Common distribution of antigenic determinants and complementation activity on matrix proteins of two vesicular stomatitis virus serotypes

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To compare the antigenic and functional domains of the matrix (M) proteins of vesicular stomatitis virus (VSV) serotypes Indiana (VSV-Ind) and New Jersey (VSV-NJ), deletion mutants and chimeras were cloned in pBSM13 and expressed as in-frame lacZ fusion proteins in Escherichia coli. Non-cross-reactive monoclonal antibodies directed to the two antigenically distinct M proteins were tested by Western blot analysis to map three epitopes of VSV-Ind M protein and four epitopes of VSV-NJ M protein. Epitope I of the VSV-Ind M protein and epitope II of the VSV-NJ M protein both mapped to the highly basic N-terminal 34 amino acids of each homotypic M protein. Epitope 2 and 3 of the VSV-Ind M protein and epitopes III and IV of the VSV-NJ M protein mapped to a region spanning amino acids 35 to 74. Epitope I of the VSV-NJ M protein mapped to a region between amino acid 75 and the C terminus. The similarity in location of the serotypically unique antigenic determinants of the two M proteins suggested that they may have a common functional domain. This hypothesis was substantiated by the finding that the two M proteins and various chimeras expressed in CV-1 cells by a recombinant vaccinia virus system were able to rescue M gene temperature-sensitive mutants of both VSV serotypes.

Vesicular stomatitis virus (VSV), the prototype rhabdovirus, consists of a membrane-enclosed helical ribonucleoprotein (RNP) core containing a single-stranded negative-sense RNA genome that encodes five structural proteins (for a review see Wagner, 1987). The non-segmented RNA genome is fully encapsidated by the nucleocapsid protein (N) to form the RNP core in association with the large (L) and phosphorylated (P) proteins which form the viral RNA-dependent RNA polymerase. The glycoprotein (G) is an integral membrane protein that constitutes the spikes protruding from the surface of the virion envelope. The matrix (M) protein, which makes up nearly one-third of the total viral protein, appears to be associated with both the envelope and the RNP core (Chong & Rose, 1993; Mancarella & Lenard, 1981). The M protein plays a central role in virion assembly and budding (Chong & Rose, 1993; Knipe et al., 1977) and in down-regulation of viral genome transcription (Carroll & Wagner, 1979; Clinton et al., 1978). The complete sequences of the VSV M proteins of the San Juan strain of the Indiana (Ind) serotype (Rose & Gallione, 1981) and the Ogden strain of the New Jersey (NJ) serotype (Gill & Banerjee, 1986) have been determined from cDNA clones. Excluding polyadenylic acid, the VSV-Ind M mRNA is 831 nucleotides long whereas the VSV-NJ M mRNA contains 758 nucleotides, but each of them encodes a protein of 229 amino acid residues. An amino acid identity of 62 % was found between the M proteins of VSV-Ind and VSV-NJ (Gill & Banerjee, 1986). A highly basic amino-terminal domain, containing eight lysine residues within the first 19 amino acid residues of the VSV-Ind M protein or seven lysine residues within the first 20 amino acid residues of the VSV-NJ M protein, followed by a proline–proline–X–tyrosine sequence is present in both the M proteins, suggesting a common function for that domain (Gill & Banerjee, 1986).

Monoclonal antibodies (MAbs) have been raised against the M protein of both VSV-Ind and VSV-NJ (Pal et al., 1985a; Ye et al., 1985). Little, if any, antibody cross-reactivity could be found between the two M proteins. Four antigenic determinants were identified for the M protein of each serotype, but epitope 4 of the VSV-Ind M protein was represented by only a single IgM antibody which could not be purified by Protein A–Sepharose CL-4B chromatography and therefore was unavailable for further study (Pal et al., 1985a). Various degrees of partial overlap were detected between epitopes 2 and 3 of the VSV-Ind M protein, as well as epitopes I and II, and I and IV of the VSV-NJ M protein. MAbs directed against epitope 1 of the VSV-Ind M protein and epitopes I and III of the VSV-NJ M protein were found to reverse the inhibition of transcription by the RNP-M complexes (Pal et al., 1985b; Ye et al., 1985). Partial and
proteins of VSV-Ind (shaded boxes) and VSV-NJ (open boxes) encoded by vectors listed on the left. All the numbered sites refer to sequence positions of amino acids. The plasmid pBSM13— was used to provide evidence for localization of epitope 1 of the gene hybrids are also designated sequentially chimeras 1, 2, 3 and 4.

Fig. 1. Stick models of the wild-type, truncated, deleted and chimeric M proteins expressed by the pBSM13—based plasmids used in these studies. An internal deletion of the VSV-Ind M gene, designated pBS-IndM(1–74/107–229), was constructed by cleaving plasmid pBS-IndM (previously designated pYL-OM79) containing the entire wild-type VSV-Ind M gene inserted at the Smal site of a Stratagene vector pBSM13— previously constructed in our laboratory (Li et al., 1988). The M gene was cleaved at nucleotides 258 and 358 with BgII, thus deleting amino acids 75 to 106 from the expressed M protein; the gap was sealed with the Klenow fragment of DNA polymerase I and T4 DNA ligase. A deletion of the VSV-Ind M gene coding for the first 74 amino acids, designated pBS-IndM(1–74), was created by digestion of the plasmid pBS-IndM with BgII at nucleotide 258 of the M gene and with KpnI at a vector site immediately downstream from the M gene, and sealing the gap with a synthetic double-stranded oligonucleotide linker providing an in-frame stop codon. A truncated VSV-Ind M gene coding for the carboxy-terminal 124 amino acids, designated pBS-IndM(106–229), was created by digestion of the M gene in the plasmid pBS-IndM with XbaI at a vector site immediately upstream from the M gene and with BgII at nucleotide 358 of the M gene; the gap was sealed with an oligonucleotide linker providing an in-frame start codon. Another truncation of the VSV-Ind M gene coding for the carboxy-terminal 160 amino acids, designated pBS-IndM(70–229), was created by insertion of a DNA fragment into pBSM13— between BamHI and EcoRI sites; the DNA fragment was amplified by PCR on the plasmid pBS-IndM using an upstream primer containing two adenines followed by the six nucleotides representing the BamHI recognition site and 22 nucleotides identical in sequence to VSV-Ind M gene nucleotides 245 to 266 with an in-frame start codon at nucleotide 249, and a downstream primer identical in sequence to the T7 promoter present on the vector downstream from the multiple cloning site. The VSV-NJ M gene (see Fig. 1) was excised with PstI from pM24, a plasmid containing the whole wild-type VSV-NJ M gene (a generous gift from A. K. Banerjee; Gill & Banerjee, 1986), and subcloned into pBSM13— linearized with PstI and designated pBS-NJM. A complete trypsin digests and Western blot analysis have provided evidence for localization of epitope 1 of the VSV-Ind M protein to a region between amino acid residues 18 and 43 (Ogden et al., 1986). By immunoblotting N-chlorosuccinimide (NCS)-cleaved fragments of the purified M protein and a trypsin-resistant M protein fragment, epitopes 2 and 3 of the VSV-Ind M protein were tentatively located between amino acid 43 and the C terminus (Ogden et al., 1986). By means of synthetic oligopeptides, epitope 1 of the VSV-Ind M protein was more precisely mapped to amino acid residues 17 to 31 (Shipley et al., 1988). The presence of a glycine residue at position 21 of the M protein of VSV-Ind was found to be essential for the recognition of epitope 1 (Shipley et al., 1988; Li et al., 1988).

None of the four epitopes of VSV-NJ M protein has been mapped and the epitopes of VSV-Ind M protein, especially epitopes 2 and 3, have been assigned only to vague map locations. It was also of interest to compare the distribution of epitopes and functional domains of the M proteins of the two VSV serotypes by using recombinant DNA techniques. To accomplish this, standard methods (Sambrook et al., 1989) were used for the construction, amplification and purification of plasmids containing VSV M genes as well as deletion mutants and chimeras created using a PCR kit (Perkin Elmer) and restriction enzymes (Gibco BRL and Boehringer Mannheim). Each of the constructs was checked for authenticity by restriction enzyme mapping, DNA sequencing and Western blotting of the expression products.

Fig. 1 illustrates by stick models the composition of the wild-type, truncated, deleted and chimeric M proteins expressed by the pBSM13—based plasmids used in these studies. An internal deletion of the VSV-Ind M gene, designated pBS-IndM(1–74/107–229), was constructed by cleaving plasmid pBS-IndM (previously designated pYL-OM79) containing the entire wild-type VSV-Ind M gene inserted at the Smal site of a Stratagene vector pBSM13— previously constructed in our laboratory (Li et al., 1988). The M gene was cleaved at nucleotides 258 and 358 with BgII, thus deleting amino acids 75 to 106 from the expressed M protein; the gap was sealed with the Klenow fragment of DNA polymerase I and T4 DNA ligase. A deletion of the VSV-Ind M gene coding for the first 74 amino acids, designated pBS-IndM(1–74), was created by digestion of the plasmid pBS-IndM with BgII at nucleotide 258 of the M gene and with KpnI at a vector site immediately downstream from the M gene, and sealing the gap with a synthetic double-stranded oligonucleotide linker providing an in-frame stop codon. A truncated VSV-Ind M gene coding for the carboxy-terminal 124 amino acids, designated pBS-IndM(106–229), was created by digestion of the M gene in the plasmid pBS-IndM with XbaI at a vector site immediately upstream from the M gene and with BgII at nucleotide 358 of the M gene; the gap was sealed with an oligonucleotide linker providing an in-frame start codon. Another truncation of the VSV-Ind M gene coding for the carboxy-terminal 160 amino acids, designated pBS-IndM(70–229), was created by insertion of a DNA fragment into pBSM13— between BamHI and EcoRI sites; the DNA fragment was amplified by PCR on the plasmid pBS-IndM using an upstream primer containing two adenines followed by the six nucleotides representing the BamHI recognition site and 22 nucleotides identical in sequence to VSV-Ind M gene nucleotides 245 to 266 with an in-frame start codon at nucleotide 249, and a downstream primer identical in sequence to the T7 promoter present on the vector downstream from the multiple cloning site. The VSV-NJ M gene (see Fig. 1) was excised with PstI from pM24, a plasmid containing the whole wild-type VSV-NJ M gene (a generous gift from A. K. Banerjee; Gill & Banerjee, 1986), and subcloned into pBSM13— linearized with PstI and designated pBS-NJM. A
truncation of the VSV-NJ M gene coding for amino acids 70 to 229, designated pBS-NJM(70-229), was constructed in the same way as pBS-IndM(70-229). Another truncation of the VSV-NJ M gene coding for amino acids 1 to 75, designated pBS-NJM(1-75), was created by inserting between the BamHl and KpnI sites of pBSM13—a DNA fragment amplified by PCR on the plasmid pBS-NJM using an upstream primer identical in sequence to that of the T3 promoter on the vector upstream from the multiple cloning site; also used was a downstream primer containing 23 nucleotides complementary to VSV-NJ M gene nucleotides 216 to 238 followed by three nucleotides introducing an in-frame stop codon, six nucleotides representing the KpnI site and two additional adenine residues. In addition, a BglII site was introduced by the PCR primers into both pBS-NJM(1-75) and pBS-NJM(70-229) at VSV-NJ M gene nucleotide 230 which corresponds to the BglII site at VSV-Ind M gene nucleotide 258.

Also shown in Fig. 1 are two chimeric M genes, designated pBS-IndM(1-74)/NJM(75-229) coding for the VSV-Ind M protein amino acids 1 to 74 and the VSV-NJ M protein amino acids 75 to 229 (also designated chimera 1), and conversely pBS-NJM(1-74)/IndM(75-229) coding for the VSV-NJ M protein amino acids 1 to 74 and the VSV-Ind M protein amino acids 75 to 229 (also designated chimera 2); these chimeras were constructed simply by digestion of pBS-IndM(1-74) and pBS-NJM(70-229) or pBS-NJM(1-75) and pBS-IndM(70-229) with BglII at a common M gene site and KpnI at a vector site downstream from the M gene and then rescaling the gaps with heterologous fragments. Another two chimeras, designated pBS-IndM(1-34)/NJM(35-229) coding for the VSV-Ind M protein amino acids 1 to 34 and the VSV-NJ M protein amino acids 35 to 229 (also designated chimera 3), and conversely pBS-NJM(1-34)/IndM(35-229) coding for the VSV-NJ M protein amino acids 1 to 34 and the VSV-Ind M protein amino acids 35 to 229 (also designated chimera 4), were constructed by insertion of two full-length chimeric M genes into pBSM13 between the sites for HindIII and EcoRI; these two full-length chimeric M genes were each made from two PCR fragments using four primers and the two M genes. Expressed proteins of these wild-type, deletion and chimeric constructs of VSV-Ind and VSV-NJ M genes provided the basis for mapping their epitopes by reactivity with MAbs. Competent Escherichia coli cells were transformed with each of the plasmids shown in Fig. 1. Expression of lacZ fusion proteins was induced with IPTG and ampicillin-resistant colonies were incubated in LB medium overnight at 37 °C by standard methods (Sambrook et al., 1989). Transformed E. coli cells from 0.4 ml of overnight cultures were pelleted, suspended in 50 μl of 1× SDS gel loading buffer, heated at 100 °C for 3 min, and subjected to SDS–PAGE in 12.5% acrylamide gels under reducing conditions along with VSV-Ind and VSV-NJ virions. Protein bands were then transferred by electroelution from the gels onto nitrocellulose sheets and detected using MAbs. It should be noted that the β-galactosidase component of these fusion proteins results in retarded gel mobility compared to that of the native M proteins.

Fig. 2 shows Western blots of the wild-type VSV M proteins and deletion mutants reactive with three MAbs and a polyclonal antibody directed to the VSV-Ind M protein (Fig. 2a to d) as well as with four MAbs directed to the VSV-NJ M protein (Fig. 2e to h). As positive controls, the native M proteins in the virions of both VSV serotypes and the M fusion proteins expressed by pBS-IndM and pBS-NJM were shown to react readily but only with the serotype-specific antibodies. As negative controls, the cells transfected with the original vector pBSM13—only expressed no proteins recognized by any of the antibodies. Whereas all four antibodies directed to the VSV-Ind M protein bound to the internal deletion mutant IndM(1-74/107-229) and to the carboxy-terminal truncation IndM(1-74), none of them was able to recognize the two amino-terminal truncations, IndM(106-229) and IndM(70-229); these data indicate that the N-terminal region comprising the first 74 amino acids of the VSV-Ind M protein contains all three epitopes, whereas the carboxy-terminal portion from amino acid 75 onwards contains none. Surprisingly, the anti-Ind M polyclonal antiserum did not contain any antibodies reactive with the C-terminal 155 amino acids of VSV-Ind M protein, suggesting that all potential antigenic determinants from amino acid 74 onwards are shielded. This postulate is supported by the finding that the anti-Ind M polyclonal antiserum did not react with the chimera 2 protein containing VSV-Ind M amino acid sequence 75 to 229 (Fig. 3d, lane 7). It should be noted that the failure to detect the truncated M proteins expressed by pBS-Ind M(106-229) or pBS-Ind M(70-229) is entirely because of failure to bind MAbs and polyclonal antibody and not owing to failure of expression (Fig. 2a to d, lanes 5 and 6); both of these truncated carboxy-terminal VSV-Ind M proteins were readily expressed as measured by labelling with [35S]methionine (data not shown).

In contrast to the VSV-Ind M protein, the VSV-NJ M protein does have an antigenic determinant on the carboxy-terminal side of amino acid 74. As shown in Fig. 2(e), NJ MAb 1 directed to epitope I reacts quite strongly with NJ M protein truncated to amino acids 70 to 229 (Fig. 2e, lane 4) but not with NJ M protein expressed by pBS-NJM(1-75) (Fig. 2e, lane 5). All of the other three VSV-NJ M protein-reactive MAbs (MAbs 4, 8 and 16 directed to epitopes II, III and IV, respectively)
reacted with the VSV-NJ M protein truncated to the first 75 N-terminal amino acids only and not to C-terminal amino acids 70 to 229 (Fig. 2f to h, lanes 4 and 5). These data clearly indicate that the major antigenic determinants of the M proteins of both VSV-Ind and VSV-NJ are located in the amino-terminal third of these proteins.

These experiments with expressed deletion mutants reveal that three epitopes each of VSV-Ind and VSV-NJ M proteins are clustered within their first 74 N-terminal amino acids. However, we were unable to segregate these epitopes by this technique because of an inability to express peptides shorter than 74 amino acids in length: for example, peptides of the 34 N-terminal amino acids were not detectable when clones with M gene cDNAs 102 nucleotides in length were expressed. However, because the MAbs directed to the M proteins of the two serotypes do not cross-react, chimeras of the two M proteins could be used to detect epitopes in two different regions within the first 74 amino acids of both the VSV-Ind and VSV-NJ M proteins.

Fig. 3 illustrates Western blots of the two wild-type VSV M proteins and four chimeric proteins expressed in E. coli reacting with each of the MAbs directed to the two serotypes (Fig. 3a to h), as well as epitope maps of the VSV-Ind and VSV-NJ M proteins based on the results of these experiments (Fig. 3i and j). Again, none of the antibodies was found to cross-react with the heterotypic M protein, which confirmed the specificity of the short segments of each chimera being responsible for recognition of each MAb. As shown here, Ind MAb 2 directed to epitope 1 of the VSV-Ind M protein reacted with chimera 1 M protein expressed by pBS-IndM(1-74)/NJM(75-229) and chimera 3 M protein expressed by pBS-IndM(1-34)/NJM(35-229) but not with chimera 2 M protein expressed by pBS-NJM(1-74)/IndM(75-229) or chimera 4 M protein expressed by pBS-NJM(1-34)/IndM(35-229) (Fig. 3a). These data show that epitope 1 of the VSV-Ind M protein maps within the N-terminal 34 amino acids (Fig. 3i), which is consistent with all the earlier reports on epitope mapping of the VSV-Ind M protein (Pal et al., 1985a; Ogden et al., 1986; Shipley et al., 1988). Ind MAb 4 directed to epitope 2 and Ind MAb 25 directed to epitope 3 of the VSV-Ind M protein both bind to M protein chimeras 1 and 4 but not to chimeras 2 and 3 (Fig. 3b and c), thus localizing both epitopes 2 and 3 of the VSV-Ind M protein to a region spanning amino acids 35 to 74 (Fig. 3i) and supporting a previous finding that these two epitopes partially overlap (Pal et al., 1985a). A polyclonal antibody directed to the VSV-Ind M protein is shown to bind to chimeras 1, 3 and 4 but not to chimera 2 containing VSV-Ind M amino acids 75 to 229 (Fig. 3d), indicating again that no epitope exists in the carboxy-terminal two-thirds of the VSV-Ind M protein (Fig. 3i). NJ MAb 1 directed to epitope I of the VSV-NJ M protein was shown to bind to M chimeras 1 and 3 but not to chimeras 2 and 4 (Fig. 3e), evidence for mapping epitope I of the VSV-NJ M protein to a region somewhere beyond amino acid 75 (Fig. 3j). NJ MAb 4 directed to epitope II of the VSV-NJ M protein binds to chimeras 2 and 4 but not to chimeras 1 and 3 (Fig. 3f), evidence for mapping epitope II of the VSV-NJ M protein to the N-terminal 34 amino acids (Fig. 3j). Two
MAbs designated NJ MAb 8 directed to epitope III and NJ MAb 16 directed to epitope IV of the VSV-NJ M protein are shown to bind to M protein chimeras 2 and 3 but not to chimeras 1 and 4 (Fig. 3g and h), thus localizing both epitopes III and IV of the VSV-NJ M protein to a region spanning amino acids 35 to 74 (Fig. 3j).

These experiments reveal an epitope map of the VSV-NJ M protein quite similar to that of the VSV-Ind M protein, except that the C-terminal region of the VSV-NJ M protein spanning amino acids 75 to 229 was recognized by a MAb whereas no epitope could be detected in the equivalent region of the VSV-Ind M protein. Epitope I of the VSV-NJ M protein is not likely to be far downstream from amino acid 75 since a previous study reported a certain degree of overlap between epitopes I and IV of the VSV-NJ M protein (Ye et al., 1985) and results presented here localized epitopes III and IV to a region spanning amino acids 35 to 74.

In order to determine the existence of any functional relationships between these two antigenically distinct but otherwise related VSV M proteins, complementation assays were performed to measure their respective capacities to rescue M gen temperature-sensitive (ts) mutants of both serotypes, VSV-Ind tsO23 and VSV-NJ tsC1. The two wild-type M genes and the four chimeric M genes were subcloned between the NcoI and EcoRI sites of pTM1, a vector which contains the bacteriophage T7 polymerase promoter and terminator sequences, as well as a cap-independent translation property (kindly provided by B. Moss; Moss et al., 1990). An NcoI site was created at the start codon of each insert by
Table 1. Comparative rescue of M gene mutants of VSV-Ind tsO23 and VSV-NJ tsC1 by transfecting plasmids expressing wild-type M proteins of VSV-Ind and VSV-NJ and by chimeras expressing different regions of VSV-Ind and VSV-NJ M proteins

<table>
<thead>
<tr>
<th>Transfecting plasmid</th>
<th>VSV-Ind tsO23</th>
<th>VSV-NJ tsC1</th>
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<tr>
<td></td>
<td>31 °C</td>
<td>39 °C</td>
</tr>
<tr>
<td>pTM1</td>
<td>8.1 x 10⁴</td>
<td>1.0 x 10⁶</td>
</tr>
<tr>
<td>pTM1-IndM</td>
<td>1.0 x 10⁴</td>
<td>5.0 x 10⁴</td>
</tr>
<tr>
<td>pTM1-NJM</td>
<td>5.3 x 10⁵</td>
<td>1.0 x 10⁷</td>
</tr>
<tr>
<td>pTM1-NJM(1-74)/IndM(75-229)</td>
<td>5.5 x 10⁶</td>
<td>5.0 x 10⁷</td>
</tr>
<tr>
<td>pTM1-IndM(1-34)/NJM(35-229)</td>
<td>6.0 x 10⁶</td>
<td>1.0 x 10⁷</td>
</tr>
<tr>
<td>pTM1-NJM(1-34)/IndM(35-229)</td>
<td>1.2 x 10⁷</td>
<td>1.0 x 10⁷</td>
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* Yield was titrated by plaque assay on L cell monolayers at 31 °C and 39 °C.
† High frequency of reversion accounted for the high titres of tsC1 at 39 °C as compared with those of tsO23.

amplifying the genes by PCR using synthetic oligonucleotide primers introducing the NcoI site to facilitate the subcloning and to optimize expression. Using the same amino acid sequence terminology as that for the pBS plasmids shown in Fig. 1, the resulting pTM1 plasmids are designated pTM1-IndM, pTM1-NJM, pTM1-NJM(1-74)/IndM(75-229), pTM1-IndM(1-34)/NJM(35-229) and pTM1-NJM(1-34)/IndM(35-229).

To perform the marker rescue experiments, subconfluent cultures of CV-1 cells grown in 60 mm plates were infected with tsO23 or tsC1 at a multiplicity of 0.1 p.f.u. per cell and allowed to adsorb for 30 min at room temperature. The inoculum was then removed and the monolayers were exposed for 30 min at 37 °C to 15 p.f.u. per cell of vTF1-6,2, a recombinant vaccinia virus expressing the T7 RNA polymerase (Fuerst et al., 1986), followed by lipofectin-assisted transfection at 39 °C with 10 gg of control or recombinant pTM1 plasmid DNA. After incubation at 39 °C for 16 h, the medium was harvested and the virus yield was titrated by plaque assay on L cell monolayers at both 31 °C and 39 °C; the washed cells were also lysed with 1 x SDS gel loading buffer and analysed by SDS-PAGE on 12.5% acrylamide gels and Western blotting to identify the relevant VSV M proteins. Western blot analysis (data not shown) revealed virtually equal levels of expression of the wild-type and chimeric M proteins, except chimera 1 which expressed less efficiently and therefore was not included in the marker rescue experiments reported here.

Table 1 shows the mean yield of progeny VSV virions from duplicate experiments, demonstrating the degree of complementation of mutants tsO23 and tsC1 by cotransfection with vectors expressing the VSV-Ind M, VSV-NJ M and three chimeric VSV M proteins. Since the complemented progeny of tsO23 and tsC1 released virions that are still genotypically temperature-sensitive, their titres obtained by plaque assay at 39 °C are considerably lower than those obtained at 31 °C. Nevertheless, both ts mutants show a relatively high rate of spontaneous reversion, considerably greater for tsC1. Titres of the media from tsO23- or tsC1-infected and mock (pTM1)-transfected cells represent the spontaneous emergence of these revertants. It was also observed that, at the restrictive temperature (39 °C) but not at the permissive temperature (31 °C), cells co-infected with the VSV mutants (especially tsO23) and vTF1-6,2 or wild-type vaccinia virus released many fewer VSV progeny virions than did cells infected with the mutants alone, indicating an inhibition of VSV replication at 39 °C by co-infecting vaccinia virus (data not shown). Table 1 shows that the expressed VSV-Ind M protein, VSV-NJ M protein, and the three chimeric M proteins are all able to rescue both tsO23 and tsC1, yielding about 100-fold more plaque-forming virions than do the mock-transfected cells. The M proteins of wild-type VSV-Ind, tsO23 and wild-type VSV-NJ can be readily distinguished by their differing mobility in SDS-PAGE (Li et al., 1988; Ye et al., 1985). Reaction of Western blots of the rescued virions with anti-VSV M protein antibodies revealed much more wild-type or chimeric M proteins in rescued virions than the parental leaky mutant M proteins (data not shown).

These results indicate that the two serotypically different VSV M proteins retain common recognition sites for both the viral membrane and RNP components of the two different serotypes of VSV. These functional domains, ostensibly essential for the assembly and budding of VSV, are presumably located at the same regions since the construction of chimeras of the M proteins did not significantly reduce the ability of the respective M proteins to complement the homotypic or heterotypic ts mutants. The antigenic domains of the two
different VSV serotypes have similar map locations on the respective M proteins.

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References


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