate is partially non-processive. It is possible that this placental enzyme is influenced considerably by the secondary structure of its substrates. It and the enzyme from yeast both hydrolyze substrates with 5'-phosphate end groups at 10-20 times the rate of those lacking 5'-phosphate end groups. The nucleolar enzyme was not highly purified because of its instability (7). The results presented showed that it is not at all discriminative with respect to 5'-end groups.

In the studies of Noble et al. (9) on the splicing of SV40 early pre-mRNA using HeLa cell nuclear extracts, the authors found a collection of RNAs which they believe resulted from protection of early pre-mRNA at specific points from an endogenous 5'→3' exoribonuclease activity. The protection occurred upstream from both the 5' splice junction and from the lariat structure and may result from binding of factors at these points. The placental enzyme is easily purified and may be very useful in studying such RNA-protein complexes.

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REFERENCES

1. Furuichi, Y., LaFiandra, A., and Shatkin, A. J. (1977) Nature 266, 235-239.
2. Shimotohno, K., Kodama. Y., Hashimoto, J., and Miura, K. (1977) Proc. Natl. Acad. Sci. USA 74, 2734-2738.
3. Stevens, A. (1978) Biochem. Biophys. Res. Commun. 81, 656-661.
4. Stevens, A. (1979) Biochem. Biophys. Res. Commun. 86, 1126-1132.
5. Stevens, A. (1980) J. Biol. Chem. 255, 3080-3085.
6. Stevens, A., and Maupin, M. K. Arch. Biochem. Biophys., in press.
7. Lasater, L. S., and Eichler, D. C. (1984) Biochemistry 23, 4367-4373.
8. Green, M. R., Maniatis, T., and Melton, D. A. (1983) Cell 32, 681-694.
9. Noble, J. C. S., Prives, C., and Manley, J. L. (1986) Nucleic Acids Res. 14, 1219-1235.
10. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475-1489.
11. Stevens, A. (1985) J. Bacteriol. 164, 57-62.
12. Stevens, A. (1974) Biochemistry 13, 493-503.
13. Donis-Keller, H., Maxam, A. M., and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527-2538.
14. Siegel, L. M., and Monty, K. J. (1966) Biochem. Biophys. Acta 112, 346-362.
15. Martin, R. G., and Ames, B. N. (1961) J. Biol. Chem. 236, 1372-1379.

16. Stevens, A. (1980) Biochem. Biophys. Res. Commun. 96, 1150-1155.
17. Nossal, N. G., and Singer, M. F. (1968) J. Biol. Chem. 243, 913-922.
18. Thomas, K. R., and Olivera, B. M. (1978) J. Biol. Chem. 253, 424-429.
19. Soeiro, R., Vaughan, M. H., Warner, J. L., and Darnell, J. E. (1968) J. Cell Biol. 39, 112-118.