JOURNAL OF VIROLOGY, Apr. 1977, p. 235-237

Vol. 22, No. 1
Printed in U.S.A.

Strandedness of Pichinde Virus RNA

WAI-CHOI LEUNG,* HARA P. GHOSH, AND WILLIAM E. RAWLS

Department of Pathology and Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada L8S 4J9

Received for publication 13 October 1976

The Pichinde virus RNA did not possess the following characteristics of eucaryotic mRNA: polyadenylic acid sequence, capped methylated structure, and ability to direct protein synthesis in vitro. Polysomal RNA extracted from cells infected with Pichinde virus reannealed with ^{32}P-labeled virus RNA, protecting about 60% of the latter against RNase digestion. The polyadenylic acid-containing polysomal RNA also reannealed to the ^{32}P-labeled virus RNA to approximately the same extent. These findings indicate that the major part of the genomic RNA of Pichinde virus is negative stranded.

Pichinde virus, a member of the arenavirus group, is an enveloped virus that contains single-stranded RNA. Five RNA species with sedimentation coefficients of 31*S*, 28*S*, 22*S*, 18*S*, and 4-5*S* have been extracted from purified virions (1, 3, 11). The 28*S* and 18*S* RNA are of host ribosomal origin (4). The mode of replication of Pichinde virus is largely unknown. The presence of ribosomes in the virions of Pichinde virus suggests that positive-stranded RNA might be present; however, the presence of an RNA polymerase suggests that the virus is negative stranded (2). As an initial step in investigating the mode of replication of Pichinde virus, the present study was undertaken to examine the virion RNA with regard to properties associated with mRNA and its relation to virus-specific polysomal RNA in infected cells.

The presence of polyadenylated sequences at the 3′ termini (8), the presence of a capped and methylated structure at the 5′ termini (6, 9, 10), and the ability to stimulate the incorporation of amino acids into an acid-precipitable product in an in vitro protein-synthesizing system were the characteristics of mRNA that were examined. We found that Pichinde virus RNA did not contain extensive polyadenylic acid [poly(A)] sequences, as indicated by the lack of binding of ^{32}P-labeled virion RNA to oligo-deoxyribosylthymine[oligo(dT)]cellulose. To minimize internal steric hindrance, the RNA was further digested with pancreatic and T1 RNases, which should yield only poly(A) sequences. Such digestion of Pichinde virus RNA failed to yield a product that would bind to oligo(dT)-cellulose. We were also unable to find evidence of capped and methylated structures by examining the products of digestion of about 10^7 cpm of ^{32}P-labeled virion RNA by *Penicillium* nuclease and nucleotidyl pyrophosphatase. Under the same experimental conditions, the presence of capped and methylated structure was demonstrated in BHK-21 cell poly(A)-containing RNA. Although Pichinde virus RNA lacked these two structural characteristics of mRNA, it could conceivably function as mRNA since species of sea urchin mRNA have no detectable terminal poly(A) sequences (5). In addition, poliovirus RNA does not contain a capped and methylated structure (7), and yet it functions perfectly as mRNA. The possible messenger function of Pinchinde virus RNA was examined by adding nonlabeled Pichinde viral RNA to a wheat germ in vitro protein-synthesizing system (13). A two- to threefold stimulation of the incorporation of [^{3}H]leucine into hot trichloroacetic acid-precipitable material was found. In contrast, vesicular stomatitis virus mRNA or Brome mosaic virus RNA showed a 60-fold stimulation. The magnitude of stimulation by Pichinde virus RNA was similar to the nonspecific stimulation observed with the addition of rRNA alone. Since the Pichinde virus contains a considerable amount of rRNA, this could account for the observed slight stimulation of [^{3}H]leucine incorporation. Furthermore, when the product synthesized in vitro by Pichinde virus RNA was examined by 7.5 to 15% polyacrylamide gradient slab gel electrophoresis, no radioactive bands with electrophoretic mobility similar to the four Pichinde virus structural polypeptides were found. The above observations provide strong evidence that the Pichinde virus RNA has no messenger function.

At 16 to 20 h after infection, polysomes were isolated from cells by velocity sedimentation of a 10,000 × *g* supernatant of cytoplasmic extract

on a linear 10 to 40% (wt/wt) sucrose gradient. Virus-specific antigen synthesis at this time could be readily detected, but virus particle production was not yet maximal. We found little difference in the polysomal profiles between polysomes from mock-infected cells and those from Pichinde virus-infected cells, except that slightly less radioactivity was consistently observed in the latter. The identity of the polysomal structure was further demonstrated by its sensitivity to EDTA. When the cell extracts were prepared and centrifuged in the presence of 20 mM EDTA, less radioactivity was found in the polysomal region of the gradient.

RNA was extracted from pooled polysomal fractions of greater than 100S. The polysomal RNAs from Pichinde virus-infected and mock-infected cells were then reannealed with ^{32}P-labeled RNA extracted from purified Pichinde virus. The virus RNA was obtained from virions that were replicated and labeled in the presence of 0.05 μg of actinomycin D per ml to suppress the labeling of 28S and 18S rRNA. In addition, the RNA was further centrifuged in a sucrose gradient to remove the low-molecular-weight 4-5S RNA. The ^{32}P-labeled virus RNA obtained was found to contain about 70% of the radioactivity in the 31S and 22S region when examined by gel electrophoresis (Fig. 1). A direct relationship was observed between the concentration of polysomal RNA in the reaction mixture and the percentage of virus RNA resistant to RNase digestion (Table 1). The highest percentage of the RNase-resistant hybrid obtained in the experiment was 62.9. Polysomal RNA from mock-infected cells did not hybridize with the 31S and 22S RNA preparation to a significant degree. To demonstrate that complementary RNA was mRNA, polyadenylated RNA was selected from the polysomal RNA preparation by passage through oligo(dT)-cellulose. There was no significant hybridization when polyadenylated RNA from mock-infected cells was mixed with the 31S and 22S RNA; however, the polyadenylated RNA from the polysome of infected cells rendered 59.8% of the 31S and 22S RNA resistant to RNase digestion (Table 1). These observations suggest that at least 60% of the virion RNA is complementary to the polysomal RNA isolated from infected cells.

These data suggest that at least 60% of the

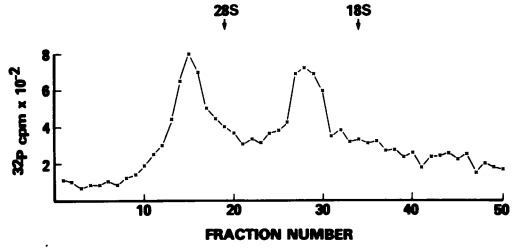

FIG. 1. *Electrophoresis of ^{32}P-labeled Pichinde viral RNA. The ^{32}P-viral RNA was labeled in vivo in the presence of 0.05 μg of actinomycin D per ml and 40 μCi of ^{32}P (as orthophosphoric acid) per ml, and phosphate-free minimal essential medium (MEM) with 1/50 volume of MEM with regular phosphate content was added. The virus was harvested and purified as described (1). RNA was extracted by phenol-chloroform (14) and centrifuged in a sucrose gradient. The high-molecular-weight RNA species were pooled and examined by electrophoresis in 2.4% acrylamide-0.5% agarose as described (4).*

TABLE 1. *Hybridization of polysomal RNA with ^{32}P-labeled Pichinde virus RNA*[a]

^{32}P-labeled Pichinde virus RNA annealed to:	RNase resistance (%)[b]
Mock-infected cell polysomal RNA (μg)	
10	0.5
25	0.2
50	0.4
100	1.0
150	2.4
Pichinde-infected cell polysomal RNA (μg)	
10	6.5
25	20.4
50	36.2
100	61.1
150	62.9
Mock-infected cell polyadenylated polysomal RNA (μg)	
0.5	0.6
1.0	1.2
2.0	0.9
Pichinde-infected cell polyadenylated polysomal RNA (μg)	
0.5	29.3
1.0	53.4
2.0	59.8

[a] The ^{32}P-labeled viral RNA was obtained according to Fig. 1 and has a specific activity of 1.5×10^5 cpm/μg.

[b] Approximately 5,000 cpm of ^{32}P-labeled virion RNA was added to various concentrations of polysomal RNA in 2× SSC (SSC = 0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.5) and 0.1% sodium dodecyl sulfate in a final volume of 0.1 ml in a capped ampoule. All samples were in duplicates. The reaction mixture was boiled for 5 min and incubated at 68°C for 18 h. The reaction mixture was then slowly cooled to room temperature. A 0.3-ml portion of 2× SSC was added to each ampoule. The samples were divided into two equal portions, one of which was treated with 50 μg of RNase A per ml and 25 U of RNase T1 for 30 min at 30°C. All samples were then acid precipitated and assayed for radioactivity. A background of 220 cpm was subtracted.

31S and 22S genomic RNA is transcribed. It should be noted that the 31S and 22S RNA constituted only about 70% of the ^{32}P-labeled viral RNA probe used; a considerable amount of radioactivity was at the 28S and 18S ribosomal RNA region. When a radioactive viral RNA probe of a greater degree of purity can be obtained, one would expect a greater percentage of reannealing with the virus-specific polysomal RNA. On the other hand, posttranscriptional cleavage of the complementary RNA may occur, and some of the cleavage product might not function as mRNA. As estimated by the radioactivity of the ^{3}H-labeled polysomal RNA, less than 1% of the total polyadenylated RNA in the virus-infected cells hybridized to the ^{32}P-labeled Pichinde virus RNA.

Previously, Carter et al. found an RNA-dependent RNA polymerase but not reverse transcriptase associated with purified Pichinde virus (2). Together with the data present in this communication, these observations strongly suggest that Pichinde virus, a member of the arenavirus group, is negative stranded.

This work was supported by grants from the Medical Research Council and the National Cancer Institute of Canada.

We are indebted to Maria F. K. L. Leung and Carol Lavery for excellent technical assistance.

LITERATURE CITED

1. **Carter, M. F., N. Biswal, and W. E. Rawls.** 1973. Characterization of nucleic acid of Pichinde virus. J. Virol. 11:61-68.
2. **Carter, M. F., N. Biswal, and W. E. Rawls.** 1974. Polymerase activity of Pichinde virus. J. Virol. 13:577-583.
3. **Carter, M. F., F. A. Murphy, J. P. Brunschwig, C. Noonan, and W. E. Rawls.** 1973. Effects of actinomycin D and ultraviolet and ionizing radiation on Pichinde virus. J. Virol. 12:33-38.
4. **Farber, F. E., and W. E. Rawls.** 1975. Isolation of ribosome-like structures from Pichinde virus. J. Gen. Virol. 26:23-31.
5. **Fromson, D., and D. P. S. Verma.** 1976. Translation of nonpolyadenylated messenger RNA of sea urchin embryos. Proc. Natl. Acad. Sci. U.S.A. 73:148-151.
6. **Furuichi, Y., M. Morgan, S. Muthukrishran, and A. J. Shatkin.** 1975. Reovirus messenger RNA contains a methylated, blocked 5'-terminal structure. Proc. Natl. Acad. Sci. U.S.A. 72:362-366.
7. **Hewlett, M. J., J. K. Rose, and D. Baltimore.** 1976. 5'-Terminal structure of poliovirus polyribosomal RNA is pUp. Proc. Natl. Acad. Sci. U.S.A. 73:327-330.
8. **Johnson, R. E., and H. R. Rose.** 1972. Correlation of messenger RNA function with adenylate-rich segments in the genomes of single-stranded RNA viruses. Proc. Natl. Acad. Sci. U.S.A. 69:1514-1516.
9. **Moyer, S. A., G. Abraham, R. Adler, and A. K. Banerjee.** 1975. Methylated and blocked 5'-termini in vesicular stomatitis virus *in vivo* mRNAs. Cell 5:59-67.
10. **Muthukrishnan, S., G. W. Both, Y. Furuichi, and A. J. Shatkin.** 1975. 5'-Terminal 7-methylguanosine in eucaryotic mRNA is required for translation. Nature (London) 255:33-37.
11. **Pederson, I. R.** 1973. Different classes of ribonucleic acid isolated from lymphocytic choriomeningitis virus. J. Virol. 11:416-423.
12. **Ramos, B. A., R. J. Courtney, and W. E. Rawls.** 1972. Structural proteins of Pichinde virus. J. Virol. 10:661-667.
13. **Roberts, B. E., and B. H. Patterson.** 1973. Efficient translation of tobacco mosaic virus RNA and rabbit globin 9S RNA in a cell-free system from commercial wheat germ. Proc. Natl. Acad. Sci. U.S.A. 70:2330-2334.
14. **Toneguzzo, F., and H. P. Ghosh.** 1976. Characterization and translation of methylated and unmethylated vesicular stomatitis virus mRNA synthesized in vitro by ribonucleoprotein particles from vesicular stomatitis virus-infected L cells. J. Virol. 17:477-491.