Heterogeneity in Nucleotide Sequences Adjacent to Poly(riboadenylic acid) in L-Cell Messenger Ribonucleic Acid†

J. L. Nichols* and Joseph J. Eiden

ABSTRACT: Digestion of L-cell mRNA with T_1 ribonuclease in high ionic strength buffer released fragments of RNA which contained the poly(A) segments and pyrimidine nucleotides adjacent to them in the RNA chain. The poly(A) containing fragments were isolated by affinity chromatography on columns of poly(U)-cellulose. Alkali hydrolysis of the fragments revealed the presence of both cytidylate and uridylate residues in addition to adenylate residues derived from the poly(A) segments. The proportions of the pyrimidine nucleotides indicated that there was, on the average, approximately three residues per poly(A) segment of chain length 200. Pancreatic ribonuclease digestion of material purified from T_1 ribonuclease digests resulted in the release of only cytidylate and uridylate residues in addition to oligoadenylates, indicating that the pyrimidine residues were not interspersed with adenylate residues. Digestion of the pyrimidine-containing poly(A) fragments with U_2 ribonuclease resulted in the degradation of the poly(A) segments and the release of components of the type (Py-)nAp. Differences in the pyrimidine composition and chain lengths of the (Py-)nAp products demonstrated that there were no common nucleotide sequences in the RNA immediately adjacent to the poly(A) segments in unfractionated L-cell mRNA. This would indicate that the nuclear poly(A) synthetases do not recognize a specific nucleotide sequence, or, alternatively, the recognition site is further removed from the terminus of the transcriptional unit.

Segments of poly(riboadenylic acid) [poly(A)] are covalently attached to messenger RNA (mRNA), heterogeneous nuclear RNA (HnRNA) (Lim and Canellakis, 1970; Edmonds *et al.*, 1971; Darnell *et al.*, 1971a,b; Lee *et al.*, 1971; Sheldon *et al.*, 1972a,b; Greenberg and Perry, 1972; Lindberg and Persson, 1972; Pemberton and Baglioni, 1972), and mitochondrial RNA (mtRNA) (Perlman *et al.*, 1973; Hirsch and Penman, 1973; Ojala and Attardi, 1974) of eukaryotic cells. Polyadenylation is probably a post-transcriptional process (Darnell *et al.*, 1971b; Jelinek *et al.*, 1973; Philipson *et al.*, 1971; Birnboim, 1973) involving nuclear poly(A) synthetases (ATP polymerases) (Edmonds and Abrams, 1960; Niessing and Sekeris, 1973). An examination of the poly(A) segments from HeLa cell mRNA and HnRNA (Molloy and Darnell, 1973) and L-cell mRNA (Eiden and Nichols, 1973) has shown these to consist of only adenylic acid. In the case of both HeLa cell mRNA and HnRNA it has further been shown that all poly(A) segments are adjacent to pyrimidine nucleotides (Molloy and Darnell, 1973).

The region of RNA which precedes the terminally located poly(A) segment may contain specific nucleotide sequences which act either as recognition sites for the poly(A) synthetases and/or represent termination signals for transcription by RNA polymerase. The development of isolation procedures based on the presence of poly(A) in the RNA (Lee *et al.*, 1971; Sheldon *et al.*, 1972b; Nakazato and Edmonds, 1972; Lindberg *et al.*, 1972) makes it possible to study nucleotide sequences which precede poly(A) segments in poly(A) containing fragments derived from intact RNA.

The present report presents the results of experiments designed to determine if L-cell mRNAs have homologous nucleotide sequences adjacent to the poly(A) segments. Although all species were found to contain one or more pyrimidine nucleotides adjacent to the poly(A) segment, the composition, length, and sequence variability indicate that if specific nucleotide sequences are involved in poly(A) synthetase recognition, they do not immediately precede the poly(A) segments. The variability in nucleotide sequences would also tend to rule out the transcription into RNA of common termination nucleotide sequences to which the poly(A) is added.

Materials and Methods

Labeling of Cells. Mouse L-cells were grown in phosphate-free medium (Joklik's modification, Grand Island Biological Co.) containing carrier-free $^{32}PO_4$ (New England Nuclear; 175 μCi/ml) as previously described (Eiden and Nichols, 1973). In some experiments labeling of cells was carried out after an initial 30-min incubation in the presence of actinomycin D (0.05 μg/ml, Calbiochem) and ethidium bromide (1 μg/ml, Calbiochem).

Cell Fractionation and Preparation of RNA. The breakage of cells and preparation of post-mitochondrial supernatants were accomplished by procedures previously described (Penman *et al.*, 1963; Vesco and Penman, 1969). Polysomes were isolated by centrifuging the post-mitochondrial supernatants for 2.5 hr through 40% sucrose in RSB[1] at 40,000 rpm in a SW 50 rotor. Polyribosomal RNA was ex-

† From the Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710. *Received March 13, 1974.* This study was supported by Grant AI 10361 from the U. S. Public Health Service.

[1] Abbreviations used are: (Py-)nAp, oligonucleotides containing a variable number of pyrimidine residues with a 3′-terminal adenylic acid residue; (A-)nPyp, oligonucleotides containing a variable number of adenylic acid residues with a 3′-terminal cytidylic or uridylic acid residue; RSB, 0.01 M NaCl-0.01 M Tris-HCl (pH 7.2)-0.0015 M $MgCl_2$.

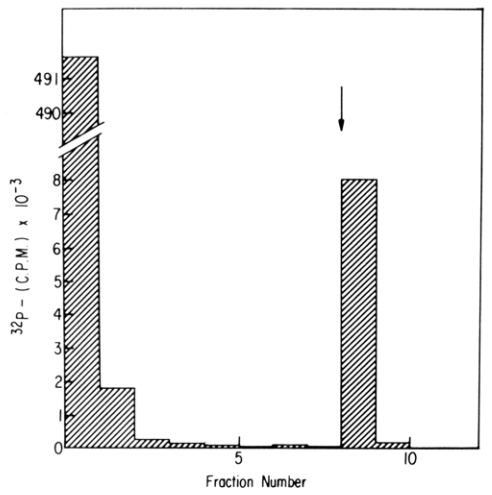

FIGURE 1: Poly(U)-cellulose affinity chromatography of a T_1 ribonuclease digest of polyribosomal RNA isolated from L-cells grown in the absence of actinomycin D (see Materials and Methods). The arrow indicates the point at which elution was begun with 0.01 M Tris-HCl (pH 7.5) at 37°.

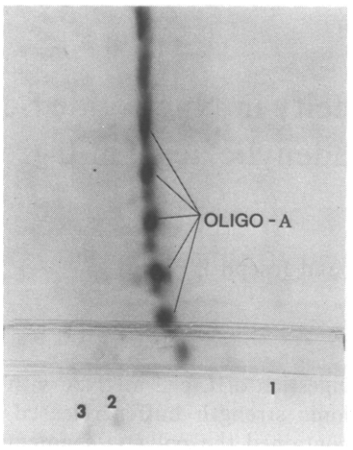

FIGURE 2: Autoradiograph of the two-dimensional fractionation of a pancreatic ribonuclease digest of fragments isolated from a T_1 ribonuclease digest of polyribosomal RNA. The fragments were isolated by poly(U)-cellulose chromatography and further purified by electrophoresis on polyacrylamide gels. Direction of electrophoresis in the first dimension was from right to left, and electrophoresis in the second dimension was from top to bottom, in relation to the photograph. (1) cytidine 3′-monophosphate; (2 and 3) uridine 3′-monophosphate and uridine 2′, 3′-cylic monophosphate, respectively. Cytidine 2′-monophosphate could not be detected in this experiment.

tracted by phenol deproteinization as previously described (Lee *et al.*, 1971).

Preparation of Poly(A) Containing RNA Fragments. Polyribosomal RNA was digested with T_1 ribonuclease (0.2 unit; Sankyo Co.) in 100 μl of a high ionic strength buffer (0.2 M NaCl–0.01 M Tris-HCl (pH 7.4)–0.01 M EDTA) for 1 hr at 37°. Digestion of polyribosomal RNA with pancreatic ribonuclease (0.2 μg; Worthington Biochemicals) was carried out for 45 min at 37° in 100 μl of high ionic strength buffer. The poly(A) fragments were isolated by chromatography on columns of poly(U)-cellulose (Sheldon *et al.*, 1972b). For this procedure the nuclease digest was added to 0.5 ml of running buffer (0.01 M Tris-HCl (pH 7.5)–0.1 M NaCl) and applied to a 1-in. column of poly(U)-cellulose contained in a pasteur pipet. The column, which had previously been equilibrated with the running buffer, was operated at 4°. Fractions of 2 ml were collected until the unbound RNA was completely washed through the column (5–8 fractions). The column was then warmed to 37° and the poly(A) containing fragments were eluted with buffer [0.01 M Tris-HCl (pH 7.5)] at the same temperature. The radioactivity in each fraction was determined by liquid scintillation counting of 25-μl aliquots. Fractions containing the poly(A) fragments were combined, the solution was made 0.1 M in NaCl, and the RNA was recovered by ethanol precipitation at −20°. Further purification of the fragments was achieved either by rechromatography on poly(U)-cellulose columns or by electrophoresis on polyacrylamide gels (Eiden and Nichols, 1973).

Digestion and Fractionation of Poly(A) Containing Fragments. Purified RNA fragments were digested with either pancreatic or T_1 ribonuclease in low ionic strength buffer (0.01 M Tris-HCl (pH 7.4)–0.001 M EDTA) according to published procedures (Sanger *et al.*, 1965). Digestion with U_2 ribonuclease (Adams *et al.*, 1969; Nichols, 1970) employed 10 μl of enzyme (2 units/ml) in buffer (0.05 M sodium acetate (pH 4.5)–0.002 M EDTA containing 0.1 mg/ml of bovine serum albumin) for 60 min at 37°. U_2 ribonuclease was kindly provided by Sankyo Co., Japan.

The enzyme digests were fractionated according to the procedures of Sanger and coworkers (Sanger *et al.*, 1965; Brownlee *et al.*, 1968).

Alkali hydrolysis was accomplished by incubation of the sample in 0.2 N NaOH for 18 hr at 37°. The resulting nucleoside monophosphates were resolved by paper electrophoresis at pH 3.5 (Sanger *et al.*, 1965).

Results

Poly(A) segments have been shown to be covalently linked only to mRNA in polyribosomal RNA preparations (Sheldon *et al.*, 1972b; Eiden and Nichols, 1973; Perry and Kelley, 1974). Thus, in order to obtain poly(A) containing fragments from the mRNA, polyribosomal RNA was digested directly with either T_1 or pancreatic ribonuclease and the poly(A) fragments were then separated from other digestion products by affinity chromatography. Figure 1 shows the fractionation of a T_1 ribonuclease digest of L-cell polyribosomal RNA on a poly(U)-cellulose column. In polyribosomal RNA preparations derived from cultures grown in the absence of actinomycin D and digested with either T_1 or pancreatic ribonuclease, the resulting poly(A) fragments accounted for approximately 2% of the total radioactivity.

Poly(A) containing fragments isolated from T_1 or pancreatic ribonuclease digests were further purified by polyacrylamide gel electrophoresis. Material eluted from the gels (approximately 100,000 cpm) was then hydrolyzed in alkali. The analysis of fragments purified from pancreatic ribonuclease digests showed only the presence of adenylic acid, indicating that within the limits of detection all messenger species had a pyrimidine residue immediately preceding the poly(A) segment. Fragments prepared from T_1 ribonuclease digests had a base composition which indicated the presence of approximately three pyrimidine residues per poly(A) segment of chain length 200 (Table I). To determine if the pyrimidine residues were interspersed with adenylic acid residues in the region preceding the poly(A) segment, fragments isolated from T_1 ribonuclease digests in high ionic strength buffer were digested with pancreatic ribonuclease in low ionic strength buffer and fractionated by two-dimensional electrophoresis (Figure 2). In addition to

TABLE I: Composition of Poly(A) Fragments Prepared from T_1 Ribonuclease Digests of Polyribosomal RNA.[a]

Nucleotide	% Expt 1	% Expt 2	% Av
Up	1.1	1.3	1.2
Cp	0.6	0.8	0.7
Gp[c]			
Ap	98.3	97.9	98.1

[a] Two different procedures were employed to obtain the data shown here. In experiment 1, the RNA was hydrolyzed in alkali and the nucleoside 2′(3′)-monophosphates were resolved by paper electrophoresis (see Materials and Methods). In experiment 2, the RNA was digested for 45 min with pancreatic ribonuclease and the digestion products were resolved by two-dimensional electrophoresis as illustrated in Figure 2. The total radioactivity in the oligoadenylates was determined and this is represented by the value for Ap. Similarly, the radioactivity in both uridine 3′-phosphate and uridine 2′,3′-cyclic phosphate is represented by the value for Up; the radioactivity in both cytidine 3′-phosphate and cytidine 2′,3′-cyclic phosphate is represented by the value for Cp. In both experiments the position of the nucleotides was determined by autoradiography and each nucleotide area was excised and counted in a toluene-based scintillation fluid. [b] The total radioactivity was 35,484 and 48,736 cpm in experiments 1 and 2, respectively. [c] The radioactivity in the guanylic acid area of the electrophoretograms in both experiments was less than 0.1% of the total.

TABLE II: The Composition of Oligonucleotides Produced by U_2 Ribonuclease Digestion of Poly(A) Fragments.[a]

Component[c]	Composition	% Total RNA Chains[b] Expt 1	Expt 2	Av
1	C-Ap[d]	32.4	21.5	26.9
4	U-Ap	30.5	18.1	24.3
5	(U,C)Ap	5.7	7.2	6.5
6	(U,C_2)Ap	0.7	1.9	1.3
7	U-U-Ap	4.7	8.3	6.5
8	(U_2C)Ap	5.5	10.0	7.8
9	(U_2,C_2)Ap	3.2	4.2	3.7
10	U-U-U-Ap	7.1	15.1	11.1
11	(U_3,C)Ap	3.2	4.2	3.7
12	(U_3,C_2)Ap	0.9	1.7	1.3
13	U-U-U-U-Ap	4.0	4.5	4.3
14	(U_4,C)Ap	1.0	2.5	1.8
15	(U_4,C_2)Ap	1.0	0.8	0.9

[a] The poly(A) fragments were isolated on poly(U)-cellulose columns following digestion of polyribosomal RNA with T_1 ribonuclease in high ionic strength buffer (see Materials and Methods). The fragments were further purified by chromatography on a second poly(U)-cellulose column. [b] The radioactivity in each component was determined by liquid scintillation spectrophotometry. The percentage of total counts in each of the components was determined after correction for chain length differences. The total radioactivity in all of these components was 8728 and 9684 cpm in experiments 1 and 2, respectively. [c] The numbers refer to the location of the components on the autoradiograph of Figure 3. [d] To clearly separate C-Ap from Ap, it was necessary to excise the area of the electrophoretogram containing these components and reelectrophoresce it on DEAE-paper at pH 3.5.

the oligoadenylates, only cytidylic and uridylic acid were present; no components of the type (A-)nPy could be detected.

The fact that the pyrimidine residues were not interspersed with adenylate residues made it possible to use the purine-specific enzyme ribonuclease U_2 (Arima *et al.*, 1968), to digest the poly(A) segments, and leave the adjacent pyrimidines unhydrolyzed. It can be seen in Figure 3 that many different components resulted from U_2 ribonuclease digestion of poly(A) fragments originally isolated from a T_1 ribonuclease digest. The composition of each of the components resulting from U_2 ribonuclease digestion was determined following alkali hydrolysis by separation and quantitation of the nucleoside monophosphates. Digestion of each of the components with pancreatic ribonuclease released adenosine monophosphate, demonstrating that this nucleotide was present at the 3′ end of each oligonucleotide; it represents, therefore, the first adenylate residue of the poly(A) segment (Table II). Cytidylate and uridylate monophosphates could not be detected on the fingerprints, even when the digestion time with U_2 ribonuclease was extended to 90 min, and for this reason it is doubtful that the U_2 ribonuclease-released components were derived from longer pyrimidine stretches adjacent to the poly(A) segments. Occassionally U-Up and U-U-U-Up could be detected. These components are likely to have been derived by U_2 ribonuclease digestion of components such as U-U-Ap and U-U-U-U-Ap. Small amounts of U-U-A-Ap and U-U-U-A-Ap, resulting from incomplete hydrolysis with U_2, could also be identified in some preparations.

RNA prepared from cultures grown in the presence of actinomycin D and ethidium bromide during the ^{32}P-labeling period was found to contain the same components in the same relative proportions as those listed in Table II. This makes it unlikely that the results could be attributed to contamination of the RNA with mt- or rRNA (Zylber *et al.*, 1969; Perry, 1963).

Discussion

The observation that phosphodiester linkages involving adenylic acid residues becomes susceptable to attack by pancreatic ribonuclease (Lane and Butler, 1959) in low ionic strength solution, but not in high ionic strength solution (Beers, 1960), has made it possible to employ conditions for nuclease digestion of RNA which ensure the isolation of intact poly(A) regions. It was previously shown that digestion of L-cell mRNA with both T_1 and pancreatic ribonuclease in high ionic strength buffer released intact poly(A) segments which contained only adenylic acid (Eiden and Nichols, 1973). Since it is known that the poly(A) segments are at the 3′ end of this and other cellular RNA molecules (Mendecki *et al.*, 1972; Molloy *et al.*, 1972; Sheldon *et al.*, 1972a; Nakazato *et al.*, 1973; Brownlee *et al.*, 1973), any nucleotides besides adenylic acid which are covalently linked to poly(A) fragments must be present at the 5′ ends of these fragments.

In the present study, fragments produced by digestion with T_1 ribonuclease, but not pancreatic ribonuclease, were

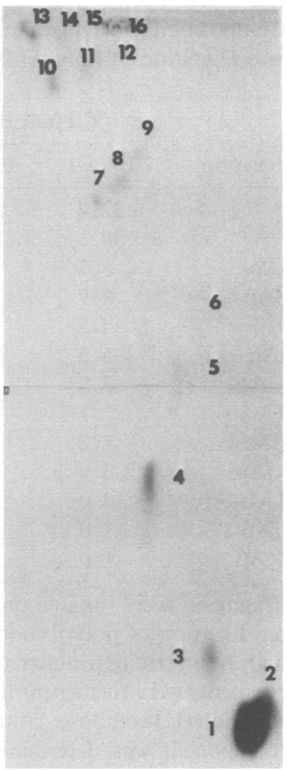

FIGURE 3: Autoradiograph of the two-dimensional fractionation of a U_2 ribonuclease digest of fragments (200,000 cpm) isolated from a T_1 ribonuclease digest of polyribosomal RNA. The fragments were isolated by poly(U)-cellulose chromatography and further purified by rechromatography on a second poly(U)-cellulose column. Direction of electrophoresis in the first dimension was from right to left, and electrophoresis in the second dimension was from top to bottom, in relation to the photograph. 1, Ap; 2, C-Ap; 3, A-Ap; 4, U-Ap; 5, (U,C)Ap; 6, (U,C_2)Ap; 7, U-U-Ap; 8, (U_2,C)Ap; 9, (U_2,C_2)Ap; 10, U-U-U-Ap; 11, (U_3,C_2)Ap; 12, (U_3,C_2)Ap; 13, U-U-U-U-Ap; 14, (U_4,C)Ap; 15, (U_4,C_2)Ap; 16, unhydrolyzed fragments.

found to contain a variety of sequences adjacent to the poly(A). Over half of the total mRNA molecules contained a single pyrimidine residue adjacent to the poly(A). These molecules terminate in the following way: 5′-G-C-poly(A)-3′ and 5′-G-U-poly(A)-3′; other mRNA molecules contained several consecutive pyrimidine residues adjacent to the poly(A) segment; the longest of these was 5′-G-(U_4,C_2)-poly(A)-3′.

All of the L-cell mRNA species have one or more pyrimidine residues immediately adjacent to the poly(A) segment. A similar situation exists in HeLa cell mRNA and HnRNA where it was found that the composition of both RNAs indicated the presence of two uridylate, and one to two cytidylate residues adjacent to the poly(A) segments (Molloy and Darnell, 1973). Proudfoot and Brownlee (1974) have determined that globin mRNA has a sequence 5′-A-U-U-G-C-poly(A)-3′ and Haff and Keller (1973) have shown that yeast RNA has a unique sequence 5′-G-C-A-A-U-poly(A)-3′. Thus, all cellular RNA species examined to date have at least one pyrimidine residue adjacent to the poly(A) segment.

Apart from the presence of a pyrimidine residue adjacent to the poly(A) segment, the diversity in nucleotide sequences found in L-cell mRNA illustrates that there are no other common features in this region of the RNA. In fact, the total number of different nucleotide sequences preceding poly(A) segments is actually larger than the 13 which could be distinguished on the basis of composition alone (Table II), since preliminary data have indicated that several of the components contain more than the one isomer.

The nucleotide sequences which precede poly(A) segments probably represent transcriptional chain termini or termini resulting from cleavage of HnRNA prior to polyadenylation (Darnell *et al.*, 1973). It is clear from the heterogeneity found in the nucleotides adjacent to the poly(A) that there is no apparent common recognition site for the poly(A) synthetases. It is still possible, however, that such a site may be constituted from pyrimidine-rich sequences, or the site could be further removed from the termini.

The knowledge of nucleotide sequences in mRNA now makes it possible to compare these with the corresponding regions in HnRNA with a view to studying the precursor–product relationship between these RNAs. Preliminary findings in this laboratory indicate that the same sequences adjacent to the poly(A) in L-cell mRNA are also present in the same relative proportions in L-cell HnRNA.

Acknowledgment

The authors wish to thank Mrs. Marie Waddell for expert technical assistance.

References

Adams, J. M., Jeppesen, P. G. N., Sanger, F., and Barrell, B. G. (1969), *Nature (London) 223,* 1009.

Arima, T., Uchida, T., and Egami, F. (1968), *Biochem. J. 106,* 609.

Beers, R. F. (1960), *J. Biol. Chem. 235,* 2393.

Birnboim, H. C. (1973), *Proc. Nat. Acad. Sci. U. S. 70,* 2189.

Brownlee, G. G., Cartwright, E. M., Cowan, N. J., Jarvis, J. M., and Milstein, C. (1973), *Nature (London), New Biol. 244,* 236.

Brownlee, G. G., Sanger, F., and Barrell, B. G. (1968), *J. Mol. Biol. 34,* 379.

Darnell, J. E., Jelinek, W. R., and Molloy, G. R. (1973), *Science 181,* 1215.

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 Abrams, R. (1960), *J. Biol. Chem. 235,* 1142.

Edmonds, M., Vaughan, M. H., and Nakazato, H. (1971), *Proc. Nat. Acad. Sci. U. S. 68,* 1336.

Eiden, J. J., and Nichols, J. L. (1973), *Biochemistry 12,* 3951.

Greenberg, J. R., and Perry, R. P. (1972), *Biochim. Biophys. Acta 287,* 361.

Haff, L. A., and Keller, E. B. (1973), *Biochim. Biophys. Acta 54,* 1611.

Hirsch, M., and Penman, S. (1973), *J. Mol. Biol. 80,* 379.

Jelinek, W., Adesnik, M., Salditt, M., Sheiness, D., Wall, R., Molloy, G., Philipson, L., and Darnell, J. E. (1973), *J. Mol. Biol. 75,* 515.

Lane, B. G., and Butler, G. C. (1959), *Can. J. Biochem. Physiol. 37,* 1329.

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) 277,* 710.

Lindberg, U., and Persson, T. (1972), *Eur. J. Biochem. 31,* 246.

Lindberg, U., Persson, T., and Philipson, L. (1972), *J. Virol. 10,* 909.
Mendecki, J., Lee, Y., and Brawerman (1972), *Biochemistry 11,* 792.
Molloy, G. R., and Darnell, J. E. (1973), *Biochemistry 12,* 2324.
Molloy, G. R., Sporn, M., Kelley, D. E., and Perry, R. P. (1972), *Biochemistry 11,* 3256.
Nakazato, H., and Edmonds, M. (1972), *J. Biol. Chem. 247,* 3365.
Nakazato, H., Kopp, D., and Edmonds, M. (1973), *J. Biol. Chem. 248,* 1472.
Nichols, J. L. (1970), *Nature (London) 225,* 147.
Niessing, J., and Sekeris, C. E. (1973), *Nature (London), New Biol. 243,* 9.
Ojala, D., and Attardi, G. (1974), *J. Mol. Biol. 82,* 151.
Pemberton, R., and Baglioni, C. (1972), *J. Mol. Biol. 65,* 531.
Penman, S., Scherrer, K., Becker, Y., and Darnell, J. E. (1963), *Proc. Nat. Acad. Sci. U. S. 49,* 654.
Perlman, S., Abelson, H., and Penman, S. (1973), *Proc. Nat. Acad. Sci. U. S. 70,* 350.
Perry, R. (1963), *Exp. Cell Res. 29,* 400.
Perry, R. P., and Kelley, D. E. (1974), *Cell 1,* 37.
Philipson, L., Wall, R. Glickman, G., and Darnell, J. E. (1971), *Proc. Nat. Acad. Sci. U. S. 68,* 2806.
Proudfoot, N. J., and Brownlee, G. G. (1974), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 38,* 179.
Sanger, F., Brownlee, G. G., and Barrell, B. G. (1965), *J. Mol. Biol. 13,* 373.
Sheldon, R., Jurale, C., and Kates, J. (1972b), *Proc. Nat. Acad. Sci. U. S. 69,* 417.
Sheldon, R., Kates, J., Kelley, D. E., and Perry, R. P. (1972a), *Biochemistry 11,* 3829.
Vesco, C., and Penman, S. (1969), *Proc. Nat. Acad. Sci. U. S. 62,* 218.
Zylber, E., Vesco, C., and Penman, S. (1969), *J. Mol. Biol. 44,* 195.

Characterization of the Steady-State Population of Messenger RNA and Its Poly(adenylic acid) Segment in Mammalian Cells†

William R. Jeffery‡ and George Brawerman*

ABSTRACT: The poly(A) containing RNA of mouse sarcoma and rabbit reticulocyte polysomes was characterized using a method based on complex formation with radioactive poly(U). EDTA treatment of reticulocyte polysomes releases the poly(A) containing material as a homogeneous particle, while the same treatment applied to sarcoma polysomes releases a highly heterogeneous population of particles. The poly(A) segments obtained from steady-state sarcoma polysomal RNA exhibit a more heterogeneous sedimentation profile than the poly(A) from newly synthesized RNA. Their average sedimentation coefficient is also lower. The estimate of the average size of the steady-state poly(A) segment of sarcoma polysomal RNA, based on the observed sedimentation coefficient, is 130 residues, compared to the value of 170 residues for 2-hr labeled poly(A). The poly(A) of reticulocyte polysomal RNA sediments more slowly than the sarcoma poly(A), and appears to consist of two size classes of about 60 and 100 residues. The steady-state poly(A)-protein complex released from RNase-digested sarcoma polysomes is also more heterogeneous than the complex bearing newly synthesized poly(A); it also has an overall lower sedimentation coefficient. The reticulocyte poly(A)-protein complex is more homogeneous and sediments more slowly than the sarcoma steady-state complex. The characteristics of steady-state poly(A) appear to be a reflection of the aging process to which mRNA is subjected after its appearance in the cytoplasm.

Eukaryotic mRNA[1] is frequently detected by virtue of its selective labeling with radioactive precursors. Short periods of isotope incorporation primarily label mRNA present in the polysomes and other cytoplasmic particles (Penman *et al.,* 1963; Perry and Kelley, 1968; Knöchel and Tiedemann, 1972). Selective labeling of mRNA can also be achieved during longer incorporation periods in the presence of low levels of actinomycin D which prevents the synthesis of rRNA (Roberts and Newman, 1966; Penman *et al.,* 1968). Isotope incorporation studies have provided much information on the metabolism of newly synthesized mRNA, but this method is not well suited for the detection of changes which may occur during the life cycle of these molecules. For instance, it has been shown that the poly(A) segment of mammalian mRNA undergoes a gradual reduction in size after its appearance in the cytoplasm (Mendecki *et al.,* 1972; Brawerman, 1973; Sheiness and Darnell, 1973). The

† From the Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111. *Received June 3, 1974.* This work was supported by research grants from the U. S. Public Health Service (GM 17973) and the National Science Foundation (GB 35436).

‡ Present address: Department of Biophysical Sciences, University of Houston, Houston, Texas 77004.

[1] Abbreviations used are: mRNP, messenger ribonucleoprotein; poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); SDS, sodium dodecyl sulfate; EDTA, disodium ethylenediaminetetraacetate, mRNA, messenger ribonucleic acid.