THE IDENTIFICATION OF THE 3′ TERMINUS OF THE 70 S RNA OF MURINE SARCOMA VIRUS (MOLONEY)

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1. Introduction

The major nucleic acid component of Oncornaviruses is a high molecular weight RNA that sediments between 60 and 70 S. Its molecular weight has been estimated to be about 10^7 daltons [1]. It can be dissociated by heat or dimethyl sulfoxide into subunits that sediment at about 35 S [2].

Little is known about the primary structure of this 60–70 S RNA. The identification of the 3′ terminus is a first approach in this analysis and may provide interesting information because the 3′ terminus is apparently important for binding the RNA dependent-DNA polymerase of these viruses [3]. Uridine has been found to be the terminal base for 70 S RNA of several C-type viruses of avian, reptilian or mammalian origin [4, 5]. By using the method used by Rajbhandary [6] of labeling the 3′ terminal nucleoside residues by periodate oxidation and subsequent reduction with sodium [^{3}H] borohydride, we have identified uridine as the major terminal nucleoside of the 60–70 S RNA of the murine leukemia–sarcoma virus complex.

2. Materials and methods

2. 1. *Preparation of viral RNA*

Virus was obtained from the supernatant fluids of a chronically-infected cell line (78 A_1 cells) [7]. Viral RNA was isolated as previously described [8]. Polyvinylsulfate (10 μg/ml) was systematically added during the course of isolation. 70 S RNA was separated from the other molecular species in a 5–20% sucrose gradient.

^{32}P-labeled virus was prepared by labeling cells for 24 hr with 20 μCi/ml of [^{32}P] ortho-phosphate carrier-free from CEA (Saclay).

2. 2. *^{3}H-labeling of 3′ terminal groups*

The reaction conditions were essentially those described by Watcher and Fiers [9]. They were modified for small amounts of RNA. All manipulations were carried out at room temperature. RNA was dissolved in 50 μl of 0.02 M Na-acetate pH 5.1. 5 μl of 0.01 M $NaIO_4$ were added. The solution was left in the dark for 1 hr. The oxidation step was stopped by 5 μl of 0.1 M ethyleneglycol. 30 min later, 1 ml of 0.5 M phosphate buffer, pH 7, was added, then, 7 to 10 mCi of [^{3}H]$NaBH_4$ (Radiochemical Centre, Amersham) dissolved in 0.1 ml of 1 M NaOH and stored at −20° (specific activity: 3 Ci/mmole). The mixture was incubated in the dark for 1.5 hr. 20 μl of cyclohexanone were added to stop the reaction.

2. 3. *Sephadex gel filtration*

The reaction mixture was filtered through a Sephadex G-25 column (27 × 2 cm) equilibrated with 0.02 M Na acetate pH 5.1 to eliminate excess borohydride. The tritiated RNA was precipitated with cold ethanol in the presence of 200 μg of carrier cellular RNA.

2. 4. *Alkaline hydrolysis and determination of ^{3}H-labeled terminal nucleoside derivatives*

[^{3}H]RNA was hydrolyzed by 15 μl of 0.5 M NaOH for 18 hr at 37°. The hydrolysate was applied to a

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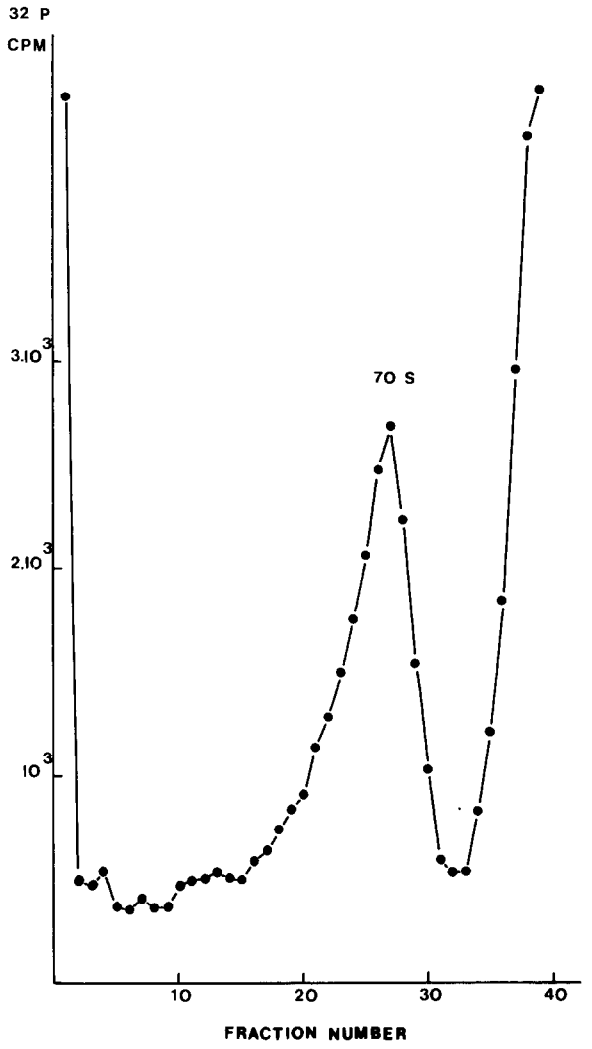

Fig. 1. Sucrose gradient sedimentation of MSV–MLV complex RNA. A trace amount of viral [^{32}P] RNA was mixed with unlabeled viral RNA. Sedimentation was performed in a 5%–20% sucrose gradient in a Spinco SW 25 rotor at 25,000 rpm for 3.5 hr at 4°. The fractions corresponding to 70 S RNA were pooled and precipitated with ethanol.

Whatmann N°1 MM paper with a mixture of trialcohol nucleoside markers and subjected to high voltage electrophoresis in a solvent consisting of pyridine, acetic acid and water [5:5:90], pH 3.5, at 3,000 V for 1.5 hr.

The four nucleoside trialcohols were visualized in UV light. The paper was cut into 2 cm × 1cm pieces and ^{3}H radioactivity was counted with 10 ml of scintillantion solution.

2. 5. *Preparation of trialcohol nucleoside derivatives*

Nucleoside trialcohols were prepared according to the method described by Wachter and Fiers [9].

3. Results

A trace amount of [^{32}P]virus was systematically mixed with unlabeled virus preparation in order to magnify the peaks of 70 S RNA during the purification procedures. Fig. 1 shows the profile of 70 S RNA purified in sucrose gradients.

The purified 70 S RNA was oxidized with metaperiodate and than reduced by sodium [^{3}H] borohydride. The product isolated from the reaction mixture was filtered through Sephadex G-25 to separate the 70 S [^{3}H]RNA and remove the bulk of non-bound ^{3}H, as shown in fig. 2.

To identify the ^{3}H-labeled 3'OH terminus, RNA was hydrolyzed by NaOH. Fig. 3 shows the typical ^{3}H distribution in the alkaline hydrolysate on the electrophoretic paper. Radioactivity remaining at the origin has been reported [10, 11] but its nature has not been elucidated.

Table 1 shows the analysis of the distribution of ^{3}H radioactivity in nucleoside trialcohols. For control purposes, we have included a comparative analy-

Table 1

3' Terminal nucleosides of viral 70 S RNA and various cell RNA's.

RNA source	% of ^{3}H radioactivity in nucleoside trialcohols			
	Adenosine	Cytidine	Guanosine	Uridine
MSV–MLV complex 70 S RNA	11	3	9	77
78 A_1 cells				
4 S RNA*	79	14	0	7
5 S RNA*	23	9	0	68
7 S RNA*	13	5	5	77
Total ribosomal RNA*	41	2	2	55

The values in the table are average values.

* The various cell RNA's were prepared according to methods described by Galibert et al. [17]. They were purified by sucrose gradients, chromatography on MAK columns and polyacrylamide gel electrophoresis.

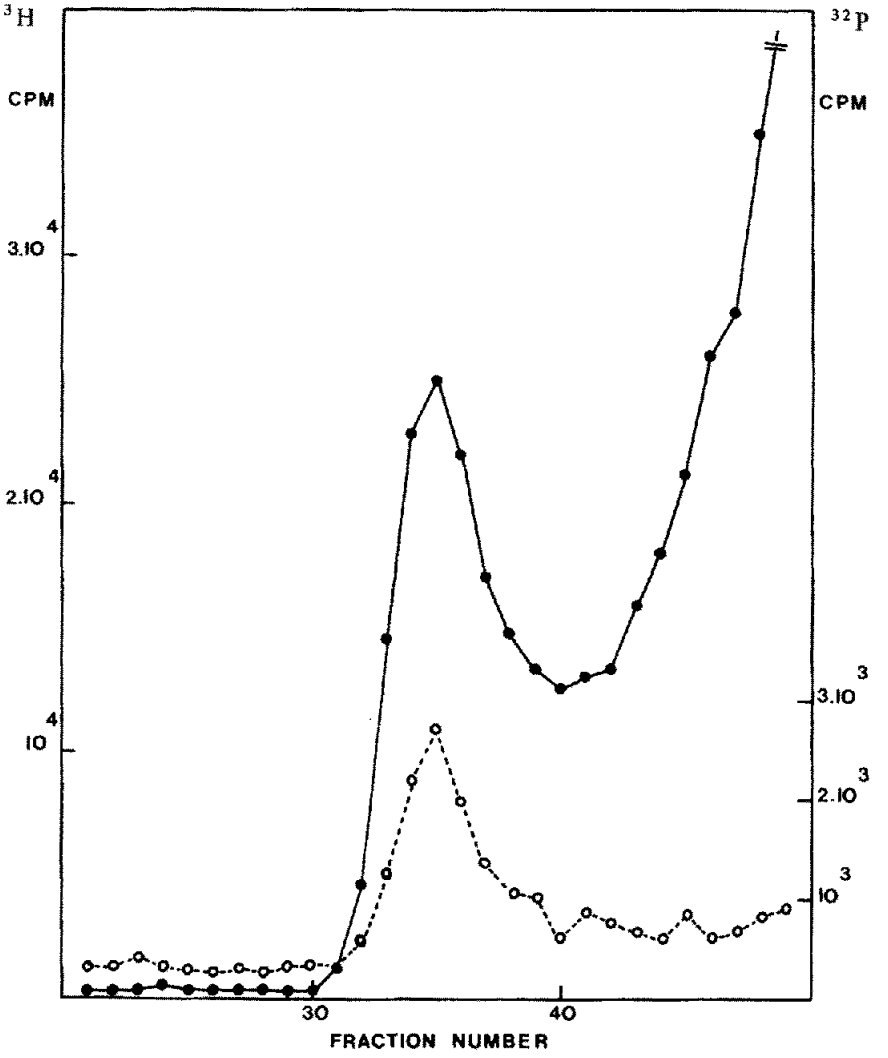

Fig. 2. Sephadex G-25 filtration of ^{3}H-labeled 70 S RNA. Reaction mixture was layered on a 2 cm × 27 cm column of Sephadex G-25 equilibrated in 0.02 M Na acetate pH 5.1 and eluted with this buffer. (•–•–•) ^{3}H radioactivity. (o- -o- -o) ^{32}P radioactivity.

sis of the 3′ termini of different RNA of 78 A_1 cells. The results for cellular RNA agree with results obtained for other cell RNA.

The determination of 3′OH terminal nucleoside of 70 S RNA was applied to three viral preparations. On each occasion about 80% of the radioactivity was found on uridine.

4. Discussion

In the present study, we have applied the technique of periodate oxidation and [^{3}H]borohydride reduction to identify the 3′ terminal nucleoside of 60–70 S RNA of MSV–MLV complex. Since the borohydride reagent interacts with many compounds [12, 13], 70 S RNA was purified in sucrose gradients from smaller RNA's and from non RNA contaminants. However traces of 8 S and 4 S RNA associated with 70 S RNA and only released after heating (unpublished results) were not removed for this study.

Control experiments made with 78 A_1 RNA's are in agreement with data reported for other cell RNA's [14–16]. tRNA ends predominantly in A but

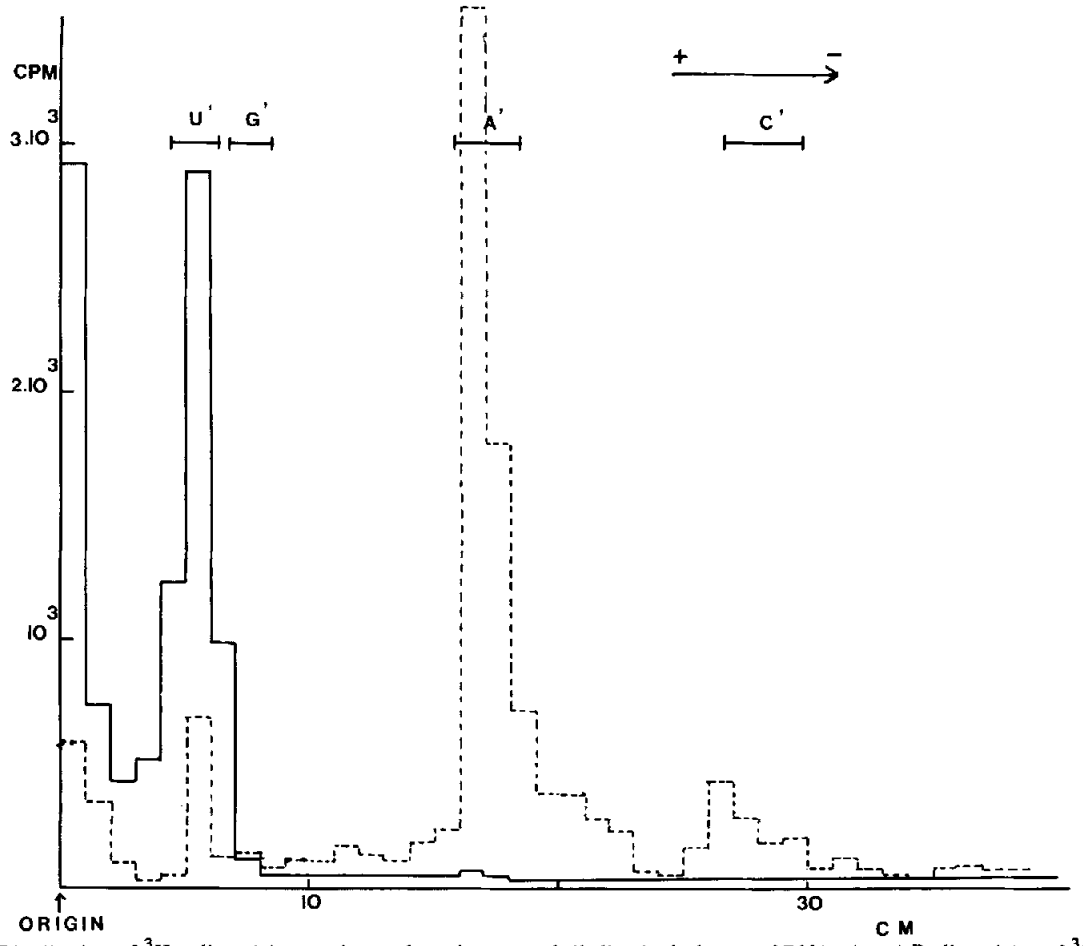

Fig. 3. Distribution of ^{3}H radioactivity on electrophoretic paper of alkaline hydrolysate of RNA. (——) Radioactivity of ^{3}H-end labeled viral 70 S RNA; (- - -) radioactivity of ^{3}H-end labeled cell tRNA.

a few C residues are present because C is the penultimat 3′ base. Some contamination by 5 S RNA can explain the presence of U since this molecular species ends in U. Some A and C are found for 5 S RNA because of 4 S RNA contamination. U is the major terminus of 7 S RNA which is linked to 28 S rRNA. The 3′ termini of 28 S and 18 S rRNA's are U and A in the nearly same proportions as described for L cells rRNA's [14]. Therefore, our method seems most efficient for identifying the 3′ terminus of RNA. Its application to 70 S RNA of MSV–MLV complex gave uridine as the major 3′ end. As we were going on this investigation, U was found to be the predominant base in RNA of several tumor viruses (for 70 S RNA and 35 S subunits) [4, 5]. Our results are in accord with them. However, a different finding was recently published by Stephenson et al. [10] who reported finding A as the terminal nucleoside of 70 S and 35 S RNA of avian myeloblastosis virus. To date, from this result which was not confirmed by Erikson, it cannot be concluded that all 70 S RNA molecules of Oncornaviruses end in U. Further studies should solve this open question.

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References

[1] P.H. Duesberg, Current topics in Microbiology and Immunology 51 (1970) 79.
[2] J.P. Bader and T.L. Steck, J. Virol. 4 (1969) 454.
[3] J.P. Leis and J. Hurwitz, J. Virol. 9 (1972) 130.
[4] H.B. Maruyama, M. Hatanaka and R.V. Gilden, Proc. Natl. Acad. Sci. U.S. 68 (1971) 1999.
[5] R.L. Erikson, E. Erikson and T.A. Walker, Virology 45 (1971) 527.
[6] U.L. Rajbhandary, J Biol. Chem. 243 (1968) 556.
[7] C. Bernard, M. Boiron and J. Lasneret, Compt. Rend. 264 (1968) 2170.
[8] R. Emanoïl Ravicovitch, C. Bernard and C.J. Larsen, Compt. Rend. 266 (1968) 1802.
[9] R. De Wachter and W. Fiers, J. Mol. Biol. 30 (1967) 507.
[10] M.L. Stephenson, Lr.L. Wirthlin, J.F. Scott and P.C. Zamecnik, Proc. Natl. Acad. Sci. U.S. 69 (1972) 1176.
[11] Y. Furuichi and K. Miura, J. Mol. Biol. 64 (1967) 507.
[12] L. J. Lewandowski, J. Content and S.H. Leppla, J. Vir. 8 (1971) 701.
[13] S.H. Leppla, B. Bjoraker and R.M. Bock, Methods in Enzymology, 12 (1968) 236.
[14] B.G. Lane and T. Tamaoki, J. Mol. Biol. 27 (1967) 335.
[15] J.A. Hunt, Biochem. J. 120 (1970) 353.
[16] B.G. Forget and S.M. Weissman, Science 158 (1967) 1695.
[17] F. Galibert, C.J. Larsen, J.C. Lelong and M. Boiron, Bull. Soc. Chim. Biol. 48 (1966) 21.