Alterations in Peptide Structure of Vesicular Stomatitis Virus Mutant and its Central Nervous System Isolate

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SUMMARY

Gradient SDS-polyacrylamide gel electrophoresis (PAGE) and proteolytic digestions were utilized to examine the virion proteins of two isolates of wild-type vesicular stomatitis virus (WT-VSV), WT_ATCC from the American Type Culture Collection and WT_GL from Glasgow, as well as temperature-sensitive (ts) mutant ts G31 and a central nervous system (CNS) isolate of ts G31 designated ts G31BP. The WT_ATCC M protein differed in electrophoretic mobility and in its tryptic or chymotryptic peptide maps from the 125I-labelled M proteins in WT_GL, ts G31 or ts G31BP. The M protein in the latter three viruses appeared identical using either tryptic or chymotryptic digestion procedures; however, limited digestion with V8 protease revealed a difference between the M protein of ts G31 and both WT_GL and ts G31BP M proteins. The L, NS and G proteins all had identical tryptic and chymotryptic peptide maps in WT_GL, ts G31 and ts G31BP virions. The N protein, however, was demonstrated to be distinctly different in the WT_GL virion when compared with the ts G31 (or ts G31BP) virion by its tryptic peptide map. In addition, limited proteolytic digestion of the 125I-labelled N proteins revealed a different peptide structure in ts G31BP compared to N proteins of ts G31 or WT_GL. The altered N protein in the CNS isolate, ts G31BP, is discussed in terms of its altered in vivo phenotype of labile viral RNA, and its potential role in the unique CNS disease associated with this virus.

INTRODUCTION

Our recent studies have described some rather striking differences in the CNS disease resulting with several ts vesicular stomatitis virus (VSV) mutants in comparison to the wild-type (WT)-VSV (Dal Canto et al., 1976a, b, 1979; Rabinowitz et al., 1976, 1977b). While infection with WT-VSV is rapidly fatal to mice (Doyle & Holland, 1973; Rabinowitz et al., 1976; Wagner, 1974), intracerebral injection of some ts mutants, including ts G31, results in a slowly progressive clinical course, characterized by hind leg paralysis and a marked spongioform myelopathy in the spinal cord of the infected animals (Dal Canto et al., 1976b; Rabinowitz et al., 1976). Ts G31, a member of genetic complementation group III, has been characterized by a reduced synthesis and/or maturation of its matrix (M) protein at elevated temperatures (Hughes et al., 1979b; Knipe et al., 1977a, b; Lafay, 1974, Printz & Wagner, 1971). However, there has been little or no evidence of a structural alteration of this membrane or envelope protein that is responsible for the ts maturation of infectious virions.

We have recently presented evidence that the ts mutant (designated ts G31BP), that is isolated from the CNS of ts G31-infected animals, is probably responsible for the progressive spongioform CNS disease (J. V. Hughes & T. C. Johnson, unpublished results). These
conclusions are based on the observations that ts G31 cannot be recovered from the infected animals during the course of the disease and that the virus recovered, ts G31BP, is capable of causing an infection of the CNS that has the same histopathology, clinical course and hind leg paralysis that was originally thought to be the result of the ts G31 mutant. Unlike either the WT-VSV or the ts G31, the CNS isolate ts G31BP has been shown to have a replicative cycle at elevated temperatures that is characterized by an altered stability of viral RNA transcripts (J. V. Hughes & T. C. Johnson, unpublished results). In the present study, we have examined the peptide structure of the five VSV proteins of ts G31 and ts G31BP in comparison with two WT-VSV isolates, WT_ATCC and WT_GL, in an attempt to link the ts phenotypic properties to alterations in protein structure.

METHODS

**Viruses.** VSV Indiana strains WT_ATCC, WT_GL and ts G31 were each plaque purified and doubly cloned as described previously (Rabinowitz et al., 1976). The ts G31BP virus was isolated from the brains of outbred Swiss mice 4 days after intracerebral injection with ts G31 (Hughes et al., 1979b) and the CNS isolate was then plaque purified and doubly cloned. Viral stocks, grown in BHK-21 cells at 31 °C, were purified on sucrose gradients (Rabinowitz et al., 1977a).

**Polyacrylamide gel electrophoresis (PAGE).** The viral proteins were separated on 0.75 mm-thick slab gels using the discontinuous SDS–buffer system of Laemmli (1970) in combination with exponential-gradient polyacrylamide gels (Kelly & Luttges, 1975). These slab gels contained a 4% acrylamide stacking layer and a 6 to 17.5% gradient resolving gel cast with an exponential change in acrylamide concentration. These gels were cast using 1 vol. 17.5% acrylamide solution and 2 vol. 6% acrylamide solution in closed mixing chambers so that the % of the acrylamide changed exponentially initially as the 6% mixed with the 17.5% solution. After the volumes were equal in both chambers of the gradient former, the chambers were opened to provide a linear gradient for the remainder of the resolving gel. Protein samples were electrophoresed at 17 mA at room temperature until the bromophenol blue tracking dye reached the bottom of the gel. Following electrophoresis, the proteins were stained with 0.2% Coomassie blue in 50% methanol, 5% acetic acid for 30 min and destained with 10% methanol. The destained gels were dried using a Savant gel dryer.

**Peptide analysis.** The individual protein bands were sliced from the dried gels with a razor blade and the slices were placed in siliconized tubes, washed extensively with 10% methanol to remove SDS and lyophilized to dryness. The proteins in each gel preparation were iodinated by adding 20 µl 0.5 M-sodium phosphate buffer pH 7.5, 100 to 150 µCi ¹²⁵I in 5 µl (13 to 17 mCi/mmol; Amersham/Searle, Arlington Heights, Ill., U.S.A.) and 5 µl chloramine T (1 mg/ml). After 30 to 45 min of incubation at 25 °C, the reactions were terminated by the addition of 1 ml sodium bisulphite (1 mg/ml). The bisulphite was removed after 15 min, and the slices were washed repeatedly with 10% methanol. The radiolabelled proteins were digested with either 12.5 µg trypsin–TPCK (270 units/mg) or 12.5 µg chymotrypsin (49.2 units/mg) (both from Worthington Biochemicals) added to the slices. Digestion was allowed to proceed at 37 °C overnight, after which 12.5 µg trypsin (or chymotrypsin) was again added and incubation was continued at 37 °C for an additional 4 h. The supernatant fluids containing the peptide digests were then removed and lyophilized to dryness.

The resulting peptides were analysed as described by Elder et al. (1977) on cellulose-coated thin-layer chromatographic TLC plates (10 × 10 cm) (MC/B Manufacturing Chemists, Cincinnati, Ohio, U.S.A.). The lyophilized peptide digests were dissolved in 20 µl buffer I (acetic acid :formic acid :water, 15 :5 :80, by vol.; pH 1.5), and 2 µl (0.1 × 10^6 to 0.5 × 10^6 ct/min) were spotted on to each plate. Electrophoresis was carried out at 4 °C in buffer I at
1000 V for 30 min, using 2% orange G and 1% acid fuchsin as tracking dyes. Following electrophoresis, the plates were dried and the peptides were chromatographed in the second dimension in buffer II (butanol : pyridine : acetic acid : water, 32.5 : 25 : 5 : 20, by vol.). After chromatography the plates were dried and the position of the radiolabelled peptides were identified with Kodak XRP-1 X-ray film and a Dupont Cronex screen to increase the intensity of the labelled peptides where radioactivity was low. When electrophoresis was carried out at pH 3.5, all procedures were the same as above, except that the running buffer consisted of acetic acid (4.5%, w/v) and pyridine (0.46%, v/v). For separation at pH 6.5, the buffer consisted of acetic acid : pyridine : water (3 : 100 : 879, by vol.). A clearer separation of the peptides was accomplished for the pH 6-5 runs if the peptides were first separated by chromatography and then by electrophoresis in the second dimension.

**Peptide mapping by limited proteolysis.** For these studies disrupted virions were iodinated as described by Doel & Brown (1978). Viral proteins (50 to 75 μg) were dissolved in 10 μl 0.5 M-tris-HCl pH 7.6, 1.5% SDS and 4 M-urea and then boiled for 30 s. Then, 10 μl 125I (0.5 to 1.0 mCi) were added, followed by 20 μl chloramine T (5 mg/ml). After a 30 min incubation at room temperature, the reaction was terminated with the addition of sodium metabisulphite (1 mg/ml). Excess reagents and unbound 125I were removed by successive cycles of acetone precipitation and centrifugation. These radioactive viral proteins were then separated by SDS–PAGE, and the individual bands were sliced out of the gels for protease digestion.

The technique developed by Cleveland et al. (1977) was employed to analyse the 125I-labelled virion proteins by limited proteolysis. Briefly, the slices from the separating SDS gel were soaked in 0.125 M-tris–HCl pH 6-8, 0.1% SDS, 1 mM-EDTA and then pushed to the bottom of the sample well on a second gel and overlaid with 10 μl of this solution with a given amount of V8 protease (Miles Laboratories, Elkhart, Ind., U.S.A.). For resolution of protease digests, a 15% polyacrylamide gel with a 3 cm stacking gel was used. Electrophoresis was performed in the normal manner until the bromophenol blue dye reached the stacking gel, when the current was turned off for 30 min. The current was then turned on again until the tracking dye reached the bottom of the resolving gel. The gels were fixed in 10% acetic acid and 5% glycerol and then dried and exposed to Kodak XRP-1 film.

**RESULTS**

**PAGE of viral proteins**

When the virion proteins from sucrose gradient-purified viruses were analysed on exponential-gradient SDS–polyacrylamide gels, the five VSV proteins were clearly separated (Fig. 1). Surprisingly, this separation system also revealed a difference in the migration of the matrix (M) protein from two different WT-VSV isolates. One isolate obtained from the American Type Culture Collection, WT_ATCC, had an M protein that migrated faster than the M protein found in all of the other VSV isolates examined. This WT-VSV (from ATCC) was initially used as a comparison to the ts G31 and ts G31BP virions. To corroborate the different mol. wt. of the M proteins, we obtained another isolate of VSV, the WT_GL, from which ts G31 was originally isolated. The WT_GL virion was then subsequently demonstrated to have an M protein that migrated on SDS gels in a similar way to the M protein of ts G31 and ts G31BP.

The migration alterations observed were not the result of minor electrophoretic differences between slab gel lanes since mixtures of the WT_ATCC and WT_GL or ts G31 resulted in two separate M proteins (Fig. 1). The migration of all five VSV proteins of ts G31BP was very similar to the proteins of ts G31 or WT_GL (not shown). In addition, mixing viral proteins of WT_GL, ts G31 and ts G31BP resulted in only single protein bands, suggesting that there were
no major alterations in the mol. wt. of the proteins. To examine more closely the differences in
the M protein, as well as to determine if there were any other structural alterations in the
other VSV proteins among any of these viruses, we next analysed the peptides obtained from
the five VSV proteins.

M protein peptides

As suggested by analysis on SDS–PAGE (Fig. 1), the M protein of the WT<sub>ATCC</sub> virion was
quite distinct from the M proteins of WT<sub>GL</sub>, ts G31 or ts G31BP (Fig. 2 and 3). Two-dimensional separation of the tryptic digests resulted in 13 peptides from all of these
viruses (Fig. 2), but peptide 9 was found only in WT<sub>ATCC</sub> and not in the other three. Peptide
14, however, was detected in WT<sub>GL</sub>, ts G31 and ts G31BP but was not detected in WT<sub>ATCC</sub>
(Ts G31BP M protein had peptides identical to ts G31 M protein, although it was not shown
in this figure.) Mixing experiments were performed with these M peptide digests to ensure that
the alterations seen were not the result of minor electrophoretic or chromatographic
differences. These results (Fig. 2d to f) suggested that the WT<sub>ATCC</sub> M protein did indeed
differ from the M proteins of the other three viruses, although no differences were detected
between the three viruses derived from the Glasgow wild-type.

Digestion by chymotrypsin confirmed both qualitative and quantitative differences in the
M protein for these viruses (Fig. 3). There were 13 labelled peptides in the digests of the
WT<sub>ATCC</sub> M protein, and only 12 in the WT<sub>GL</sub>, ts G31 and ts G31BP digested M proteins. The
WT<sub>ATCC</sub> M protein contained two peptides (numbers 4 and 5) not present in the WT<sub>GL</sub>, ts
G31 or ts G31BP. The latter three viruses did, however, contain one peptide (number 14) not
present in the chymotryptic digests of the WT<sub>ATCC</sub> M protein. The peptide patterns for WT<sub>GL</sub>,

**Fig. 1.** Virion polypeptides separated on exponential-gradient gels. Purified virions were electrophoresed
as described in the text: 1, ts G31; 2, WT<sub>GL</sub>; 3, WT<sub>ATCC</sub>; 4, WT<sub>ATCC</sub> and ts G31; 5, WT<sub>GL</sub> and WT<sub>ATCC</sub>.
Fig. 2. Tryptic peptide maps of the M protein. The M proteins from the various virions were sliced from the dried polyacrylamide gels, iodinated with \( {^{125}}I \), then treated with trypsin as described in the text. The peptides were spotted on to TLC plates (in the lower left corner) and separated first by high-voltage electrophoresis and then by liquid chromatography as indicated in (a). The TLC plates were then exposed to Kodak XRP-1 film for various lengths of exposure time. (a) WT\textsubscript{ATCC} M peptides; (b) WT\textsubscript{GL} M peptides; (c) ts G31 M peptides; (d) WT\textsubscript{ATCC} and ts G31 M peptides; (e) WT\textsubscript{ATCC} and WT\textsubscript{GL} M peptides; (f) ts G31 and WT\textsubscript{GL} M peptides. The dotted circle in (b) and (c) denotes a peptide found in WT\textsubscript{ATCC} but not found in the WT\textsubscript{GL} or ts G31 M proteins; also, peptide 14 was found only in the WT\textsubscript{GL} and ts G31 virions.
Fig. 3. Chymotryptic peptide maps of the M protein. M protein peptides were prepared and separated as described in the legend to Fig. 2, except that chymotrypsin was used to digest the proteins. (a) WT<sub>ATCC</sub> M peptides; (b) WT<sub>GL</sub> M peptides; (c) ts G31 M peptides. The dotted circles in (b) and (c) denote expected peptides from the WT<sub>ATCC</sub> M peptide maps; peptide 14 was unique to WT<sub>GL</sub> and ts G31 and not found in WT<sub>ATCC</sub> M peptides.

Fig. 4. Limited protease digestion of the M protein. 125I-labelled M proteins from WT<sub>GL</sub>, ts G31 and ts G31BP were subjected to limited digestion with V8 protease as described in the text. The concentration of the V8 used is listed above each lane (either 1 or 10 µg). Peptides 8 and 11 appear to be present only in ts G31 M protein digests. Since the rather heavily labelled peptides close to 8 and 11 partially interfered with the visualization of these peptides, a portion of the dried polyacrylamide gel (indicated by dotted lines) was cut out and re-exposed to X-ray film for a longer period of time. Peptides 8 and 11 were found only in ts G31 and these peptides accumulated only when high levels of protease (greater than 1 µg) were used.

ts G31 and ts G31BP M proteins were very closely related to each other and no detectable differences were seen in chymotryptic digests of a number of independently isolated M proteins.

We have further examined the peptide structure of the M protein in the WT<sub>GL</sub>, ts G31 and ts G31BP virions by utilizing the partial proteolytic digestion technique described by Cleveland <i>et al.</i> (1977). When 125I-labelled M proteins were digested with the protease V8, there were two peptide differences between the M proteins of the WT<sub>GL</sub> and ts G31 viruses (Fig. 4). These peptides (numbers 8 and 11) only appeared as minor species using high
Fig. 5. Tryptic peptide maps of N protein. The N protein from WTGL (a, c) and ts G31 (b, d) were isolated, iodinated and digested with trypsin as described in Fig. 2. The labelled peptides were then separated by electrophoresis at either pH 1.9 (a, b) or pH 6.5 (c, d) followed by chromatography. Peptides with numbers encircled denote peptides unique to WTGL; peptides with squares around the numbers designate the unique ts G31 peptides; and dotted circles indicate areas of missing peptides. Since some peptide spots were not clearly discerned as unique peptides in photographic reproduction, the peptide spots themselves were encircled. The N protein tryptic peptides from ts G31BP are identical to the ts G31 N peptides (not shown).

concentrations of V8 protease and were more clearly discerned by an extended exposure of a portion of the dried gel of the V8 protease digest (Fig. 4). The M protein of ts G31BP appeared to yield peptide species similar to those of the WTGL, rather than the original ts G31.

N protein peptides

In previous reports from our laboratory we have presented evidence suggesting that ts G31BP virus has a temperature-dependent defect which is associated with viral RNA metabolism (Hughes et al., 1979b; Hughes & Johnson, 1980). We were, therefore, particularly interested in obtaining the peptide maps of the proteins associated with the VSV nucleocapsid, i.e. N, NS and L, and assessing the possibility that a detectable change may be seen in ts G31BP when compared with ts G31, as well as with WTGL.

The tryptic digests of the N protein resulted in a number of labelled peptides, with 12 to 13 peptides resolved by electrophoresis at pH 1.9 and 17 to 18 peptides resolved at pH 6.5 (Fig. 5). These peptide maps demonstrated clear differences between the N protein of WTGL and
Fig. 6. Limited protease digestion of N protein. 125I-labelled N proteins from WTGL, ts G31 and ts G31BP were subjected to limited protease digestion with V8 protease as described in the text. The concentration (µg) of the V8 used is listed above each lane. Peptide 4 (starred) appears to be present only in ts G31BP N protein digests. (The long exposure times necessary to allow visualization of some of the minor peptide products do not allow detection of peptide 4 as a separate product.) However, further titration of protease between 0.5 and 0.1 µg demonstrated clearly that this peptide is unique to ts G31BP N protein.

those of ts G31 or ts G31BP (Fig. 5; ts G31BP had an identical peptide structure to ts G31 in this total digestion procedure). Although it is not entirely clear why separation at pH 6.5 would give a better resolution (17 to 18 peptides), this number of labelled peptides would appear to be almost the maximum number for the N protein (i.e. there are 18.7 residues of tyrosine per N protein; Brown & Prevec, 1978). Chymotryptic digests of the N protein demonstrated no differences in the peptides of ts G31 and ts G31BP, although the WTGL N protein did have some unique peptides (data not shown).

Examination of the N protein by limited digestion with V8 protease did, however, reveal a difference between the ts G31BP N protein and the N protein of either ts G31 or WTGL (Fig. 6). Over a wide range of protease concentrations, there was one distinct peptide in the ts G31BP N protein which was found at low concentrations of enzyme. This peptide was not found in ts G31 or WTGL (Fig. 6). Titration of the amount of enzyme used for digestion over a smaller range (0.08 to 0.5 µg) also suggested that this peptide was unique to ts G31BP and would only accumulate in detectable amounts at relatively low concentrations of enzyme. All other digestion products appeared to be identical for ts G31, ts G31BP and WTGL.

NS protein peptides

The NS protein has also been analysed for differences in either the tryptic or the chymotryptic peptide maps. No differences were detected with either tryptic or chymotryptic digests of WTGL, ts G31 or ts G31BP NS proteins (data not shown). The NS peptides migrated in a similar manner and had comparable intensities of labelling whether trypsin or chymotrypsin was used as the digesting enzyme or whether the peptides were resolved by electrophoresis at pH 3.5 or 1.9.

L protein peptides

The L protein, a rather large polypeptide which has been estimated to have a mol. wt. of 160 x 10^3 to 190 x 10^3, demonstrated a very complex pattern of tryptic- or chymotryptic-derived peptides (data not shown). There were approx. 30 125I-labelled tryptic or chymotryptic peptides resolved in this separation system, although a number of these do not appear to be intensely labelled by 125I. Repeated analyses of the L peptides from WTGL indicated that this polypeptide was very similar to the L protein peptides from ts G31 and ts G31BP.
Tryptsin digestion of the G glycoprotein produced 17 distinguishable peptides from each of the WTGL, ts G31 and ts G31BP G proteins, with no apparent differences between them. Similar results were obtained with chymotryptic digests of the G protein, where 15 to 16 peptides were resolved with essentially no differences between the three viruses (data not shown).

DISCUSSION

Our recent studies (Hughes et al., 1979a, b) have examined the RNA and protein metabolism of ts G31, a virus which upon intracerebral injection results in a unique CNS disease with hind limb paralysis, and a slowly progressive status spongiosus in the spinal cord (Dal Canto et al., 1976a, b, 1979; Rabinowitz et al., 1976, 1977b). This unique disease is probably the result of the virus recovered from the CNS, ts G31BP, which appears to have a number of properties not characteristic of the initial ts G31 mutant (Hughes et al., 1979b; Hughes & Johnson, 1980). The peptide analyses described in this paper were designed to compare the proteins of the initial ts G31 mutant and its CNS isolate, ts G31BP, with each other, as well as with the WT-VSV, in order to correlate the ts defects in RNA and protein metabolism and hopefully to associate the CNS disease with alterations in particular VSV proteins.

When we examined the proteins of the WT-VSV, a surprising difference in the electrophoretic mobility and peptide structure of the M protein from two different isolates was found (Fig. 1 to 3). The different peptide structure of WTGL from WT ATCC does not apparently result in any phenotypic differences and appears to be maintained in ts G31 and ts G31BP, the mutants derived from WTGL.

Analysis of the viral proteins by gradient SDS–PAGE revealed a difference in the electrophoretic migration of the M protein, the M proteins of the WTGL appearing to be larger than that of the WT ATCC, with possibly six to ten more amino acid residues in the latter M proteins. This difference might be the result of either an alteration at the level of the RNA genome (with a new termination point for transcription or translation), or an alteration in post-translational maturation involving cleavage, or perhaps phosphorylation of the M protein (Clinton et al., 1978).

Although a number of studies have reported that ts G31 is associated with decreased accumulation and/or functioning of the M protein at the level of the cell membrane (Printz & Wagner, 1971; Lafay, 1974; Knipe et al., 1977a, b; Hughes et al., 1979b; Dal Canto et al., 1979), the total digests with trypsin and chymotrypsin did not allow detection of any structural alterations. However, V8 protease digestion procedures have demonstrated differences between the ts G31 and WTGL M proteins (Fig. 4). These peptide alterations, which are similar to results of other investigators (M. E. Reichman, personal communication), may underlie the temperature-sensitive defect associated with the M protein in ts G31. Ts G31 (and ts G31BP virions) also differed from WTGL in the N protein (Fig. 5). It is possible that some other protein alterations, i.e. as in the M protein, might be present but not detectable by the methods we have employed; for example, there may be alterations in peptides not labelled by 125I, or altered peptides may not have undergone changes detectable with our separation techniques. Even though there are apparent molecular changes in both the M and N proteins of ts G31, this virion complements with mutants of groups I, II, IV and V, but not III, suggesting the only phenotypic lesion in ts G31 is in the M protein.

Since we recently proposed that the ts G31BP virion appears to be responsible for the unique spongioform myelopathy resulting from intracerebral injection of ts G31 (Hughes et al., 1979b; J. Hughes & T. Johnson, unpublished results), we were particularly interested to determine which peptides may have been altered in this CNS isolate. It appears that this
The viron is quite closely related to the initial ts G31, with the L, NS and G proteins appearing identical in ts G31BP and ts G31. The V8 proteolytic digestion of the M protein indicated that the ts G31BP peptide was very similar to the WTGL M peptides and differed from ts G31, suggesting that the M protein in ts G31BP may have reverted to the WTGL M protein. The alteration in the structure of the N or nucleocapsid protein, as detected by V8 digestion, also apparently occurred during the infection process in the mouse CNS. As expected for a multiple mutant, ts G31BP did not complement with any mutants that we have tested from the VSV complementation groups I, II, III, IV and V (data not shown). We have demonstrated two phenotypic properties of ts G31BP that may be attributed to an altered N protein and may be responsible for its ts defect. The mutant ts G31BP appears to have an altered RNA:N protein interaction in its nucleocapsid structure as detected by an abnormally large hyperchromatic shift upon heating, as compared to WTGL or ts G31 (Hughes & Johnson, 1980). In addition, ts G31BP-infected cells incubated at elevated temperatures, 39 or 37 °C, display a lability of newly made viral RNA (J. V. Hughes and T. C. Johnson, unpublished results). The ability of the N protein to protect the viral RNA from RNase digestion in vitro is already well established. Perhaps this mutant, ts G31BP, will enable us to examine the possible role played by N protein in the stability of newly made viral RNA in the infected cell.

The radiolabelling and separation techniques employed in this study have provided a relatively rapid and reproducible method for examining structural differences in all of the VSV polypeptides, even for those normally present at low concentrations, i.e. L and NS. These procedures have allowed us to detect an alteration in a single protein (N) in the CNS isolate, ts G31BP, that may be responsible for the altered phenotype of this virus as well as a change in the M protein that may be a reversion to WTGL. The alteration of these viral proteins may also be potentially responsible for the unique spongiform myelopathy and hind limb paralysis which results from the intracerebral infection that is initiated by ts G31. As ts G31 has never been re-isolated from the CNS, it has been important to establish the relationship of ts G31BP to ts G31. These studies will potentially lead to a much better understanding of, not only the alterations in viral genomes that occur in the CNS, but also the roles of both viron in the pathological response of the host.

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Altered peptides of VSV


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