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Polyriboadenylate Sequences at the 3′-Termini of Ribonucleic Acid Obtained from Mammalian Leukemia and Sarcoma Viruses

[RNA-tumor viral RNA/poly(A) detection and location/3′-OH exoribonuclease hydrolysis/polynucleotide phosphorylase phosphorolysis]

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ABSTRACT The location of poly(A) sequences in the RNA of mammalian RNA-tumor viruses was determined by enzymatic analyses. The 56-64S viral genomic RNAs, the 20-40S viral subunit RNAs, and the 4-5S poly(A) sequences excised from these viral RNAs were subjected to either hydrolysis with a 3′-OH specific exoribonuclease from Ehrlich ascites tumor cells or phosphorolysis from the 3′-termini with polynucleotide phosphorylase from *Micrococcus luteus*. Purified adenosine-labeled poly(A) fragments, excised from genomic viral RNAs by RNase A and T_1 digestion, were hydrolyzed with the 3′-OH specific exoribonuclease for various periods of time. Poly(U) filter binding studies of the residual poly(A) indicated that 97% of the poly(A) fragments were hydrolyzed. Adenosine-labeled genomic and subunit viral RNAs and excised poly(A) fragments were phosphorolyzed from their 3′-termini for various periods of time with polynucleotide phosphorylase. The degree of phosphorolysis was monitored by poly(U) filter binding studies, and CCl_3COOH insolubility and solubility determinations. There was an initial preferential rate of phosphorolysis of the poly(A) sequences of genomic and subunit viral RNAs as compared to the total adenosine-labeled viral RNAs. The data from these two different enzymatic mechanisms of action indicated conclusively that the poly(A) sequences were located at the 3′-termini of genomic and subunit viral RNAs.

Poly(A) sequences are quite ubiquitous in nature. They have been found in the RNA of some oncogenic and nononcogenic viruses (1-13) but not in others (14-16), in the messenger RNA (mRNA) synthesized by some DNA and RNA viruses and yeast cells (17-24), and in the mRNA and heterogenous nuclear RNA of mammalian cells (25-28) with the exception of histone mRNA (29). They have not been found in either the mRNA of bacterial cells (30) or the ribosomal RNA (rRNA) of mammalian cells (13, 31).

The poly(A) sequences of mRNA from mammalian cells (27, 28, 32), of RNA from nononcogenic viruses (1, 7), and of mRNA from DNA viruses (18) are covalently linked to the 3′-termini; however, the exact location of the covalently linked poly(A) sequences in the RNA of RNA-tumor viruses had not been definitively established. In these studies, we determined the location of the poly(A) sequences in the RNA of mammalian RNA-tumor viruses by enzymatic analyses.

MATERIALS AND METHODS

Cell Cultures and Growth Conditions. 3T3 cells chronically infected with a murine leukemia virus isolate (MLV-IC) obtained from the Moloney leukemia-sarcoma virus complex, and sarcoma positive helper positive (S+H+) cells were used in these studies. All cells employed were mycoplasma negative. The growth and maintenance of these cells have been described (13).

Radioisotopic Labeling of Viruses. The isotopic labeling with [2,8-^{3}H]adenosine (25-50 μCi/ml, 3-6 hr) and purification of the viruses have been described (13).

Extraction of [2,8-^{3}H]Adenosine Labeled RNA from Virions. Labeled viral RNA was extracted from virions with sodium dodecyl sulfate and phenol mixtures as described (13, 33).

Separation and Fractionation of Extracted Viral RNA. The extracted viral RNA was separated by rate zonal sedimentation in isokinetic sucrose gradients and fractionated as described (13).

Viral RNA Dissociation. Viral genomes were dissociated into subunit RNA by either heat (75-85° for 3 min), dimethyl sulfoxide treatment (25° for 30 min), or 1.1 M formaldehyde treatment (65° for 15 min). Dissociated viral RNAs were ethanol precipitated and separated by rate zonal sedimentation as described (13).

Detection of Poly(A) Sequences in the RNA of RNA-Tumor Viruses. Three different techniques were utilized to determine if the [2,8-^{3}H]adenosine-labeled viral RNA extracted from RNA-tumor viruses contained poly(A) sequences: (*a*) binding to Millipore filters (34); (*b*) binding to poly(U) GFC filters (35); and (*c*) RNase A and RNase T_1 resistance (9, 10, 13, 36, 37). [^{3}H]Polyribonucleotides and [^{14}C]*Escherichia coli* DNA were used as controls.

Enzymatic Analyses to Determine the Location of the Poly(A) Sequences of the Viral RNA. Both exoribonuclease from Ehrlich ascites tumor cells (32, 38) and polynucleotide phosphorylase from *Micrococcus luteus* (39, 40) were used in these studies to determine if the poly(A) sequences of the viral RNA are located at the 3′-OH termini. The exoribonuclease cleaves from the 3′-OH terminus of RNA (32) and polynucleotide phosphorylase, in the presence of excess cold PO_4^{-3}, cleaves from the 3′-terminus of the RNA by phosphorolysis (40). Reaction conditions for both the ascites exoribonuclease hydrolysis (32) and polynucleotide phosphorylase phosphorolysis (40) have been described.

Polyacrylamide Gel Electrophoresis of [^{3}H]Poly(A) Sequences. ^{3}H-Labeled poly(A) sequences, excised and purified from [2,8-^{3}H]adenosine-labeled S+H+(HGT-1) viral RNA were electrophoresed as described by Perry *et al.* (30).

Abbreviations: MLV-IC, a murine leukemia virus isolate obtained from the Moloney leukemia-sarcoma virus complex; S+H+, sarcoma positive helper positive.

TABLE 1. *Poly(A) sequences of mammalian leukemia and sarcoma viral RNA*

[2,8-^{3}H]Adenosine-labeled viral RNA*	RNase A & T$_1$ Resistant Fragments: Fragments (%)†	Alkaline hydrolysis of fragments (%)‡
MLV-IC (64S)	6 ± 0.5	100
MLV-IC (30–40S)	9 ± 0.5	—
S+H+(HTG-1) (56S)	10 ± 1	98 ± 0.5
S+H+(HTG-1) (20–40S)	10 ± 1	—

* [2,8-^{3}H]Adenosine-labeled viral RNA (1000–5000 cpm) was used per test. A minimum of nine independent tests were performed for each of the experimental conditions.

† *RNase A and T$_1$ resistance studies.* RNase A and T$_1$ assays (1 ml) were performed in both 2 × SSC (0.3 M NaCl–0.03 M Na$_3$ citrate, pH 7.2) and 0.1 × SSC (0.015 M NaCl–0.0015 M Na$_3$ citrate) that contained 2,000–20,000 cpm of radioactive polyribonucleotide or nucleic acid in 5 μl, and RNase A (2–20 μg) + RNase T$_1$ (50–150 units). Incubation was at 37° for 2 hr.

% resistance to RNase A and T$_1$ digestion

$$= \frac{(2 \times \text{SSC cpm} - 0.1 \times \text{SSC cpm})}{\text{input cpm}} \times 100.$$

‡ RNase A and T$_1$ resistance studies followed by treatment of the RNase A and T$_1$ resistant nucleic acid fragments with 0.5 N NaOH for 10 min at 100°.

Radioisotopes. [2,8-^{3}H]Adenosine (5–25 Ci/mmol) and $H_3{}^{32}PO_4$ (carrier free) were purchased from either New England Nuclear Corp. or Amersham/Searle Corp. [^{3}H]Polyribonucleotides (A, C, G, U) were purchased from either Miles Laboratories or Schwarz/Mann. [2-^{14}C]Thymidine-labeled *E. coli* DNA (46 μCi/mg of DNA) was purchased from Amersham/Searle.

Enzymes. Exoribonuclease specific for 3'-OH termini was isolated from the nuclei of Ehrlich ascites tumor cells and characterized as described (38). Polynucleotide phosphorylase (no. 0429, lot no. 3093I) was purchased from P-L Biochemicals, Inc.; pancreatic RNase A (no. WRAF9LB), DNase free of RNase (no. WDPC9LB), and Micrococcal nuclease (no. 4797) were purchased from Worthington Biochemicals, Inc., and RNase T$_1$ (no. 556785) was purchased from Calbiochem.

Buffers, Chemicals, and Reagents. Buffers, chemicals, and reagents used in these studies have been described (13, 32, 40).

RESULTS

Poly(A) sequences of mammalian leukemia and sarcoma viral RNA

RNase A and RNase T$_1$ resistance studies indicated that poly(A) sequences comprised 6–9% and 10% of the [2,8-^{3}H]-adenosine-labeled MLV-IC and S+H+(HTG-1) viral RNA respectively (Table 1). The RNase A and RNase T$_1$ resistant nucleic acid fragments were treated with 0.5 N NaOH for 10-min at 100° to determine if the resistance to enzymatic hydrolysis was due only to poly(A) sequences because [2,8-^{3}H]adenosine can be incorporated into both DNA and RNA and because approximately 2% DNA has been detected in the nucleic acid of some RNA-tumor viruses (41, 42).

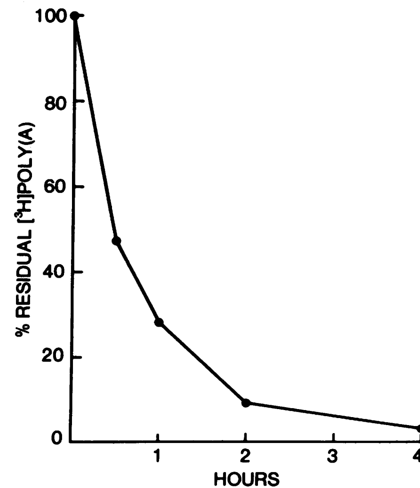

FIG. 1. Hydrolysis of [2,8-^{3}H]adenosine-labeled poly(A) sequences with a 3'-OH specific exoribonuclease from Ehrlich ascites tumor cells. [2,8-^{3}H]Adenosine-labeled S+H(HTG-1) 56S viral RNA (about 3.4 × 10^5 cpm, specific activity about 1900 cpm/μg of RNA) was treated as described by Molloy *et al.* for HeLa and L-Cell mRNA (32) with a combination of RNase A (2 μg/ml) and RNase T$_1$ (50 units/ml) for 1 hr to excise the poly(A) sequences. After digestion, the [^{3}H]poly(A) sequences were re-extracted with sodium dodecyl sulfate–chloroform–phenol and precipitated in 70% ethanol in the presence of highly purified bovine serum albumin carrier. The [^{3}H]poly(A) sequences were re-dissolved in 50 mM NaCl–1 mM EDTA buffer and passed through a small column of Sephadex G25 which was equilibrated with 50 mM NaCl–1 mM EDTA. Reaction mixture (0.2–0.5 ml) contained: 40 mM Tris·HCl at pH 7.4 at 25°, 4 mM $MgCl_2$, 0.3 mM dithiothreitol, 40 μg/ml of purified RNase free bovine serum albumin, 1 unit of ascites 3'-OH specific exoribonuclease, and about 2500 cpm of [^{3}H]poly(A) sequences. Incubation was at 37°. Radioactivity was determined after the indicated periods of incubation of binding of residual [^{3}H]poly(A) to poly(U)GFC filters.

Ninety-eight to 100% of the RNase A and RNase T$_1$ resistant fragments of [2,8-^{3}H]adenosine-labeled MLV-IC and S+H+(HTG-1) viral RNA, respectively, was degraded by alkaline treatment; therefore, the resistance of these fragments was because of their poly(A) composition and not to DNA. These poly(A) sequences are covalently linked to the viral RNA subunits because 20–40S viral RNA subunits isolated from heat, dimethyl sulfoxide and formaldehyde dissociated viral genomes also contained 9–11% RNase A and RNase T$_1$ resistant poly(A) sequences. Poly(U) filter binding analyses indicated that 53 ± 10% of these subunit RNAs contained poly(A) sequences. Having detected covalently linked poly(A) sequences in the RNA of these RNA-tumor viruses, it became of great interest to locate their position.

The position of the poly(A) sequences in the RNA of RNA-tumor viruses

Enzymatic studies used to determine the position of the poly(A) sequences present in the RNA of RNA-tumor viruses included hydrolysis with a 3'-OH specific exoribonuclease from Ehrlich ascites tumor cells (32) and phosphorolysis from the 3'-termini with polynucleotide phosphorylase (PNPase) from *Micrococcus luteus* (40). Hydrolysis of [2,8-^{3}H]poly(A) sequences from S+H+(HTG-1) 56S viral RNA with the 3'-OH specific exoribonuclease was monitored by binding of the residual poly(A) to poly(U) GFC filters. The data in Fig. 1

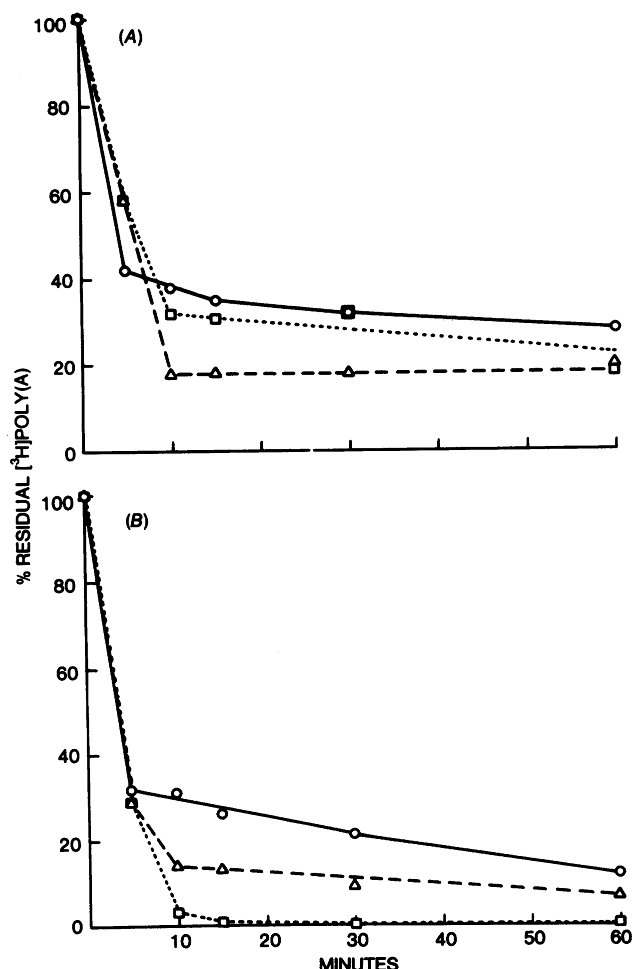

FIG. 2. Phosphorolysis of [*2,8*-^{3}H] adenosine labeled S+H+(HTG-1) and MLV-IC viral RNA by PNPase. [*2,8*-^{3}H]Adenosine-labeled viral RNA extracted from [^{3}H]S+H+(HTG-1) viral RNA (specific activity about 500–600 cpm/μg of RNA) and from [^{3}H]MLV-IC viral RNA (specific activity about 150–300 cpm/μg of RNA) were treated with polynucleotide phosphorylase as described by Chan and Singer but without the $H_3{}^{32}PO_4$ (40). The assay mixture (0.1 ml) contained: 0.1 M Tris·HCl, pH 8.3 at 25°; 10 mM K_2HPO_4; 5 mM $MgCl_2$; 1 mM EDTA; 20 μg of purified RNase free bovine serum albumin; [2,8-^{3}H]adenosine-labeled viral RNA (1,000–11,000 cpm in 5 μl per test); and 0.75 units of polynucleotide phosphorylase. Radioactivity was determined after the indicated periods of incubation by binding of the residual [^{3}H]poly(A) to poly(U)GFC filters. Each time point on the graph represents the average radioactivity from a minimum of six individual tests. (*A*), [^{3}H]S+H+(HTG-1) viral RNA: O——O, 56S genomic RNA; Δ——Δ, 28–32S subunit RNA; □——□, excised 4–5S poly(A) sequences. (*B*), [^{3}H]MLV-IC viral RNA; O——O, 64S genomic RNA; Δ——Δ, 34–35S subunit RNA; □——□, synthetic poly(A) control.

show that 97% of the poly(A) sequences were hydrolyzed from the 3′-OH termini.

Because of the 3′-OH specificity of this exoribonuclease, these studies indicated that the RNase A and RNase T_1 excised [*2,8*-^{3}H]poly(A) sequences were located at the 3′-OH termini of the viral RNA subunits.

[*2,8*-^{3}H]Adenosine-labeled viral genomic and subunit RNA and excised poly(A) sequences from both S+H+(HTG-1) and MLV-IC viruses were cleaved by phosphorolysis (Fig.

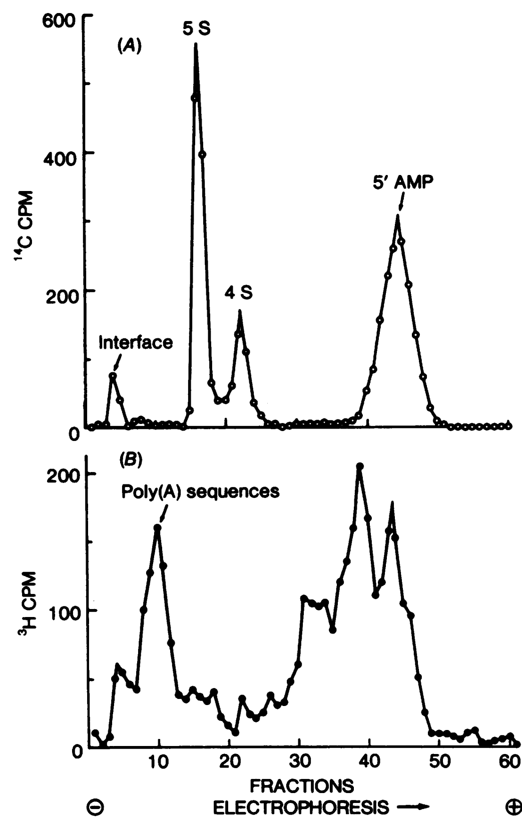

FIG. 3. Polyacrylamide gel electrophoresis of [^{3}H]poly(A) sequences excised from [*2,8*-^{3}H]adenosine-labeled S+H+(HTG-1) viral RNA. (*A*) The marker gel contained ^{14}C-labeled low molecular weight components from L cell ribosomes and [^{14}C]5′-AMP (New England Nuclear Corp.). (*B*) [^{3}H]Poly(A) sequences (1600 cpm), excised and purified from [*2,8*-^{3}H]adenosine-labeled S+H+(HTG-1) viral RNA (specific activity about 1900 cpm/μg of RNA) as described in Fig. 1, were electrophoresed as described by Perry *et al.* (30) on 10-cm gels consisting of a 1-cm segment of 2.7% gel and a 9-cm segment composed of 14% gel. Electrophoresis was for 3 hr at 5 mA per gel.

2*A* and 2*B*). Sixty-eight percent of the poly(A) sequences of 64S MLV-IC viral RNA were phosphorlyzed; likewise, 58% of the poly(A) sequences of the 56S S+H+(HTG-1) viral RNA was phosphorolyzed after 5 min, whereas only 10% of the total adenosine-labeled S+H+(HTG-1) viral RNA was made CCl_3COOH soluble. However, polynucleotides phosphorylase phosphorolysis is progressive from the 3′-termini and after 5 min, some of the internal adenosine-labeled nucleotides were also phosphorolyzed because 17, 45, 85, and 88% of the labeled 56S viral RNA was made CCl_3COOH soluble at 10, 15, 30 and 60 min, respectively. The initial preferential rate of PNPase phosphorolysis of the poly(A) sequences indicated conclusively that they were located at the 3′-termini of S+H+(HTG-1) 56S viral RNA.

Estimation of the molecular weight of the 3′-OH poly(A) sequences

The molecular weight of [*2,8*-^{3}H]poly(A) sequences from S+H+(HTG-1) viral RNA was estimated by polyacrylamide gel electrophoresis (Fig. 3*A* and *B*). These poly(A) sequences exhibited a sharp peak migrating slightly slower than the 5S rRNA marker (Fig. 3*A*). Also observed in the electrophoretogram is radioactivity in the mononucleotide region and in

regions of slightly slower mobilities. From these studies, the molecular weight of the poly(A) sequences was estimated to be 1×10^5 which is quite similar to the poly(A) fragments isolated from L-cell mRNA (32).

DISCUSSION

These enzymatic studies show that the RNAs of MLV-IC and S+H+(HTG-1) infectious RNA-tumor viruses contained poly(A) sequences covalently linked to the genomic and subunit RNAs at their 3′-termini.

The purified adenosine-labeled poly(A) fragments, excised from viral genomic RNA by RNase A and T_1 digestion, were hydrolyzed for various time periods with the 3′-OH specific exoribonuclease. Subsequent poly(U) filter binding studies of the residual poly(A) indicated that 53, 72, 91, and 97% of these fragments were hydrolyzed at 0.5, 1, 2, and 4 hr, respectively (Fig. 1). These results with the excised poly(A) fragments depend upon the clearly established 3′-OH specificity of the exoribonuclease employed (32). Poly(U) filter binding studies indicated that 63 ±10% of the 56–64S viral genomic RNAs and 53 ± 10% of the 20–40S viral subunit RNAs contained poly(A) sequences. Since a majority of the viral subunits contained poly(A) sequences, they all must be at the 3′-OH termini of their respective subunits.

Adenosine-labeled genomic and subunit viral RNA and excised poly(A) sequences were phosphorolyzed from their 3′-termini for various periods of time (Fig. 2). Phosphorolysis was monitored by poly(U) filter binding studies of the residual poly(A) and CCl_3COOH insolubility and solubility studies of the total adenosine radioactivity. 64S MLV-IC and 56S S+H+(HTG-1) viral genomic RNAs were phosphorolyzed 68% and 58%, respectively, after 5 min, whereas only 10% of the total adenosine-labeled S+H+(HTG-1) viral RNA was made CCl_3COOH soluble. The initial preferential rate of polynucleotide phosphorylase phosphorolysis of the poly(A) sequences when compared to the total adenosine radioactivity indicated that the poly(A) sequences were located at the 3′-termini of the viral RNAs. These enzymatic analyses with 3′-OH specific exoribonuclease (32) and polynucleotide phosphorylase (40) indicated conclusively a 3′-terminus position for the poly(A) sequences of genomic and subunit viral RNAs.

What are the function(s) of poly(A) sequences covalently linked to the RNA of RNA-tumor viruses? Some investigators have suggested that the poly(A) sequences may be involved in the synthesis of viral DNA by reverse transcriptase (8, 9, 11). Now that it has been determined that the poly(A) sequences are located at the 3′-OH termini of genomic and subunit RNAs, the possibilty that the poly(A) sequences could be the binding sites for reverse transcriptase increases. However, the poly(A) sequences themselves are apparently not transcribed into tracts of poly(dT) (43). Poly(A) sequences may also be important to the structural integrity of the genomic RNA of RNA-tumor viruses, as has been suggested by others (9–11), by complementary hydrogen bonding between the poly(A) sequences and possible uridine-rich segments (2, 44) in the viral RNA but some investigators have not been able to detect poly(U) tracts in either the genomic or subunit RNA of RNA-tumor viruses (45). Therefore, the absolute function(s) for these covalently linked poly(A) sequences at the 3′-OH termini of genomic and subunit viral RNAs is apparently unknown.

Note Added in Proof. During the review of this manuscript, the authors became aware of two other publications on this subject: Stephenson, M. L., Scott, J. F. & Zamecnik, P. (1973) *Biochem. Biophys. Res. Commun.* **55,** 8–16 and Rho, H. M., & Green, M. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 2386–2390.

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1. Yogo, Y. & Wimmer, E. (1972) *Proc. Nat. Acad. Sci. USA* **69,** 1877–1882.
2. Yogo, Y. & Wimmer, E. (1973) *Nature New Biol.* **242,** 171–174.
3. Armstrong, J. A., Edmonds, M., Nakazato, H., Phillips, B. A. & Vaughan, M. H. (1972) *Science* **176,** 526–528.
4. Eaton, B. T. & Faulkner, P. (1972) *Virology* **50,** 865–873.
5. Johnson, R. E. & Base, H. T. (1972) *Biochem. Biophys. Res. Commun.* **46,** 712–718.
6. Johnson, R. E. & Base, H. R. (1972) *Proc. Nat. Acad. Sci. USA* **69,** 1514–1516.
7. Donaghue, T. P. & Faulkner, P. (1973) *Nature New Biol.* **246,** 168–170.
8. Gillespie, D., Marshall, S. & Gallo, R. C. (1972) *Nature New Biol.* **236,** 227–231.
9. Green, M. & Cartas, M. (1972) *Proc. Nat. Acad. Sci. USA* **69,** 791–794.
10. Lai, M. M. C. & Duesberg, P. H. (1972) *Nature* **235,** 383–386.
11. Ross, J., Tronick, S. R. & Scolnick, E. M. (1972) *Virology* **49,** 230–235.
12. Gillespie, D., Takemoto, K., Reberts, M. & Gallo, R. C. (1973) *Science* **179,** 1328–1330.
13. Phillips, L. A., Hollis, V. W., Jr., Bassin, R. H. & Fischinger, P. J. (1973) *Proc. Nat. Acad. Sci. USA* **91,** 3002–3006.
14. Ehrenfeld, E. & Summers, D. F. (1972) *J. Virol.* **10,** 683–688.
15. Ward, R., Banarjee, A. K., LaFiandra, A. & Shatkin, A. J. (1972) *J. Virol.* **9,** 61–69.
16. Stoltzfus, C. M. & Banerjee, A. K. (1972) *Arch. Biochem. Biophys.* **152,** 733–743.
17. Kates, J. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35,** 743–752.
18. Sheldon, R., Kates, J., Kelley, D. E. & Perry, R. P. (1972) *Biochemistry* **11,** 3829–3834.
19. McLaughlin, C. S., Warner, J. R., Edmonds, M. Nakazato, H. & Vaughan, M. H. (1973) *J. Biol. Chem.* **248,** 1466–1477.
20. Philipson, L., Wall, R., Glickman, G. & Darnell, J. E. (1971) *Proc. Nat. Acad. Sci. USA* **68,** 2806–2809.
21. Raskas, H. J. & Bhaduri, S. (1973) *Biochemistry* **12,** 920–925.
22. Weinberg, R. A., Ben-Ishai, Z. & Newbold, J. E. (1972) *Nature New Biol.* **238,** 111–113.
23. Bachenheimer, S. L. & Roizman, B. (1972) *J. Virol.* **10,** 875–879.
24. Silverstein, S., Bachenheimer, S. L., Frenkel, N. & Roizman, B. (1973) *Proc. Nat. Acad. Sci. USA* **70,** 2101–2104.
25. Greenberg, J. R. & Perry, R. P. (1972) *J. Mol. Biol.* **72,** 91–98.
26. Edmonds, M., Vaughan, M. H., Jr. & Nakazato, H. (1971) *Proc. Nat. Acad. Sci. USA* **68,** 1336–1340.
27. Darnell, J. E., Jelinek, W. R. & Molloy, G. R. (1973) *Science* **181,** 1215–1221.
28. Jelinek, W., Adesnik, M., Salditt, M., Sheiness, D., Wall, R., Molloy, G., Philipson, L. & Darnell, J. E. (1973) *J. Mol. Biol.* **75,** 515–532.
29. Adesnik, M., Salditt, M., Thomas, W. & Darnell, J. E. (1972) *J. Mol. Biol.* **71,** 21–30.

30. Perry, R. P., Illey, D. E. & LaTorre, J. (1972) *Biochem. Biophys. Res. Commun.* **48**, 1593–1600.
31. Hunt, J. A. (1970) *Biochem. J.* **120**, 353–363.
32. Molloy, G. R., Sporn, M. B., Kelley, D. E. & Perry, R. P. (1972) *Biochemistry* **11**, 3256–3260.
33. Perry, R. P., LaTorre, J., Kelley, D. E. & Greenberg, J. R. (1972) *Biochim. Biophys. Acta* **262**, 220–226.
34. Lee, S. Y., Mendecki, J. & Bramerman, G. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1331–1335.
35. Sheldon, R., Jurale, C. & Kates, J. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 417–421.
36. Beers, F., Jr. (1960) *J. Biol. Chem.* **235**, 2393–2398.
37. Sato-Asano, K. (1959) *J. Biochem.* **46**, 31–37.
38. Sporn, M. B., Lazarus, H. M., Smith, J. M. & Henderson, W. R. (1969) *Biochemistry* **8**, 1698–1706.
39. Singer, M. F., Hilmoe, R. J. & Grunberg-Manago, M. (1960) *J. Biol. Chem.* **235**, 2705–2712.
40. Chou, J. Y. & Singer, M. F. (1970) *J. Biol. Chem.* **245**, 995–1004.
41. Bismal, N., McCain, B. & Benyesh-Melnick, M. (1971) *Virology* **45**, 697–706.
42. Levinson, W., Bishop, J. M., Quintrell, N. & Jackson, J. (1970) *Nature* **227**, 1023–1025.
43. Reitz, M., Gillespie, D., Saxinger, W. C., Robert, M. & Gallo, R. C. (1972) *Biochem. Biophys. Res. Commun.* **49**, 1216–1224.
44. Riman, J. (1966) *Biochem. Biophys. Res. Commun.* **25**, 447–453.
45. Marshall, S. & Gillespie, D. (1972) *Nature New Biol.* **240**, 43–45.