Tinoco, I., Jr., and Cantor, C. R. (1970), *Methods Biochem. Anal. 18*, 81.
Uhlenbeck, O. (1972), *J. Mol. Biol. 65*, 25.
Uhlenbeck, O., Baller, J., and Doty, P. (1970), *Nature (London) 225*, 508.
Vournakis, J. N., and Scheraga, H. A. (1966), *Biochemistry 5*, 2997.
Watanabe, K., and Imahori, K. (1971), *Biochem. Biophys. Res. Commun. 45*, 488.
Warshaw, M. M., and Tinoco, I. Jr. (1966), *J. Mol. Biol. 19*, 29.
Willick, G. E., and Kay, C. M. (1971), *Biochemistry 10*, 2216.
Yaniv, M., and Barrell, B. G. (1969), *Nature (London) 222*, 278.

Localization of Polyadenylic Acid Sequences in Messenger Ribonucleic Acid of Mammalian Cells†

George R. Molloy,‡ Michael B. Sporn,§ Dawn E. Kelley,¶ and Robert P. Perry*,¶

ABSTRACT: The location of the poly(A) sequences in the mRNA of cultured L and HeLa cells was determined by submitting the mRNA, or poly(A) fragments excised from the mRNA, to hydrolysis with a highly purified exoribonuclease specific for 3′-OH termini. Analysis of the reaction products by a variety of means indicated that most and possibly all of the poly(A) sequences are at the 3′-OH termini of the mRNAs.

Polyadenylic acid is present in the large heterogeneous nuclear RNA (HnRNA)[1] and the relatively smaller mRNA of mammalian cells, as well as in vaccinia virus mRNA and adenovirus specific nuclear and polyribosomal RNA (Hajivassiliov and Brawerman, 1966; Edmonds and Caramela, 1969; Kates, 1970; Lim and Canellakis, 1970; Darnell *et al.*, 1971a,b; Edmonds *et al.*, 1971; Lee *et al.*, 1971; Burr and Lingrel, 1971; Philipson *et al.*, 1971). The poly(A) region is approximately 200 nucleotides long and is covalently linked to the RNA. Recent experiments by Darnell *et al.* (1971b) and Philipson *et al.* (1971) indicate that the poly(A) is added posttranscriptionally to the nuclear RNA, suggesting that the poly(A) is located near a terminus of the nuclear RNA molecules.

The following question arises. Are the poly(A) sequences in mRNA located at a terminus, and if so, at which one? Kates (1970) has presented evidence derived from chemical end-group analysis indicating that the poly(A) region is located at the 3′-OH end of *in vitro* synthesized vaccinia virus RNA. Burr and Lingrel (1971) have shown by base sequence analysis that oligonucleotides derived from the 3′-OH terminus of rabbit hemoglobin mRNA consist of five to six adenine nucleotides. These oligonucleotides were produced by digestion with pancreatic ribonuclease under low ionic strength conditions in which poly(A) is partially hydrolyzed (Beers, 1960), and thus might be derivatives of a larger poly(A) sequence described by Lim and Canellakis (1970). In the present study we have investigated the location of the large poly(A) sequences in the mRNA of cultured mammalian cells using a highly purified nuclear exoribonuclease specific for 3′-OH termini.

The nuclear exoribonuclease is a processive enzyme (RNA molecules are hydrolyzed to completion before the enzyme dissociates from them) which attacks the 3′-OH end of an RNA molecule and produces only 5′-mononucleotides (Lazarus and Sporn, 1967; Lazarus *et al.*, 1968). An RNA molecule with a phosphate in the 3′-terminal position cannot be degraded by the enzyme (Sporn *et al.*, 1968). The enzyme appears to prefer substrates with relatively little helix content, and has been observed to hydrolyze poly(A) and rapidly labeled (presumably mRNA-like) RNA at roughly comparable rates (Lazarus and Sporn, 1967). If the poly(A) region of mRNA is located at or near the 3′-OH end of the molecule, then incubation of mRNA with the exoribonuclease, under conditions approaching enzyme excess, should result in the disappearance of poly(A) at a rate much faster than that of total RNA. Moreover, poly(A) fragments excised from mRNA with pancreatic ribonuclease should be susceptible to exoribonuclease digestion only if they have come from the 3′-OH end. The results of such experiments with mRNA from mouse L cells and HeLa cells[2] provide strong evidence that

† From the Institute for Cancer Research, Philadelphia, Pennsylvania, from the Department of Biological Sciences, Columbia University, New York, New York, and from the National Cancer Institute, National Institutes of Health, Bethesda, Maryland. *Received February 16, 1972.* The research was supported in part by Grants GB-15397 and GB-8497 from the National Science Foundation; CA-11159-03, CA-06927, and RR-05539 from the U. S. Public Health Service; and 410-3070-2037 from the American Cancer Society; and an appropriation from the Commonwealth of Pennsylvania. Dr. George R. Molloy is a postdoctoral fellow of the American Cancer Society.

‡ Department of Biological Sciences, Columbia University, New York, N. Y. 10027.
§ National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014.
¶ The Institute for Cancer Research, Philadelphia, Pa. 19111.

[1] Abbreviation used is: HnRNA, heterogeneous nuclear RNA.

[2] L cell and HeLa cell mRNA contained poly(A) regions of essentially the same size as judged by polyacrylamide gel analyses of poly(A) fragments excised from the mRNA with T1 and pancreatic ribonuclease. However, in order to produce poly(A) fragments of maximum size, the ribonuclease digestion had to be carried out at a higher salt concentration (0.27 N NaCl) for L cell mRNA than that which is suitable for HeLa mRNA (0.1 M NaCl); digestion of the L cell mRNA at 0.1 M NaCl resulted in considerable degradation of the poly(A). Whether this difference in ribonuclease susceptibility is due to some subtle differences in the structures of the two kinds of mRNA is presently not clear.

most if not all of the poly(A) sequences are at the 3′-OH termini.

Experimental Procedures

HeLa Cell Polyribosomal RNA. Cell culture conditions and procedures for cell fractionation, polyribosome preparation and release of polyribosomal RNA with EDTA were as previously reported (Soeiro and Darnell, 1970).

L Cell Polyribosomal RNA. Conditions of cell culture and polyribosome purification were as previously described (Schochetman and Perry, 1972). RNA was extracted from the polyribosomes using 0.5% sodium dodecyl sulfate and phenol–chloroform (1:1) as described elsewhere (Perry *et al.*, 1972).

Exoribonuclease. The exoribonuclease was isolated from Ehrlich ascites tumor cell nuclei as described previously (Lazarus and Sporn, 1967), and further purified by phosphocellulose and hydroxylapatite column chromatography using continuous gradient elution at pH 7.4. Peak activity of the enzyme was eluted between 0.25 and 0.30 M NaCl on phosphocellulose and between 0.25 and 0.30 M potassium phosphate on hydroxylapatite.

Incubations were carried out at 37° in 0.2- to 0.5-ml volumes containing 1 unit/ml of exonuclease, 40 mM Tris-chloride (pH 7.4), 4 mM $MgCl_2$, 0.3 mM dithiothreitol, 40 mM potassium phosphate, and 40 μg/ml of bovine serum albumin. Exonuclease units were determined with commercial poly(A) as described previously (Lazarus and Sporn, 1967). The other details of the experiments are given in the figure captions.

Results

The exoribonuclease preparation used in these experiments was essentially free of endonuclease contamination. This was verified by submitting a sample of 45S preribosomal RNA to hydrolysis with the enzyme and examining the partially hydrolyzed products by gel electrophoresis (Perry and Kelley, 1972). Such an analysis indicated that this enzyme preparation produced less than 1 endonucleolytic break/3000 exonucleolytic cleavages. The absence of endonuclease was also indicated by the following experiment (Figure 1). Messenger RNA of uniform size, prepared by zonal centrifugation in a sucrose density gradient, was exposed to 1 unit/ml of the exoribonuclease (the highest concentration possible with the available enzyme preparations) for varying periods, and the size distribution of the residual partially hydrolyzed RNA examined on 2.8% acrylamide gels. It is seen that there are essentially no small fragments produced during the early stages of the reaction; this would not be the case if there were a significant amount of endonucleolytic activity.

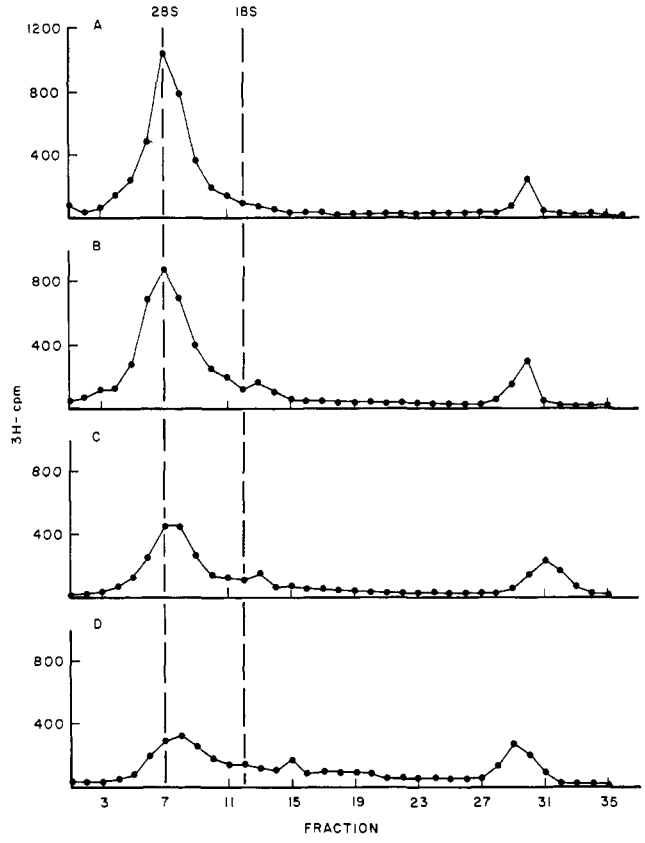

FIGURE 1: Evaluation of the amount of endonuclease contamination in the nuclear exoribonuclease preparation. 1.5 × 10^8 HeLa cells at 3 × 10^6 cells/ml were exposed to 0.05 μg/ml of actinomycin D for 30 min prior to labeling for 3 hr with 10 mCi of [^{3}H]adenosine (20.9 Ci/mmole). Polyribosomes were isolated, treated with EDTA, and sedimented through sucrose gradients [15–30% (w/w) in 0.01 M Tris, pH 7.4, 0.01 M NaCl, and 0.01 M EDTA]. The material sedimenting between 30 and 70 S was extracted with phenol–chloroform (1:1) at pH 8 according to Lee *et al.* (1971) and subjected to zonal sedimentation [15–30% sucrose (w/w) in 0.01 M Tris (pH 7.4), 0.01 M NaCl, 0.01 M EDTA, and 0.2% sodium dodecyl sulfate; SW 40 rotor, 9 hr, 38,000 rpm, 25°]. RNA sedimenting at approximately 23 S was precipitated with ethanol, redissolved, passed throuh Sephadex G-25 equilibrated with 0.05 M NaCl and 0.001 M EDTA, and exposed to the exoribonuclease. At 0, 15, 30, and 60 min, aliquots of the reaction mixture were removed, adjusted to 0.001 M EDTA–0.5% sodium dodecyl sulfate, and applied to a 2.8% polyacrylamide gel. The samples were allowed to run 5 cm at 3 mA/gel. [^{14}C]Uridine-labeled HeLa rRNA was applied to each gel as a marker. A, B, C, and D represent samples taken at 0, 15, 30 and 60 min. During these intervals 0, 10, 46, and 54% of the mRNA, respectively, was rendered acid soluble. It should be noted that mRNA with a sedimentation coefficient of about 23 S has a mobility on polyacrylamide gels corresponding to the 28S rRNA species.

To examine the location of the poly(A) sequences a similar incubation was performed, and the amount of residual poly(A) was measured after varying degrees of hydrolysis. Two different methods were used to assay for the amount of residual poly(A): (i) analysis on polyacrylamide gels of poly(A) fragments excised from the mRNA by digestion with pancreatic and T1 ribonuclease (Darnell *et al.*, 1971a) and (ii) selective adsorption of the residual mRNA substrate to Millipore filters (Lee *et al.*, 1971). It may be seen in Figure 2 that the quantity of poly(A), seen as a peak of material migrating in the 7–10S region of the gel, is continuously reduced during the course of hydrolysis, eventually being diminished by 75% after 53% of the total RNA has been hydrolyzed. A comparison of the reduction in poly(A) determined with the polyacrylamide gel and Millipore filter techniques is presented in Table I. Both assays are in qualitative agreement, and show a faster rate of hydrolysis of the poly(A) than that of total RNA, therefore suggesting an enrichment of poly(A) sequences at the 3′-OH end of the mRNA molecules. However, one would expect a more rapid decline in poly(A) content relative to the overall hydrolysis if all the mRNA molecules contained poly(A) at their 3′-OH ends (*cf.* Figure 3), and *if these regions were being simultaneously removed by the exoribonuclease.* This latter assumption is probably not valid for this experiment, since, as seen in Figure 1, there are some unhydrolyzed substrate molecules which persist until quite late in the reaction. This suggests

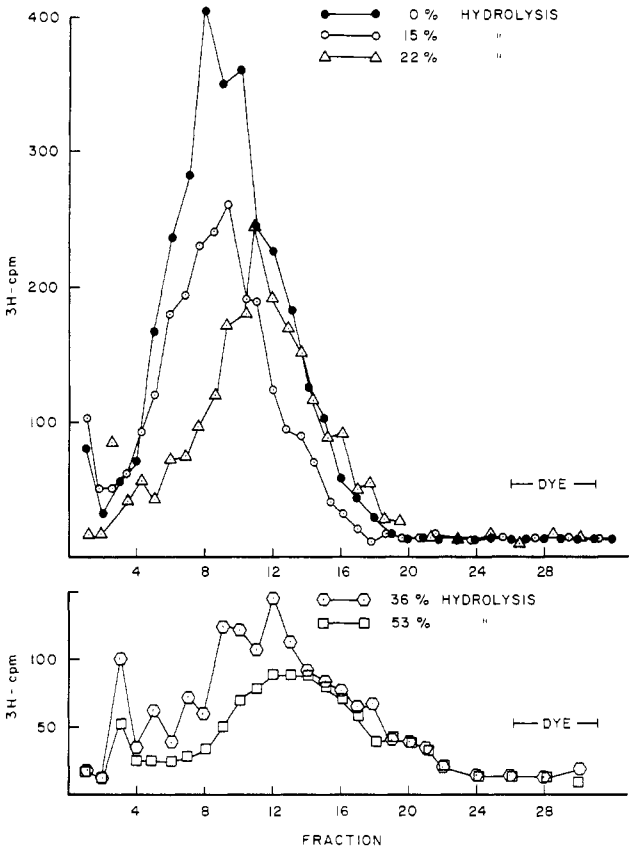

FIGURE 2: Profile of poly(A) segments on polyacrylamide gels after exposure of HeLa mRNA to the nuclear exoribonuclease. [^{3}H]-Adenosine-labeled mRNA, approximately 23 S in size, was prepared and incubated with exoribonuclease as in Figure 1. At intervals during the reaction aliquots were removed and adjusted to 0.01 M EDTA. A portion of each aliquot was used to determine the amount of trichloroacetic acid precipitable counts per minute and the counts per minute binding to Millipore filters (see Table I). The remainder of the aliquot was adjusted to 0.10 M NaCl, and pancreatic RNase (2 μg/ml) plus T1 RNase (10 units/ml) were added for 30 min at 37°. The RNase-resistant RNA [poly(A)] was bound and subsequently eluted from Millipore filters according to Lee *et al.* (1971) and ethanol precipitated. The poly(A) together with a dye marker was analyzed on 10% polyacrylamide gels according to Darnell *et al.* (1971b).

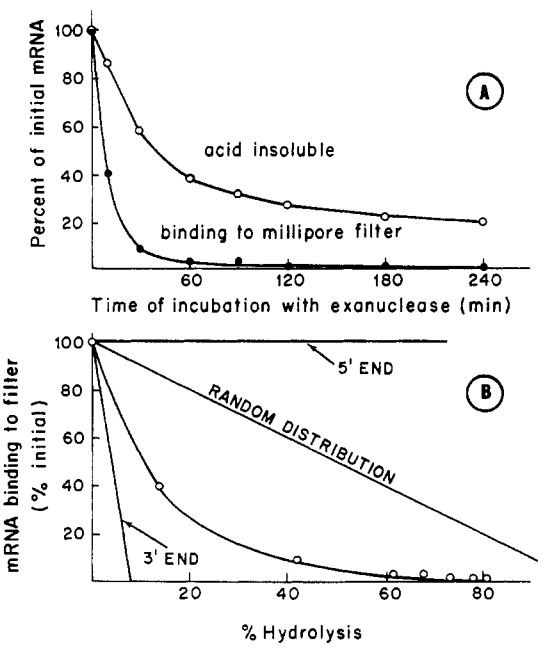

FIGURE 3: Relative decrease in total acid-insoluble mRNA and in mRNA capable of binding to Millipore filters as a function of time of incubation with exoribonuclease (A) and of per cent total hydrolysis (B). L cells were labeled 22 hr with ^{32}P (2 μCi/ml). ^{32}P-Labeled RNA (specific activity 14,000 cpm/μg) was extracted from polyribosomes and passed through a Millipore filter (Lee *et al.* 1971), and the poly(A)-containing mRNA, which is selectively adsorbed, was eluted from the filter with six 0.5-ml portions of 0.01 M Tris buffer (pH 7.4), containing 0.1% sodium dodecyl sulfate. NaCl was added to a final concentration of 0.1 M; the mRNA was precipitated with ethanol, redissolved, passed through Sephadex G-25 equilibrated with 0.05 M NaCl and 0.001 M EDTA, and exposed to the exoribonuclease. The concentration of total RNA in the reaction mixture was estimated to be about 7 μg (20 nmoles) per ml. At the indicated times, aliquots were withdrawn from the reaction, diluted into a buffer containing 0.01 M Tris (pH 7.4), 0.1 M NaCl, 0.001 M EDTA, and 0.1% Sarkosyl and extracted with phenol-chloroform (1:1). One-half of the material was assayed for trichloracetic acid precipitable counts per minute and the other assayed for counts per minute binding to Millipore filters. The theoretical curves in (B) are based on a poly(A) segment of approximately 7 $\times$ 10^4 in an mRNA molecule of 10^6.

that all molecules are not being synchronously attacked and, therefore, that the enzyme was probably not truly in excess. The presence of an undetermined amount of unlabeled rRNA along with the mRNA may have contributed to this condition.

Another experiment was performed which helped remove these ambiguities. In this experiment we used an mRNA preparation which was (i) steady-state labeled with ^{32}P so that an approximate specific activity could be estimated, and (ii) purified by adsorption and elution from Millipore filters (Lee *et al.*, 1971). Analysis of this mRNA preparation by acrylamide gel electrophoresis indicated that it consisted of large molecules (0.5 $\times$ 10^6 to 2.0 $\times$ 10^6 molecular weight), and that it was not significantly contaminated with rRNA. This mRNA was incubated with the nuclear exoribonuclease at an enzyme:substrate ratio of about 50 units/μmole of nucleotide, which according to previous experiments (Perry and Kelley, 1972) represents enzyme excess for a population of mRNA molecules of the size used. The residual fraction of acid-insoluble mRNA and of mRNA capable of binding to Millipore filters [poly(A)-containing mRNA] was measured and plotted as a function of incubation time (Figure 3A). It is seen that the decrease in poly(A)-containing mRNA is much more rapid, especially during the initial stages of hydrolysis, than the reduction of total acid-precipitable mRNA. Figure 3B depicts the per cent reduction of poly(A)-containing mRNA as a function of total per cent hydrolysis. The rates of reduction that would be observed if the poly(A) sequences were all at the 5′ terminus, were randomly distributed, or

TABLE I: Per Cent Hydrolysis of Total HeLa mRNA and Poly(A) by Nuclear Exoribonuclease.

Min	% Total Hydrolysis	% Hydrolysis of Poly(A)	
		Gel	Millipore
0	0	0	0
10	15	24	35
30	22	38	55
60	36	56	67
120	53	75	81

TABLE II: Base Composition of L Cell ^{32}P-Labeled mRNA and of 5′-Mononucleotides Released from the mRNA upon Digestion with the Nuclear Exoribonuclease.[c]

Nucleotide	Overall Base Composition of mRNA (%)[a]	Acid-Soluble Exoribonuclease Product (%)[b]
GMP	28	13
UMP	29	13
AMP	25	54
CMP	18	19

[a] Determined from a KOH hydrolysate of [^{32}P]mRNA substrate. [b] Twenty-two per cent of total radioactivity in mRNA was rendered acid soluble. [c] Base compositions were determined by thin-layer chromatography on polyethyleneimine cellulose as described elsewhere (Kelley and Perry, 1971).

were all at the 3′ terminus of the mRNA molecules are shown for comparison. The fact that most of the poly(A) is removed during the early stages of the exoribonuclease reaction indicates that most poly(A) sequences are indeed located near the 3′-OH end of the mRNA molecules.

The gel electrophoretic profile of the mRNA used in this experiment is shown in Figure 4 along with a profile of the partially hydrolyzed product remaining after 20% of the total nucleotides was rendered acid soluble. The absence of a significant quantity of small RNA fragments in this product attests to the purity of the exonuclease preparation, and clearly demonstrates that the removal of poly(A) described above is achieved without significant endonuclease activity.

Additional evidence that the poly(A) sequences are near the 3′-OH terminus comes from an analysis of the 5′-mononucleotides produced when the ^{32}P-labeled mRNA was incubated with exoribonuclease. After an incubation in which 22% of the total RNA was hydrolyzed it was found that 54% of the acid-soluble radioactivity was recovered as AMP with the remainder being distributed more or less uniformly in the other three ribonucleotides (Table II). By comparison the overall AMP content of this mRNA is only 25%.

The failure of the experimental data of Figure 3 to follow the theoretical curves expected if *all* poly(A) were at the 3′-OH end and the presence of ribonucleotides other than AMP in the acid-soluble hydrolysate could mean that there was still some asynchrony in the exoribonuclease reaction in spite of the relatively high enzyme:substrate ratio used. On the other hand, it could mean that there are some poly(A) sequences internal to the 3′-OH terminus. The latter possibility was examined by determining the proportion of poly(A) segments which have free 3′-OH termini. Advantage was taken of the fact that the nuclear exoribonuclease, while effective against substrates with 3′-OH termini, does not hydrolyze molecules that contain a 3′-phosphate terminal group. With enzyme from the same batch used in these experiments, it was observed that commercial poly(A) treated with low levels of micrococcal nuclease (0.1 μg/ml) to produce poly(A) fragments with 3′-phosphate end groups was totally insensitive to exoribonuclease (Sporn *et al.*, 1968). Therefore, poly(A) fragments, derived from mRNA by treatment with pancreatic ribonuclease, should be hydrolyzed by the nuclear exoribonuclease if they have come from the 3′-OH terminus, whereas they should be resistant if they have come from internal regions, in which case they would have a 3′-phosphate terminus. In the experiments shown in Figure 5 about 90% of the L cell poly(A) and 73% of the HeLa poly(A) was susceptible to hydrolysis by the exoribonuclease.

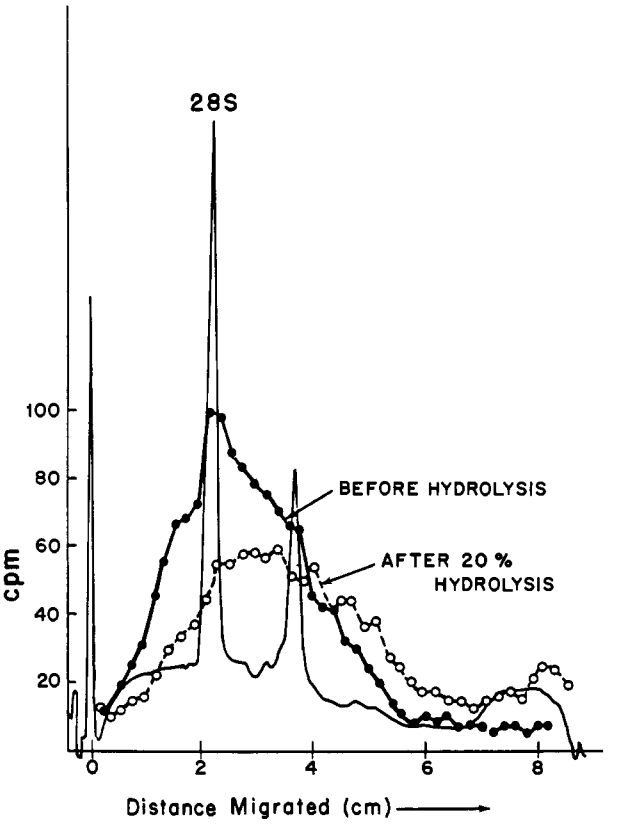

FIGURE 4: Polyacrylamide gel profiles of ^{32}P-labeled mRNA before and after hydrolysis. The mRNA used in Figure 3 was hydrolyzed with the exoribonuclease in an experiment similar to that described in Figure 3 (150 units/μmole) until 20% of the radioactivity was rendered acid soluble. The reaction mixture was made 1% in sodium dodecyl sulfate and 15% in sucrose and layered directly onto 2.7% polyacrylamide gels. Electrophoresis was carried out as described previously (Perry and Kelley, 1968). (—●—) Initial mRNA substrate before hydrolysis; (--○--) partially hydrolyzed mRNA after 20% hydrolysis. All of the radioactivity in the initial substrate was accounted for by the sum of that in partially hydrolyzed RNA plus the 20% in 5′-mononucleotides.

Inclusion of alkaline phosphatase in one set of reactions rendered the remaining poly(A) sensitive to enzymatic hydrolysis, but also resulted in an increased rate of hydrolysis. This rate increase, also found when commercial poly(A) was used as a substrate, was probably due to contamination of the alkaline phosphatase (a commercial preparation derived from *E. coli*) with a trace amount of endonuclease. Indeed, in a separate experiment the alkaline phosphatase was observed to cause partial breakdown of 45S preribosomal RNA.

Discussion

The foregoing results indicate that the large majority (at least 90% of the L cell and at least 73% of the HeLa) poly(A) sequences is located at the 3′-OH termini of their mRNA molecules. This conclusion depends on the contention that the exonuclease is virtually free of contamination with endonuclease and phosphatase. The absence of endonuclease

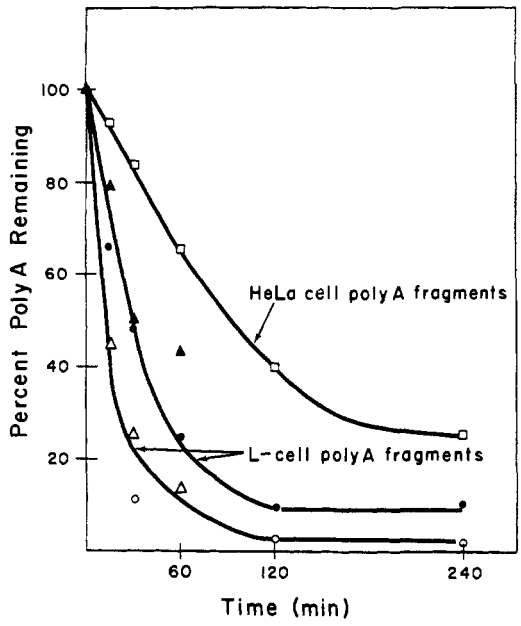

FIGURE 5: The susceptibility of poly(A) fragments derived from mRNA to hydrolysis by nuclear exoribonuclease. L cell mRNA was obtained as described in Figure 3 from cells treated 30 min with 0.08 μg/ml of actinomycin D and labeled for 1 hr with [^{3}H]adenosine (10 μCi/ml, 15.5 Ci/mmole) in the presence of the drug. Poly(A) fragments were prepared by treating mRNA with pancreatic RNase (2 μg/ml) for 60 min at 37° in 0.27 M NaCl. After phenol–chloroform (1:1) extraction, the RNA was bound and eluted from Millipore filters (Lee *et al.*, 1971). HeLa mRNA poly(A) was prepared according to Darnell *et al.* (1971a) and the five peak fractions migrating in the 7–10S region of a 15% acrylamide gel were selected for the exoribonuclease experiment. The L cell poly(A) fragments were incubated for 30 min at 24° in a solution containing 40 mM Tris (pH 7.4), 4 mM $MgCl_2$, 0.3 mM dithiothreitol, and 40 μg/ml of bovine serum albumin with (○, △) or without (●, ▲) 50 μg/ml of *E. coli* alkaline phosphatase (EC 3.1.3.1, BAFP: Worthington Biochemical Corp.) and then for various times with 1 unit/ml of ascites nuclear exoribonuclease. Circles and triangles represent separate experiments. The HeLa fragments (□) were incubated with 0.5 unit/ml of exoribonuclease under otherwise similar conditions. Poly(A) was assayed either by adsorption to Millipore filters or acid insolubility. Both assay methods gave similar results.

activity was demonstrated in several ways, including an experiment (Figure 4) performed with a high ratio of enzyme: substrate comparable to those used in the other experiments. The absence of phosphatase activity was demonstrated by the complete inability of the enzyme to act on poly(A) molecules containing 3′-PO_4 end groups (Sporn *et al.*, 1968). Moreover, the exonuclease digestions were performed at 40 mM PO_4, a concentration found to be inhibitory to alkaline phosphatase (unpublished experiments).

The relatively small portion of poly(A) which was not rendered acid soluble by the exoribonuclease could represent partially hydrolyzed fragments or a fraction of the poly(A) molecules which possess 3′-phosphate terminal groups. The results with alkaline phosphatase appear to favor the latter possibility. The minor fraction of exoribonuclease-resistant poly(A) could be: (a) an artifact of the poly(A) preparation, (b) poly(A) not located at the 3′-OH terminus, or (c) the result of an *in vivo* enzymatic modification of the poly(A) terminus.

The present finding that a large variety of mRNA molecules have poly(A) sequences at their 3′-OH ends is relevant to the concept of mRNA processing. The previous demonstration (Darnell *et al.*, 1971b) that poly(A) is added posttranscriptionally to HnRNA suggests that the poly(A) sequences are probably located at the ends of the HnRNA molecules, and more recent results (Sheldon *et al.*)[8] indicate that they are, in fact, at the 3′-OH ends. If we assume that these poly(A) segments are the same ones that eventually appear in mRNA, then our results imply that HnRNA → mRNA conversion involves conservation of an RNA segment near the 3′-OH terminus, and the elimination of nucleotides at the 5′-P(PP) end of the HnRNA molecules. This is in contrast to the processing of mammalian rRNA in which there is a loss of nucleotides at the 3′-OH end of the 45S ribosomal precursor (Perry and Kelley, 1972).

Acknoweldgments

We thank Drs. James E. Darnell, Milton Adesnik, and Jay R. Greenberg for helpful discussions during the course of this investigation and Mr. William Thomas for excellent technical assistance.

References

Beers, R. F. (1960), *J. Biol. Chem. 235*, 2393.
Burr, H., and Lingrel, J. B. (1971), *Nature (London), New Biol. 233*, 41.
Darnell, J. E., Philipson, L., Wall, R., and Adesnik, M. (1971b), *Science 174*, 507.
Darnell, J. E., Wall, R., and Tushinski, R. J. (1971a), *Proc. Nat. Acad. Sci. U. S. 68*, 1321.
Edmonds, M., and Caramela, M. G. (1969), *J. Biol. Chem. 244*, 1314.
Edmonds, M., Vaughan, M. H., Jr., and Nakazato, H. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1336.
Hajivassiliov, A., and Brawerman, G. (1966), *J. Mol. Biol. 20*, 1.
Kates, J. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35*, 743.
Kelley, D. E., and Perry, R. P. (1971), *Biochim. Biophys. Acta 238*, 357.
Lazarus, H. M., and Sporn, M. B. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1386.
Lazarus, H. M., Sporn, M. B., and Bradley, D. F. (1968), *Proc. Nat. Acad. Sci. U. S. 60*, 1503.
Lee, S. Y., Mendecki, J., and Brawerman, G. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1331.
Lim, L., and Canellakis, E. S. (1970), *Nature (London) 227*, 710.
Perry, R. P., and Kelley, D. E. (1972), *J. Mol. Biol.* (in press).
Perry, R. P., and Kelley, D. E. (1968), *J. Cell. Physiol. 72*, 235.
Perry, R. P., LaTorre, J., Kelley, D. E., and Greenberg, J. R. (1972), *Biochim. Biophys. Acta. 262*, 220.
Philipson, L., Wall, R., Glickman, R., and Darnell, J. E. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 2806.
Schochetman, G., and Perry, R. P. (1972), *J. Mol. Biol. 63* (in press).
Soeiro, R., and Darnell, J. E. (1970), *J. Cell. Biol. 44*, 467.
Sporn, M. B., Lazarus, H. M., Smith, J. M., and Henderson, W. R. (1968), *Biochemistry 8*, 1698.

[8] Submitted for publication.