Comparison of 6/94 Virus and Sendai Virus RNA by RNA-RNA Hybridization

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The genomic RNA of 6/94 virus, an agent isolated from the brains of multiple sclerosis patients, was studied for sequence homology by RNA-RNA hybridization with closely related Sendai virus and another paramyxovirus virus, Newcastle disease virus. It was found that the genomic RNA of 6/94 virus hybridizes equally as well to the virus-specific 18S RNA found in Sendai-infected cells as that of Sendai virus.

ter Meulen et al. have isolated a virus from cultures of human brain cells, obtained from regions adjacent to fresh plaque areas of two multiple sclerosis patients and fused with CV-1 cells in the presence of lysolecithin (6). This virus, called 6/94 virus, was classified as a member of the type I parainfluenzae on the basis of both immunological (6) and morphological evidence (4). More recently, virion polypeptides of 6/94 virus were found to be remarkably similar to those of Sendai viruses when analyzed by polyacrylamide gel electrophoresis (L. J. Lewandowski, F. S. Lief, M. A. Verini, M. M. Pienkowski, V. ter Meulen, and H. Koprowski, submitted for publication; D. Waters and L. J. Lewandowski, submitted for publication). Conversely, 6/94 virus is more effective in fusing cells than Sendai virus (Lewandowski et al., submitted for publication; M. Pienkowski, H. Koprowski, and L. J. Lewandowski, Abstr. Annu. Meet. Amer. Soc. Microbiol. p. 243, 1973) and displays higher hemolytic activity (Lewandowski et al., submitted for publication). To examine more closely the degree of similarity between 6/94 and Sendai virus, we investigated the sequence homology of their viral genomes by RNA-RNA hybridization, using the RNA of Newcastle disease virus (NDV), another type I parainfluenza virus, as a control.

In both Sendai- and NDV-infected cells treated with actinomycin D and labeled for short periods with radioactive ribonucleosides, several virus-specific RNA species (50S, 35S, 22S, and 18S) were identified by sucrose gradient analysis (1, 2). Of these, by far the most predominant species, which sediments at 18S, was shown to be completely complementary to

its homologous virion RNA (1, 2) but not to heterologous parainfluenza virion RNA (1). We therefore isolated the genomic RNA of 6/94 virus and examined whether or not it contained sequences homologous to Sendai RNA due to its ability to hybridize to Sendai 18S complementary RNA (cRNA) (see legend to Fig. 1). As shown in Fig. 1, the 48S RNA of 6/94 virus was equally as effective as that of Sendai in protecting Sendai 18S cRNA against ribonuclease digestion after annealing, whereas that of NDV, as previously shown (1), demonstrated no detectable sequence homology by this test.

In the case of NDV, Bratt and Robinson have shown that the 18S cRNA is complementary to approximately 50% of the viral genome (2). Although no comparable data in the case of the 18S RNA found in Sendai-infected cells exists, it seems likely that this RNA is complementary to an equivalent amount of the viral genome in view of the similar pattern of virus-specific RNAs found in both Sendai- and NDV-infected cells (1). If this is so, then the viral genomes of 6/94 and Sendai are at least 50% homologous, no statement being possible about that portion of the viral genome that is not transcribed into 18S RNA.

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FIG. 1. Hybridization of parainfluenza viral RNAs to [3 H]Sendai 18S-cRNA. [3 H]Sendai 18S-cRNA was prepared as follows. Three-day-old confluent cultures of MDBK cells in 75-cm 2 Falcon tissue culture flasks were infected with 10 mean egg infective doses per cell of Sendai virus (Harris strain). After 45 min at 30 C, the infecting medium was removed and replaced with 15 ml of Dulbecco modified Eagle medium containing 2% dialyzed calf serum. At 18 h postinfection (30 C), 10 ml of medium was removed and 50 µg of actinomycin D was added to the remaining 5 ml of medium, followed by 100 µCi of [3 H]uridine (25 mCi/µmol) 90 min later. After a further 60 min of incubation, the cells were harvested by trypsinization and washed once with phosphate-buffered saline, and the RNA was isolated by phenol extraction at 60 C (7). The RNA was recovered from the aqueous phase by ethanol precipitation and dissolved in buffer A (0.5 M NaCl, 0.01 M Tris buffer [pH 7.6], 0.1% SDS), and the solution (3 ml) was applied to a column (0.5 by 4 cm) of thymidine polynucleotide cellulose (3) equilibrated with buffer A at room temperature. After washing with 3 ml of buffer A followed by 3 ml of the same buffer but containing 0.1 M NaCl, the column

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was eluted with 0.01 M Tris buffer (pH 7.6) containing 0.1% SDS. Fractions containing the radioactivity eluted at low salt (third wash) were pooled, and the RNA was recovered by ethanol precipitation, dissolved in 0.2 ml of water, and centrifuged through a sucrose gradient (5 to 23% in 50 mM LiCl, 10 mM Tris-hydrochloride [pH 7.5], 4 mM EDTA, and 0.1% SDS) in the Spinco rotor SW41 at 38,000 rpm for 3 h and 45 min (7 C). The major peak of radioactivity, sedimenting at 18S and representing about 40% of the load, was isolated, and the RNA was recovered by ethanol precipitation and dissolved in distilled water. For hybridization assays, each tube contained 500 counts/min of [3 H]18S cRNA plus varying amounts of either Sendai, 6/94, or NDV 48S viral RNA (isolated as previously described [5]) as indicated, in a total volume of 0.5 ml of $2 \times$ SSC. The tubes were sealed, heated for 4 min at 125 C, quickly cooled in ice water, and then annealed for 1 h at 78 C. The tubes were then opened, and 0.5 ml of $1.5 \times$ SSC containing 33 µg of ribonuclease A was added and incubated for 30 min at 25 C. Carrier rRNA (30 µg) was added, and the RNA was precipitated with 6% trichloroacetic acid and collected on membrane filters (Millipore Corp.); 3 H radioactivity was counted by liquid scintillation.