JOURNAL OF VIROLOGY, Mar. 1989, p. 1162–1173
0022-538X/89/031162-12$02.00/0

Vol. 63, No. 3

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

MINORU AYATA, AKIKO HIRANO, AND TIMOTHY C. WONG*

Department of Microbiology, University of Washington School of Medicine, Seattle, Washington 98195

Received 17 August 1988/Accepted 16 November 1988

Biken strain, a nonproductive measles viruslike agent isolated from a subacute sclerosing panencephalitis (SSPE) patient, contains a posttranscriptional defect affecting matrix (M) protein. A putative M protein was translated in vitro with RNA from Biken strain-infected cells. A similar protein was detected in vivo by an antiserum against a peptide synthesized from the cloned M gene of Edmonston strain measles virus. By using a novel method, full-length cDNAs of the Biken M gene were selectively cloned. The cloned Biken M gene contained an open reading frame which encoded 8 extra carboxy-terminal amino acid residues and 20 amino acid substitutions predicted to affect both the hydrophobicity and secondary structure of the gene product. The cloned gene was expressed in vitro and in vivo into a 37,500 M_r protein electrophoretically and antigenically distinct from the M protein of Edmonston strain but identical to the M protein in Biken strain-infected cells. Chimeric M proteins synthesized in vitro and in vivo showed that the mutations in the carboxy-proximal region altered the local antigenicity and those in the amino region affected the overall protein conformation. The protein expressed from the Biken M gene was unstable in vivo. Instability was attributed to multiple mutations in both the amino and carboxy regions. A surprising number of mutations in both the coding and noncoding regions of the Biken M gene were identical to those in an independently isolated SSPE virus strain with a similar defect. These results offer insights into the basis of the defect in Biken strain and pose intriguing questions about the evolutionary origins of SSPE viruses in general.

Measles virus typically causes acute and highly contagious infections in children and young adults. On rare occasions, a few individuals might develop a chronic infection in the central nerous system (CNS) years after exposure in a condition known as subacute sclerosing panencephalitis (SSPE) (41). Unlike acute measles virus infections, SSPE is always fatal but noncontagious. Typically, measles virus-related antigens and RNA can be detected in the CNS of SSPE patients, but infectious virus can rarely be isolated. Successful isolation has occasionally been accomplished by cocultivating the infected CNS materials with permissive primate cells. The virus thus isolated may continue to replicate in a cell-associated manner and produce no extracellular virions, or it may become productive after in vitro passages (45). However, only the nonproductive strains retain neurovirulence in experimental animals (33, 42). Therefore, the basis of nonproductive infection, a hallmark of SSPE, is of great interest.

Studies have linked nonproductive infections by SSPE strains to abnormal expression of several viral proteins. The most common defects affect the matrix (M) protein, which forms the intermediary layer between the ribonucleoprotein core and the outer lipid envelope of the virion (12, 24). Serum samples from SSPE patients frequently lack antibody activities against M protein despite having high activities against other measles virus proteins, and brain materials from SSPE patients often contain no detectable M protein (1, 11, 17, 18, 46). Biochemical analyses have revealed possible defects affecting transcription, translation, or stability of the M protein in different cases (2, 7, 10, 40). More recent studies indicate that in some cases of SSPE, both M protein and antibodies specific for M protein can be detected (2, 14, 27, 31, 32). Furthermore, abnormalities in other viral proteins, especially the envelope-associated hemagglutinin (H) and fusion (F) proteins, have also been observed (2, 27). Current evidence suggests that an abnormality in any one of several viral proteins could affect virus maturation and might contribute to a nonproductive infection. A clear knowledge of the putative viral defects is important for understanding the basis of this type of infections.

Biken strain is a measles viruslike agent that was isolated from an SSPE patient by cocultivating the CNS materials with human embryonic lung (HEL) cells (43). The agent is nonproductive and spreads from cell to cell by fusion. Biochemical analysis of the Biken agent has thus far yielded conflicting data. In an early study, nonproductive infection was attributed to abnormality in H function (5). Lin and Thormar later suggested that the defect was due to lack of M protein (28). In contrast, by immunofluorescence techniques, Johnson et al. detected low levels of M protein in Biken strain-infected cells (23). However, this observation was not confirmed by a later study involving 14 independent monoclonal antisera (39).

In this study, we used biochemical, recombinant DNA, and immunological approaches to identify and define a defect affecting the M protein of Biken strain. The results show that Biken strain indeed encodes an M-related protein. However, the Biken M gene is riddled with mutations predicted to affect both the hydrophobicity and secondary structure of the gene product. By DNA-mediated gene transfer, the cloned Biken M gene produces a structurally altered M protein with a half-life 16 times shorter than that of the M protein of Edmonston strain measles virus. By expressing chimeric M proteins containing different Biken mutations, the structural alterations and instability of the protein were attributed to multiple mutations in the amino

* Corresponding author.

and carboxy regions. The mutations in the Biken M gene were surprisingly similar to those in an independently isolated SSPE virus strain with a similar defect. These results shed light on the possible mechanism of the defect in the Biken M protein and pose interesting questions about the origins of SSPE virus strains.

MATERIALS AND METHODS

Cells and viruses. Biken strain isolated in Japan by cocultivating infected brain materials from an SSPE patient with HEL cells (43) was a kind gift from S. Ueda, Osaka University, Osaka, Japan. The chronically infected Biken-HEL cells produced no extracellular virions and were propagated by occasionally being replenished with fresh HEL cells in Eagle minimal essential medium supplemented with 10% fetal calf serum. Edmonstron strain measles virus was propagated in Vero cells or African green monkey kidney (CV-1) cells in the same medium. Simian virus SV40-transformed CV-1 (COS) cells have been described previously (15).

Antisera. The GM antiserum against total measles virus proteins was also a kind gift from S. Ueda and was originally prepared by immunizing a female African green monkey with CV-1 cells infected with Nagahata strain measles virus (44). The GM serum recognized all the structural proteins of both Nagahata and Edmonston strain measles virus.

Mouse monoclonal antibodies were prepared against affinity column-purified Nagahata strain measles virus antigens by using a previously described procedure (21). Hybrid cell lines producing measles virus-specific antibodies were identified by an indirect immunofluorescence assay, and the antibody specificities were determined by radioimmune precipitation. Specific antibodies were harvested either from the cell culture fluid or from ascites fluid from syngeneic animals which received the hybridoma cells.

Region-specific polyclonal antisera were prepared against peptides expressed from cloned DNA in bacteria. To generate the M-BC antiserum against the entire Edmonston M protein, we inserted the *Bgl*II-*Cla*I fragment from cDNA clone pcD-M2i (48) into a procaryotic expression vector containing an inducible *trp*E promoter (36) (see Fig. 6). The hybrid protein containing *trpE* and M sequences produced in *E. coli* HB101 was purified by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis (SDS-PAGE) and injected subcutaneously into a female New Zealand White rabbit in two 200- to 300-μg doses administered 2 weeks apart. Sera collected at 1-week intervals after the second injection were tested by radioimmune precipitation. The M-BE and M-EE antisera against the amino and carboxy regions of the Edmonston M protein were prepared by the same method with DNA fragments spanning the *Bgl*II and the 5′-proximal *Eco*RI sites or the two *Eco*RI sites in pcD-M2i, respectively (48) (see Fig. 6). Similarly, the N-EE antiserum was prepared against a peptide encoded by the sequence between two *Eco*RI sites in the N gene of Edmonston strain (S. Castaneda and T. Wong, submitted for publication).

Protein and RNA analyses. Cellular proteins were labeled, immunoprecipitated, and analyzed by SDS-PAGE as previously described (48), except that CL-4B protein A-Sepharose (Pharmacia) instead of fixed *Staphylococcus aureus* (Calbiochem-Behring) was used to recover the immune complexes in some experiments.

DNA probes specific for the N, P, M, F, and H genes of measles virus were prepared from full-length cDNA clones (47, 48; T. Wong, G. Wipf, M. Ayata, and A. Hirano, manuscript in preparation). RNA was prepared for Northern (RNA) blot analysis as described previously (48), except that electrophoresis was performed in a 1% agarose gel containing formaldehyde instead of methylmercuric hydroxide (29).

Novel approach for selective cloning of specific full-length cDNA. A novel simple method was devised to selectively obtain full-length cDNA representing any gene for which the 5′ sequence is known or can be deduced from other sources such as genomic sequence. The vector pTZ18RX, generated by inserting a *Xho*I site at the original *Sma*I site in pTZ18R (Pharmacia, Inc.), was linearized at the *Pst*I site, tailed with oligo(dT), and recut with *Xba*I (Fig. 1). The gel-purified oligo(dT)-tailed vector (2.8 μg or 1.4 pmol) was annealed with 5 μg of poly(A)$^+$ RNA, isolated from Biken-HEL cells when cytopathic effects were maximal, to prime the synthesis of the first cDNA strand in a fashion similar to the Okayama-Berg procedure (34). The first cDNA strand was synthesized at 37°C for 1 h in 50 μl of a reaction mixture containing 50 mM Tris hydrochloride (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM each dATP, dGTP, dCTP, and TTP, 50 U of RNasin (Promega Biotec), and 1,000 U of reverse transcriptase from Moloney murine leukemia virus (Bethesda Research Laboratories, Inc.). Reaction products were extracted with phenol-chloroform, precipitated with ethanol, and suspended in Tris hydrochloride (pH 8.0) containing 1 mM EDTA. A portion (10%) of the reaction products was treated with 50 mM NaOH at 65°C for 1 h to hydrolyze RNA. After neutralizing with Tris hydrochloride (pH 8.0) and HCl, 1 pmol of a synthetic oligonucleotide (5′-AGGAGCAAAGTGATTGCCTC-3′) corresponding to the 5′ mRNA terminus of the M gene of Edmonston strain was added and annealed to full-length M-specific first-strand cDNA by being heated to 65°C for 2 min and slowly cooled to room temperature (Fig. 1). The mixture was adjusted to 100 μl, containing 50 mM Tris hydrochloride (pH 7.4); 5 mM $MgCl_2$; 5 mM dithiothreitol; 50 μg of bovine serum albumin per ml; 50 μM each dATP, dGTP, dCTP, and TTP; and 10 U of the Klenow fragment of *Escherichia coli* DNA polymerase I. Second-strand synthesis was carried out at 15°C overnight. The vector-cDNA was circularized by T4 DNA ligase and transformed into competent *E. coli* x1776 or DH5 (29). Clones containing the Biken M gene were identified by colony hybridization with a DNA probe prepared from the Edmonston M gene. Full-length clones were identified by regeneration of an *Xba*I site at the 5′ end of the cDNA insert and confirmed by sequence analysis.

Structural analysis of the cloned gene. Sequence analysis was carried out by a dideoxynucleotide-induced chain termination method in both directions (38), with overlapping deletion clones generated by digestion with either *Exo*III nuclease (19) or specific restriction endonucleases.

Hydropathy and secondary protein structure were deduced from sequence information by using criteria described by Kyte and Doolittle (26) and Chou and Fasman (13), respectively, and a GenePro computer program (Riverside Scientific Enterprises, Seattle, Wash.).

Construction of chimeric M genes. To generate chimeric M genes for in vitro expression, the *Eco*RI sites in the vector sequences of parental clones pTZ-EM1 and pTZ-BM1, which contained the full-length M genes of Edmonston and Biken strains, respectively, were first destroyed. DNA fragments from the *Bgl*II site to the 5′-proximal *Eco*RI site, between the two *Eco*RI sites, and from the 3′-proximal *Eco*RI site to a *Hin*dIII site in the vector were isolated from the parental clones by gel electrophoresis and ligated with

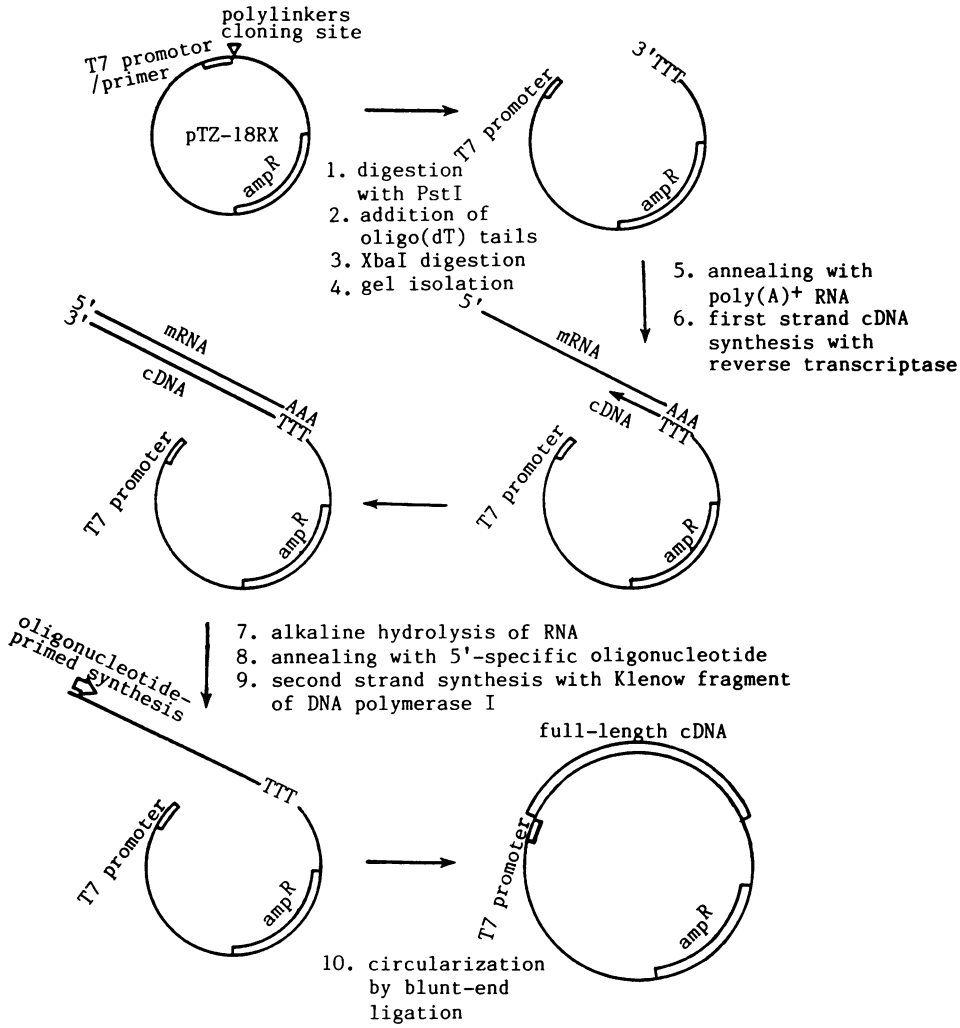

FIG. 1. Simple method for selective cloning of expressible full-length cDNAs from specific genes (see Materials and Methods).

the appropriate recipient clone to generate a series of chimeric genes containing different Edmonston and Biken sequences (see Fig. 8). For in vivo expression, the entire series of chimeric M genes were released from the pTZ vectors by digestion with *Bam*HI and *Hin*dIII and inserted into the *Bam*HI site downstream of the SV40 early promoter in the pcD vector (34) by using a *Bam*HI linker (see Fig. 9).

In vivo expression of cloned genes. Cultures containing 1.5 $\times$ 10^6 COS cells (15) were transfected with 20 μg of cloned DNA as previously described (48). Cellular proteins were labeled for 6 h with 25 to 30 μCi of [^{35}S]methionine (3,000 Ci/mmol; Du Pont; NEN Research Products) at 45 h post-transfection and analyzed as described above.

In vitro expression of cloned genes. After the plasmids were linearized at the *Hin*dIII site downstream of the cDNA inserts, RNA was transcribed in vitro by T7 RNA polymerase from the cDNA clones which contained an integral T7 promoter (30) (Fig. 1). Equal amounts (1 μg) of RNA were translated in rabbit reticulocyte lysates (Promega Biotec) in the presence of [^{35}S]methionine (25). Equal amounts of proteins standardized by scintillation counts of trichloroacetic acid-precipitated radioactivity were immunoprecipitated with antisera and analyzed by SDS-PAGE.

RESULTS

Posttranscriptional defect affecting M protein of Biken strain. We first compared the overall gene expression of Biken strain with wild-type (Edmonston strain) measles virus at the protein and RNA levels. Edmonston strain-infected CV-1 cells produced the N (nucleoprotein), P (phosphoprotein), M, F_1 (cleavage product of F), and H measles virus proteins detectable by the appropriate monoclonal antisera (Fig. 2A, lanes N, P, M, F, and H, respectively). All the corresponding proteins except M were detected in Biken strain-infected human embryonic lung cells (Biken-HEL) (Fig. 2B, lanes N, P, M, F, and H, arrowheads). (The autoradiogram for Biken-HEL cells was overexposed to show the lack of detectable M protein. The minor bands near the expected position of M protein indicated by asterisks are of cellular origin.) Similarly, an antiserum against total measles viral proteins (GM serum [44]) which recognized the H, P, N, F, and M proteins in Edmonston strain-infected cells (Fig. 2A, lane GM) also failed to detect M protein in Biken-HEL cells (Fig. 2B, lane GM). To rule out possible host cell differences, we also examined the viral proteins in

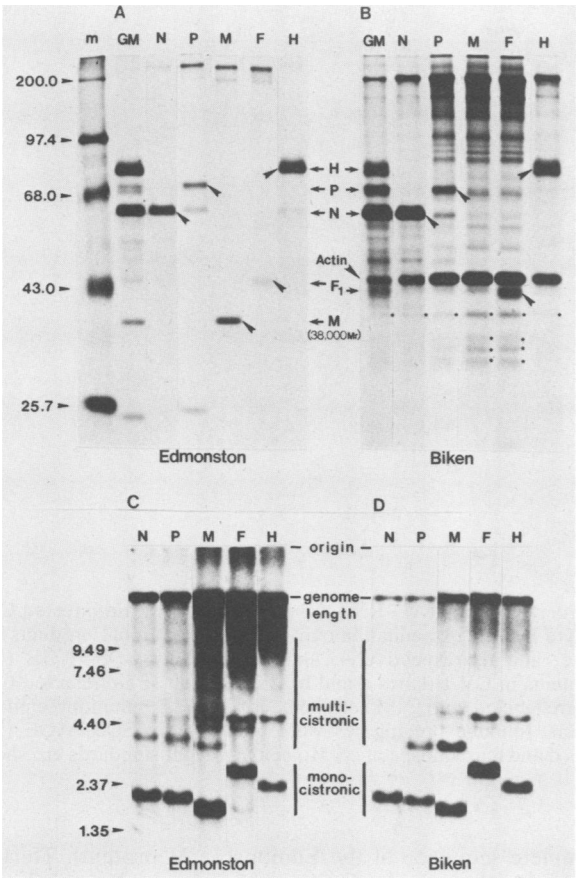

FIG. 2. Viral proteins and RNAs in Biken strain-infected cells. Proteins in Edmonston strain-infected CV-1 cells (A) or Biken-HEL cells (B) were labeled with [^{35}S]methionine for 12 h and immunoprecipitated with the GM antiserum against total measles viral proteins (lane GM), or with monoclonal antibodies against the N (lane N), P (lane P), M (Cl.99, lane M), F (lane F), and H (lane H) proteins of measles virus. Immune precipitates were analyzed by SDS-PAGE (10% polyacrylamide). The first lane represents molecular weight standards (shown in thousands). Total RNA purified from Edmonston strain-infected CV-1 cells (C) and Biken-HEL cells (D) was resolved by electrophoresis in a 1% agarose gel containing formaldehyde, transferred onto nitrocellulose filter paper, and hybridized successively with ^{32}P-labeled DNA probes specific for the N, P, M, F, or H gene of Edmonston strain (shown in lanes with the same letters). Size markers are shown in kilobases.

Biken strain-infected CV-1 or Vero cells. Again, M protein was not detected by these antisera (data not shown).

The apparent absence of M protein in Biken-HEL cells was not due to a transcriptional defect, since the accumulated levels of monocistronic RNAs, multicistronic readthrough RNAs, and genome-length RNAs were comparable in both Biken-HEL and Edmonston strain-infected cells, as shown by Northern blot analysis with five measles virus gene-specific DNA probes, including M (Fig. 2C and D, lanes N, P, M, F, and H). Although we did not test for expression from the L gene, which encodes the presumed RNA polymerase (4), active transcription from the other viral genes implied a functional polymerase activity. Thus, the apparent defect in M protein of Biken strain occurs at a posttranscriptional level.

Translation of an M-related protein from Biken-HEL RNA in vitro. The failure to detect M protein despite high levels of M-specific RNA in Biken-HEL cells could be due to a translational defect. Alternatively, it was equally possible that the Biken M protein assumed an antigenically altered conformation which was not recognized by the antisera. To distinguish between these two possibilities, polyadenylated RNA was prepared from Biken-HEL and Edmonston strain-infected CV-1 cells and translated in vitro in rabbit reticulocyte lysates. To maximize the chance of detecting a possible M protein, a peptide containing nearly the entire M protein of Edmonston strain was overproduced in bacteria from cloned DNA spanning the *Bgl*II to *Cla*I sites of the Edmonston M gene and used to generate a polyclonal antiserum called M-BC (see Fig. 6 and Materials and Methods). Another antiserum similarly prepared against the N protein of Edmonston strain served as a control (N-EE serum).

Three major proteins were immunoprecipitated by the GM antiserum from the translational products of RNA from Edmonston strain-infected cells (Fig. 3A, lanes a and b). Two of these proteins were identified as the M and N proteins by the M-BC and N-EE antisera, respectively (Fig. 3A, lanes c and d, respectively). In contrast, the M protein was not immunoprecipitated by the GM antiserum from the in vitro-translated products of Biken-HEL RNA (Fig. 3B, lanes a and b). To our surprise, the M-BC antiserum immunoprecipitated a 37,500 M_r protein from the same translational products (Fig. 3B, lane c, arrowhead). Immunoprecipitation of this protein was specific, since the N-EE antiserum recognized the N protein but not the 37,500 M_r protein (Fig. 3B, lane d). These results provided the first hint that Biken strain might produce an M-related protein.

Biken-HEL cells produce an altered M protein in vivo. To see whether the putative M protein was actually produced in vivo, we immunoprecipitated the intracellular proteins in Biken-HEL cells with the M-BC antiserum. This serum clearly precipitated a low but detectable amount of a 37,500 M_r protein in Biken-HEL cells (Fig. 3C, lane f) which was not present in uninfected CV-1 or HEL cells (Fig. 3C, lanes a, b, g, and h). Since this protein was not recognized by the GM antiserum (Fig. 3C, lane e), it might represent an antigenically altered M protein. The apparent low level of this protein could be due to inefficient recognition by the antiserum. Alternatively, additional defects might affect the expression or stability of the protein. Furthermore, it remained possible that the 37,500 M_r protein was a cross-reacting cellular product induced by Biken virus infection.

Selective cloning of the Biken M gene by a novel approach. To allow definitive identification and detailed analysis of the putative Biken M protein, we developed a simple general method to obtain full-length cDNAs from specific genes. This method involved the use of an oligo(dT)-tailed vector to synthesize the first cDNA strand from poly(A)$^+$ RNA from Biken-HEL cells. The second DNA strand was selectively primed by a synthetic oligonucleotide specific for the 5′ terminus of the Edmonston M gene (Fig. 1) (see Materials and Methods). The vector and primer were designed to regenerate an *Xba*I recognition site only if the second strand was correctly primed on a full-length first-strand cDNA. These features simplied the production and identification of the desired full-length cDNA clones. All the M-specific clones initially identified by hybridization with a DNA probe prepared from the Edmonston M gene were found to contain full-length cDNAs of the Biken M gene.

Structure of the Biken M gene. The sequence of a full-length clone (pTZ-BM1) showed that the Biken M gene is 1,462 nucleotides (nt) long excluding the poly(A) tract, identical in length to the M gene of the Edmonston strain

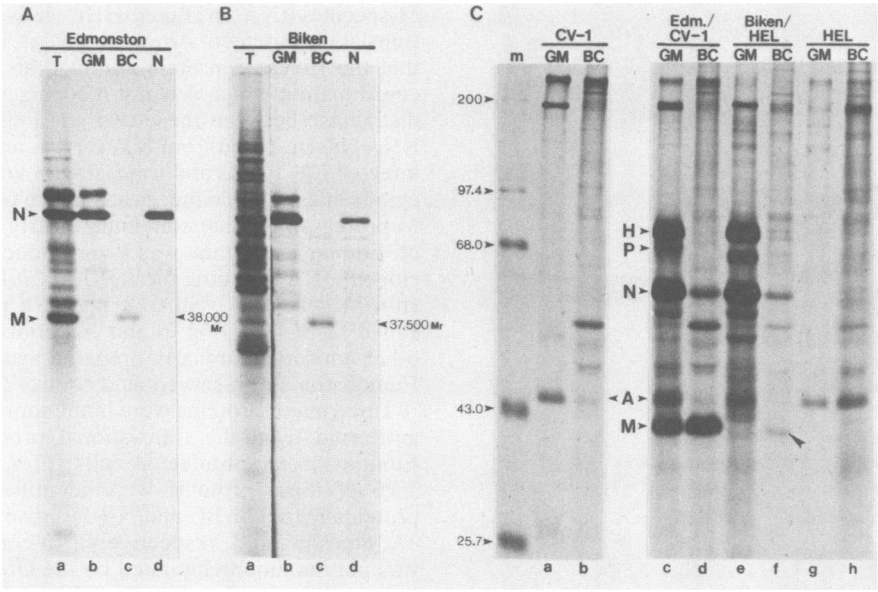

FIG. 3. Putative Biken M protein detected in vitro and in vivo. One microgram of poly(A)$^+$ RNA from Edmonston strain-infected CV-1 cells (A) or Biken-HEL cells (B) was translated in vitro in rabbit reticulocyte lysates. An equal amount of the translational products was immunoprecipitated with the GM, M-BC, or N-EE antisera (lanes b, c, and d, respectively), and analyzed by SDS-PAGE (10% polyacrylamide) along with the total translational products (lane a). (C) Proteins in CV-1 (lanes a and b), Edmonston strain-infected CV-1 (lanes c and d), Biken-HEL (lanes e and f), and HEL (lanes g and h) cells were labeled with [^{35}S]methionine for 4 h and immunoprecipitated with GM (lanes a, c, e, and g) or M-BC (lanes b, d, f, and h) antiserum. Immune precipitates were analyzed by SDS-PAGE (10% polyacrylamide). (The 50,000 to 60,000 M_r bands above the M protein in lanes d and f are nonspecific.) Molecular weight standards are shown in thousands.

previously determined from full-length cDNA clone pcD-M2i (48). The Biken M gene contains an open reading frame beginning at the same translational start ATG codon (nt 33) as in the Edmonston M gene (3). However, a T-to-C transition at position 1038 abolishes the normal translational termination codon and lengthens the protein-coding region by 8 triplet codons (Fig. 4). Translation is predicted to terminate at position 1062, where another C-to-T transition has generated a TGA codon (Fig. 4). As a result, the Biken M gene potentially encodes a protein which contains 343 instead of 335 amino acid residues, with a calculated molecular weight of 38,249. Downstream of the new termination codon is a long 3′ untranslated region with two potential overlapping open reading frames preceded by ATG codons. However, as in the Edmonston M gene, these open reading frames are not followed by translational termination codons.

The M genes of Biken and Edmonston strains differ in 65 positions, of which 40 lie in the protein-coding sequence and 25 lie in the normally untranslated 3′ region (Fig. 4). Thus, the overall sequences differ by about 4.4%, and the 3′ untranslated regions are more diverged, differing by about 6.1%. Some mutations are not found in all the Biken M cDNA clones analyzed and probably represent genetic variation characteristic of SSPE virus strains (see below). A surprising number of mutations are shared with another SSPE virus strain recently analyzed (Fig. 4, arrowhead) (10). The significance of this is discussed below (see Discussion).

Predicted changes in the Biken M protein. On the basis of parameters described by Chou and Fasman (13), the amino half of the Edmonston M protein encoded between the *Bgl*II and the 5′-proximal *Eco*RI sites is predicted to contain several beta-sheet structures (Fig. 5, parallel bars) and a potential alpha helix (overlapping circles) interrupted by multiple potential beta turns (crosses; see reference 3 for the complete sequence of the Edmonston M protein). Thirteen of the 20 amino acid substitutions in the predicted Biken M protein occur in this amino region. With the exception of Leu-17, all the amino-proximal substitutions decrease the hydropathy values according to a scale proposed by Kyte and Doolittle (26), resulting in a noticeable shift toward hydrophilicity in the hydropathy profile of this region. For instance, the inverted hydrophilic peak between Pro-42 and Leu-50 is exaggerated, and the hydrophobic peaks around Ser-54, Pro-76, and especially Asn-142 and Thr-147 are all compromised (Fig. 5). Leu-17, the only substitution in the amino region with increased hydropathy, occurs only in two of four full-length cDNA clones analyzed and might simply reflect genetic variation common in SSPE virus strains (10).

In addition to their effect on hydrophobicity, the substitutions by Pro-42 and Pro-76 near two potential beta turns are predicted to extend the regions involved in the turns as a result of the turn-promoting character of proline (Fig. 5, crosses) (37). Furthermore, the Lys-89–to–Glu-89 substitution might extend a potential alpha helix as a result of the helix-forming potential of glutamic acid (Fig. 5, overlapping circles). These amino-proximal mutations might alter the secondary protein structure and, with the reduced hydrophobicity described above, could drastically affect the folding and tertiary structure of the protein.

By contrast, most of the substitutions in the carboxy region encoded between the two *Eco*RI sites do not markedly affect the hydrophobicity of the protein. Perhaps the most nonconservative mutations in that region are Ala-192 and Ala-209, which might affect a potential beta-sheet structure and a potential alpha helix, respectively (Fig. 5, parallel bars and overlapping circles, respectively). However, none of the carboxy-proximal mutations affects the predicted beta turn nor generates new potential turns. The extra carboxy-

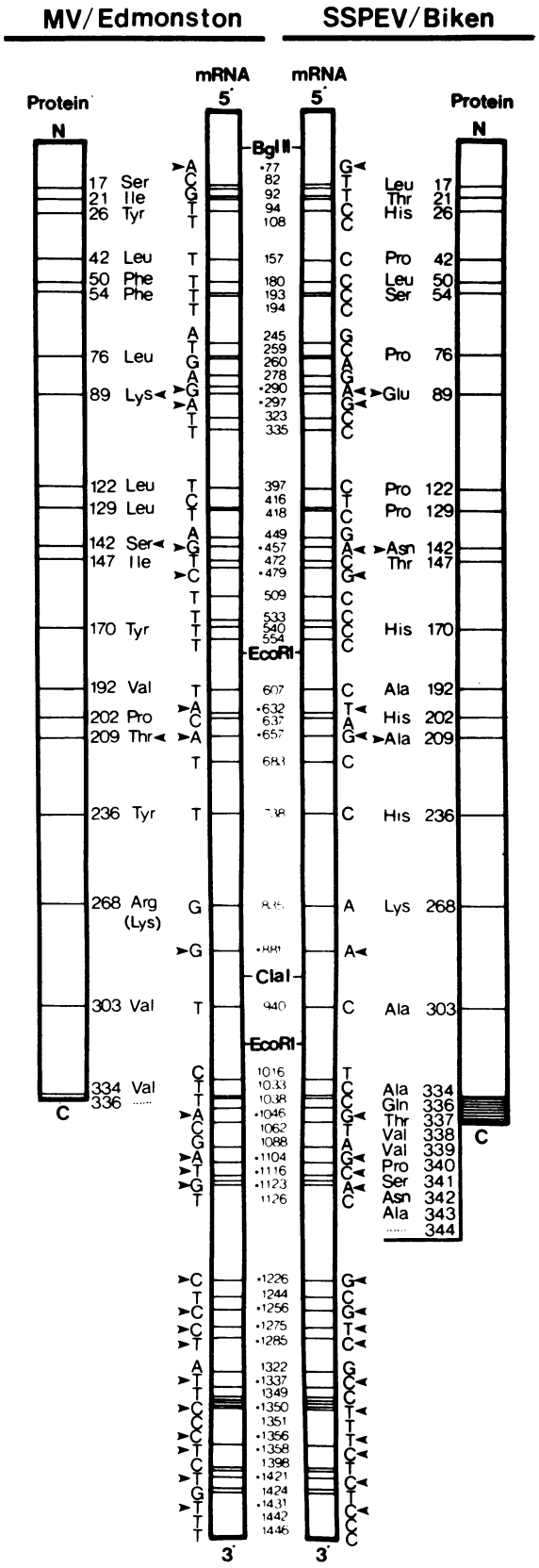

FIG. 4. Sequence comparison between the Biken and Edmonston M genes. The complete sequence of the Biken M gene in full-length clone pTZ-BM1 was compared with pcD-M2i (48). The sequence shows the mRNA strand with the first adenine residue corresponding to the 5' terminus of the mRNA as nucleotide 1. Nucleotide differences are identified by their nucleotide numbers. The protein-coding regions are shown as open boxes next to the cDNAs. Predicted amino acid substitutions are identified by their amino acid residue numbers. The apparent mutation at Lys-268 reflects variation among different virus stocks, since the Edmonston M gene sequenced by Bellini et al. and Cattaneo et al. contains a lysine at this position (3, 8). Mutations shared between Biken and IP-3 strains are indicated by arrowheads (see Discussion).

terminal residues in the predicted Biken M protein are either hydrophobic (Val-338, Val-339, Pro-340, and Ala-343) or polar (Gln-336, Thr-337, Ser-341, and Asn-342). As discussed below, these residues also affect the overall nature of the protein.

In vitro and in vivo expression of an antigenically altered M protein from the cloned Biken M gene. The predicted Biken M protein has a molecular weight higher than that of the Edmonston M protein. However, the putative M protein in Biken-HEL cells migrated faster than the Edmonston M protein (Fig. 3). Since mRNAs of some SSPE virus strains have been found to be heterogeneous (10), it was important to ascertain whether the cloned gene in fact encoded the Biken M protein. RNA was transcribed in vitro from pTZ-BM1 and pTZ-EM1 containing the M genes of Biken and Edmonston strains, respectively (see Materials and Methods), and the proteins translated from these RNAs in rabbit reticulocyte lysates were analyzed by SDS-PAGE.

The cloned M genes of Edmonston and Biken strains both produced a single protein in vitro (Fig. 6A, lanes a and h). The Biken M gene product indeed migrated slightly faster than the Edmonston M gene product. Furthermore, the Edmonston M gene product was immunoprecipitated by the GM, M-BC, and monoclonal Cl.99 antisera (Fig. 6A, lanes b, c, and f, respectively), but the Biken M gene product was recognized only by the M-BC antiserum but not by the GM and Cl.99 antisera (Fig. 6A, lanes j, i, and m, respectively), just as observed in Biken-HEL cells. As expected, neither product was recognized by the anti-N serum (Fig. 6A, lanes g and n, respectively).

To test whether the Biken M gene also produced the same protein in vivo, the cloned gene was placed under the control of the early promoter of SV40 (pcD-BM1) and transfected into SV40-transformed CV-1 (COS) cells (15). Expression was compared with that of an equivalent plasmid containing the Edmonston M gene (pcD-PM-M). Cells transfected with the Edmonston M gene produced a 38,000 M_r protein immunoprecipitated by the GM, M-BC, and Cl.99 antisera (Fig. 6B, lanes a, b, and e, respectively). In contrast, cells transfected with the Biken M gene expressed a low but detectable level of 37,500 M_r protein, which was recognized only by the M-BC antiserum (Fig. 6B, lanes f, g, and j).

These experiments positively identify both the cloned Biken M gene and the 37,500 M_r M protein in Biken-HEL cells and show that the antigenic alterations in the Biken M protein are virus encoded.

To localize the antigenic alterations, we used two additional antisera, M-BE and M-EE, directed against the amino and carboxy halves of the Edmonston M protein, respectively (see Materials and Methods). Both of these antisera immunoprecipitated the Edmonston M protein synthesized in vitro (Fig. 6A, lanes d and e) and in vivo (Fig. 6B, lanes c and d). However, neither antiserum recognized the Biken M protein synthesized in vitro (Fig. 6A, lanes k and l) or in vivo (Fig. 6B, lanes h and i). Therefore, both the amino and

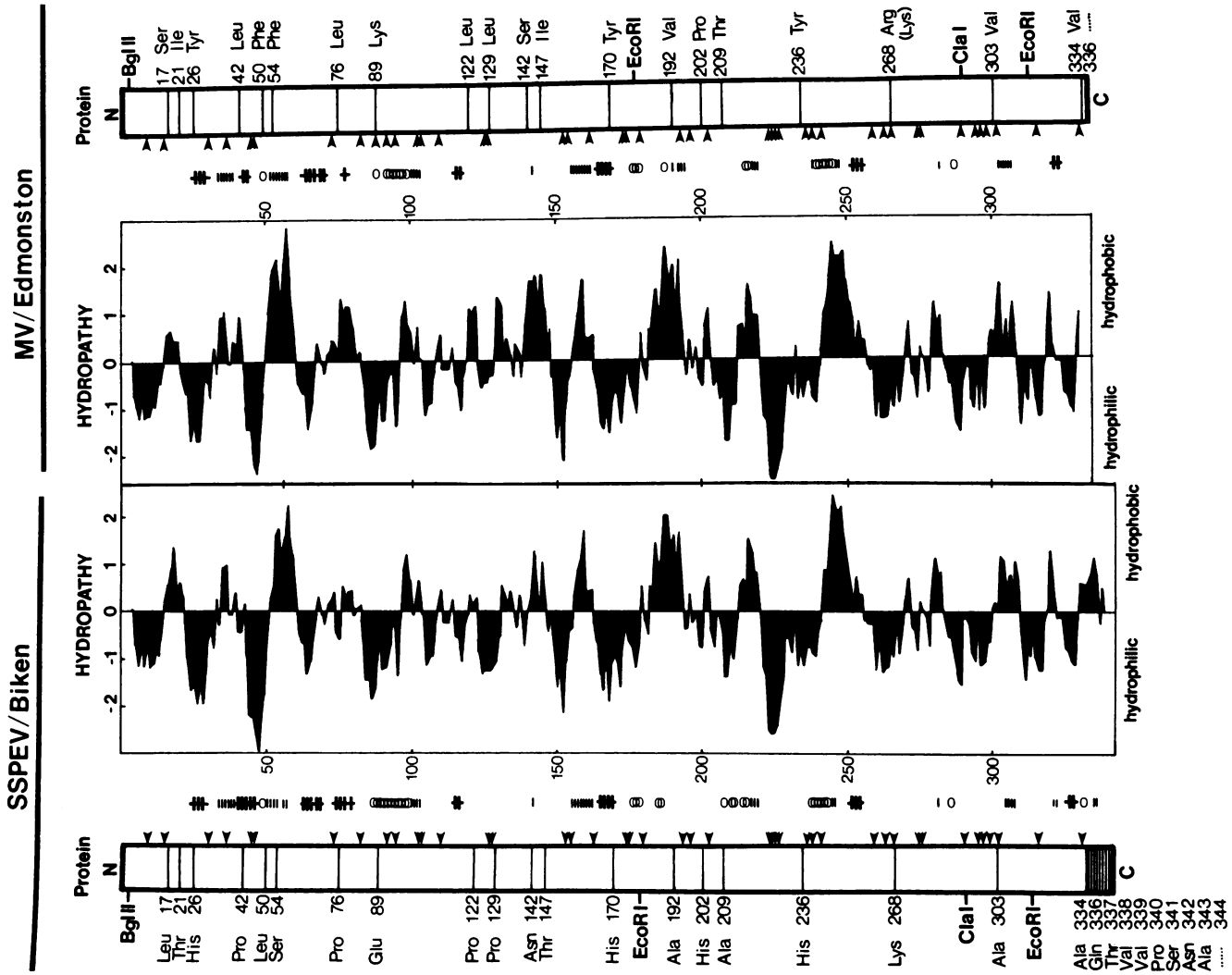

FIG. 5. Predicted hydropathy and secondary structure of the Biken M protein. Open boxes represent the proteins predicted from the Edmonston and Biken M genes showing the identities and positions of amino acid substitutions. The hydropathy profiles were deduced by a GenePro computer program based on a scale developed by Kyte and Doolittle (26). Potential beta-sheet structures (IIIII), alpha helices (OOOOO), and beta turns (+++++) were deduced by using parameters described by Chou and Fasman (13). Locations of the conserved lysine and arginine residues are indicated by arrows.

carboxy halves of the Biken M protein are antigenically altered.

Instability of the Biken M protein in vivo. The M-BC antiserum, which efficiently recognized the Biken M protein translated in vitro, could barely detect the same protein in vivo (compare Fig. 3B, lane c, with Fig. 3C, lane f, and compare Fig. 6A, lane j, with Fig. 6B, lane g). Since the Biken M RNA seemed to be translated efficiently, we compared the half-lives of the M proteins expressed in vivo from the cloned Edmonston and Biken M genes in a pulse-chase experiment.

COS cells transfected with pcD-PM-M or pcD-BM1 (Fig. 6) were pulse-labeled with [^{35}S]methionine for 30 min and chased with unlabeled medium. The intracellular half-life of the Edmonston M protein was about 8 h (Fig. 7, lanes a to d). In contrast, the Biken M protein had a half-life of less than 30 min (Fig. 7, lanes e to h). This explains the discrepancy between the levels of the Biken M protein detected by the M-BC antiserum in vitro and in vivo.

Differential effects of the amino and carboxy mutations on the structure of the M protein. To directly examine the mutational effects on protein structure rather than stability, we constructed chimeric M genes expressible in vitro into chimeric proteins which contained different Edmonston and Biken sequences (Fig. 8). Clone pTZ-EMW/Ba contained the 5′-proximal substitutions predicted to markedly affect both hydrophobicity and secondary structure of the protein (Fig. 5 legend). Clone pTZ-EMW/Bb contained the less disruptive 3′-proximal substitutions (Fig. 8). Clone pTZ-EMW/Bc encoded a protein with the extra carboxy-terminal residues. Clones pTZ-BMW/Ea, pTZ-BMW/Eb, and pTZ-BMW/Ec were designed to test the combined effects of more than one of these mutated regions (Fig. 8). RNAs transcribed from these chimeric genes were translated in vitro, and identical amounts of the radioactively labeled proteins were tested with the different antisera (see Materials and Methods).

The different mutations markedly affected the electrophoretic mobility of the M protein. The amino mutations retarded the electrophoretic mobility (Fig. 8, column T, rows b

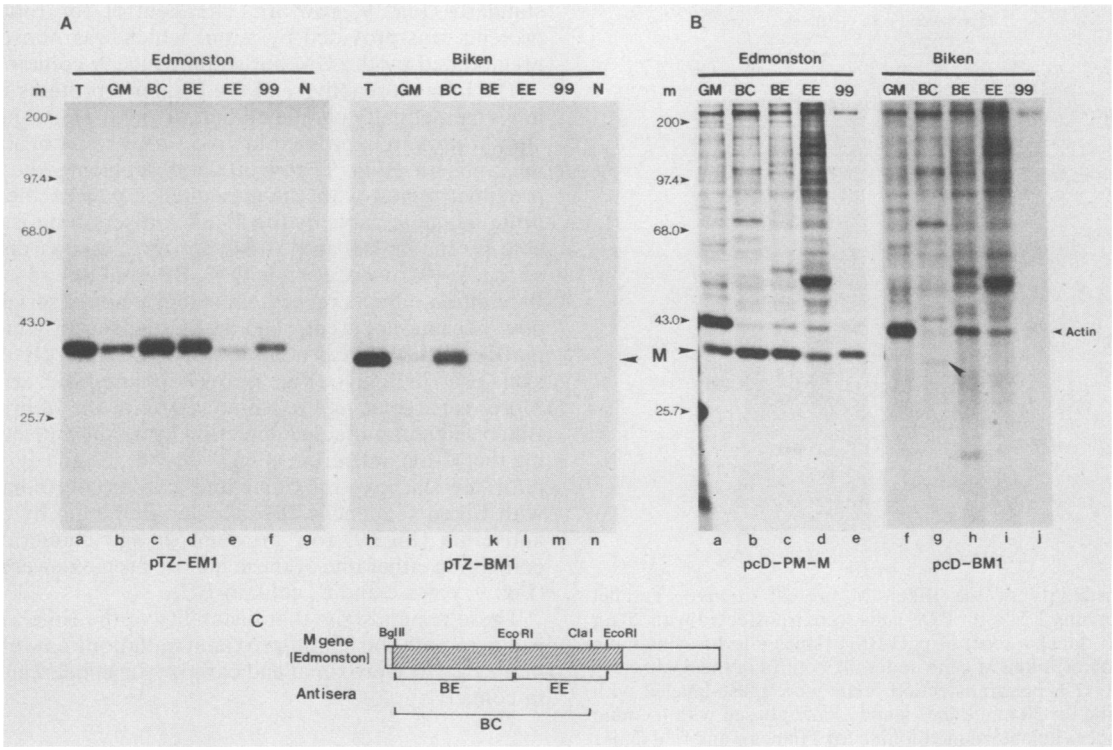

FIG. 6. In vitro and in vivo expression of the cloned Biken M gene. (A) Plasmids pTZ-EM1 and pTZ-BM1 containing the full-length M genes of Edmonston (lanes a to g) and Biken strain, respectively (lanes h to n), were linearized at the *Hin*dIII site downstream of the M gene and transcribed into RNA in vitro by using the integral T7 promoter. One microgram of the in vitro-generated RNA was translated in rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Equal amounts of the translated proteins were immunoprecipitated with the GM (lanes b and i), M-BC (lanes c and j), M-BE (lanes d and k), M-EE (lanes e and l), Cl.99 (lanes f and m), or N-EE antiserum (lanes g and n). Immune precipitates were compared with the total translational products (lanes a and h) by SDS-PAGE (10% polyacrylamide). (B) Amounts of 20 μg of pcD-PM-M or pcD-BM1 plasmid containing the Edmonston (lanes a to e) or Biken M gene (lanes f to j), respectively, driven by the SV40 early promoter were transfected into 1.5×10^6 COS cells. Transfected cells were labeled with 30 μCi of [^{35}S]methionine for 6 h beginning from 45 h posttransfection. Equal amounts of the labeled cell lysates were immunoprecipitated with the GM (lanes a and f), M-BC (lanes b and g), M-BE (lanes c and h), M-EE (lanes d and i), or Cl.99 antiserum (lanes e and j) and analyzed by SDS-PAGE (10% polyacrylamide). (C) Polyclonal antiserum M-BC was prepared against a peptide encoded by almost the entire protein-coding sequence (▨) between the *Bgl*II and *Cla*I sites in pcD-M2i. Amino- and carboxy-specific polyclonal antisera M-BE and M-EE were directed against peptides encoded between the *Bgl*II and *Eco*RI sites, or between the two *Eco*RI sites, respectively (see Materials and Methods).

and g; migration from left to right). Unexpectedly, the added carboxy-terminal residues increased the mobility (Fig. 8, column T, rows d and e). Chimeric proteins which contained both the amino- and carboxy-terminal mutations, including the Biken M protein itself, migrated with an intermediate mobility slightly higher than that of the Edmonston M protein (Fig. 8, column T, rows f and h). Only the mutations in the carboxy-proximal region (excluding the terminus) did not affect mobility (Fig. 8, column T, rows a and c). These results suggest that both the amino- and carboxy-terminal mutations affect the mobility of the Biken M protein, possibly by altering the protein conformation.

Definitive evidence was provided by immunoprecipitating equal amounts of these in vitro-synthesized proteins. The Edmonston M protein from the parental clone was recognized by all the antisera (Fig. 8, row a, columns GM, BC, BE, EE, and 99). Replacing the carboxy half of the M protein with Biken sequence resulted in a chimeric protein undetectable by the carboxy-specific M-EE antiserum but still detectable by the amino-specific M-BE antiserum (Fig. 8, row c, columns EE and BE, respectively). In contrast, replacing the amino half of the M protein with Biken sequence rendered the protein unrecognizable not only by the amino-specific M-BE antiserum but also by the M-EE antiserum (Fig. 8, row b, columns BE and EE, respectively). Since all the chimeric proteins are recognized by the M-BC antiserum (Fig. 8, column BC), the mutations affected the antigenicity and not the protein synthesis. Thus, the carboxy-proximal mutations, excluding the extra carboxy-terminal residues, affected mainly the local antigenicity, and those in the amino half affected antigenicity both locally and in a distal region. Furthermore, a chimeric protein with the extra carboxy-terminal residues was also inefficiently recognized by the M-EE antiserum (Fig. 8, row d, column EE). These results provide strong evidence that the amino- and carboxy-terminal mutations alter the overall protein conformation, as predicted from the nature of the amino acid substitutions (Fig. 5 legend).

Instability of the Biken M protein in vivo is attributed to multiple mutations in both the amino and carboxy regions. To further investigate the effects of the Biken mutations in vivo, the entire series of chimeric M genes were placed under the control of the SV40 early promoter and transfected into COS cells (see Materials and Methods). Northern blot analysis showed that all the transfectants produced equivalent amounts of M-specific RNA (data not shown). Proteins expressed from the chimeric genes in parallel cultures were analyzed by immunoprecipitation and SDS-PAGE. Each

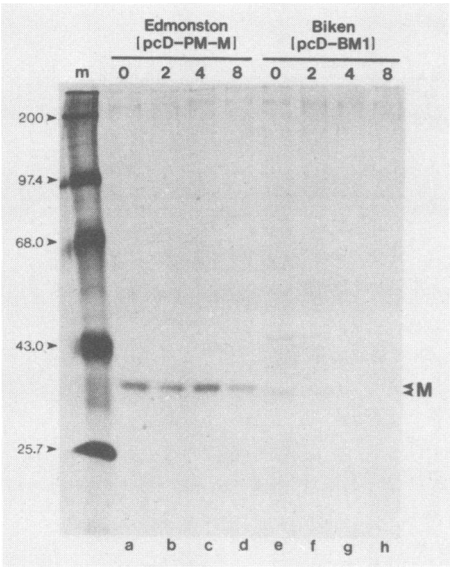

FIG. 7. Instability of the Biken M protein in vivo. Parallel cultures containing 1.5×10^6 COS cells were transfected with 20 μg of pcD-PM-M (lanes a to d) or pcD-BM1 (lanes e to h), containing the Edmonston or Biken M gene under the control of the SV40 early promoter. At 42 h posttransfection, cells were pulse-labeled with [^{35}S]methionine for 30 min (lanes a and e) and chased with medium containing excess unlabeled methionine for 2 (lanes b and f), 4 (lanes c and g), or 8 (lanes d and h) h. Labeled proteins were immunoprecipitated with the M-BC antiserum and analyzed by SDS-PAGE (10% polyacrylamide). Molecular weight standards are shown in thousands.

column in Fig. 9 shows the different chimeric proteins immunoprecipitated with the same antiserum as compared with a parallel transfection with the Edmonston M gene as a standard (Fig. 9, row a). The control for total cellular proteins was provided by actin, which was nonspecifically precipitated by the GM antiserum (Fig. 9, column GM).

The immunoreactivity of the chimeric proteins expressed in vivo essentially paralleled that in vitro. Thus, the Edmonston M protein expressed in vivo was also recognized by all the antisera (Fig. 9, row a), and replacing the carboxy-proximal region with Biken sequence caused the chimeric protein undetectable by the M-EE antiserum but still detectable by the M-BE and M-BC antisera, just as observed in vitro (Fig. 9, row c, columns EE, BE, and BC, respectively). In addition, effects on protein stability not observed in vitro now became apparent. The M-BC antiserum which recognized the Biken M protein in vitro could hardly detect the same protein in vivo (Fig. 9, row h, column BC, arrowhead). Moreover, replacing the amino region of the M protein with Biken sequence affected detection by all the antisera, including the M-BC antiserum (Fig. 9, row b). Similarly, replacing both the carboxy-proximal and carboxy-terminal regions with Biken sequences also affected detection by the M-BC antiserum (Fig. 9, row e), even though chimeric proteins containing either one of these mutated regions were detected (Fig. 9, rows c and d, column BC).

These results show that instability of the Biken M protein is due to both the amino-proximal mutations as well as those in the carboxy-proximal and carboxy-terminal regions acting in concert.

DISCUSSION

The present study shows that Biken strain-infected cells produce a structurally altered M protein as a result of multiple mutations in the M gene. As demonstrated with the chimeric proteins synthesized from the cloned genes in vitro, the amino- and carboxy-terminal mutations markedly alter the overall protein conformation, while the carboxy-proximal mutations affect mainly the local antigenicity. Acting in

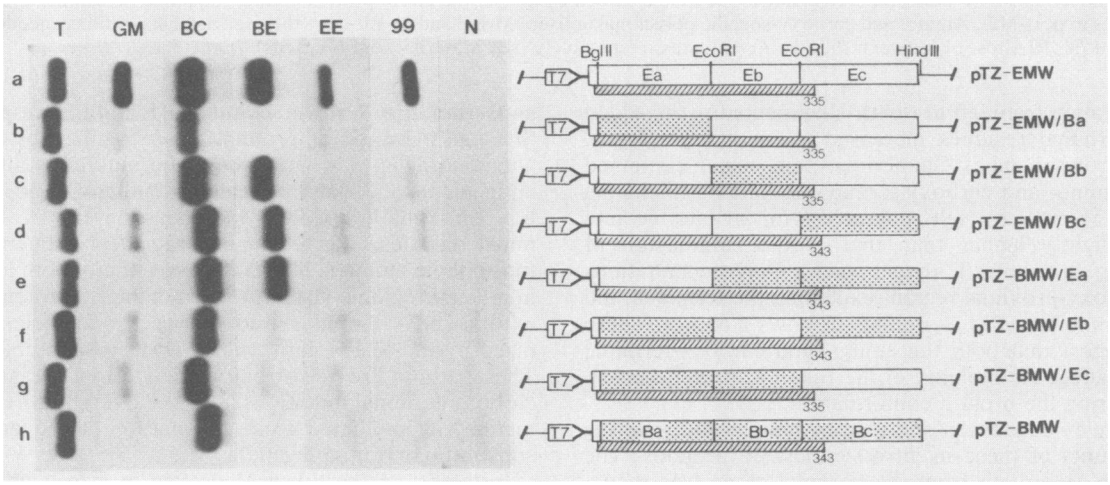

FIG. 8. Antigenicity of chimeric M proteins synthesized in vitro. Chimeric M genes containing the Biken mutations affecting the amino (*Bgl*II to *Eco*RI), carboxy-proximal (*Eco*RI to *Eco*RI), or carboxy-terminal (*Eco*RI to *Hin*dIII) region were constructed by swapping different regions between pTZ-EMW and pTZ-BMW, which were identical to pTZ-EM1 and pTZ-BM1, respectively (Fig. 6), except that the *Eco*RI site in the vector has been destroyed (see Materials and Methods). Symbols: ▭, Edmonston strain-derived DNA sequences; ▩, sequences derived from the Biken strain; ▨, regions encoding the chimeric proteins. The number of amino acid residues is shown for each protein. These chimeric genes were linearized at the *Hin*dIII site and transcribed into RNA in vitro by using the integral T7 promoter. One microgram of the in vitro-synthesized RNA was translated in reticulocyte lysates in the presence of [^{35}S]methionine, and equal trichloroacetic acid-precipitable counts of the translational products were immunoprecipitated with the GM, M-BC, M-BE, M-EE, Cl.99, and N-EE antisera and analyzed by SDS-PAGE (10% polyacrylamide). Each column shows the proteins precipitated by the same antiserum resolved in the same gel (migration from left to right). Total translational products before immunoprecipitation are shown in column T.

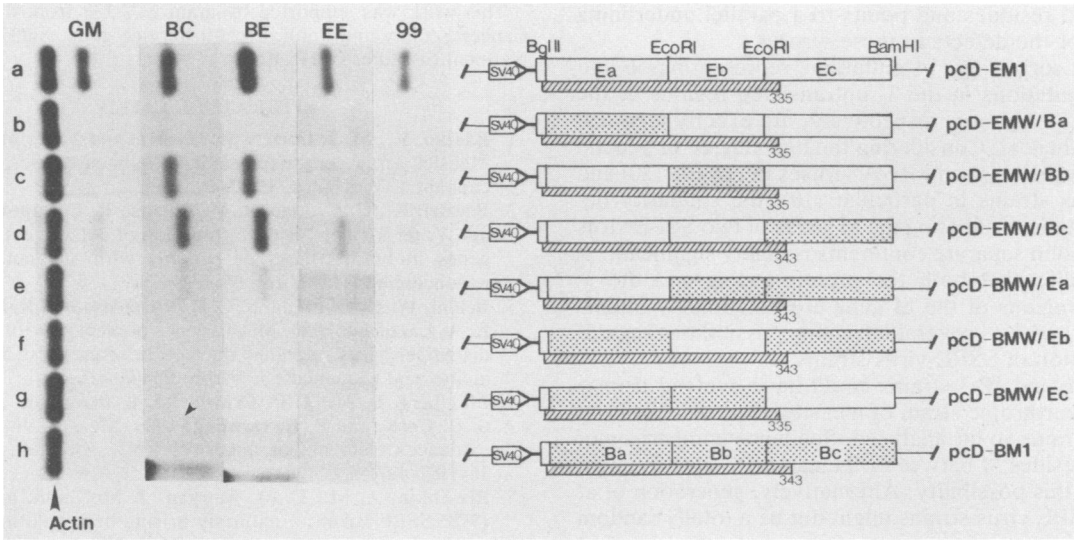

FIG. 9. Effects of Biken mutations on chimeric M proteins expressed in vivo. The entire series of chimeric M genes shown in Fig. 8 was placed under the control of the SV40 early promoter in the pcD vector (see the legend to Fig. 8 for description of the chimeric constructs). Equal amounts (20 μg) of the chimeric M genes were transfected into cultures containing 1.5×10^6 COS cells. Proteins were labeled with 30 μCi of [^{35}S]methionine for 6 h starting at 45 h posttransfection. Equal amounts of the labeled lysates were immunoprecipitated with the GM, M-BC, M-BE, M-EE, and Cl.99 antisera and analyzed by SDS-PAGE (10% polyacrylamide). Each vertical column shows the chimeric M proteins precipitated by the same antiserum resolved in the same gel (migration from left to right). Cellular actin nonspecifically precipitated by the GM antiserum served as an internal control for total labeled proteins.

concert, these mutations render the Biken M protein highly unstable in vivo. These results provide strong evidence that the defects in the Biken M protein are virus encoded and are not due to host cell factors. These observations explain the apparent pleomorphic nature of the defect in Biken strain. In retrospect, detection of the Biken M protein in some but not other studies could be due to a combination of the instability and altered immunoreactivity of the Biken M protein (23, 28, 39).

Comparison between the M proteins of Biken and Edmonston strains provides significant insights into possible mechanisms of the defect in Biken strain. As pointed out by Bellini et al. (3), the Edmonston M protein contains a number of closely spaced arginine and lysine clusters which are associated with hydrophilic regions likely to be exposed on the external surfaces of the protein (e.g., Arg-45–Lys-46, Arg-154–X–Arg-156, Arg-175–Arg-176, Arg-225–Arg-226–Lys-227–Lys-228, Lys-265–X–X–Lys-268, and Arg-293–X–X–X-Arg-297–X–Arg-299 [Fig. 5, arrowheads]). Interspersed among these charged residues are a number of hydrophobic regions, many of which associated with potential beta-sheet structures (e.g., Fig. 5, parallel bars spanning amino acid residues 33 to 38, 51 to 57, 100 to 103, 156 to 163, 191 to 195, 218 to 220, 246 to 247, and 303 to 308). The charged arginine and lysine pairs, the hydrophobic beta-sheet structures, and the beta-turn-promoting proline and glycine residues are the most highly conserved features among the M proteins of paramyxoviruses (3). Perhaps a specific M-protein conformation is required for proper interaction with other charged components during virus maturation. Although most of the arginine and lysine pairs are conserved in the Biken M protein, the hydrophobicity and secondary structure of the protein are altered. We hypothesize that these alterations affect the folding of the Biken M protein into a functional tertiary form. Indeed, such conformational changes are demonstrable both in vitro and in vivo in the chimeric proteins (Fig. 8 and 9).

It is possible that some of the sequence alterations are unrelated to the nonproductive phenotype and simply reflect genetic variations or mutations acquired during in vitro passages of Edmonston and Biken strains. However, the Edmonston M gene differs by less than 0.5% from the M genes of other lytic strains of measles virus analyzed, including a recent street isolate (9), suggesting that the M genes of lytic strains do not diverge quickly even in vitro. Furthermore, Cattaneo et al. recently characterized an SSPE virus strain, IP-3, originally isolated in the United States, which also contains a posttranslational defect affecting the stability of M protein (6, 8, 40). The IP-3 M gene contains 45 mutations, representing an overall mutational frequency of about 3%. However, 22 (50%) of those mutations are exactly identical to the ones affecting the Biken M gene (nt 77, 290, 297, 457, 479, 632, 657, 881, 1046, 1104, 1116, 1123, 1226, 1256, 1275, 1285, 1337, 1350, 1356, 1358, 1421, and 1431 [Fig. 4, arrowheads]). Eight of these shared mutations occur in the protein-coding region; five are silent, and three lead to amino acid substitutions. Remarkably, all three common amino acid substitutions occur at positions predicted to greatly alter the hydrophobicity or secondary structure of the Biken M protein (Glu-89, Asn-142, and Ala-209 [Fig. 4 and 5 legends]). In fact, Glu-89 and Ala-209 are found in multiple SSPE virus strains analyzed to date, including those cloned directly from brain tissues (9; M. Ayata, unpublished observation). Equally significant, 23% of the silent mutations in the Biken M gene (nt 77, 245, 278, and 290) and 27% of the silent mutations in the IP-3 M gene (8) occur at nucleotides encoding either lysine or arginine, even though these residues constitute only 13% of the total peptide. In fact, two of these silent mutations (nt 77 and 290, corresponding to Lys-15 and Lys-86, respectively) are shared between the Biken and IP-3 M genes (Fig. 4). The similarity between the mutations in the M proteins of these two SSPE virus strains supports the postulated significance

of the affected residues and points to a parallel underlining mechanism for the defects in these viruses.

Even more surprising, 14 mutations representing 56 and 61% of the mutations in the 3′ untranslated regions of the Biken and IP-3 M genes, respectively, are exactly identical (Fig. 4, arrowheads). Considering the high degree of genetic variation in negative-strand RNA viruses in general (20) and in SSPE virus strains in particular (10), the similarity between the mutations affecting the M genes of two SSPE virus strains isolated in separate continents is highly significant. It not only implies that both the protein-coding and the 3′ untranslated regions of the M gene are functionally important, but also suggests several intriguing possibilities regarding the evolution of SSPE virus strains.

First, Biken and IP-3 strains could have evolved from a preexisting neurotropic strain of measles virus distinct from all the lytic strains so far analyzed. Sequence comparison of other genes besides M between Biken and IP-3 strains might shed light on this possibility. Alternatively, generation of at least some SSPE virus strains might not be a totally random degenerative process as currently viewed. Instead, certain combinations of mutations might actually predispose the virus for chronic CNS infections and are positively selected for. Such a hypothesis could explain the rarity of SSPE more easily than one invoking a simple loss of viral function. It may be noteworthy that two of the three amino acid substitutions shared between the Biken and IP-3 M proteins (Glu-89 and Asn-142) are also found in the M protein of canine distemper virus (3), a particularly neurotropic relative of measles virus. Third, the conservation between the Biken and IP-3 M genes could also be explained by invoking a second selectable function unrelated to virus maturation in either the M protein or the primary nucleotide sequence. In the rhabdovirus vesicular stomatitis virus, the M protein apparently acts as a suppressor for viral RNA transcription besides serving a maturation function (35). Whether the measles virus M protein serves a similar function is not known.

While a causal relationship between abnormality in M protein and SSPE remains to be established, the defect in the Biken M protein appears to be tightly associated with the nonproductive phenotype. We have recently isolated a panel of clonal cell lines from Biken strain-infected CV-1 cells (a kind gift from S. Ueda). Some of those cell lines began to produce low levels of extracellular virions. Concomitantly, the M protein in the productive clones became detectable by antisera previously unable to detect the Biken M protein (A. Hirano, unpublished results). A similar phenomenon has also been observed in two phenotypic revertants of the IP-3 strain (8). These observations mirror those in previous reports that chronic CNS infection by measles virus in hamsters was accompanied by disappearance of detectable M protein (22) and that progression of SSPE in a patient correlated with a drastic reduction in the M protein level (16). The present results do not exclude the possibility that additional or alternative changes in other viral components could also contribute to chronic infections in some SSPE cases (2, 27). However, the accumulated evidence strongly indicates a role for the M protein in at least some cases, as originally proposed by Hall and Choppin (11, 17).

ACKNOWLEDGMENTS

We thank Shigeharu Ueda for providing the Biken-HEL cells and GM antiserum; Gregory Wipf for preparing the M-BC, M-BE, and M-EE antisera; David Powell for assistance in DNA sequencing; and Sharon Castaneda for critical reading of the manuscript.

This work was supported by grant MV-238 from the American Cancer Society and Public Health Service grant AI23732 from the National Institutes of Health.

LITERATURE CITED

1. **Baczko, K., M. J. Carter, M. Billeter, and V. ter Meulen.** 1984. Measles virus gene expression in subacute sclerosing panencephalitis. Virus Res. **1**:585–595.
2. **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.
3. **Bellini, W. J., G. Englund, C. D. Richardson, S. Rozenblatt, and R. A. Lazzarini.** 1986. Matrix genes of measles virus and canine distemper virus: cloning, nucleotide sequences, and deduced amino acid sequences. J. Virol. **58**:408–416.
4. **Blumberg, B. M., J. C. Crowley, J. I. Silverman, J. Menonna, S. D. Cook, and P. C. Dowling.** 1988. Measles virus L protein evidences elements of ancestral RNA polymerase. Virology **164**:487–497.
5. **Breschkin, A. M., E. M. Morgan, J. McKimm, and F. Rapp.** 1979. SSPE-Biken: a naturally arising hemagglutination-defective mutant of measles virus. J. Med. Virol. **4**:67–80.
6. **Burnstein, T., L. B. Jacobsen, W. Zeman, and T. T. Chen.** 1974. Persistent infection of BSC-1 cells by defective measles virus derived from subacute sclerosing panencephalitis. Infect. Immun. **10**:1378–1382.
7. **Carter, M. J., M. M. Willcocks, and V. ter Meulen.** 1983. Defective translation of measles virus matrix protein in a subacute sclerosing panencephalitis cell line. Nature (London) **305**:153–155.
8. **Cattaneo, R., A. Schmid, M. 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.
9. **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 viruses in human brain infections. Cell **55**:255–265.
10. **Cattaneo, R., A. Schmid, G. Rebmann, K. Baczko, V. ter Meulen, W. J. Bellini, S. Rozenblatt, and M. A. Billeter.** 1986. Accumulated measles virus mutations in a case of subacute sclerosing panencephalitis: interrupted matrix protein reading frame and transcription alteration. Virology **154**:97–107.
11. **Choppin, P. W.** 1981. Measles virus and chronic neurological diseases. Ann. Neurol. **9**:17–20.
12. **Choppin, P. W., and R. W. Compans.** 1975. Reproduction of paramyxoviruses, p. 95–187. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 4. Plenum Publishing Corp., New York.
13. **Chou, P. Y., and G. D. Fasman.** 1974. Conformational parameters for amino acids in beta sheet, and random coil regions calculated from proteins. Biochemistry **13**:211–222.
14. **Dhib-Jalbut, S., H. F. McFarland, E. S. Mingioli, J. L. Sever, and D. E. McFarlin.** 1988. Humoral and cellular immune responses to matrix protein of measles virus in subacute sclerosing panencephalitis virus. J. Virol. **62**:2483–2489.
15. **Gluzman, Y.** 1981. SV40-transformed simian cells support the replication of early SV40 mutants. Cell **23**:175–182.
16. **Haase, A. T., D. Gantz, B. Eble, D. Walker, L. Stowring, P. Ventura, H. Blum, S. Wietgrefe, M. Zupancic, W. Tourtelotte, C. J. Gibbs, Jr., E. Norrby, and S. Rozenblatt.** 1985. Natural history of restricted synthesis and expression of measles virus genes in subacute sclerosing panencephalitis. Proc. Natl. Acad. Sci. USA **82**:3020–3024.
17. **Hall, W. W., and P. W. Choppin.** 1979. Evidence for lack of synthesis of the M polypeptide of measles virus in brain cells in subacute sclerosing panencephalitis. Virology **99**:443–447.
18. **Hall, W. W., R. A. Lamb, and P. W. Choppin.** 1979. Measles and subacute sclerosing panencephalitis virus proteins: lack of antibodies to the M protein in patients with subacute sclerosing panencephalitis. Proc. Natl. Acad. Sci. USA **76**:2047–2051.
19. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III

creates targeted breakpoints for DNA sequencing. Gene **28:** 351–359.

20. **Holland, J., K. Spindler, F. Horodyski, E. Grabau, S. Nichol, and S. VandePol.** 1982. Rapid evolution of RNA genomes. Science **215:**1577–1585.
21. **Ikuta, K., H. Homma, K. Maotani, S. Ueda, S. Kato, and K. Hirai.** 1982. Monoclonal antibodies specific to and cross-reactive with Marek's disease virus and herpesvirus of turkeys. Biken J. **25:**171–175.
22. **Johnson, K. P., E. Norrby, P. Swoveland, and D. R. Carrigan.** 1981. Experimental subacute sclerosing panencephalitis: selective disappearance of measles virus matrix protein from the central nervous system. J. Infect. Dis. **144:**161–169.
23. **Johnson, K. P., E. Norrby, P. Swoveland, and D. R. Carrigan.** 1982. Expression of five antigens in cells infected with wild-type and SSPE strains of measles virus: correlation with cytopathic effects and productivity of infections. Arch. Virol. **73:**255–262.
24. **Kingsbury, D. W.** 1985. Orthomyxo- and paramyxoviruses and their replication, p. 1157–1178. *In* B. N. Fields (ed.), Virology. Raven Press, New York.
25. **Krieg, P. A., and D. A. Melton.** 1984. Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs. Nucleic Acids Res. **12:**7057–7070.
26. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157:**105–132.
27. **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.
28. **Lin, F. H., and H. Thormar.** 1980. Absence of M protein in a cell-associated subacute sclerosing panencephalitis virus. Nature (London) **285:**490–492.
29. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Melton, D. A., P. A. Krieg, M. R Rebagliati, R. 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.
31. **Norrby, E., K. Kristensson, W. J. Brzosko, and J. G. Kapsenberg.** 1985. Measles virus matrix protein detected by immune fluorescence with monoclonal antibodies in the brain of patients with subacute sclerosing panencephalitis. J. Virol. **56:**337–340.
32. **Ohara, Y., M. Tashiro, S. Takase, and M. Homma.** 1985. Detection of antibody to M protein of measles virus in patients with subacute sclerosing panencephalitis: a comparative study on immunoprecipitation. Microbiol. Immunol. **29:**709–723.
33. **Ohuchi, M., R. Ohuchi, and M. Homma.** 1981. Mode of subacute sclerosing panencephalitis (SSPE) virus infection in tissue culture cells. III. Neurovirulence of cell-free SSPE viruses of Niigata-1, Kitaken-1, and Biken strains. Microbiol. Immunol. **25:**887–893.
34. **Okayama, H., and P. Berg.** 1983. A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. Mol. Cell. Biol. **3:**280–289.
35. **Pal, R., B. W. Grinnell, R. M. Snyder, and R. R. Wagner.** 1985. Regulation of viral transcription by the matrix protein of vesicular stomatitis virus probed by monoclonal antibodies and temperature-sensitive mutants. J. Virol. **56:**386–394.
36. **Pape, L. K., T. J. Koerner, and A. Tzagoloff.** 1985. Characterization of a yeast nuclear gene (MST1) coding for the mitochondrial threonyl-$tRNA_1$ synthetase. J. Biol. Chem. **260:**15362–15370.
37. **Richardson, J. S.** 1981. The anatomy and taxonomy of protein structure. Adv. Protein Chem. **34:**167–339.
38. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
39. **Sato, T. A., A. Fukuda, and A. Sugiura.** 1985. Characterization of major structural proteins of measles virus with monoclonal antibodies. J. Gen. Virol. **66:**1397–1409.
40. **Sheppard, R. D., C. S. Raine, M. B. Bornstein, and S. A. Udem.** 1986. Rapid degradation restricts measles virus matrix protein expression in a subacute sclerosing panencephalitis cell line. Proc. Natl. Acad. Sci. USA **83:**7913–7917.
41. **ter Meulen, V., J. R. Stephenson, and H. W. Kreth.** 1983. Subacute sclerosing panencephalitis, p. 105–142. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 18. Plenum Publishing Corp., New York.
42. **Thormar, H., P. D. Mehta, and H. R. Brown.** 1978. Comparison of wild-type and subacute sclerosing panencephalitis strains of measles virus. Neurovirulence in ferrets and biological properties in cell cultures. J. Exp. Med. **148:**674–691.
43. **Ueda, S., Y. Okuno, Y. Okuno, Y. Hamamoto, and H. Ohya.** 1975. Subacute sclerosing panencephalitis (SSPE): isolation of a defective variant of measles virus from brain obtained at autopsy. Biken J. **18:**113–122.
44. **Ueda, S., M. Takahashi, T. Kurimura, Y. Minekawa, N. Suzuki, K. Yamanishi, K. Baba, Y. Okuno, T. Sasada, K. Onishi, T. Konobe, K. Takaku, Y. Yamada, T. Tanami, F. Kusano, Y. Hayakawa, and T. Kurose.** 1972. Development of extremely attenuated live measles virus vaccine (CAM-EX). Biken J. **15:**173–177.
45. **Wechsler, S. L., and H. C. Meissner.** 1982. Measles and SSPE viruses: similarities and differences. Prog. Med. Virol. **28:**65–95.
46. **Wechsler, S. L., H. L. Weiner, and B. N. Fields.** 1979. Immune response in subacute sclerosing panencephalitis: Reduced antibody response to the matrix protein of measles virus. J. Immunol. **123:**884–889.
47. **Wong, T. C., and A. Hirano.** 1986. Functional cDNA library for efficient expression of measles virus-specific gene products in primate cells. J. Virol. **57:**343–348.
48. **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.