Properties of a HeLa Cell 3’ Exonuclease Specific for Degrading Poly(A) Tails of Mammalian mRNA*

(Received for publication, March 9, 1992)

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A HeLa cell 3'-exonuclease with properties of a mammalian mRNA poly(A) tail-removing enzyme has been characterized. The exonuclease shows high specificity for the poly(A) tail, and it is single strand-specific and requires a 3'-hydroxyl group for its activity. During degradation 5'-AMP is liberated as a product, and a 3'-OH group is left on the last adenosine residue of the remaining poly(A) tail. The activity is inhibited by 5'-AMP and can be competed by poly(A)-containing mRNA or poly(A). Based on these findings we propose a reaction pathway for poly(A) tail removal catalyzed by the HeLa cell poly(A) tail-specific 3’ exonuclease.

For several years the function of the poly(A) tail of eukaryotic mRNA has been unknown. Recent studies have suggested that the poly(A) tail plays an important role during translation, and it has been postulated that the length of the poly(A) tail determines the translational capacity of an mRNA (see Refs. 1-4 for recent reviews and references). Polyadenylation of mRNA has been extensively studied, and it has been shown that two independent steps, RNA cleavage and AMP addition, take place during the reaction (5-7). The length of the poly(A) tail is regulated by two competing reactions, addition and removal. AMP addition is catalyzed by a poly(A) polymerase associated with an additional factor (see Ref. 8 for a recent review). Poly(A) tail removal, deadenylation, has recently been studied in oocytes of Xenopus laevis (9, 10) and in mammalian cells (see Refs. 11-13 and references therein). In oocytes of X. laevis an activity exists that removes the poly(A) tail of certain mRNAs without affecting the mRNA body. It has been suggested that this activity together with the AMP addition reaction determines the length of the poly(A) tail and thereby regulates stage-specific gene expression in developing eggs of X. laevis (9, 10).

We have recently identified an activity in HeLa cells having properties of a mammalian poly(A) tail-removing enzyme (14).

In this paper we report on the characterization of this HeLa cell poly(A) tail-removing activity. We show that the activity liberates 5’-AMP as a product, and we propose a reaction pathway for poly(A) tail removal. Poly(A) degrading activities have previously been identified in several organisms (11, 15-22). The relationship between these activities and the HeLa cell poly(A) tail-removing activity is discussed.

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* This work was supported by the Swedish Natural Research Council and Marcus Borgström Foundation grants from Uppsala University. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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**MATERIALS AND METHODS**

**Cells and Preparation of Cell Extracts**

HeLa cells were grown in Dulbecco's minimal essential medium supplemented with 10% newborn calf serum and harvested in the exponential phase (5 x 10^6 cells/ml). Nuclear extract was prepared essentially according to Dignam et al. (23) with the modifications of Moore and Sharp (7). The nuclei were extracted with 1 ml of buffer C (20 mM HEPES (KOH), 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT, 50 mM KCl, and pH 8.2) per milliliter of collected nuclei. The nuclear extract was dialyzed against buffer D (20 mM HEPES (KOH), 100 mM KCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT, and 20% glycerol, pH 8.2) in dialysis tubing with molecular weight cut-off at 6000-8000 (Spectra/Por 1 132650) for 5 h. After dialysis the nuclear extract was frozen in liquid nitrogen and stored at -70°C. The nuclear extract contained approximately 13 mg of protein/ml. Protein concentration was determined using the Bio-Rad protein assay kit (500-0001) and bovine γ-globulin as reference.

**Fractionation**

DEAE-Sephacel Chromatography—A DEAE-Sephacel ion exchange column (Pharmacia LKB Biotechnology Inc. 17-0500) with a bed volume of 100 ml and diameter of 5 cm was equilibrated with buffer D (see above) at a flow rate 9 cm/h. Approximately 325 mg (25 ml) of nuclear extract was thawed, centrifuged at 20,000 x g for 10 min and applied to the column at flow rate 9 cm/h. Three successive step elutions were performed with buffer D containing 0.17, 0.34, and 1.0 M KCl. The column was washed with 250 ml of the current elution buffer before the next step elution was performed. Aliquots of the eluate were assayed for protein. Protein that eluted in the flowthrough fraction and each step fraction was combined as fractions I (2.4 mg/ml in 36 ml), II (1.7 mg/ml in 28 ml), III (2.4 mg/ml in 32 ml), and IV (1.1 mg/ml in 20 ml). Fraction I was frozen directly and fractions II, III, and IV were dialyzed against buffer D for 5 h. Fractions were frozen in liquid nitrogen and stored at -70°C.

**Cellulose Phosphate P11 Chromatography**—Cellulose phosphate P11 (Whatman 4071010) was freshly prepared, according to the protocol obtained from the manufacturer, before every separation. A column with a bed volume of 0.8 ml and a diameter of 1 cm was equilibrated with buffer D at flow rate 38 cm/h. Subsequently the column was blocked with 3 column volumes of buffer D supplemented with 0.2 mg/ml of methylated bovine serum albumin (Calbiochem 455451), washed with five column volumes of buffer D containing 0.5 M KCl, and finally equilibrated with buffer D. Fraction II (7.5 mg) was applied to the cellulose phosphate P11 column at a flow rate of 38 cm/h. The flowthrough fraction was collected and named fraction IIIF (0.6 mg/ml in 5 ml). Subsequently, the column was eluted by a linear gradient of 0.1-0.5 M KCl and 1-mI fractions were collected. Fractions were frozen in liquid nitrogen and stored at -70°C. Heparin-Sepharose Chromatography—Heparin-Sepharose CL-6B (Pharmacia 17-0467-01) was prepared according to the protocol obtained from the manufacturer. A column with bed volume of 1 ml and a diameter of 1 cm was equilibrated with buffer D at a flow rate of 38 cm/h. Fraction II (2.7 mg) was applied to the Heparin-Sepharose column at a flow rate of 38 cm/h. The flowthrough fraction was collected and named fraction IIHF (0.2 mg/ml in 3 ml). Subsequently, the column was eluted by a linear gradient 0.1-0.5 M NaCl.
Poly(A) Tail Removal

KC1 and 1-ml fractions were collected. Fractions containing 3'-exonuclease were identified, pooled, and named IIFHG (0.15 mg/ml in 3 ml). Fraction IIFHG was dialyzed against buffer D for 5 h, frozen in liquid nitrogen, and stored at -70 °C.

SDS-Polyacrylamide Gel Electrophoresis

SDS-polyacrylamide (acrylamide:bisacrylamide, molar ratio 30:0.8) gels (4 and 10% acrylamide in spacer and separation gels, respectively) were prepared according to Laemmli (24) using a Mini-Protean II gel apparatus (Bio-Rad 125BR). The protein sample to be separated was precipitated by addition of 1 volume of acetone at -20 °C followed by centrifugation at 12,000 × g for 30 min at 4 °C. The obtained pellet was dissolved in 10 µl of sample buffer (50 mM Tris-HCl pH 6.8, 2 mM Na3EDTA, 1% (w/v) SDS, 1% (v/v) β-mercaptoethanol, 8% (v/v) glycerol, 0.025% (w/v) bromphenol blue) and separated by gel electrophoresis. The resulting gel was fixed and stained (50% (v/v) methanol, 10% (v/v) acetic acid, and 0.1% (w/v) Coomassie Brilliant Blue), washed (5% (v/v) methanol and 10% (v/v) acetic acid), and finally soaked in 10% (v/v) glycerol.

Preparation of RNA Substrates

RNA substrate L3(A50) capped at the 5' end was synthesized by in vitro transcription using T3 RNA polymerases (Promega no. P208C) and plasmid pT3L3(A50) (14), digested with NsiI, as DNA template. Uniformly labeled RNA substrates were generated by inclusion of [α-32P]UTP (Du Pont-New England Nuclear NE-007X, 40 Ci/mmol in the transcription mixture), [α-32P]ATP (Du Pont-New England Nuclear NE-003H, 5 Ci/mmol in the transcription mixture) during transcription. L3(54) RNA substrate was synthesized by in vitro transcription using T3 RNA polymerases and plasmid pT3L3 (25), digested with RsaI as DNA template in the presence of [α-32P]UTP (Du Pont-New England Nuclear NE-007X, 40 Ci/mmol in the transcription mixture). Transcribed RNA was purified according to Moore and Sharp (7). 3'-end-labeled RNA was obtained by ligation (26) of [5'-32P]pCp (Du Pont-New England Nuclear NE-018A, 3000 Ci/mmol) to RNA substrate in 20 µl at 25 µM ATP, 10 µg/ml bovine serum albumin, 3.3 mM DTT, 10% (v/v) dimethyl sulfoxide, 825 units/ml RNAguard (Pharmacia 27-0815), 1200 units/ml RNA ligase (Pharmacia 27-0883-02), and 2.5 pmol/ml RNA substrate. Nonincorporated pCp was removed by G-50-spin column chromatography.

Assay Conditions

Conditions for in vitro deadenylation were: 1 mM MgCl2, 2.5% (v/v) polyvinyl alcohol (Sigma P-8136, M, 10,000), 200 mM KC1, 0.15 units of RNAguard, 5-20 fmol of RNA substrate, 20-4876 (v/v) buffer (20 mM HEPES (KOH), 0.2 mM EDTA, 0.5 mM DTT, and 25% glycerol, pH 8.2) and protein fraction (14). Reaction volume was 15
Identification of 5'-AMP as a reaction product. A, L3(Aoo) RNA substrate labeled by $[^{32}P]ATP$ was incubated in conditions for poly(A) tail removal in the presence of 5 pl of fraction IIFHG. Reactions were terminated at indicated time points in min. Reacted RNA was purified and subjected to gel electrophoresis. The resulting autoradiograph is shown. $S$ and $P$ denote the location of RNA substrate and product, respectively. $B$, a small aliquot (2 pl) of the reactions described above was removed at indicated time points and analyzed by one-dimensional TLC using solvent III (see "Materials and Methods"). The developed TLC plate was subjected to autoradiography. $Ori$ and $P$ indicate the location of origin of separation and mononucleotide product, respectively. $C$, L3(Aoo) RNA substrate labeled by $[^{32}P]ATP$ was incubated in conditions for poly(A) tail removal in the presence of 5 pl of fraction IIFHG for 30 min. 2 pl of the reaction was analyzed by two-dimensional TLC using solvents I and II (see "Materials and Methods") followed by autoradiography. First and second dimensions are indicated by 1 and 2, respectively. The location of marker nucleotides are shown. $Ori$ represents origin of separation.

or 25 pl, and incubations were performed at 30 °C. RNA substrate labeled by $[^{32}P]UTP$ was used unless noted. ATP (Pharmacia 27-2056-01), 7'-AMP (Sigma A-1752), 2'-AMP (Sigma A-9396), 3'-AMP (Sigma A-0386), 2',3'-cAMP (Sigma A-9376), 3',5'-cAMP (Sigma A-6885), poly(A) (Sigma P-9403), poly(U) (Sigma P-9528), tRNA (Sigma R-9001), and mRNA were included at the indicated concentrations. mRNA was obtained from HeLa cells and purified by oligo(dT) cellulose chromatography (27). Reactions were terminated, and RNA was purified according to Moore and Sharp (7). RNA was analyzed by electrophoresis in 20% polyacrylamide (19:1 acrylamide/bisacrylamide)-7 M urea gels followed by autoradiography of the resulting gel. Partially processed RNA molecules were purified by elution from 10% polyacrylamide (19:1 acrylamide/bisacrylamide)-7 M urea gels (27).

**T1 and T2 Ribonuclease Digestion**

T1 RNase digestion of RNA was performed in 25 pl at 25 mM Tris-HCl (pH 7.8), 50 mM NaCl, 10 mM MgCl$_2$, 300 mg/ml bovine serum albumin, 0.2 mM $\beta$-mercaptoethanol, 10 mg/ml tRNA, and 400 units/ml T1. RNAse (Calbiochem 656785) for 30 min at 37 °C. Treated RNA was purified by phenol/chloroform extraction and precipitated by ethanol. RNA was analyzed by electrophoresis in 20% polyacrylamide (19:1 acrylamide/bisacrylamide)-7 M urea gels followed by autoradiography of the resulting gel. T2 RNase digestion of RNA was performed in 10 pl at 50 mM NaCl, 2.5 mM HEPES, pH 7.6, 0.25 mM EDTA, and 125 units/ml T2. RNAse (Calbiochem 656865) for 30 min at 37 °C. The reaction was directly analyzed by thin-layer chromatography.

**Thin-Layer Chromatography**

Chromatography on PEI-cellulose F plates (Merck 5579) was performed according to Konarska et al. (28). Liberated product from deadenylation reactions or T1 RNase digestions were analyzed by two-dimensional thin-layer chromatography in standard chambers using: isobutyric acid, concentrated NH$_4$OH, H$_2$O; 577/38/385 (v/v) as first-dimension solvent (I) and saturated (NH$_4$)$_2$SO$_4$, 1 M sodium acetate, isopropanol: 80/18/12 (v/v) as second-dimension solvent (II). After the first-dimension separation the plates were left to dry at room temperature for 8 h to let the isobutyric acid evaporate. As markers adenosine (Nutritional Biochemicals Corporation), 5'-AMP, 2'-AMP, 3'-AMP, 2',3'-cAMP, and 3',5'-cAMP were used. Position of markers were detected by UV light (Mineralight lamp UVG-54, Ultra-Violet Prod. Inc.). Radioactive molecules were detected by autoradiography of the resulting PEI cellulose F plate. Quantitation of deadenylation activity was performed by one-dimensional chromatography using 0.75 M K$_2$HPO$_4$, pH 3.5 (H$_2$PO$_4$), as solvent (III).

**Quantitation**

Deadenylation activity was quantified as follows: L3(Aoo) RNA substrate labeled by $[^{32}P]ATP$ was incubated in conditions for deadenylation reactions. Reactions were analyzed by one-dimensional thin-layer chromatography (TLC) using solvent III, and the resulting PEI cellulose F plate was scanned by a 400 S PhosphorImager (Molecular Dynamics). The fraction of released $[^{32}P]AMP$ was determined. Knowing the specific activity of $[^{32}P]AMP$ in the RNA substrate the amount (in moles) of released AMP was calculated.

**RESULTS**

Poly(A) Tail Removal in Vitro—The RNA substrates used in this study are depicted in Fig. 1A. The poly(A) tail-removing activity was purified from HeLa cell nuclear extract by DEAE-Sephacel chromatography followed by cellulose phosphate P11 and heparin-Sepharose fractionation (Fig. 1B). The relative purity of the activity was calculated by determining the amount of liberated mononucleotide as a function of added amount of protein. Purification was estimated to be 100-fold relative to the nuclear extract. To investigate the protein content of each fraction containing poly(A) tail-removing activity we fractionated by SDS-polyacrylamide gel electrophoresis poly(A) tail-removing activity releasing 2 pmol of AMP per min (Fig. 1C). The calculated purity and the complexity of polypeptides as visualized by gel electrophoresis were in agreement. The conditions for poly(A) tail removal were determined by titrating monovalent (K$^+$ and...
patterns of L3(54) RNA and L3(Asn) RNA labeled by inclusion of \([\alpha-^{32}P]UTP\) and RNA ligase. A, partially deadenylated 3' end-labeled L3(A30) RNA was separated by 10% polyacrylamide gel electrophoresis. B, RNA separated in A (see above) was treated with T1 RNase. Digested RNA was recovered and subjected to 20% polyacrylamide gel electrophoresis. T1 RNase-digested L3(54) RNA (lane 1), L3(A30) (lane 2), and 3' end-labeled partially deadenylated L3(A30) (lane 3) were separated. The resulting autoradiograph is shown. The RNase T1 digestion patterns of L3(54) RNA and L3(A30) RNA labeled by inclusion of \([\alpha-^{32}P]UTP\) and \([\alpha-^{32}P]ATP\) during transcription, respectively, have been described (14, 34). The obtained patterns are as expected. The arrows and the numbers to the left indicate the locations and the sizes of RNA fragments. C, partially deadenylated 3' end-labeled L3(A30) RNA (A, lane 3) was digested with T1 RNase and analyzed by two-dimensional TLC using solvents I and II (see "Materials and Methods") by autoradiography. First and second dimensions are indicated by I and 2, respectively. The location of marker nucleotides are shown. Ori represents origin of separation.

Na+ and divalent (Mg2+ and Mn2+) cations at pH 8.2. The optimal conditions were found to be 200 mM for K+ and 150 mM for Na+ (Fig. 2A). Polya(T) tail removal was inhibited at high concentrations of monovalent cations. Divalent cations were required for poly(A) tail removal. The optimal was observed that Mg2+ could not be replaced by Mn2+ (data not shown).

5' AMP Is Liberated during Poly(A) Tail Removal in Vitro—To characterize the product which is liberated during poly(A) tail removal in vitro, we incubated L3(A30) RNA (labeled by inclusion of \([\alpha-^{32}P]ATP\) during in vitro transcription) under conditions for poly(A) tail removal. Reactions were terminated after 0, 5, 10, 20, 40, 60, 80, and 120 min of incubation, and the reaction products were investigated by denaturing polyacrylamide gel-electrophoresis or by TLC. Gel electrophoresis of the products (Fig. 3A) showed that the L3(A30) RNA was shortened during incubation. We have not been able to detect oligonucleotides corresponding to oligo(A) when analyzing denadenylation reactions by gel electrophoresis. The TLC analysis (Fig. 3B) showed that mononucleotides accumulated during the reaction. Thus, this analysis showed that while the RNA substrate was degraded at the 3' end (14) mononucleotides were released.

To investigate the structure of the released mononucleotide we incubated the same RNA substrate for 30 min under conditions for poly(A) tail removal and characterized the released mononucleotide by two-dimensional TLC. Nonradioactive 5'-AMP, 3'-AMP, 2'-AMP, 2':3'-cAMP, and 3':5'-cAMP were included as markers during TLC. After the chromatogram was developed the positions of the marker nucleotides were identified by ultraviolet light, and the position of the released radioactively labeled mononucleotide was identified by fluorography. Fig. 3C shows that the radioactively labeled mononucleotide comigrated with 5'-AMP during TLC. Taken together these results show that the poly(A) tail removal activity liberates 5'-AMP.

A Hydroxyl Group Is Present at the 3' End of the Partially Deadenylated RNA—To investigate the structure of the 3' end of partially deadenylated RNA we incubated nonradioactive L3(A30) RNA substrate in deadenylating conditions for 35 min, and the RNA product was purified and subjected to gel electrophoresis. The partially deadenylated L3(A30) RNA was located in the gel, eluted, and subsequently labeled at the 3' end by \([5'-^{32}P]pCp\) using RNA ligase (26). The labeled RNA (Fig. 4A, lane 3) was digested to completion with T1 RNase or T2 RNase. The T1 RNase-digested material was purified and fractionated by gel electrophoresis. Fig. 4B shows that the T1 RNase digestion pattern that was obtained. This was the expected pattern if \([5'-^{32}P]pCp\) was ligated to 3' ends located in the poly(A) tail. Thus, we conclude that the 3' ends of the partially deadenylated L3(A30) RNA were located in the poly(A) tail in agreement with previous results (14). Furthermore, we conclude that the partially deadenylated L3(A30) RNA contained a 3'-hydroxyl group that was recognized by RNA ligase. The T2 RNase-digested material.
poly(A) tail removal at approximately 10-times molar excess was investigated by TLC analysis using nonradioactive 5'-Competitor. Reacted RNA was purified and subjected to gel electrophoresis. The resulting autoradiographs are shown. In lanes P and S L3(A\text{a}) and L3(A\text{a}) RNAs were fractionated, respectively, A addition of poly(A) and poly(U) RNA. Numbers above lanes indicate added amounts of competitors expressed as femtomoles. In lane \(-\), competitor was omitted. B, addition of tRNA and poly(A)-containing mRNA. Numbers above lanes indicate added amounts of competitor given in nanograms. 30 ng of tRNA and mRNA correspond to approximately 1200 and 45 fmol, respectively.

was investigated by TLC analysis using nonradioactive 5'-AMP, 3'-AMP, 2'-AMP, and adenosine as markers. Fig. 4C shows that the released radioactive nucleotides comigrated with 3'-AMP. This is the expected result after T2 RNase digestion if the partially deadenylated L3(A\text{a}) RNA was terminated by a 3'-hydroxyl group since the radioactive phosphate in [5'-\text{32}P]pCP will be transferred to the 3' hydroxyl group of the terminal adenosine residue.

Inhibition of Poly(A) Tail Removal in Vitro—Poly(A) tail removal was performed in the presence of increasing amounts of 5'-AMP, 3'-AMP, 2'-AMP, and ATP. Fig. 5 shows that 5'-AMP and ATP were the two most efficient inhibitors. We ascribe the inhibitory effect of ATP to the Mg\textsuperscript{2+} chelating property of ATP since addition of Mg\textsuperscript{2+} to a final concentration of 4 mM restored the activity in the presence of 3 mM ATP (data not shown). At high concentrations 3'-AMP, 2',3'-cyclic AMP, and 3',5'-cyclic AMP also inhibited poly(A) tail removal (Fig. 5A).

The inhibitory effects of poly(A), tRNA, and poly(A)-containing mRNA during deadenylation in vitro were investigated. Poly(A)-containing mRNA and poly(A) inhibited poly(A) tail removal at approximately 10-times molar excess (Fig. 6, A and B), while tRNA inhibited the reaction at approximately 500-times molar excess (Fig. 6B). To test if poly(A) duplexed with poly(U) was degraded by the 3'-exonuclease we added increasing amounts of poly(U) to in vitro deadenylation reactions. Fig. 6A shows that poly(U) completely inhibited poly(A) tail removal at a 1:1 molar ratio, suggesting that the 3'-exonuclease is single strand-specific.

**DISCUSSION**

In this paper we report on the characterization of a HeLa cell 3'-exonuclease which removes poly(A) tails of eukaryotic mRNA (14). Based on the results presented in this paper and in a previous report (14) we propose the following reaction pathway for poly(A) tail removal.

\[5'-(pX)(pA)_{n}pApA_{i+1}3' \rightarrow 5'-(pX)(pA)_{n}pApA_{i+1}3' + pA_{i+1}\]

where \(pX\) represents any of the four nucleotides, \(pA\) represents adenosine residues, and \(n\) and \(i\) are integral numbers.

We base this conclusion on the following observations: (i) efficient poly(A) tail removal requires a 3'-end-located poly(A) tail ended by a 3'-hydroxyl group (14); (ii) poly(A) tail removal does not require any specific sequences in the mRNA body (14); (iii) a partially deadenylated poly(A) RNA is terminated by a 3'-located hydroxyl group (Fig. 4); (iv) partially deadenylated RNA is substrate for further deadenylation (Fig. 3 and Ref. 14); (v) poly(A) tail removal is mediated by a 3'-exonucleolytic activity since the 3' end of the RNA substrate is degraded (14), mononucleotides are liberated (Fig. 3), and oligo(A) cannot be detected during in vitro deadenylation; (vi) the released nucleotide is 5'-AMP (Fig. 3), and 5'-AMP is an efficient inhibitor of in vitro deadenylation (Fig. 5); (vii) poly(A) and poly(A) tail-containing mRNA compete efficiently in the reaction (Fig. 6, A and B).

Exoribonuclease activities degrading poly(A) have previously been identified in several organisms including both mammals and plants (11, 15–22). Many of these activities liberate 5'-AMP as mononucleotide product. These activities thus resemble the activity that we have found in HeLa cell nuclei. However, the previously identified exoribonucleases differ from the activity we have described in many respects. It has, most importantly, previously not been shown that these exoribonucleases exclusively degrade the poly(A) tail of an mRNA leaving the mRNA body intact (14). It is difficult to determine the relationship between the enzyme we have investigated and those previously described since enzyme sources, assay conditions, and RNA substrates differ. Lazarus and Sporn (19) identified and characterized exoribonuclease from Ehrlich ascites tumor cells and mouse cells. This exoribonuclease has many characteristics in common with the HeLa cell 3'-exonuclease we have described, e.g., it is mainly nuclear, shows high specificity for poly(A) and liberates 5'-AMP. However, the mouse exonuclease is active in the presence of Mn\textsuperscript{2+}. Kwan (18) described a cytoplasmic HeLa cell 3'-exonuclease which is inhibited by 50 mM NaCl and degrades ssRNA, mRNA, rRNA, and poly(C) in addition to poly(A). Thus, this cytoplasmic activity differs in cation requirement and RNA substrate specificity. A calf thymus 2',3'-exonuclease, specific for poly(A) (EC 3.1.13.4), has been extensively purified and characterized by Schröder and colleagues (20, 30, 31). Compared to the activity we describe, this exonuclease differs since it degrades poly(A) in poly(A):poly(U) duplexes and is active in the presence of Mn\textsuperscript{2+}. Kumagai et al. (17, 32) identified and purified a rat liver microsomal exonuclease which degrades poly(A). This enzyme differs from the HeLa cell 3'-exonuclease we have found since it is associated with microsomes and differs in substrate specificity, i.e., it degrades poly(C) and poly(U) in addition to poly(A). Abraham and Jacob (15) have characterized a rat liver 3'-exonuclease activity specific for poly(A) which is associated with poly(A) polymerase activity. The poly(A) polymerase activity in HeLa cell nuclear extract...
fractionates in DEAE-Sephael fraction I and is bound to cellulose phosphate P11 (33). Thus, the 3′-exonuclease activity we have detected appears not to be associated with poly(A) polymerase in HeLa cells.

Acknowledgments—We thank U. Pettersson, P. A. Sharp, E. Bridge, and C. Hemström for valuable suggestions and discussions throughout the completion of this work. We are grateful to A.-C. Thuresson for technical assistance, to Hans Friberg for growing HeLa cells, and to Dr. M. M. Konarska for technical advice.

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