NOTES

Genome RNA Terminus Conservation and Diversity among Vesiculoviruses

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Sequence analysis of the RNA genome termini of various vesiculovirus standard and defective interfering (DI) particles demonstrated that some virus regulatory sequences and domains of virus N protein are highly conserved while others show considerable divergence. Clearly, distinct RNA signal sequences and protein-coding regions of these virus genomes have quite different evolutionary pressures or constraints. Terminal regions of DI-particle RNA genomes of these viruses were found to possess self-complementary stems at the RNA termini, demonstrating the conservation of this DI-particle structural feature throughout the vesiculovirus group. A high degree of conservation of the 3'-terminal sequences of recent and historic isolates of vesicular stomatitis virus New Jersey was also demonstrated.

The vesiculoviruses have previously been grouped on the basis of morphology and various degrees of serological cross-reactivity, although some inconsistencies exist (5, 6, 12, 42). Considerable information is available concerning the structure of vesicular stomatitis virus (VSV) Indiana RNA

sequences critical for the control and regulation of virus functions (4, 10, 11, 15, 19, 22, 23, 26, 43). Terminal RNA genome sequences have also been suggested to play an important role in controlling the interaction between standard particles and DI particles (18, 24, 25, 33, 35) and have

FIG. 1. Diagrammatic representation of the terminal structures of the DI-particle RNA genomes of Piry, Chandipura, and Cocal viruses. Solid bars indicate minus-sense RNA homologous to standard-particle RNA. Open bars indicate complementary plus-sense RNA. Arrows indicate the nucleotide length of the terminal self-complementary stem structures. N indicates an ambiguous nucleotide.

genomes, their relationship to the structure of defective interfering (DI) particles, and their interaction with these particles (24, 31, 33, 35). The terminal regions of the RNA genomes of VSV standard and DI particles contain se-

quences critical for the control and regulation of virus functions (4, 10, 11, 15, 19, 22, 23, 26, 43). Terminal RNA genome sequences have also been suggested to play an important role in controlling the interaction between standard particles and DI particles (18, 24, 25, 33, 35) and have

been shown to accumulate base changes during *in vitro* persistent virus infections or serial high-multiplicity-infection passages of virus, indicating that a relatively rapid evolution of these regions can occur (32, 44).

A detailed analysis of the terminal noncoding and coding RNA genome sequences of standard and DI particles of various members of the VSV group was undertaken to

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FIG. 2. (A) Comparison of sequences of cDNA transcripts of 3' termini of genome RNAs of VSV Indiana (IND), Cocal virus (CO), VSV NJ, Ogden strain (NJ), Piry virus (PIRY), and Chandipura virus (CHAND). These regions were sequenced as described previously (31) by using cDNA oligonucleotide primers synthesized on the basis of published sequences of the 3' termini of the genome RNAs of VSV Indiana (CGAAGACAAACAAACC) and NJ (CGAAGACAAAAAACC) (29, 40) and on the basis of the leader RNA sequences of Cocal (CGAAGACAAACAAACC), Chandipura, and Piry (CGAAGAAAAACAAACC) viruses as published and corrected (Cocal and Chandipura sequences having been inadvertently switched) (13; Dan Kolakofsky, personal communication). The sequences shown represent the leader, spacer (S), and N-gene-noncoding regions. (B) Comparison of deduced sequences of 5' termini of genome RNAs of the viruses shown in panel A. The sequences shown represent the leader, spacer (S), and L-gene-noncoding regions. Dashes indicate nucleotides which are homologous to those in the VSV Indiana sequence. Base substitutions, insertions, and deletions relative to VSV Indiana are indicated. The five-times-repeated A encapsidation signal (4) is indicated by dots, and the postulated virus NS protein interaction site is also indicated (19, 22). Regions of apparent extensive sequence homology are indicated by solid bars, while regions of apparent extensive sequence diversity are indicated by open bars. The asterisk indicates the previously published genome RNA 5'-terminal sequence of VSV NJ, Ogden strain (21), which is shown for comparison. N indicates an ambiguous nucleotide. Sequence comparisons were performed by using the nucleic acid homology programs of Goad and Kanehisa (14).

ascertain the extent of natural evolution of these regulatory and protein-coding RNA regions and to determine the precise interrelationship of these viruses.

VSV Indiana serotype, Mudd-Summers strain (from John Mudd), was originally obtained from cattle in Indiana in 1925 (7). VSV new Jersey (NJ) serotype, Ogden strain (from F. Schaffer), was originally isolated from cattle in Ogden, Utah, in 1949 (6). Vesiculoviruses Cocal (sometimes referred to as Indiana 2 virus) (20), Chandipura (Nagpur strain) (2), and Piry were obtained from the American Type Culture Collection. Their identities were verified by a virus neutralization assay with antisera prepared against these viruses. Standard-

particle 5'-terminal genome sequences of the various VSVs were derived by direct enzymatic or chemical RNA sequencing of the 5' and 3' termini (respectively) of purified genome RNAs of the DI particles of these viruses, as described previously (9, 17, 28, 31, 34). The validity of assuming the DI- and standard-particle 5' termini to be identical has been verified by direct sequencing of numerous VSV Indiana DI-particle RNA termini (24, 31, 33, 35) and was further verified by successfully sequencing the RNA 5' terminus of Chandipura virus standard particles (data not shown). The DI-particle RNA genomes of the various VSVs had self-complementary terminal sequences, consistent with their

IND	ATGTCGTGTTACAGTCAAGAGAATCATTGACAACACAGTCATAGTTCCAAAACCTCTGCAATGAGGATCCAGTGGAAATACCCGGCAGATTACTTC
CO	---G---C---T---G---AC-CG-G---TG-TT-G--G--TACA-----T-----T-----T-----T-----T---C-----
NJ	---G--CC-----T-----A-TG--T-CA-A--TCAG--T-----A--G--C-----G--A--G--T---T-----
PIRY	-----GT-C--G---C-C--TTCAAC-GGG--CCAGG-T--GG-GGCTT-G-----G-GC--A--C-----A---TT--T--T--G-----T
CHAND	---AG-TC-CA---ATTCT-C--TTC-ACAGGTCA-ACAG-CTCCGTGTGC-----A-----A--C-----G-TT--A-GG-CA-T----
IND	MetSerValThrValLysArgIleIleAspAsnThrValIleValProLysLeuProAlaAsnGluAspProValGluTyrProAlaAspTyrPhe
CO	- AlaAla - - - - Val - - AspSer - - Thr - - Phe -
NJ	- AlaPro - - - - - - - AsnAspSerIle - Gln -
PIRY	- - - Ser - - - - - SerThrGly - GlnVal - AlaAla - - - Ser - - - - - Phe - - Glu - -
CHAND	- - SerGln - PheCys - SerThrGlyGlnThrValSerValCys - - - - - - - - - - Phe - GlyAlaPhe -

	160	180	200	220	240
IND	AGAAATCAAGGAGATTCCTCTTTACATCAATACTACAAAAAGTTTGTCAGATCTAAGAGGATATGTCTACCAAGGCCTC				
CO	-A--GG---CGA--C--C--GG-G-----C--C-----G---C--G-G--CT-G-----G--C--T-----				
NJ	GA---TAAC-CCA-C--AGTAT-A--TG-G--C--C-A-GTGGCAC-AAAT---T-G---C---C--A-----G--				
PIRY	G _C -----C-G-- _A -----C--GG-G-T---TCA---CGCTTGGAC---GACA-A--TC-CCAG-----G--TATG--AT-G				
CHAND	-CTCC-AATGCCAG--AA--GACGGTGT-AC---AAGA-GG-GACAGA--T--GTC				
IND	ArgLysSerLysGluIleProLeuTyrIleAsnThrThrLysSerLeuSerAspLeuArgGlyTyrValTyrGlnGlyLeu				
CO	LysArg - ArgAsp - - Val - - - - - - - - - - Ala -				
NJ	GluAsnAsnThrAsn - Val - - Val - - LysValAla - Asn - - - Ala - - - - - - - - - - - - - - - - -				
PIRY	Ala - AsnGlyLys - - ValPhe - Ser - ArgLeuAsp - Asp - - - Gln - - - Met - -				
CHAND	ThrProAsnAlaArgLys - ThrValLeuHisLysGluGlyAspArg - GluSer				

FIG. 3. Comparison of sequences of cDNA transcripts of 3' termini of N-protein-coding regions of VSV Indiana (IND), Cocal virus (CO), VSV NJ, Ogden strain (NJ), Piry virus (PIRY), and Chandipura virus (CHAND). The sequences are numbered from the 3' termini of the virus genome RNAs. The deduced amino acid sequences are presented directly below the nucleic acid sequences for this region. Dashes indicate homology to the VSV Indiana sequence. Amino acid regions of apparent extensive sequence homology are indicated by the solid bar, while amino acid regions of apparent extensive sequence diversity are indicated by the open bar.

having copy-back-type structures (Fig. 1), like the majority of VSV Indiana DI-particle RNAs analyzed (24, 35). The conservation of this general structural feature of DI-particle genomes of numerous vesiculoviruses must provide a strong selective advantage to these particles to maintain their interfering abilities. The Cocal virus DI particles reported here have a complementary stem structure of only 44 nucleotides, making them the smallest VSV DI particle stem structures thus far observed. The Cocal virus minus-sense leader RNA may also be small.

A summary of the deduced sequences of the terminal RNA noncoding regions of the various standard VSVs is shown in Fig. 2. The presence of base substitutions, insertions, and deletions relative to Indiana can be seen. The 5'- and 3'-terminal 18 nucleotides appear to be highly con-

served. The five-times-repeated A residue every third base which has been suggested to be the signal for the encapsidation of virus RNA (4) is conserved at the 5' termini of both the plus- and minus-sense virus RNAs in all cases except Cocal virus, in which a single A-to-G change is present at the 5' end of the genome RNA. Clearly, the uninterrupted presence of this signal sequence cannot be an absolute prerequisite for the efficient encapsidation of virus RNA. The remainder of the leader transcript-coding regions appear to be highly divergent, including the sequence region in which virus polymerase (NS) protein interaction occurs (19, 22) and which is probably involved in the initiation of RNA transcription and replication. The lack of conservation of this site at the 3' termini of both the minus- plus-sense virus RNAs is interesting. As the proteins of these viruses show

FIG. 4. Comparison of sequence of cDNA transcripts of 3' termini of VSV NJ strains. W82-3 represents the identical sequence of three VSV NJ isolates (Idaho 1, New Mexico, and California) isolated at the early, mid, and late stages (respectively) of a major VSV epizootic in 1982 to 1983 in the western United States. Ogden represents the sequence of VSV NJ, Ogden strain. M represents the previously published sequence of VSV NJ, Missouri strain (30). The deduced amino acid sequences are presented directly below the nucleic acid sequences. Dashes indicate sequence homology to the W82-83 sequence. X indicates an undetermined base.

numerous migrational differences upon polyacrylamide gel analysis (13; data not shown), coevolution of both the NS protein and its binding site may have occurred in these viruses. The spacer sequence at the 3' terminus between the leader transcript-coding region and the nucleocapsid (N) protein gene is also highly conserved, being AAAU in all cases except VSV NJ, which has the very similar sequence AAAAU. The VSV NJ RNA 5'-terminal sequence previously published (21) and shown here for comparison would appear to be unusual with regard to the extent of base insertion in this region relative to that of VSV Indiana. The signal sequence AGUUUUUUUCA for the termination of transcription and for polyadenylation of the virus large (L) protein mRNA (41) is highly conserved in each case, whereas the adjacent noncoding region near the termini of the L protein mRNA appears to be highly divergent.

We compared the evolution of these noncoding regulatory sequences with that of a virus protein-coding RNA region. The RNA sequence and the predicted amino acid sequence of the amino terminus of the virus N protein are shown in Fig. 3. Adjacent regions of apparent sequence conservation and divergence can be seen, particularly at the amino acid level. It is interesting to note that Piry virus has three nucleotides inserted relative to VSV Indiana, keeping the reading frame in phase and resulting in an amino acid insertion relative to VSV Indiana. The wide range of conservation and diversity of both noncoding regulatory RNA sequences and protein-coding RNA sequences among these members of the vesiculovirus group indicate that these regions must have quite different functional and evolutionary constraints.

Previous RNA hybridization annealing studies have suggested that there is very little nucleotide sequence homology

between members of different serotypes within the vesiculovirus group (38, 39). The direct sequence analysis reported here demonstrated that at least 50% RNA sequence homology existed among the viruses over the virus genome region analyzed, confirming the correct grouping of these viruses in the vesiculovirus subgroup of *Rhabdoviridae*. The high degree of sequence homology that exists between VSV Indiana and Cocal RNA termini correlates well their ability to interact at the level of virus transcription and replication (3, 8, 36, 37). The degree of RNA genome terminus sequence homology among the various VSVs also correlates well with the degree of antigenic relatedness exhibited by these viruses (5, 6, 12, 42), suggesting that these viruses may have evolved from a common ancestor by mutational drift rather than by recombinational rearrangements.

Owing to the sequence divergence observed between viruses of different VSV serotypes, we examined the extent of divergence of these regions within members of the same serotype. The VSV NJ serotype has been divided into at least two subtypes (38). In addition, RNase T₁ fingerprint analysis has suggested that considerable genetic variation can occur among recent field isolates of VSV NJ (S. T. Nichol, submitted for publication). However, sequencing of three recent VSV NJ isolates revealed no nucleotide differences throughout the genome RNA region analyzed (Fig. 4), despite the fact that these viruses were isolated up to 15 months apart and from different geographical locations. Comparison of these sequences with those of two older laboratory strains of VSV NJ (Ogden and Missouri) representing the two different NJ subtypes also demonstrated the relatively high degree of conservation of these sequences (Fig. 4) (1). These results are in good agreement with the results of sequence analyses of VSVs serially passaged or

maintained in persistent DI-particle-mediated infections (32). A different situation may exist in persistent VSV infections of L cells, in which virus genome RNA 3'-terminal mutations can accumulate relatively rapidly (44). The sequence conservation and diversity data presented have the potential to provide VSV group-, serotype-, and subtype-specific nucleic acid diagnostic probes which would be useful in the rapid differential diagnosis of vesicular diseases of livestock, which are of economic importance throughout the Americas (16, 27).

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