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Irreversible Modification of Measles Virus RNA In Vitro by Nuclear RNA-Unwinding Activity in Human Neuroblastoma Cells

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The matrix (M) gene of a measles virus (MV) variant passaged in IMR-32 human neuroblastoma cells displays numerous uridine-to-cytosine transitions called biased hypermutation. Using an in vitro assay, we show that IMR-32 cells contain high levels of an activity which unwinds and irreversibly alters the base pairing of double-stranded RNA synthesized from the M gene of MV. This activity is found exclusively in the cellular nucleus and is present at a lower level in African green monkey kidney Vero cells. Experiments with mixed cell extracts suggest that the low activity in Vero cells is not due to inhibitory factors. These findings support the hypothesis that this RNA-modifying and -unwinding activity is responsible for biased hypermutation of MV strains that infect the central nervous system. Possible functions of this neural cell activity and implications for central nervous system disorders are discussed.

Measles, the second most common cause of mortality in infants and children in the world, is responsible for a million or more deaths annually. The causative agent, measles virus (MV), has also been implicated in various forms of central nervous system (CNS) disorders. Acute and subacute infections of the CNS cause, respectively, acute measles inclusion body encephalitis and subacute sclerosing panencephalitis (SSPE) (15, 20, 27). In addition, host immune response to MV can lead to measles allergic encephalitis (14).

MV strains recovered from patients with CNS infections are often genetically distinguishable from those that cause acute measles, especially in the viral genes encoding matrix (M), fusion (F), and hemagglutinin (H) proteins (1–3, 7, 9, 11, 17, 33). Some of the MV strains that cause CNS infections show intriguing unidirectional uridine (U)-to-cytosine (C) transitions known as biased hypermutation (8, 32). When the M gene of an acute MV (Nagahata strain) is compared with that of its likely descendant virus (Biken strain) isolated in the same locale from a patient with SSPE, biased hypermutation accounts for nearly all the missense mutations which alter the structure and properties of the SSPE viral M protein (1, 33). This suggests that biased hypermutation plays a significant role in the evolution of SSPE virus.

An interesting hypothesis suggests that biased hypermutation in MV RNA is a manifest of a cellular enzyme which causes deamination of adenosine (A) residues in double-stranded (ds) RNA (5, 16). This activity, which has been found in a variety of eukaryotic cells, unwinds dsRNA by changing A residues into inosines (I) (4, 19a, 29). Since an I residue can base pair with C, an A-to-I substitution in the minus-sense MV genome would be expressed as a U-to-C transition in the plus-sense viral mRNA. However, except for the similarly biased nature of hypermutation in MV RNA and deamination by the cellular RNA-modifying and -unwinding activity, there has been no evidence that causally links together these two phenomena.

We previously observed that the M gene of an SSPE virus (Yamagata strain) that was passaged in IMR-32 human neuroblastoma cells displayed numerous additional U-to-C transitions that were not found in the M gene of another stock of the same virus that was passaged in African green monkey kidney Vero cells (32). The extra hypermutated residues occurred within a well-defined 5′ region in the M gene of the virus passaged in neuroblastoma cells. This pattern of mutation cannot be easily explained by random errors in viral RNA synthesis but is compatible with an exogenous RNA-modifying activity acting locally on the viral RNA. To elucidate the basis of this phenomenon, we investigated whether an increased level of the putative RNA-modifying and -unwinding activity was present in IMR-32 neuroblastoma cells compared with that in Vero cells.

Vero cells were grown in minimal essential medium with 10% newborn calf serum. IMR-32 cells were grown in the same medium supplemented with 1% nonessential amino acids. Since mammalian RNA-modifying and -unwinding activities are cell cycle dependent (28), cells were synchronized by maintaining them overnight in medium containing 2% newborn calf serum and stimulated by increasing the serum concentration to 10% for 12 h before harvest. Whole-cell extracts were prepared by a previously described procedure (18) modified for the RNA-unwinding assay (4). Cells were swollen for 20 min on ice in 4 volumes of the packed cells (PCV) of solution A (100 mM Tris-HCl [pH 7.9], 1 mM EDTA, 5 mM dithiothreitol [DTT]) and homogenized with a Dounce homogenizer. The whole-cell lysates were gently mixed with 4 PCV of solution B (50 mM Tris-HCl [pH 7.9], 10 mM $MgCl_2$, 2 mM DTT, 25% sucrose, 50% glycerol). One PCV of saturated $(NH_4)_2SO_4$ was added dropwise, and the mixture was kept on ice for 30 min with gentle stirring. The mixture was centrifuged at 40,000 rpm for 3 h in a Beckman SW55 rotor. To the collected supernatant, solid $(NH_4)_2SO_4$ was added to 0.33 g/ml. After the solid $(NH_4)_2SO_4$ dissolved, 0.01 ml of 1 N NaOH per g of $(NH_4)_2SO_4$ was added, and the mixture was stirred for 30 min. The precipitated proteins were pelleted at 15,000 × *g* for 20 min in a Beckman JA20 rotor and resuspended in 0.05 volume of TGKED (50 mM Tris-HCl [pH 7.8], 0.5 mM $MgCl_2$, 50 mM KCl, 0.1 mM EDTA, 0.5 mM DTT, 25% glycerol). After dialyzing overnight at 4°C against 50 volumes of TGKED with two changes of buffer, the cell extracts were centrifuged at 10,000 × *g* for 10 min in a Beckman JA20 rotor. Aliquots of the supernatant were quickly frozen in liquid nitrogen and stored at −80°C.

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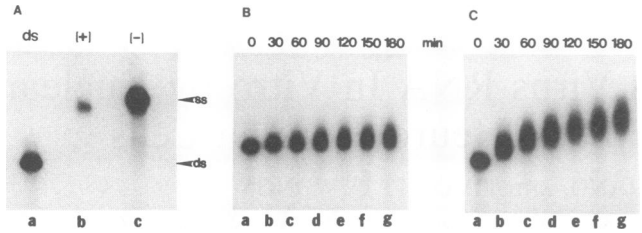

FIG. 1. Unwinding of MV RNA in Vero and IMR-32 neuroblastoma cell extracts. ^{32}P-labeled dsRNA (A, lane a) formed between the sense (A, lane b) and antisense (A, lane c) RNA synthesized from the 5′ region of Edmonston strain MV M gene was incubated in Vero (B) or IMR-32 (C) whole-cell extract for 0 to 180 min. The RNA recovered from the extract was analyzed along with the unreacted RNA (A) by electrophoresis in a nondenaturing 4% polyacrylamide gel. ss, single stranded.

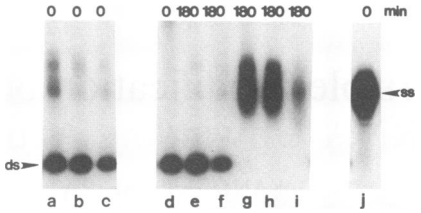

FIG. 2. Irreversible modification of the unwound MV RNA. ^{32}P-labeled dsRNA was incubated with IMR-32 whole-cell extract for 0 min (lanes a to c) or 180 min (lanes g to i). Parallel samples were incubated for the same time with IMR-32 cell extract that had been heated at 58°C for 10 min (lanes d to f). One of the samples in each set was recovered by proteinase K treatment, phenol-chloroform extraction, and ethanol precipitation (lanes a, d, and g, respectively). The other samples were treated with proteinase K, ethanol precipitated, and reannealed at 45°C for 15 h (lanes b, e, and h) or denatured at 90°C for 3 min and then reannealed (lanes c, f, and i). The samples were analyzed along with the antisense M RNA (lane j) in a nondenaturing 4% polyacrylamide gel. Incompletely annealed sense and antisense RNA was not removed by RNase treatment in this experiment and is visible in the 0-min samples (lanes a to c). ss, single stranded.

To facilitate future comparison with naturally occurring hypermutation in MV, we chose a 5′ region of the Edmonston strain MV M gene (up to the *Eco*RI site at nucleotide 567 [34]) as a substrate for studying the RNA-modifying activity in vitro. This region contains numerous U residues that are potential targets for hypermutation in neuroblastoma cells (32). Sense and antisense 5′ M RNAs were synthesized in vitro from subclones of the pGEM-M2i plasmid (34) by use of the SP6 and T7 bacteriophage promoters (19). The sense and antisense strands were labeled with [^{32}P]UTP at a 1:10 ratio in specific activity. After repeated ethanol precipitation in 2.5 M NH_4 acetate to remove unincorporated UTP, equal molar amounts of the two RNA strands were hybridized at 45°C for 15 h in 40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid] buffer (pH 6.7) containing 0.4 M NaCl, 1 mM EDTA, and 80% formamide. Overhanging vector sequences in the RNA duplex were trimmed by RNase treatment, and the duplex was recovered by phenol-chloroform extraction and ethanol precipitation. The annealed RNA duplex can be resolved from the sense and antisense RNA strands by electrophoresis in a nondenaturing gel (Fig. 1A, lanes a, b, and c, respectively).

The RNA-unwinding assay was done in 15-μl reaction mixtures containing 40 μg of Vero or IMR-32 cellular proteins, 50 ng of ^{32}P-labeled dsRNA, 50 mM Tris-HCl (pH 7.8), 0.5 mM $MgCl_2$, 50 mM KCl, 50 mM EDTA, 0.5 mM DTT, 25% glycerol, and 10 μg of tRNA. After the mixture was incubated at 37°C for various times, the reaction was stopped by the addition of 2 μl of 0.5 M EDTA. The RNA samples were digested with proteinase K, precipitated with ethanol, and electrophoresed in a 4% nondenaturing polyacrylamide gel.

When the dsRNA was incubated with whole Vero cell extract, a very slight shift in electrophoretic mobility in the RNA was detected after prolonged incubation (e.g., 120 to 180 min; Fig. 1B, lanes e to g). By contrast, a marked shift in electrophoretic mobility of the dsRNA was observed within 30 min of incubation with IMR-32 whole-cell extract (Fig. 1C, lanes a and b), and the mobility of the duplexes continued to decrease during 180 min of incubation (Fig. 1C, lanes c to g).

To rule out the possibility that the mobility shift was due to formation of RNA-protein complexes that were resistant to proteinase, after incubation with IMR-32 whole-cell extract, the proteinase K-treated RNA was further purified by phenol-chloroform extraction and analyzed by electrophoresis in a nondenaturing polyacrylamide gel. The phenol-chloroform-purified RNA from the 180-min sample migrated with retarded mobility compared with the 0-min sample (Fig. 2, lanes g and a, respectively). Phenol-chloroform extraction did not change the pattern of mobility shift in the RNA. Therefore, the mobility shift was not due to formation of RNA-protein complexes but was due to unwinding of the RNA substrate. To determine whether the unwound RNA could reanneal, we recovered the RNA from the 0- and 180-min samples and either hybridized the RNA directly or heat denatured the RNA and rehybridized it. The RNA recovered from the 0-min sample reannealed completely into dsRNA, regardless of whether the RNA was hybridized directly or heat denatured before reannealing (Fig. 2, lanes b and c, respectively). By contrast, neither the nondenatured nor the heat-denatured RNA recovered from the 180-min sample could reanneal completely into dsRNA (Fig. 2, lanes h and i, respectively). This indicates that base pairing of the dsRNA was irreversibly altered by the IMR-32 cell extract. The heat-denatured RNA did not reanneal efficiently after 180 min of incubation in the IMR-32 cell extract (Fig. 2, lane i), probably because the partially unwound RNA was randomly nicked by cellular RNases and could not reassociate into duplexes of the same size. The RNA-modifying and -unwinding activity was completely inactivated by subjecting the IMR-32 cell extract to 58°C for 10 min (Fig. 2, lanes d to f). The dsRNA recovered from the inactivated cell extract was unmodified and could be denatured and reannealed into fully ds form (Fig. 2, lanes e and f).

These results indicate that IMR-32 human neuroblastoma cells contain significantly higher levels of an RNA-modifying and -unwinding activity than Vero cells. This activity is heat sensitive, and it irreversibly alters the base pairing of dsRNA. A portion of the A residues in the unwound RNA were converted into I residues, and this activity was titratable with excess dsRNA (data not shown). These properties are characteristic of RNA-modifying and -unwinding activities previously found in *Xenopus* oocytes and some mammalian cells (4, 29).

To test whether the low RNA-modifying and -unwinding activity in Vero cells was due to the presence of nonspecific inhibitors such as proteases, we attempted to block the inhibitors with bovine serum albumin (BSA). However,

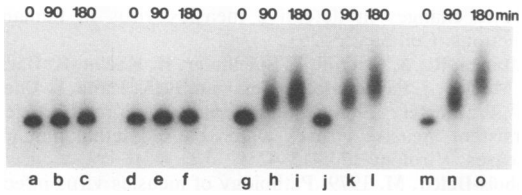

FIG. 3. RNA-modifying and -unwinding activities in mixed cell extracts. ^{32}P-labeled dsRNA was incubated for 0, 90, or 180 min in whole-cell extracts containing 40 μg of proteins from Vero cells (lanes a to c), IMR-32 cells (lanes g to i), or a mixture containing 40 μg of proteins each from Vero and IMR-32 cells (lanes m to o). Parallel reactions were done with the Vero or IMR-32 cell extracts mixed with 40 μg of BSA (lanes d to f and j to l, respectively). RNA was recovered and analyzed as described in the legend to Fig. 1.

addition of BSA to the Vero cell extract did not increase the RNA-modifying and -unwinding activity (Fig. 3, lanes a to f). We also tested whether Vero cells contained excess factors that inhibited the RNA-modifying and -unwinding activity. A mixed extract containing equal amounts of proteins from IMR-32 and Vero cells was found to be as efficient in RNA unwinding as IMR-32 cell extract alone (Fig. 3, lanes m to o and g to i, respectively). BSA did not enhance the RNA-modifying and -unwinding activity in the IMR-32 cell extract (Fig. 3, lanes j to l). These results show that Vero cells do not contain excess factors that inhibit the RNA-modifying and -unwinding activity. The high activity in IMR-32 cells likely reflects an elevated level of the putative enzyme.

We further investigated the intracellular localization of the RNA-modifying and -unwinding activity. Vero and IMR-32 cells were homogenized in 3 PCV of a hypotonic buffer (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.9], 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride). The nuclei were collected by centrifugation at 3,300 × *g* for 15 min and resuspended in 0.5 volume of the packed nuclei of LSB (50 mM Tris-HCl [pH 7.9], 0.5 mM $MgCl_2$, 20 mM KCl, 0.1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM DTT, 25% glycerol). An equal volume of HSB (same as LSB except with 0.3 M KCl) was added dropwise, and the nuclear materials were extracted by stirring for 30 min at 4°C. The extracted nuclei were removed by centrifugation at 25,000 × *g* for 30 min, and the supernatant was dialyzed against several changes of 50 volumes of MSB (same as LSB except with 50 mM KCl) until the conductivity of the extract and that of LSB were equal. The extract was centrifuged at 25,000 × *g* for 20 min, and the supernatant was aliquoted, frozen in liquid nitrogen, and stored at −80°C.

Cytoplasmic extracts were prepared from the same cells by adding 0.11 volume of a 10× buffer containing 0.3 M HEPES (pH 7.9), 1.4 M KCl, and 30 mM $MgCl_2$ to the first postnuclear fraction. The mixture was centrifuged at 100,000 × *g* for 60 min. The supernatant (S-100) was dialyzed against 50 volumes of MSB until the conductivity of the extract equaled that of 50 mM KCl. The S-100 fraction was centrifuged at 25,000 × *g* for 20 min, and the supernatant was aliquoted and tested for RNA-modifying and -unwinding activity.

Both the nuclear and cytoplasmic fractions of Vero cells lacked a significant level of RNA-modifying and -unwinding activity (Fig. 4C and D, respectively). The dsRNA was slowly unwound in the nuclear fraction from Vero cells after a very long (120-min) incubation, as indicated by the degradation of the partially unwound substrates by cellular

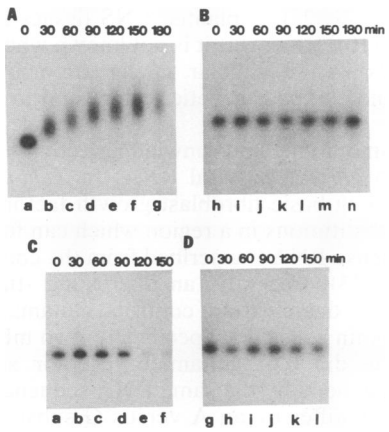

FIG. 4. Nuclear localization of the RNA-modifying and -unwinding activity in IMR-32 neuroblastoma cells. ^{32}P-labeled dsRNA was incubated with nuclear or cytoplasmic extract containing 20 μg of proteins from IMR-32 cells (A and B, respectively) or Vero cells (C and D, respectively). At the times indicated, RNA was recovered from the cell extracts and analyzed as described in the legend to Fig. 1.

RNases (Fig. 4C, lanes e and f). Including excess amounts of tRNA in the reaction preserved the partially unwound substrates (Fig. 1B). More important, a high level of RNA-modifying and -unwinding activity was found in the nuclear extract of IMR-32 cells (Fig. 4A). The cytoplasmic fraction of IMR-32 cells caused no shift in the mobility of the RNA duplexes (Fig. 4B). Therefore, the RNA-modifying and -unwinding activity is localized exclusively in the cellular nucleus.

The finding of high RNA-modifying and -unwinding activity in IMR-32 cells provides further support for the hypothesis that this activity is responsible for hypermutation of the Yamagata strain SSPE virus propagated in that cell line (32). However, the nuclear localization of this activity poses intriguing questions about its mode of action in vivo. How might the genomic RNA of MV, which replicates in the cytoplasm, be subject to modification by a cellular nuclear activity? In *Xenopus* oocytes, the RNA-modifying and -unwinding activity which is normally confined to the nuclei is released into the cytoplasm when the nuclear membrane breaks down during meiosis (4). One would predict similar leakage of this activity into the cytoplasm when mammalian somatic cells undergo mitosis. It is also possible that integrity of the cellular nuclear membrane is disrupted at a late stage of MV infection.

If neurons in the CNS also contain RNA-modifying and -unwinding activity predominantly in the nuclei, mitosis-dependent release of the activity into the cytoplasm is not expected, since neurons do not divide after maturation. How might MV strains that infect the CNS be modified by this activity? It has long been recognized that cellular nuclei may play a role in MV infection (for a review, see reference 31). MV yield is reduced by almost 100-fold in enucleated cells (12). All the nonglycosylated MV proteins are detected in the nuclei (30), and MV RNA can be recovered from the nuclear fraction of MV-infected cells (6, 25). Electron microscopy studies demonstrated MV nucleocapsids not only in the cytoplasm, but also in the nuclei, especially at a late stage of infection (21, 24). It is particularly noteworthy that accumulation of intranuclear viral nucleocapsids is a common characteristic of chronic infections by MV and SSPE viruses in

cultured cells (21, 22), explanted CNS tissues (23, 24), and SSPE brains (10). However, it is not known whether the MV nucleocapsids in the cellular nuclei are active in RNA replication and can pass genetic changes on to the progeny virus.

The RNA-modifying and -unwinding activity may modify cellular RNA as well as viral RNA. In *Xenopus* oocytes, mRNA encoding basic fibroblast growth factor is modified by A-to-G substitutions in a region which can form a duplex with an antisense RNA transcribed from the complementary DNA strand (13). Recently, an interesting study suggests that adenosine deamination controls glutamate-gated ion channels in brain cells (26). Specifically, two mRNA classes which encode different glutamate receptor subtypes are found to be encoded by the same DNA sequences. The two mRNA classes differ by an A versus G substitution corresponding to a glutamine versus arginine difference in the predicted channel-forming region of the protein, which influences the properties of the channel (26). Thus, a defect in regulation of the RNA-editing process can potentially affect ion transport and may lead to neuronal disorders. These observations raise the possibility that adenosine-deaminating enzymes serve important regulatory functions in the CNS. Understanding the action and control of the RNA-modifying and -unwinding activity in neural cells may not only shed light on CNS infection by MV but may also offer insights into possible roles of this activity in neuronal functions and dysfunctions.

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REFERENCES

1. **Ayata, M., A. Hirano, and T. C. Wong.** 1989. Structural defect linked to nonrandom mutations in the matrix gene of Biken strain subacute sclerosing panencephalitis virus defined by cDNA cloning and expression of chimeric genes. J. Virol. **63**:1162–1173.
2. **Ayata, M., A. Hirano, and T. C. Wong.** 1991. Altered translation of the matrix genes in Niigata and Yamagata neurovirulent measles virus strains. Virology **180**:166–174.
3. **Baczko, K., U. G. Liebert, M. Billeter, R. Cattaneo, H. Budka, and V. ter Meulen.** 1986. Expression of defective measles virus genes in brain tissues of patients with subacute sclerosing panencephalitis. J. Virol. **59**:472–478.
4. **Bass, B. L., and H. Weintraub.** 1988. An unwinding activity that covalently modifies its double-stranded RNA substrate. Cell **55**:1089–1098.
5. **Bass, B. L., H. Weintraub, R. Cattaneo, and M. A. Billeter.** 1989. Biased hypermutation of viral RNA genomes could be due to unwinding/modification of double-stranded RNA. Cell **56**: 331.
6. **Carter, C., F. L. Black, and A. Schluederberg.** 1973. Nucleus-associated RNA in measles virus-infected cells. Biochem. Biophys. Res. Commun. **54**:411–416.
7. **Cattaneo, R., A. Schmid, M. A. Billeter, R. D. Sheppard, and S. A. Udem.** 1988. Multiple viral mutations rather than host factors cause defective measles virus gene expression in a subacute sclerosing panencephalitis cell line. J. Virol. **62**:1388–1397.
8. **Cattaneo, R., A. Schmid, D. Eschle, K. Baczko, V. ter Meulen, and M. A. Billeter.** 1988. Biased hypermutation and other genetic changes in defective measles virus in human brain infections. Cell **55**:255–265.
9. **Cattaneo, R., A. Schmid, P. Spielhofer, K. Kaelin, K. Baczko, V. ter Meulen, J. Pardowitz, S. Flanagan, B. K. Rima, S. Udem, and M. A. Billeter.** 1989. Mutated and hypermutated genes of persistent measles viruses which caused lethal human brain diseases. Virology **173**:415–425.
10. **Dubois-Dalcq, M.** 1979. Pathology of measles virus infection of the nervous system; comparison with multiple sclerosis. Int. Rev. Exp. Pathol. **19**:101–135.
11. **Enami, M., T. A. Sato, and A. Sugiura.** 1989. Matrix protein of cell-associated subacute sclerosing panencephalitis viruses. J. Gen. Virol. **70**:2191–2196.
12. **Follett, E. A. C., C. R. Pringle, T. H. Pennington, and P. Shirodaria.** 1976. Events following the infection of enucleate cells with measles virus. J. Gen. Virol. **32**:163–175.
13. **Kimelman, D., and M. W. Kirschner.** 1989. An antisense mRNA directs the covalent modification of transcript encoding fibroblast growth factor in Xenopus oocytes. Cell **59**:687–696.
14. **Kipps, A., G. Dick, and J. W. Moodie.** 1983. Measles and the central nervous system. Lancet **ii**:1406–1410.
15. **Kristensson, K., and E. Norrby.** 1986. Persistence of RNA viruses in the central nervous system. Annu. Rev. Microbiol. **40**:159–184.
16. **Lamb, R. A., and G. Dreyfuss.** 1989. Unwinding with a vengeance. Nature (London) **337**:19–20.
17. **Liebert, U. G., K. Baczko, H. Budka, and V. ter Meulen.** 1986. Restricted expression of measles virus proteins in brains from cases of subacute sclerosing panencephalitis. J. Gen. Virol. **67**:2435–2444.
18. **Manley, J. L., A. Fire, M. Samuels, and P. A. Sharp.** 1983. In vitro transcription: whole-cell extract. Methods Enzymol. **101**: 568–582.
19. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12**:7035–7056.

19a. **Morrissey, L. M., and K. Kirkegaard.** 1991. Regulation of a double-stranded RNA modification activity in human cells. Mol. Cell. Biol. **11**:3719–3725.

20. **Norrby, E., and M. N. Oxman.** 1990. Measles virus, p. 1013–1044. *In* B. N. Fields (ed.), Virology, vol. 1. Raven Press, New York.
21. **Oyanagi, S., V. ter Meulen, M. Katz, and H. Koprowski.** 1971. Comparison of subacute sclerosing panencephalitis and measles viruses: an electron microscope study. J. Virol. **7**:176–187.
22. **Oyanagi, S., V. ter Meulen, D. Muller, M. Katz, and H. Koprowski.** 1970. Electron microscopic observations in subacute sclerosing panencephalitis brain cell cultures: their correlation with cytochemical and immunocytological findings. J. Virol. **6**:370–379.
23. **Raine, C. S., L. A. Feldman, R. D. Sheppard, and M. B. Bornstein.** 1969. Ultrastructure of measles virus in cultures of hamster cerebellum. J. Virol. **4**:169–181.
24. **Raine, C. S., L. A. Feldman, R. D. Sheppard, and M. B. Bornstein.** 1971. Ultrastructural study of long-term measles infection in cultures of hamster dorsal-root ganglion. J. Virol. **8**:318–329.
25. **Schluederberg, A., and S. Chavanich.** 1974. The role of the nucleus in measles virus replication. Med. Microbiol. Immunol. **160**:85–90.
26. **Sommer, B., M. Kohler, R. Sprengel, and P. H. Seeburg.** 1991. RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. Cell **67**:11–19.
27. **ter Meulen, V., J. R. Stephenson, and H. W. Kreth.** 1983. Subacute sclerosing panencephalitis. Compr. Virol. **18**:105–159.
28. **Wagner, R. W., and K. Nishikura.** 1988. Cell cycle expression of RNA duplex unwindase activity in mammalian cells. Mol. Cell. Biol. **8**:770–777.
29. **Wagner, R. W., J. E. Smith, B. S. Cooperman, and K. Nishikura.** 1989. A double-stranded RNA unwinding activity introduces structural alterations by means of adenosine to

inosine conversions in mammalian cells and Xenopus eggs. Proc. Natl. Acad. Sci. USA **86**:2647–2651.
30. **Wechsler, S. L., and B. N. Fields.** 1978. Intracellular synthesis of measles virus-specified polypeptides. J. Virol. **25**:285–297.
31. **Wechsler, S. L., and H. C. Meissner.** 1982. Measles and SSPE viruses: similarities and differences. Prog. Med. Virol. **28**:65–95.
32. **Wong, T. C., M. Ayata, A. Hirano, Y. Yoshikawa, H. Tsuruoka, and K. Yamanouchi.** 1989. Generalized and localized biased hypermutation affecting the matrix gene of a measles virus strain that causes subacute sclerosing panencephalitis. J. Virol. **63**:5464–5468.
33. **Wong, T. C., M. Ayata, S. Ueda, and A. Hirano.** 1991. Role of biased hypermutation in evolution of subacute sclerosing panencephalitis virus from progenitor acute measles virus. J. Virol. **65**:2191–2199.
34. **Wong, T. C., G. Wipf, and A. Hirano.** 1987. The measles virus matrix gene and gene product defined by in vitro and in vivo expression. Virology **157**:497–508.