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Nucleotide Sequence of a cDNA Clone Encoding the Entire Glycoprotein from the New Jersey Serotype of Vesicular Stomatitis Virus

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The nucleotide sequence of the mRNA encoding the glycoprotein from the New Jersey serotype of vesicular stomatitis virus (VSV) was determined from a cDNA clone containing the entire coding region. The sequence of 12 5'-terminal noncoding nucleotides present in the mRNA but not in the cDNA clone was determined from a primer extended to the 5' terminus of the mRNA. The mRNA is 1,573 nucleotides long (excluding polyadenylic acid) and encodes a protein of 517 amino acids. Only six nucleotides occur between the translation termination codon and the polyadenylic acid. Short homologies between the untranslated termini of this mRNA and the mRNAs of the Indiana serotype were found. The predicted protein sequence was compared with that of the glycoprotein of the Indiana serotype of VSV and with the glycoprotein of rabies virus, using a computer program which determines optimal alignment. An amino acid identity of 50.9% was found for the two VSV serotypes. Approximately 20% identity was found between the rabies virus and VSV New Jersey glycoproteins. The positions and sizes of the transmembrane domains, the signal sequences, and the glycosylation sites are identical in both VSV serotypes. Two of five serine residues which were possible esterification sites for palmitate in the glycoprotein from the Indiana serotype are changed to glycine residues in the glycoprotein from the New Jersey serotype. Because the glycoprotein of the New Jersey serotype does not contain esterified palmitate, we suggest that one or both of these residues are the probable esterification sites in the glycoprotein from the Indiana serotype.

The two classical serotypes of vesicular stomatitis virus (VSV) are termed Indiana and New Jersey (VSV_{Ind} and VSV_{NJ}, respectively). However, it is clear that there has been considerable antigenic variation among the viruses classified within each of these serotypes (3). Viruses within these two groups can be distinguished by specific neutralizing antiserum which is directed against the single glycoprotein species (G) which forms spikes protruding from the viral envelope. (9). Analysis of the antigenic regions in the G proteins of these two serotypes by using monoclonal antibodies has shown that there are at least four epitopes in both the VSV_{Ind} and the VSV_{NJ} G proteins (10). One monoclonal antibody made against the VSV_{Ind} G protein was reported which would recognize the G protein from both serotypes, indicating that the proteins do have some homology (11). This antibody was only capable of neutralizing VSV_{Ind}.

We previously determined the nucleotide sequences for the VSV_{Ind} mRNAs encoding the N, NS, M, and G proteins, as well as the predicted sequences of each protein (7, 19). Because we are especially interested in the structure and evolution of viral membrane proteins, we have extended our sequence analysis to the mRNA encoding the G protein from VSV_{NJ}. To do this we isolated a cDNA clone of the mRNA encoding the protein and determined its complete nucleotide sequence. We have compared the predicted amino acid sequence of this G protein with that of the G protein from VSV_{Ind} determined previously and with the G protein of rabies virus (1), a distant relative of VSV in the rhabdovirus group. Knowledge of the complete amino acid sequences of glycoproteins from two VSV serotypes should permit a detailed analysis of the antigenic structure of these molecules.

MATERIALS AND METHODS

Cells and virus. The Ogden strain of VSV_{NJ} (provided by Alice Huang) was grown on BHK-21 cells adapted to suspension culture (provided by Amiya Banerjee). Published procedures were used for virus growth and purification (8). The yield of virus was 8 to 10 mg of virus protein per 6×10^8 infected cells (1 liter) for both VSV serotypes.

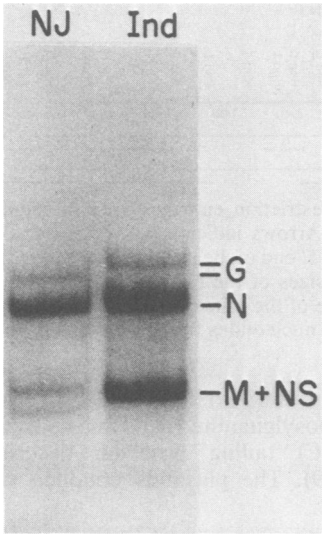

FIG. 1. Autoradiogram showing reverse transcripts of VSV$_{NJ}$ and VSV$_{Ind}$ mRNAs. DNAs were labeled with [α-^{32}P]dCTP and analyzed by electrophoresis on an alkaline agarose gel. Correspondence of the cDNAs to mRNAs is indicated.

Synthesis of mRNA and cDNA and cloning of the cDNA. VSV mRNAs were synthesized in vitro and purified by chromatograpy on oligodeoxythymidylic acid cellulose as described previously (19). Procedures for synthesis and purification of the single- and double-stranded cDNA copies were identical to those reported previously, with the following exception. After nuclease S1 digestion to cleave the single-stranded DNA loop, $ZnCl_2$ and unincorporated deoxynucleoside triphosphates remaining from the second-strand synthesis reaction were removed by chromatography on Sephadex G-50. The excluded material was precipitated with ethanol before electrophoresis on an acrylamide gel. The chromatography step prevented the formation of an insoluble precipitate which otherwise traps DNA at the top of the gel.

DNA sequence analysis. One cDNA clone containing the entire coding sequence of the VSV$_{NJ}$ G-protein mRNA (pNJG6) was used for all sequence analyses. DNA sequence analysis was by the method of Maxam and Gilbert (12). Virtually the entire sequence was determined on both DNA strands. The 5'-^{32}P-labeled 20-nucleotide primer used for sequencing the extreme 5' end of the VSV$_{NJ}$ G mRNA was isolated as follows. Approximately 100 μg of pNJG6 DNA was digested with *Hin*fI and alkaline phosphatase, and the 435-nucleotide fragment spanning the junction of the pBR322 sequences with the 5' G mRNA sequence (to position 163 in the G mRNA sequence) was purified by gel electrophoresis on a 6% polyacrylamide gel. A 423-nucleotide fragment of pBR322 copurified with this fragment. The two fragments were labeled with [^{32}P]ATP and polynucleotide kinase and then digested with *Hha*I. This digestion left three labeled fragments of 423 nucleotides (uncut pBR322 fragment), 135 nucleotides (pBR322 side of the 435-nucleotide fragment), and the 20-nucleotide primer fragment extending from position 143 to position 163 in the G mRNA sequence. These fragments were separated on a 12% sequencing gel after denaturation to separate the 20-nucleotide primer from its 25-nucleotide complementary strand generated by the staggered cutting of the restriction endonucleases. The 20-nucleotide primer fragment (2×10^6 cpm) was eluted by soaking the gel slice in water and precipitated with ethanol in the presence of 15 μg of total VSV$_{NJ}$ mRNA synthesized in vitro.

Primer extension with reverse transcriptase was as described previously (7). Greater than 80% of the primer was extended to a length of 163 nucleotides. The sequence of this material was then determined.

Labeling of VSV with [^{3}H]palmitic acid. A confluent layer of about 10^6 L cells on a 5-cm tissue culture dish was infected with VSV (multiplicity of infection = 50) and labeled in 4 ml of Dulbecco modified minimal essential medium supplemented with 1 mCi of [^{3}H]palmitic acid, 10% tryptose phosphate (Difco Laboratories, Detroit, Mich.), 5% fetal calf serum, nonessential amino acids, pyruvate, and 1% dimethyl sulfoxide [^{3}H]palmitic acid was dried and dissolved in dimethyl sulfoxide (1 mCi/0.04 ml) before addition to the medium. After 16 h, the medium was harvested from each plate and clarified by centrifugation at 12,000 $\times$ g. Virus was pelleted from the medium by centrifugation at 40,000 $\times$ g for 1 h.

Materials. Restriction endonucleases were from New England Biolabs, Beverly, Mass., or Bethesda Research Laboratories, Bethesda, Md. Polynucleotide

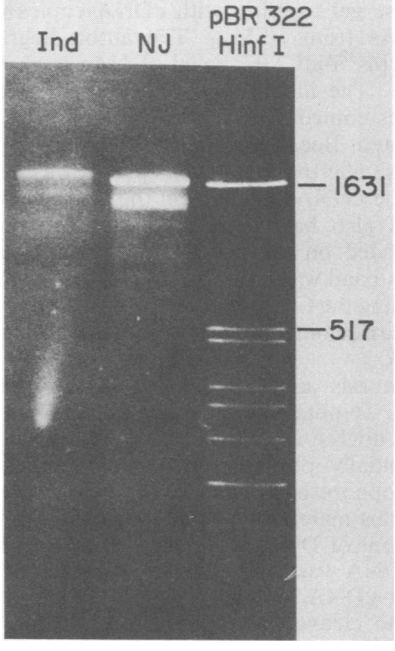

FIG. 2. Electrophoresis of double-stranded cDNA after nuclease S1 treatment. The double-stranded cDNA copy of the VSV$_{NJ}$ G mRNA was electrophoresed on a 6% polyacrylamide gel beside markers of cDNA of the G mRNA from VSV$_{Ind}$. Size markers of pBR322 DNA cleaved with *Hin*fI were also included as indicated.

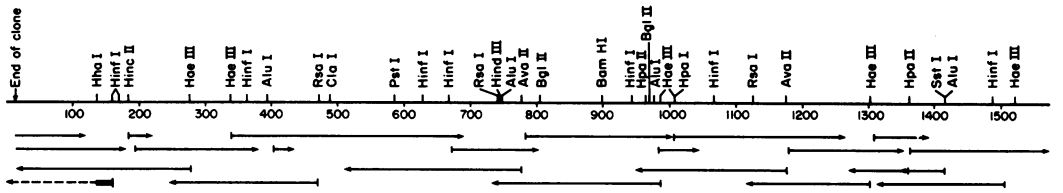

FIG. 3. Restriction map of the insert from pNJG6. Sites for restriction enzymes used in sequencing are shown. The left end corresponds to the 5′ end of the G mRNA. Arrows indicate the regions and directions sequenced. Numbers are in hundreds of nucleotides beginning at the 5′ end of the mRNA. Terminal *Pst*I sites are at the junctions of pBR322 sequences with the dG:dC tails. The sizes of the tails are not indicated. The 5′-terminal sequence of the mRNA was determined from the sequence of the primer (indicated by the heavy line) extended to the 5′ end of the mRNA. The DNA in pNJG6 lacks 12 nucleotides from the 5′ end of the mRNA.

kinase was from Boehringer Mannheim, Indianapolis, Ind. [^{32}P]ATP (crude grade) was from ICN, Irvine, Calif. Reagents used in mRNA synthesis and cDNA cloning were as described previously (19). [^{3}H]palmitic acid was from New England Nuclear Corp., Boston, Mass.

RESULTS

Identification of the first-strand cDNA. To obtain a full-length cDNA copy of the mRNA encoding the glycoprotein from VSV_{NJ}, we first synthesized cDNA copies of the mRNAs produced in vitro by the virion-associated RNA polymerase of the virus. These cDNA copies were subjected to electrophoresis on an alkaline agarose gel together with cDNA copies of the mRNAs from VSV_{Ind}. The autoradiogram of these [α-^{32}P]dCTP-labeled cDNAs is shown in Fig. 1. The identities of the full-length cDNA species copied from the mRNAs of VSV_{Ind} are indicated. Because the proteins of VSV_{NJ} have sizes similar to those of VSV_{Ind} (2), we expected that the cDNAs made from the VSV_{NJ} mRNAs would also have similar sizes. We therefore proceeded on the assumption that the largest cDNA band was a full-length copy of the mRNA encoding the G protein (which was later verified) and carried out synthesis of the second strand of cDNA.

Synthesis and cloning of double-stranded cDNA. To obtain a double-stranded cDNA copy of the mRNA as close as possible to full length, we initially purified the first-strand DNA by electrophoresis on an alkaline agarose gel and used this material as a template for the Klenow fragment of DNA polymerase I. The 3′ end of the cDNA was allowed to "self-prime" second-strand cDNA synthesis, and S1 nuclease was used to cleave the single-stranded loop. DNA obtained was then purified by electrophoresis on an acrylamide gel. Figure 2 shows a photograph of the gel stained with ethidium bromide. A stained band of double-stranded cDNA from VSV_{NJ} G mRNA just smaller than the double strand from the VSV_{Ind} G mRNA is clearly visible. This DNA was eluted from the gel and inserted at the *Pst*I site of pBR322 by using the deoxyribosylguanine (dG) and deoxyribosylcytosine (dC) "tailing" procedure described previously (19). The plasmids obtained which had

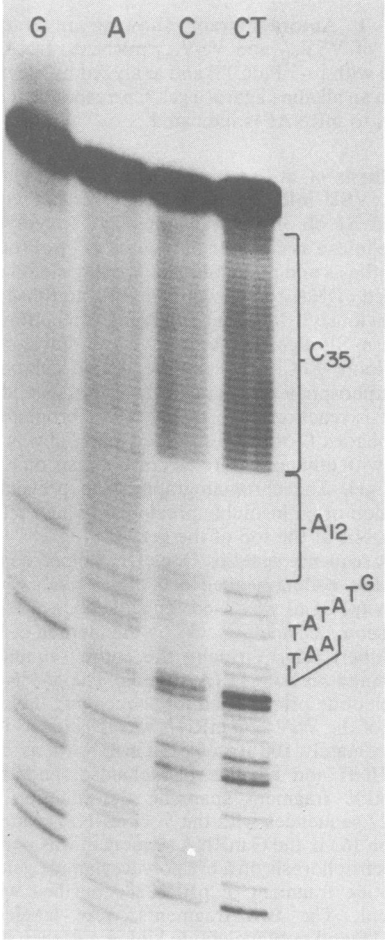

FIG. 4. Sequencing gel showing the region of the pNJG6 insert DNA corresponding to the 3′ end of the G-protein mRNA. The stop codon for protein synthesis is indicated, as are the six noncoding nucleotides between the stop codon and the poly(A) sequence.

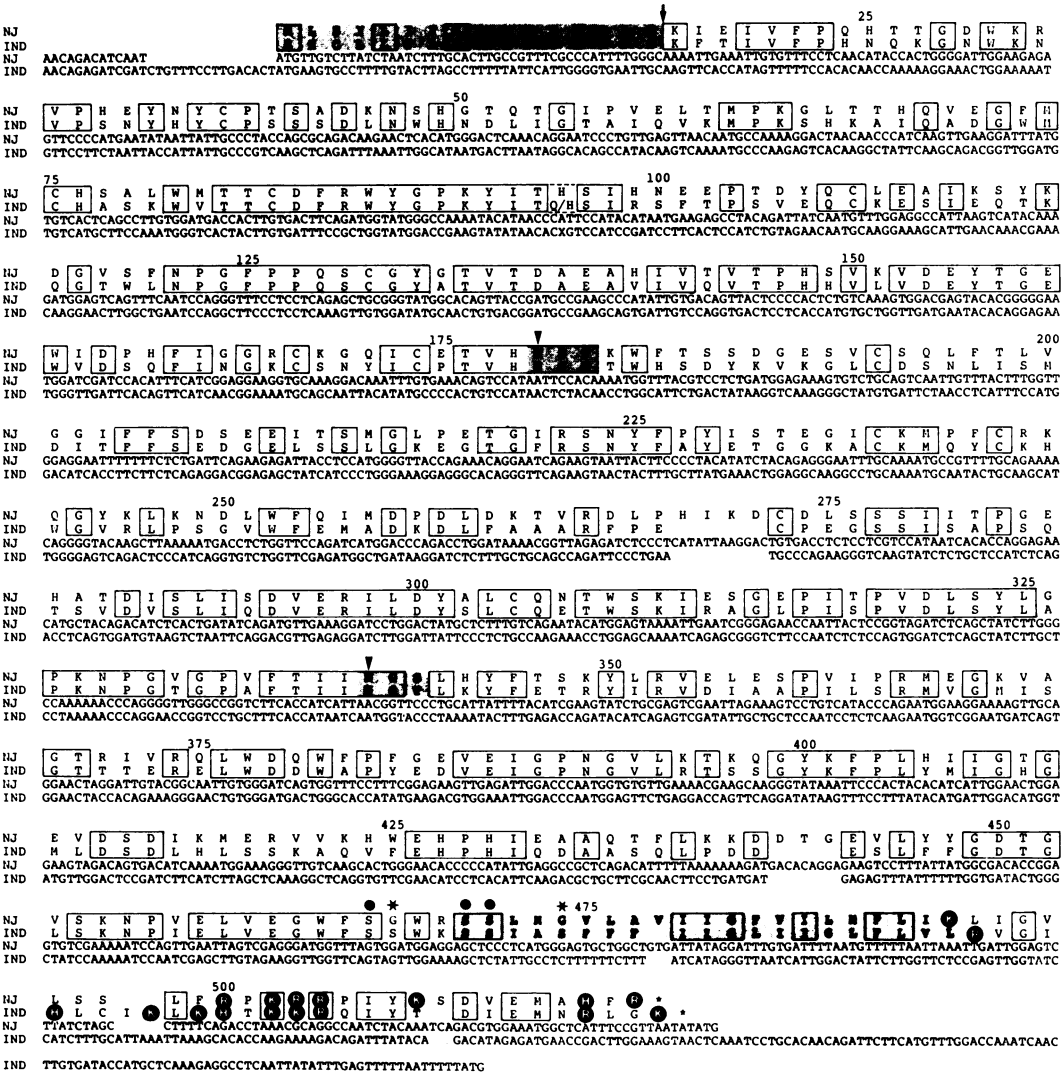

FIG. 5. Nucleotide and predicted protein sequences of the VSV$_{NJ}$ G mRNA and G protein compared with the G mRNA and predicted protein sequences from VSV$_{Ind}$. The protein sequences were aligned using the computer program described by Rose et al. (18). Homologies are boxed. NH_2-terminal signal sequences are shaded, as are the two glycosylation sequences and the hydrophobic transmembrane domains at the COOH terminus. Charged residues in the hydrophilic COOH-terminal tail are shaded also. The serine residues which are probable sites for esterification of palmitate in the VSV$_{Ind}$ glycoprotein (and which are glycine residues at the corresponding positions of the VSV$_{NJ}$ glycoprotein) are indicated by stars above the sequences. Solid circles above the sequence indicate serine residues shared between the two proteins within the possible region of palmitate esterification. The letter codes for the amino acids are as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine.

inserts at the *Pst*I site were screened by digestion with *Pst*I to determine the size of the inserted DNA. The plasmid containing the largest insert (about 1,600 nucleotides) was designated pNJG6 and chosen for sequence analysis.

Sequence determination. The nucleotide sequence of the insert DNA was determined almost entirely on both DNA strands as shown in Fig. 3. This sequence extended from position 13 [preceded by oligo(dG)$_{15}$] to nucleotide 1573 in the mRNA which precedes the polyadenylic acid [poly(A)]. An autoradiogram of the DNA sequencing gel showing the sequence corresponding to the 3′ end of the mRNA is shown in

Fig. 4. The heteropolymeric sequence is followed by A_{12} (derived from the oligodeoxythymidylic acid primer), indicating that the 3′ mRNA sequence was contained in the clone, and G_{35} generated by the dC tailing of the insert DNA. Because the nuclease S1 treatment of the double-stranded DNA results in the loss of at least a few nucleotides corresponding to the 5′ end of the mRNA, we determined how many additional nucleotides were actually present at the 5′ end of the mRNA.

The 5′-^{32}P-labeled primer indicated in Fig. 3 was hybridized to total VSV_{NJ} mRNA and extended with reverse transcriptase. The extended primer yielded a discrete band after gel electrophoresis, suggesting that it had been extended to the 5′ end of the mRNA. The nucleotide sequence of the elongated material was determined and was found to extend 12 nucleotides beyond the sequence present in the clone. This sequence must correspond to the 5′ end of the mRNA because it contained the complement of the known 5′ mRNA sequence (AACAG, reference 6) at its 3′ end. The complete mRNA sequence is shown in Fig. 5.

Absence of palmitate. The G protein from VSV_{Ind} is known to contain one to two molecules of palmitic acid (21) probably esterified to one or more of the five serine residues at amino acid positions 459, 460, 463, 464, and 467 (19). These positions are within or just on the NH_2-terminal side of the transmembrane domain. The G protein from VSV_{NJ} has glycine residues in place of the serine residues at positions 460 and 467 (Fig. 5). Because it was known that the Concan strain of VSV_{NJ} could not be labeled with [^{3}H]palmitate (H. P. Ghosh, personal communication), we attempted to label the G protein from the Ogden strain of VSV_{NJ} (the sequence reported here) with [^{3}H]palmitate. The results of this experiment are shown in Fig. 6. Equal amounts of virus protein from both serotypes labeled with [^{3}H]palmitate were subjected to electrophoresis on a polyacrylamide gel. The stained gel was photographed (Fig. 6A) and subjected to fluorography (Fig. 6B). The results reveal strong and specific labeling of the VSV_{Ind} G protein and no labeling of the VSV_{NJ} G protein.

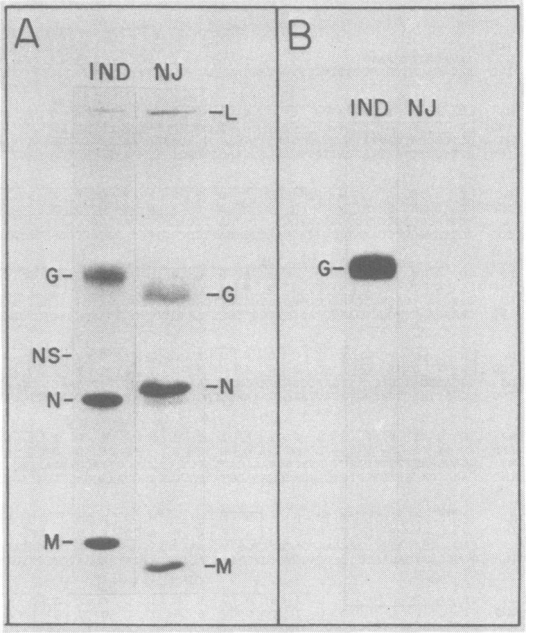

FIG. 6. Analysis of VSV_{Ind} and VSV_{NJ} proteins labeled with [^{3}H]palmitic acid. VSV (ca. 20 μg of viral protein) which had been grown in medium containing [^{3}H]palmitic acid was subjected to electrophoresis on a sodium dodecyl sulfate-polyacrylamide gel. The stained gel was photographed (A) and subjected to fluorography for 4 h (B).

DISCUSSION

Features of the nucleotide sequence. The nucleotide sequence shown in Fig. 5 corresponds to the complete sequence of the mRNA encoding the G protein from VSV_{NJ} without the 5′ cap or 3′ poly(A). There is a single open reading frame for translation beginning with the initiation codon ATG (residues 14 to 16) and terminating with TAA (residues 1565 to 1567) only six nucleotides before the beginning of the poly(A). The predicted protein sequence is 517 amino acids long (calculated molecular weight = 58,163, without carbohydrate) as compared with 511 for the G protein of VSV_{Ind}. The homology between the two predicted protein sequences (discussed below) indicates clearly that the VSV_{NJ} G protein is encoded in this reading frame. The 5′-noncoding region is only 13 nucleotides, whereas the 3′-noncoding region is only 6 nucleotides. The corresponding regions of the VSV_{Ind} mRNA are 29 and 97 nucleotides, respectively.

The five mRNAs encoded by VSV_{Ind} begin with the sequence $m^7G^{5'}ppp^{5'}$AmACAGNNAUC and terminate with UAUG-poly(A) (17). Our results show that this homology also extends to the VSV_{NJ} G mRNA. The nucleotide sequence homology in the coding region (aligned by the protein sequences [Fig. 5]) is 54%. This result is consistent with the report of little exact homology between the nucleotide sequences of VSV_{NJ} and VSV_{Ind} detectable by nucleic acid hybridization (16). This is true because the homology is interrupted by nonhomologies. Indeed, the longest continuous match is only 16 nucleotides at nucleotides 974 to 989. The lack of strong nucleotide homology in regions of amino acid identity is due almost exclusively to third-position changes in codons.

Features of the protein sequence. The protein sequence predicted by the single open reading frame in the mRNA was compared with the VSV$_{Ind}$ protein sequence by using a computer program which determines an optimal alignment for the two proteins (5). This alignment is shown in Fig. 5 along with the two nucleotide sequences. The overall identity of amino acids in the optimal alignment is 50.9%.

At the NH_2 terminus there is a hydrophobic domain aligned with the signal sequence of the VSV$_{Ind}$ G protein. The cleavage site for the signal sequence in the VSV$_{NJ}$ G protein presumably occurs at the same site as in the VSV$_{Ind}$ G protein. Analysis of the G protein from VSV$_{NJ}$ has shown that it contains two asparagine-linked complex oligosaccharides (15). Glycosylation must occur at residues 179 and 340 because these are the only potential glycosylation sequences of the form Asn-X-Ser/Thr (14). The two sites are aligned precisely with the glycosylation sites in the VSV$_{Ind}$ G protein. Near the COOH terminus there is a domain of 20 amino acids which are hydrophobic or nonpolar, and this domain is aligned with the 20-amino-acid membrane spanning domain of the VSV$_{Ind}$ G protein.

The 28 residues between the hydrophobic domain and the COOH terminus contain eight basic amino acids and only two acidic residues. The highly basic character of this "cytoplasmic" region of the protein is similar to the comparable 29-amino-acid cytoplasmic domain of the VSV$_{Ind}$ protein. Although the amino acid homology is not exact in this region, there is a conservation of charge distribution. Conservation of amino acid character in this domain of the protein suggests that it plays an important role in virus replication. This domain might interact with internal virion proteins (presumably M or N) during virus assembly. Our recent results on in vitro mutagenesis of the G gene suggest that it may also be an important signal directing G-protein transport to the plasma membrane before virus assembly.

The signal sequence and the transmembrane domains of both glycoproteins are among the least conserved regions in terms of amino acid identity, yet they have retained their hydrophobic character. This suggests that the exact amino acid sequence is relatively unimportant as long as hydrophobicity is maintained.

Cysteine residues are known to be the most highly conserved amino acids. Indeed, all 12 of the cysteine residues in the VSV$_{NJ}$ G protein are aligned with cysteine residues in the VSV$_{Ind}$ G protein. A high degree of proline conservation is also common and is noted here. In addition to conservation of specific amino acids, we note that the two proteins contain some regions of high amino acid identity. For example, the regions from amino acids 80 to 98 and 122 to 144 contain 17 and 20 identities, respectively. However, it is also notable that the longest perfect sequence identity between the two proteins is only 14 residues. Much of the variability in influenza viral glycoprotein sequences (hemagglutinin) apparently reflects the evolutionary selection of mutant proteins which can escape recognition by the immune system of the host (22). The antigenic regions in these proteins have been mapped to highly variable regions in the protein sequence which are known to be exposed on the protein. Therefore, it seems likely that the antigenic regions of the VSV G proteins will also lie within highly variable, relatively hydrophilic (exposed) regions. The epitope recognized by monoclonal antibodies which cross-reacts with both serotypes presumably falls within a conserved domain (11). The availability of the protein sequences should help to elucidate the locations of the epitopes involved in both virus neutralization and cross-reactivity.

A partial protein sequence has been reported for the N protein of VSV$_{NJ}$ and compared with the sequence of the N protein from VSV$_{Ind}$ (13). Although only about 20% of the N protein was compared, it seems significant that the homology found was 70.8%, considerably greater than the 50.9% homology between the glycoproteins of these two serotypes. Greater conservation of the N-protein sequence as compared with the G protein is also consistent with previous peptide mapping data showing that the G-protein sequence varies more rapidly than the sequences of the other proteins (2, 4). These results are not surprising because G protein is the type-specific VSV antigen, whereas N protein is the group-specific antigen.

The absence of palmitate esterification to the VSV$_{NJ}$ G protein is especially interesting in light of the sequence differences between the VSV$_{NJ}$ and VSV$_{Ind}$ G proteins at the serine residues which are the probable esterification sites of palmitate in the VSV$_{Ind}$ G protein. It seems likely that one or both of the serine residues of VSV$_{Ind}$ that are glycine residues in the VSV$_{NJ}$ sequence (Fig. 5) are the site(s) of palmitate esterification in the VSV$_{Ind}$ G protein. The absence of these acceptor residues in VSV$_{NJ}$ would prevent esterification. Alternatively, other sequence changes in the VSV$_{NJ}$ G protein sequence could prevent palmitate esterification.

The esterification of palmitate to proteins has been a modification in search of a function. It has been proposed that this modification might be an essential marker for the transport of G protein to the plasma membrane (23) or could be important in VSV budding (20). The absence of

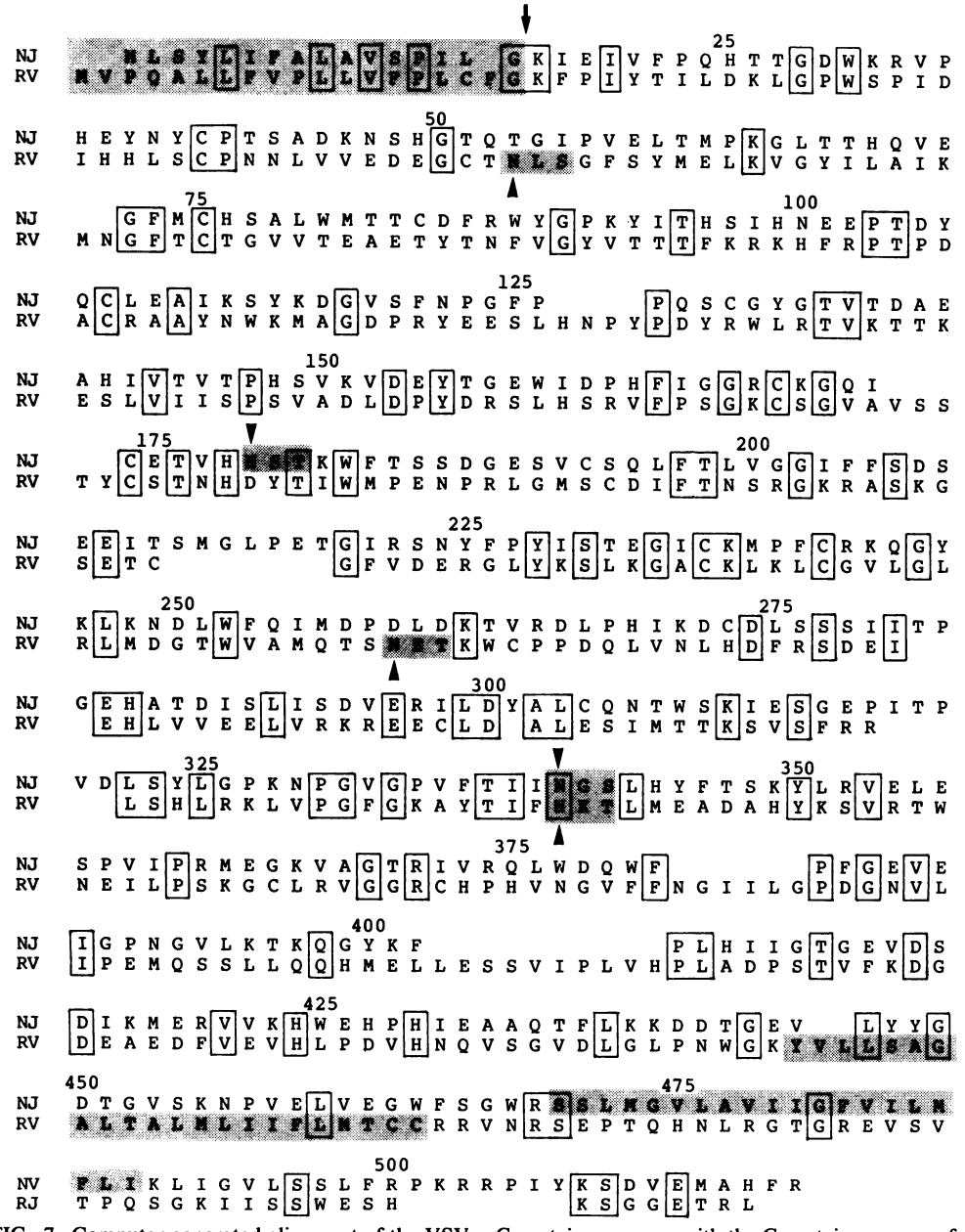

FIG. 7. Computer-generated alignment of the VSV_{NJ} G-protein sequence with the G-protein sequence from the ERA strain of rabies virus (1). The alignment was carried out as described previously (18). The entire sequence of each protein, including the signal sequence, is shown. Identities are boxed. Both the signal sequences and the transmembrane domains are shaded, as are the putative glycosylation signals Asn-X-Ser and Asn-X-Thr. Arrows indicate the three putative glycosylation sites in rabies virus G protein and the two putative glycosylation sites in the VSV_{NJ} G protein.

this modification in the VSV_{NJ} G protein indicates that the modification is certainly not critical for all VSV strains. In fact, we found that the yield of VSV_{NJ} was not significantly different (8 to 10 mg of virus protein per 6×10^8 cells) from that obtained in a parallel infection with VSV_{Ind}. Our analysis does not, of course, rule out the possibility that some other fatty acid has been esterified to the VSV_{NJ} G protein.

Homology with the rabies virus glycoprotein. We have reported previously that the VSV_{Ind} G protein shows a significant homology to the

rabies virus G protein when compared using a computer program which can determine a statistical significance for the protein match. This result supports the idea that there was a common ancestor of the two viruses. We therefore anticipated that a similar degree of relatedness would be observed when the VSV$_{NJ}$ G proteins and the rabies virus G proteins were compared.

Figure 7 shows the computer-generated alignment of these two proteins. Indeed, the extent of the identity is 21%, almost the same as the 20% identity reported for VSV$_{Ind}$ and rabies virus. More importantly, the statistical significance of the match was comparable to that obtained in the match between the VSV$_{Ind}$ G and rabies virus G proteins (18). This similar level of homology provides further support for the notion that rabies virus and the two VSV serotypes share a common ancestor. As noted in the comparison between VSV$_{Ind}$ and rabies virus G proteins, there is a strong conservation of cysteine residues and an alignment of one of the two glycosylation sites in VSV$_{NJ}$ G protein with one of the three in rabies virus G protein.

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