

Nucleotide Composition of the Ribonucleic Acid of Rabies Virus

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The nucleotide composition of the ribonucleic acid of three strains of rabies virus was determined and found to be similar to that of vesicular stomatitis virus.

The rabies virion is characterized as having bullet-shaped morphology with dimensions of 80 by 180 nm, a lipoprotein envelope, a helical ribonucleoprotein, and a single-stranded ribonucleic acid (RNA) genome (11, 12). Rabies and vesicular stomatitis virus (VSV) share basic chemical and physical characteristics and are classed as rhabdoviruses. Two recent reviews summarize the general properties of these and other members of the rhabdovirus group (4, 7).

The primary structures of the RNA genomes of rabies and VSV reflect, in the most fundamental sense, the degree to which these two viruses are related. This paper reports the base composition analysis of the RNA of three strains of rabies virus and compares their base composition to that of VSV RNA.

The Flury high-egg passage (HEP), Pitman-Moore (PM), and ERA strains of rabies virus were propagated in BHK-21 cell monolayers (11-13). The multiplicity of infection in each case was 0.5 to 2.0 plaque-forming units per cell. The Indiana strain of VSV was also propagated in BHK-21 cells. Immediately after infection, $^{32}\text{PO}_4^{3-}$ (carrier-free, New England Nuclear Corp., Boston, Mass.) was added to the virus-infected cell cultures to give a concentration of 10 to 20 μCi per ml.

Radioactive virus of each strain analyzed was concentrated and purified from 1 to 1.5 liters of cell-free tissue culture fluid by the following procedure. The virions were concentrated by precipitation with zinc acetate, followed by resuspension in a solution of disodium ethylenediaminetetraacetate (EDTA; reference 11). The virus suspension was next sedimented onto a 100% glycerol cushion by centrifugation at $80,000 \times g$ for 90 min at 5 C in an SW-27 Spinco rotor. The virus was collected from the glycerol interface, residual virus being washed off in 0.1 M NaCl, 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.4, 0.01 M EDTA

(NTE buffer). The opalescent, glycerol-containing virus suspension was finally subjected to isopycnic centrifugation (under conditions as defined above) for 16 hr to separate the virions from soluble materials and cell debris. The HEP and PM strains of rabies virus banded at a buoyant density of 1.17 g/cm³ after sedimentation into a gradient composed of 25 to 50% sucrose in NTE buffer. ERA virus banded as a zone between 1.19 and 1.17 g/cm³.

^{32}P -RNA was extracted from the banded virus with phenol containing 1.0% sodium dodecyl sulfate (SDS), precipitated with ethanol, and isolated by velocity centrifugation in a sucrose gradient. Figure 1 depicts the sedimentation of the viral RNA isolated from three strains of rabies virus. Each viral RNA sedimented as a 45S species, in agreement with data reported by Sokol et al. (12). PM virus preparations may contain a second viral RNA component (Fig. 1c) sedimenting at 18S. Experiments to determine whether this smaller RNA is contained in a defective PM particle are in progress. No significant amount of such an RNA was extracted from HEP or ERA virus. The genomes of HEP and ERA have, in fact, been shown to coelectrophore in polyacrylamide gels with only one RNA species being detected (Bishop, Aaslestad, and Clark, *manuscript in preparation*). When VSV viral RNA was banded in a sucrose gradient, a 45S and a 30S species were observed. Only the complete viral genome (45S RNA) has been employed in the analysis of base composition.

The density gradient fractions containing the $^{32}\text{PO}_4^{3-}$ -labeled viral RNA were pooled. Alkaline hydrolysis and subsequent electrophoretic separation of the labeled nucleotides were performed by the method of Sebring and Salzman (10), except that electrophoresis was carried out in 0.05 M citrate buffer (pH 3.5) with 4,000 v for 150 min. Nucleotide spots were visualized by exposing X-ray film to the dried electropherogram.

The radioactivity contained in each nucleotide spot was determined by liquid scintillation spectrometry. In addition to electrophoretic separation of the viral nucleotides, chromatography on Dowex-1-formate, as described by Bautz and Hall (2), was also performed on hydrolysates of HEP and VSV RNA.

Table 1 summarizes the base composition

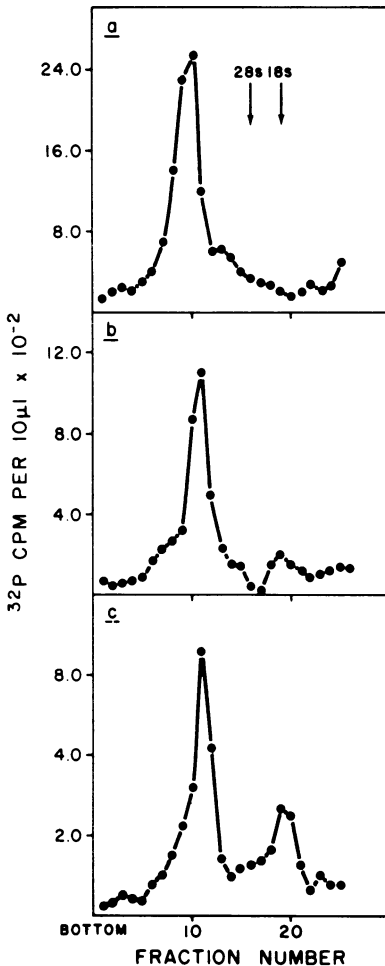


FIG. 1. Sucrose density gradient centrifugation of rabies virus RNA. Viral RNA, labeled with $^{32}\text{PO}_4^{3-}$, extracted from HEP (a), ERA (b), and PM (c) strains of rabies virus, was dissolved in NTE buffer, containing 0.1% SDS, after ethanol precipitation. Each RNA species was then layered on a 5 to 25% sucrose gradient prepared in the same buffer. Centrifugation was performed in an SW-41 Spinco rotor at $194,000 \times g$ for 120 min at 20 C. Each tube was fractionated from the bottom, and samples of each fraction were assayed for radioactivity after precipitation by trichloroacetic acid and entrapment onto membrane filters. ^3H -labeled BHK-21 cell ribosomal RNA served as a sedimentation marker.

analysis of the RNA of three strains of rabies virus and VSV. Each strain of rabies virus is characterized as having uridylic acid in greatest abundance (28.1 to 29.5%). Although there is a slight difference between HEP RNA and that of the other two rabies strains, ERA and PM, with respect to the nucleotide present in least amount, no marked differences between strains were detected. The average compositional analysis of HEP is 26.4% adenylic acid, 29.4% uridylic acid, 21.1% guanylic acid, and 23.1% cytidylic acid. VSV RNA, although having a slightly greater content of uridylic acid than rabies RNA, is judged generally similar to rabies RNA. The VSV base analysis is in close agreement with the data of Huppert et al. (5) and Mudd and Summers (9). The base analysis published by Brown et al. (3) for VSV RNA differs in that adenylic acid was slightly more abundant than uridylic acid (29.3 versus 28.7%).

Although the base composition of rabies virus and VSV are equivalent, differences have been observed in their gene products. Three structural peptides have been detected by polyacrylamide gel electrophoresis in solubilized VSV virions (6, 14), whereas Sokol et al. (13) have found at least four peptides in rabies viruses. Mudd and Summers (8), however, detected four structural peptides in VSV which have molecular weights quite similar to the rabies virus structural peptides. The presence of an RNA-dependent RNA polymerase in VSV (1) and the apparent absence of this enzyme in rabies (Aaslestad, Bishop, and Sokol, unpublished data) may be another fundamental difference. A measure of relationship between rabies and VSV, more sensitive than base composition, would be the degree of hybridization between the in vitro RNA product of the VSV RNA polymerase and the genomes of VSV and rabies. Experiments utilizing this approach are in progress.

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LITERATURE CITED

- Baltimore, D., A. S. Huang, and M. Stampfer. 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. II. An RNA polymerase in the virion. Proc. Nat. Acad. Sci. U.S.A. 66:572-576.
- Bautz, E. K. F., and B. D. Hall. 1962. The isolation of T₄-specific RNA on a DNA-cellulose column. Proc. Nat. Acad. Sci. U.S.A. 48:400-408.
- Brown, F., S. J. Martin, B. Cartwright, and J. Crick. 1967. The ribonucleic acids of the infective and interfering components of vesicular stomatitis virus. J. Gen. Virol. 1:479-486.

TABLE 1. Base composition of rabies virus and vesicular stomatitis virus (VSV) RNA

RNA	No. of determinations and method ^a	Amt of nucleotide ^b (moles/100 moles)			
		Adenylic acid	Uridylic acid	Guanylic acid	Cytidylic acid
Rabies HEP.....	3, c	26.3 ± 0.5	29.3 ± 0.3	20.6 ± 1.0	23.8 ± 0.9
Rabies HEP.....	7, e	26.4 ± 0.9	29.5 ± 0.3	21.6 ± 1.5	22.5 ± 0.8
Rabies ERA.....	4, e	25.5 ± 0.8	28.7 ± 0.4	24.1 ± 1.2	21.7 ± 0.8
Rabies PM.....	4, e	26.7 ± 0.6	28.1 ± 1.4	22.8 ± 0.8	22.4 ± 1.5
VSV.....	4, c	26.0 ± 0.8	33.3 ± 1.6	17.2 ± 1.5	23.5 ± 0.6
VSV.....	3, e	27.4 ± 0.9	30.3 ± 0.1	21.0 ± 0.1	21.5 ± 0.6

^a Method for nucleotide separation was Dowex-1-formate chromatography (c) or paper electrophoresis (e).

^b Base composition is calculated as the fraction of ³²PO₄³⁻ determined for each nucleotide divided by the total amount of radioactivity found in the four nucleotides. Standard deviations are given.

- Howatson, A. F. 1970. Vesicular stomatitis and related viruses. p. 195-256. In K. M. Smith, M. A. Lauffer, and F. B. Bang (ed.), *Advances in virus research*, vol. 16. Academic Press Inc., New York.
- Huppert, J., M. Rosenbergo, L. Gresland, and L. Harel. 1967. Properties of RNA from vesicular stomatitis virus, p. 463-468. In J. S. Colter and W. Paranchych (ed.), *The molecular biology of viruses*. Academic Press Inc., New York.
- Kang, C. Y., and L. Prevec. 1969. Proteins of vesicular stomatitis virus. I. Polyacrylamide gel analysis of viral antigens. *J. Virol.* 3:404-413.
- Matsumoto, S. 1970. Rabies virus, p. 257-301. In K. M. Smith, M. A. Lauffer, and F. B. Bang (ed.), *Advances in virus research*, vol. 16. Academic Press Inc., New York.
- Mudd, J. A., and D. F. Summers. 1970. Protein synthesis in vesicular stomatitis virus-infected HeLa cells. *Virology* 42:328-340.
- Mudd, J. A., and D. F. Summers. 1970. Polysomal ribonucleic acid of vesicular stomatitis virus-infected HeLa cells. *Virology* 42:958-968.
- Sebring, E. D., and N. P. Salzman. 1964. An improved procedure for measuring the distribution of P³²O₄³⁻ among the nucleotides of ribonucleic acid. *Anal. Biochem.* 8:126-129.
- Sokol, F., E. Kuwert, T. J. Wiktor, K. Hummeler, and H. Koprowski. 1968. Purification of rabies virus grown in tissue culture. *J. Virol.* 2:836-849.
- Sokol, F., H. D. Schlumberger, T. J. Wiktor, and H. Koprowski. 1969. Biochemical and biophysical studies on the nucleocapsid and on the RNA of rabies virus. *Virology* 38:651-665.
- Sokol, F., D. Stanček, and H. Koprowski. 1971. Structural proteins of rabies virus. *J. Virol.* 7:241-249.
- Wagner, R. R., T. C. Schnaitman, R. M. Snyder, and C. A. Schnaitman. 1969. Protein composition of the structural components of vesicular stomatitis virus. *J. Virol.* 3:611-618.