

Vesicular Stomatitis Virus

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Historical Perspective

Vesicular stomatitis viruses (VSVs) are transmitted naturally by arthropods to a broad range of animal species. A clinically significant acute disease is manifest in domesticated animals, notably cattle, horses, and pigs, and it is characterized by fever and the appearance of vesicular lesions in the mouth, tongue, udder teats, and hoof coronary bands. Symptoms are therefore similar to those following infection with the aphthovirus, foot-and-mouth disease virus (FMDV) and, consequently, rapid diagnosis is important in livestock. VSV infection was first described in the USA in 1916, following an epidemic in cattle and horses. However, reports from 1862 describe a clinically similar disease in army horses during the American Civil War. Today the virus is distributed throughout the Americas and is enzootic in Central America. In Panama, estimates suggest that up to 29% of the human population has been exposed to the virus as judged by the presence of neutralizing antibodies. Infection of humans can result in a mild febrile illness, but is generally asymptomatic. VSVs have been described rarely outside the Western Hemisphere.

Taxonomy and Classification

VSVs have a nonsegmented negative-sense RNA genome and are assigned to the order *Mononegavirales*. Within this order, they are assigned to the family *Rhabdoviridae*, based upon their characteristic ‘bullet’ shape. Within the rhabdovirus family, they are further assigned to the genus *Vesiculovirus*. A list of the nine currently recognized species assigned to the genus is provided in Table 1, along with their geographic distribution and sources of the virus in nature. The *Eighth Report of the International*

Committee on the Taxonomy of Viruses lists an additional 19 members tentatively assigned to this genus. The type species is *Vesicular stomatitis Indiana virus*. Vesicular stomatitis Indiana virus (VSIV) has been widely studied in laboratories as a prototype of all the *Mononegavirales* and will be the primary focus of this article. Much is known about the replication and molecular biology of VSIV. VSIV was isolated following an outbreak of a vesicular disease of cattle in Richmond, Indiana, in 1925. The infectious agent was maintained by serial passage in animals and eventually became the Indiana serotype of VSV. *Vesicular stomatitis New Jersey virus* is also classified as a species in the genus *Vesiculovirus*. It was isolated following an outbreak in cattle in 1926 and is serologically and genetically distinct from VSIV. Vesicular stomatitis Alagoas virus (VSAV) was isolated from domesticated animals in Alagoas Brazil during an outbreak of VSV.

Structure of VSV

Particles

A schematic of VSIV is shown in Figure 1 along with an electron micrograph showing virus particles. The particles appear bullet-shaped and are approximately 180 nm long and 70 nm in diameter. The particles comprise 74% protein, 20% lipid, 3% RNA, and 3% carbohydrate. The virus possesses a lipid envelope that is decorated with trimeric spikes of the 67 kDa attachment glycoprotein (G). The interior of the particle contains a ribonucleoprotein (RNP) core of the genomic RNA complexed with the viral nucleocapsid (N) protein. This N-RNA core is associated with the RNA-dependent RNA polymerase (RdRp), the viral components of which are a 241 kDa large (L) protein and a tetramer of a 29 kDa accessory

Table 1 Geographic distribution and sources of natural isolation of viruses representing the nine recognized species in the genus *Vesiculovirus*

Virus	Geographic distribution	Source
Carajas virus (CJSV)	Brazil	Phlebotomine sandflies
Chandipura virus (CHPV)	India, Nigeria	Mammals, sandflies
Cocal virus (COCV)	Argentina, Brazil, Trinidad	Mammals, mosquitoes, mites
Isfahan virus (ISFV)	Iran, Turkmenistan	Sandflies, ticks
Maraba virus (MARAV)	Brazil	Phlebotomine sandflies
Piry virus (PIRYV)	Brazil	Mammals
Vesicular stomatitis Alagoas virus (VSAV)	Brazil, Columbia	Mammals, sandflies
Vesicular stomatitis Indiana virus (VSIV)	Americas	Mammals, mosquitoes, sandflies
Vesicular stomatitis New Jersey virus (VSNJV)	Americas	Mammals, mosquitoes, midges, blackflies, houseflies

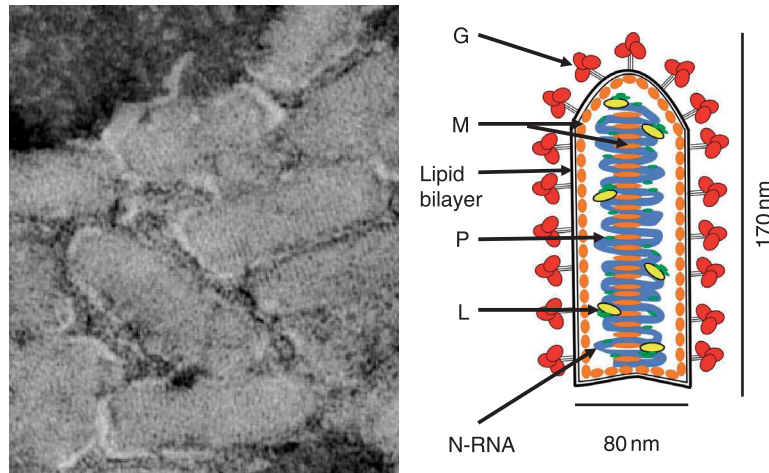


Figure 1 Electron micrograph of VSV particles and a schematic illustration of the virion. At left, a negative-stained image of a group of virus particles is shown. At right, a schematic illustration of the virion is shown along with the dimensions of a viral particle. N-RNA, nucleocapsid protein coated RNA; P, phosphoprotein; M, matrix protein; G, attachment glycoprotein; L, large polymerase subunit. Kindly provided by David Cureton, Harvard Medical School.

phosphoprotein (P). Together, these form the internal helical nucleocapsid. Another major structural component of the virus particles is the 26 kDa matrix (M) protein, which is located below the membrane and associated with the nucleocapsid. The approximate composition of the particle is one molecule of RNA, 1200 copies of N, 500 copies of P, 1800 copies of M, 1200 copies of G, and 50 copies of L. The role of these proteins in viral replication is described in more detail below.

Genome

VSV has a nonsegmented, negative-sense RNA genome of 11 161 nt. The genome comprises a 50 nt 3' leader region (le), five genes that encode in order the N, P, M, G, and L proteins, and a 59 nt 5' trailer region (Figure 2). The 5' and 3' ends of the genome are not modified and contain a 5' triphosphate and 3' hydroxyl. A key feature of all mononegaviruses is that the RNA genome is not found naked within infected cells. Instead, it is present as a ribonucleoprotein complex, in which it is completely covered by the viral nucleocapsid protein. This N-RNA template, associated with the viral L and P proteins, comprises the transcription-competent core of the virus, and delivery of this complex is required to initiate the infectious cycle. Consequently, in contrast to positive-sense RNA viruses, the naked RNA is not infectious.

Viral Replication Cycle

The replication cycle (Figure 3) should be considered a continuum of events. However, it is convenient to divide the cycle into the following three stages.

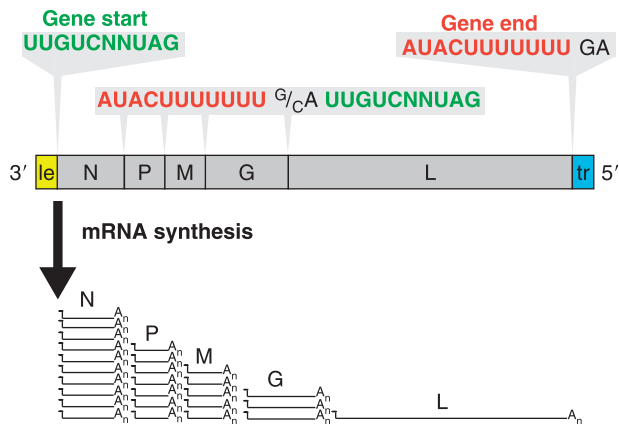


Figure 2 A schematic illustration of the VSV genome highlighting the polymerase regulatory elements. The genome is represented 3'–5' as a series of boxes, comprising le = leader region, N = nucleocapsid, P = phospho, M = matrix, G = glyco, L = large polymerase, and tr = trailer region. The conserved *cis*-acting elements that regulate polymerase activity during mRNA synthesis are shown for emphasis. Colors: green, the conserved residues of the gene-start sequence; red, the conserved residues of the gene-end sequence; and black, the nontranscribed residues of the gene junction. The leader and trailer regions also contain key elements that regulate polymerase activity and serve as promoters as described in the text.

Attachment, Entry, and Uncoating

Attachment of VSV to host cells is mediated by the glycoprotein which binds to the surface of cells. Given that VSV G can mediate infection of almost all cells in culture, either the receptor for VSV must be widely distributed, or the virus may be able to utilize multiple surface molecules for attachment. Phosphatidylserine (PS) was long thought to be the receptor for VSV but

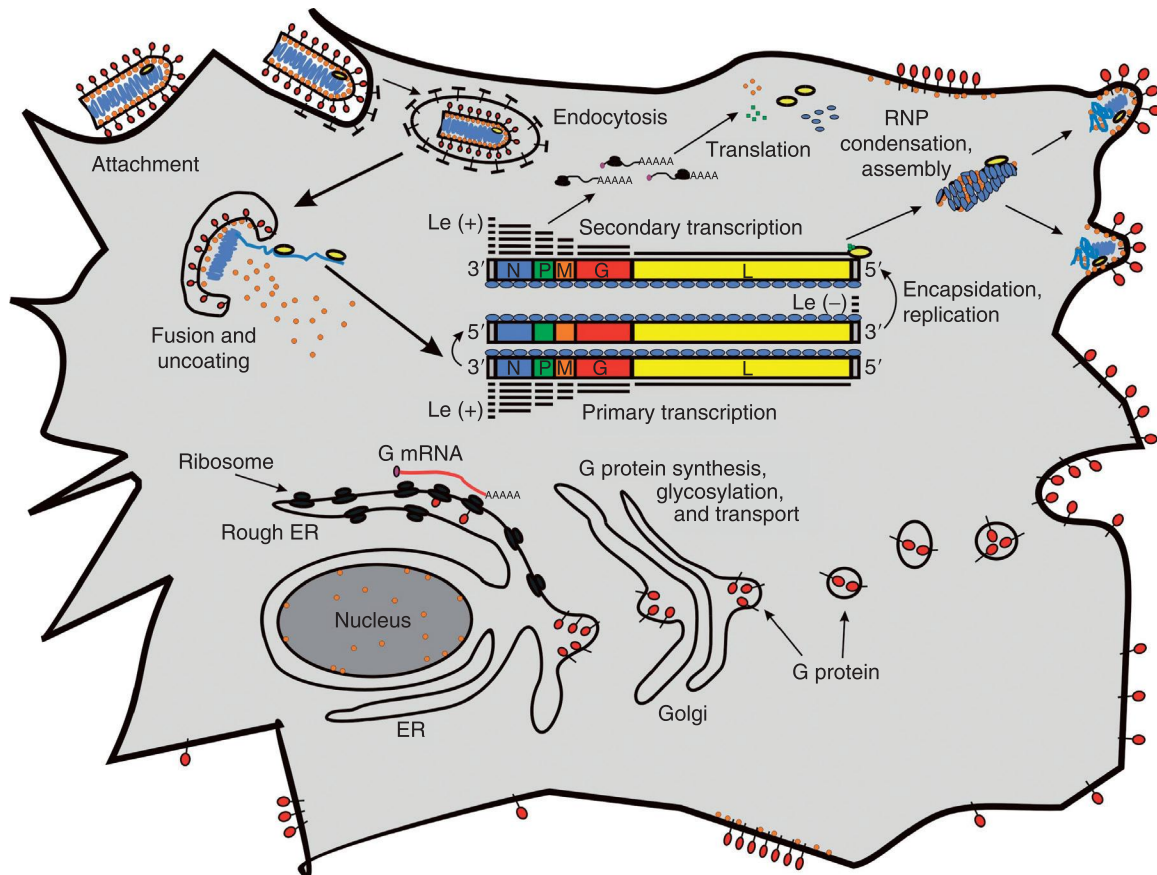


Figure 3 A schematic illustration of the replication cycle of VSV. The replication cycle (described in the text) is depicted showing attachment of virus to the cell, internalization, release of the viral core into the cytoplasm, primary viral mRNA synthesis, mRNA translation, genomic replication, secondary viral mRNA synthesis, assembly, and budding of infectious particles. Kindly provided by David Cureton, Harvard Medical School.

recent studies have questioned this finding. Importantly, the PS binding site in VSV G is internal to the trimer in its prefusion form. Following attachment to the cell, the virus is internalized via clathrin-dependent endocytosis and delivered to an early endosome. The pH threshold necessary to trigger the conformational alterations in VSV G that promote fusion of the viral and cellular membranes is approximately 6.2. This pH is reached in the early endosome, and viral and cellular membranes fuse to release the transcription-competent RNP core into the cytoplasm of the cell. Recent work has called into question this conventional view of the VSV entry pathway and has posited a new model for viral entry. In this model, fusion and RNP release are spatially and temporally separated. Specifically, the low pH encountered during endocytic transport triggers fusion and the delivery of the RNP core into an intraluminal vesicle within an endosomal carrier vesicle. A subsequent (cell-mediated) fusion event is then required to fuse the membrane of the intraluminal vesicle with the limiting membrane of the cell and deliver the RNP into the cytoplasm to initiate

the infectious process. Irrespective of the precise route by which virus enters the cell, the end result is the delivery of the transcription-competent core into the cytoplasm. This is accompanied by the release of M protein, which can migrate to the nucleus where it plays a role in inhibiting host gene expression.

Gene Expression

Following the delivery of the transcription-competent core into the cytoplasm, RNA synthesis can begin. The replication cycle of VSV is entirely cytoplasmic, occurring efficiently in enucleated cells. The demonstration that purified VSV particles contain a functional RdRp that is active *in vitro* has led to major advances in our understanding of viral gene expression. During RNA synthesis, the polymerase uses the encapsidated genomic RNA as template in two distinct reactions: (1) transcription of five mRNAs that encode the N, P, M, G, and L proteins; and (2) replication to yield full-length antigenomic, and then genomic RNA strands. Our current

understanding of gene expression is summarized as follows. In response to a specific promoter element that is provided by sequences within the 3' leader region and the conserved residues of the first (N) gene-start (3'-UUGUCNNUAG-5') sequence, the RdRp initiates mRNA synthesis. Synthesis commences at the N gene-start sequence and generates an mRNA that is capped, methylated, and polyadenylated, each of these reactions occurring co-transcriptionally. Termination at the end of the N gene is achieved by the polymerase recognizing a highly conserved sequence element referred to as the gene-end 3'-AUACUUUUUUG/C-5'. This sequence signals the polymerase to stutter on the U₇ tract to generate the polyA tail, and leads to termination of mRNA synthesis. Termination at the end of the N gene is essential for the polymerase to be able to transcribe the P gene. A poorly understood event that is localized to a short region at the N-P gene junction results in the synthesis of approximately 30% less P mRNA than N mRNA. This sequential and polar synthesis of the viral mRNAs continues through the entire genome and provides a gradient of viral mRNA synthesis such that N > P > M > G > L. These products of mRNA synthesis are illustrated in [Figure 2](#).

Among the notable steps of mRNA synthesis are the unusual mechanisms by which the 5' and 3' ends of the RNA are formed. Each stage of mRNA cap formation is distinct from those employed in other systems. Specifically, the 5' end of the pppApApCpApG mRNA is capped by an unusual ribonucleotidyltransferase activity that transfers the monophosphate RNA onto GDP derived from GTP to form the GpppApApCpApG mRNA cap structure. In contrast, all other capping reactions are catalyzed by an RNA guanylyltransferase that transfers GMP onto the 5' end of a diphosphate RNA through a reaction that involves a covalent enzyme GMP intermediate. The VSV L protein is responsible for this novel reaction, which appears to involve a covalent intermediate between L and the viral mRNA. The resulting GpppApApCpApG cap structure is then methylated at guanine N-7 and ribose 2'-O positions to yield 7^mGpppA^mpApCpApG. These activities are also provided by the L protein, and again differ from conventional mRNA cap methylation reactions. For VSIV, the two enzymatic activities have been shown to share a single binding site for the methyl donor, S-adenosyl-L-methionine (SAM). In contrast, other cap methylation reactions are normally executed by two distinct proteins with separate binding sites for the methyl donor SAM. Formation of the 3' end of the RNA is also unusual. Specifically, polyadenylation occurs in a pseudo-templated fashion in which the polymerase complex reiteratively transcribes the conserved U tract present at the end of each VSV gene.

The viral mRNAs are efficiently translated by the host translation machinery, but how they compete with cellular mRNAs for translation is not well understood. Viral protein synthesis is essential for replication of the genomic RNA. Ongoing translation provides a continuous supply of soluble N protein that drives the encapsidation of the nascent RNA chain. This process is intimately linked to genomic RNA replication which first results in the production of a full-length encapsidated complementary antigenome RNA. This antigenome can then serve as template to produce more progeny genomic RNAs for use as templates for further mRNA synthesis in a process referred to as secondary transcription.

Precisely how the different polymerase activities are regulated in infected cells remains poorly understood. Two functionally distinct pools of polymerase have been purified from cells. One initiates internally at the N gene-start sequence and functions as the viral transcriptase. A second complex initiates at the 3' end of the genome and functions as the viral replicase. These complexes are reported to differ in their composition such that the transcriptase comprises the viral P and L proteins, together with several cellular proteins including translation elongation factor-1 α , heat shock protein 60, and the host cell RNA guanylyltransferase. In contrast, the replicase is reported to comprise the viral N, P, and L proteins.

In addition to the species of RNA described above, two short leader RNAs are generated during RNA synthesis: a 47 nt Le+ from the 3' end of the genomic RNA and a 45 nt Le- from the 3' end of the antigenomic RNA. The function of these RNAs is poorly understood, although a role for the Le+ in the shutoff of host gene expression has been described. A long-standing model for the regulation of RNA synthesis in VSV postulates that polymerase initiates all RNA synthesis at position 1 of the genome, and during synthesis of Le+ a crucial regulatory decision is made to either terminate leader and initiate mRNA synthesis at the N gene start, or alternatively to read through the leader-N gene junction and synthesize the full-length antigenome. The obligatory requirement for protein synthesis to provide a source of N protein to encapsidate the nascent RNA during genome replication led to the suggestion that N protein availability switches polymerase activity from transcriptase to replicase.

In recent years, there has been an accumulation of evidence that conflicts with this model. Specifically, a VSV mutant containing a single amino acid change in the template-associated N protein produces an excess of N mRNA over Le+ *in vitro*, suggesting that polymerase can synthesize N independently of Le+. In another series of experiments, recombinant VSVs, containing a 60 nt gene inserted between the leader region and the N gene, were employed. The recombinant viruses were examined to determine the effect of altering the potential number of

ultraviolet (UV)-induced dimers between adjacent uracil residues. Such dimers block progression of the polymerase. These experiments showed that changing the UV sensitivity of the Le+ had no effect on the sensitivity of the 60 nt mRNA in infected cells, suggesting that polymerase can initiate synthesis internally at the first gene start. In addition, two separate pools of polymerase can be isolated from infected cells, one that initiates internally at the N gene start and the second that initiates at the 3' end of the genome. These findings support the hypothesis that mRNA synthesis can initiate independently of leader synthesis, and show that polymerase function is not simply switched by N protein levels. However, viral gene expression is controlled, it results in the exponential amplification of the input genomic RNA, yielding progeny genomes that can be assembled into infectious particles, the next phase of the viral replication cycle.

Assembly and Budding

Assembly of infectious virus particles involves many critical interactions. Our current understanding of this intricate process is that the matrix (M) protein complexes with the RNP core and represses transcription of viral mRNAs. This condensed RNP complex acquires a lipid envelope that has been modified by the insertion of an externally oriented glycoprotein. Details of how the RNP is transported to the site of budding and how the matrix interacts with and condenses the RNP are poorly understood. The M protein is only found associated with RNPs at sites of viral budding, and how genomic RNPs (rather than antigenomic RNPs) are specifically selected for budding is unclear. The M protein contains two 'late domains' (PTAP and PPPY motifs) that appear to be critical in the late phase of the assembly-release pathway. Amino acid substitutions in the PPPY motif result in the accumulation of bullet-shaped virions that are stalled at a late stage of virus budding. This motif, and similar motifs in proteins from other enveloped viruses, target virus for budding through interaction with components of the endosomal sorting complex required for transport or ESCRT pathway. The release of infectious particles completes the replication cycle and provides progeny virions for infection of the next cell.

The kinetics of the viral replication cycle are rapid. In mammalian cells in culture, one infectious particle can produce 10 000 infectious progeny within 8 h. While much remains to be explored about the biology of VSV, it is one of the best understood animal viruses. Studies on VSV should continue to prove informative in understanding how enveloped viruses enter and bud from their host cells, and how nonsegmented negative-strand RNA viruses (mononegaviruses) express their genetic information.

Functions of the Viral Proteins

Nucleocapsid protein

The N protein coats the viral genomic RNA and the positive-sense antigenomic replicative intermediate to form ribonucleoprotein (RNP) complexes. The interaction with the N protein renders the RNA resistant to cleavage by ribonucleases. It is in this form that the RNA is presented to the polymerase to serve as template. The N protein comprises 422 amino acids and has a molecular weight of approximately 48 kDa. The crystal structure of a complex of 10 molecules of N protein bound to 90 nt of RNA has been solved, revealing that N has a bilobed structure with RNA bound between the lobes. The structure indicates that N protein must be either transiently displaced or substantially remodeled during copying of the RNA genome by the viral polymerase.

Phosphoprotein

P is a multiply phosphorylated acidic protein of 265 amino acids that functions as an essential polymerase cofactor and plays an additional role in maintaining N protein in a soluble form necessary for RNA encapsidation. Sequence analysis has identified three domains of P. An acidic N-terminal domain (domain I) of 150 amino acids contains phosphorylation sites at Ser 60, Thr 62, and Ser 64. Phosphorylation of these residues by the host casein kinase II leads to the oligomerization of P protein and together with the large polymerase protein L, assembly of the polymerase complex. Domain I is separated from domain II by a highly variable hinge region comprising residues 150–210. Domain II (residues 210–244) contains additional phosphorylation sites at Ser 226, Ser 227, and Ser 233 that appear to be important for RNA replication. Domain III is basic and comprises the C-terminal 21 amino acids. A crystal structure of a fragment comprising amino acids 107–177 of the P protein has been solved, providing evidence that P protein functions as a tetramer. In addition to P, two proteins (C and C') are produced from the P gene. These proteins are small (55 and 65 amino acids, respectively), are highly basic, and have not been detected in virus particles. Recombinant viruses that are unable to produce C and C' replicate normally in cultured mammalian cells. The role of these proteins is unclear but may be important for infections in insects and/or mammalian hosts *in vivo*.

Matrix protein

The matrix (M) protein is the major structural component of virus particles. M is a small 229-amino-acid multifunctional protein. It condenses viral RNPs and drives budding of virus particles from the host plasma membrane. In addition, M downregulates host gene expression by directly interacting with Rae 1, thus inhibiting nuclear transport. The crystal structure of amino acids 48–229 of

the matrix protein has been solved and reveals regions of M that may be required for membrane association. Two N-terminal truncations of M are generated in infected cells by initiation of translation at methionines 33 and 51. A recombinant virus engineered to ablate the expression of these two alternate forms of M shows a modest reduction in cytopathic effect in culture cells.

Glycoprotein

The attachment glycoprotein (G) is a trimer present on the surface of the virion. It is responsible for attachment of virus to cells and promotes fusion of the viral and cellular membranes during endocytosis. The protein is synthesized as a 511-amino-acid polypeptide that is co-translationally inserted into membranes in the endoplasmic reticulum (ER). An N-terminal 16-amino-acid signal sequence is cleaved from the nascent polypeptide upon insertion. A 20-amino-acid hydrophobic sequence acts as the membrane anchor and serves as a stop-transfer signal during ER translocation. The remaining 29 amino acids of G remain as a cytoplasmic tail. Co- and post-translational modifications result in N-glycosylation of two asparagine residues in the ectodomain, and palmitoylation of a cysteine in the cytoplasmic domain. The role of palmitoylation in infection is unclear as some VSV strains lack this modification. However, glycosylation permits association of G with calnexin, an ER-resident chaperone, and mutations that prevent glycosylation result in G protein aggregation. Mature G protein is selectively transported to the basolateral surface of polarized epithelial cells. At the plasma membrane, VSV G clusters in microdomains that are the sites of virus assembly and budding. The determination of the crystal structure of a fragment of the G protein in both pre- and postfusion conformations has provided new insight into the entry process. The structures show that each G protein monomer contains two hydrophobic fusion loops that insert into the target cell membrane. Substantial rearrangements of the G protein that are driven by the low pH environment encountered in the endocytic pathway drive fusion of the viral and cellular membranes.

Large polymerase protein

The large polymerase protein (L) comprises 2109 amino acids. This 241 kDa multifunctional protein is responsible for template binding, ribonucleotide polymerization, and the co-transcriptional modification of the 5' and 3' terminus of the viral mRNAs so that they are capped and methylated. To date, structures are not available for any portion of the L protein. Amino acid sequence alignments of the L genes of representative members of the families *Rhabdoviridae*, *Paramyxoviridae*, and *Filoviridae* have led to the identification of six regions of sequence conservation (CRI–CRVI), separated by regions of no or low sequence homology. These conserved regions are thought to

represent the functional domains of L protein. CRIII contains clearly identifiable motifs found in all polymerases and in which alterations to a universally conserved aspartic acid residue eliminate polymerase activity in reconstructed RNA synthesis assays. CRVI functions as a messenger RNA cap-modifying enzyme. Amino acid substitutions to this region disrupt mRNA cap methylation at both the guanine N-7 and ribose 2'-O positions of the cap structure. The regions of L involved in other modifications have not yet been assigned. As described above, L protein has been reported to associate with a number of host proteins, including translation elongation factor-1 α , heat shock protein 60, and the host RNA guanylyltransferase. However, the functional significance of these interactions is not yet certain.

Host Range and Transmission

In nature, VSVs can infect a broad range of animals including mammals and invertebrates. Overt disease is typically only seen in cattle, horses, and swine, but there is evidence of natural infection in a range of wild ruminants, ungulates, carnivores, marsupials, and rodents. Infection can be transmitted directly from animal to animal, but this requires an abrasion or a means of introducing the virus below the skin. Humans can also be infected by contact with vesicular lesions or the saliva of infected animals, resulting in an influenza-like illness. Aerosol transmission has also been reported in humans. Insect vectors appear to be important in natural transmission of the virus. VSVs have been isolated on more than 40 occasions from hematophagous insects including mosquitoes, biting midges (*Culicoides* spp.), phlebotomine sand flies (*Lutzomyia* spp.), and black flies (*Simulium* spp.). In addition, VSV has been experimentally transmitted to mice from infected mosquitoes and between insects through co-feeding. Susceptible insect hosts are also capable of transovarial transmission and these infected progeny have been shown to infect mammalian hosts. Recovery of vesicular stomatitis New Jersey virus (VSNJV) from non-biting flies suggests that mechanical transmission may occur. There is also evidence that grasshoppers (*Melanoplus sanguinipes*) can be infected experimentally, and cattle that ingest infected grasshoppers can develop disease.

Consistent with the ability to infect a broad range of experimental animals, VSV replicates efficiently in a variety of cell lines of vertebrate and invertebrate origin. The Syrian hamster kidney cell line, BHK-21, is commonly used to generate viral stocks of high titer (10^9 – 10^{10} pfu ml⁻¹). VSV can also infect and replicate to high titers in cultured cells derived from insects, reptiles, and fish, and replication can occur in several insect cell lines. Infection of mammalian, avian, and some insect cells results in a cytopathic effect (CPE). In fibroblasts, this is

seen as an increase of membrane blebbing followed by extensive cell rounding. The M protein is responsible for these changes in infected cells. A noncytopathic infection occurs in some insect cells including lines derived from *Aedes aegypti*, *Aedes albopictus* (C6/36), and *Drosophila melanogaster*. Experimentally, the virus has been shown to express its genetic information in spheroblasts derived from the yeast, *Saccharomyces cerevisiae*, and to produce infectious virions in embryonic cells derived from the nematode *Caenorhabditis elegans*. Thus, the host requirements for viral replication are provided by an extremely broad range of eukaryotes.

Evolution

Several principles of evolutionary biology have been explored using VSV as a model system. The viral polymerase has an error rate of 10^{-3} to 10^{-5} per nucleotide per round of replication. Given the genome size is 11 161 nt, each time the genome is copied, on average, at least one nucleotide change is introduced. These mutations are typically deleterious, others will be neutral, and some may offer a selective advantage. Consequently, this high mutation rate contributes to VSV's ability to rapidly evolve and adapt to environmental changes. The high mutation rate also begs the question as to why VSV does not mutate itself out of existence. Rather than existing as a single defined sequence, the virus exists as a swarm of mutant sequences around a consensus sequence or a viral quasispecies. Critically, it is this quasispecies that is the biologically relevant target for selection.

Experiments in which the diversity of the viral population is artificially restricted by performing serial plaque-to-plaque transfers (thus restricting the infectious virus population to 1 on each transfer), rapidly result in fitness losses. Such an accumulation of deleterious mutations in populations of asexual organisms lacking compensatory mechanisms such as sex or recombination was predicted by Muller in a concept referred to as Muller's ratchet. This principle has been nicely illustrated in studies of VSV, which is not known to undergo homologous recombination. Studies with VSV have also illustrated other fundamental principles of population genetics. Work by the laboratory of John Holland demonstrated that VSVs of equal fitness could coexist for several generations, but one population would then rapidly outgrow the other. This demonstrated that populations accumulate mutations and, infrequently, mutations provide a selective advantage which leads to its outgrowth. Importantly, the mutation rates in both populations were the same, reflecting an intrinsic property of the viral polymerase. This is referred to as the Red Queen effect. This is a hypothesis proposed by the evolutionary biologist Leigh van Valen in reference to Lewis Carroll's book *Through the Looking Glass*, in which

the Red Queen comments to Alice that "it takes all the running you can do, to keep in the same place."

Despite the intrinsically high polymerase error rate, VSVs isolated in nature can show a remarkable genetic stability. For example, sequence analysis of viruses from different hosts and different years has shown that those isolated from multiple hosts within the same region over a period of decades were more closely related than viruses isolated in the same year, but from a different ecological zone. This suggests that ecological factors are a major driving force in the evolution of VSV. Consistent with this, viruses from similar ecological zones 800 km apart were found to be more closely related than viruses from different ecological zones 25 km apart. VSV evolution is unusual in this apparent lack of a 'molecular clock' in which the genome sequence does not show a clear relationship with year of isolation.

The evolutionary origins of VSVs are uncertain but the conservation of gene order and sequence similarity to other members of the *Mononegavirales* indicate they diverged from a common ancestor. Although the number of genes differ, their order is maintained across the *Mononegavirales* as 3' N-P-M-G-L 5'. The significance of this gene order has been elegantly tested in engineered VSIV recombinants by shuffling the order of the three central genes (P, M, and G), and by moving the N and G genes. Remarkably, all gene orders yield infectious virus, and the replication kinetics of several recombinant viruses in which the P, M, and G genes were shuffled were similar to those of the wild-type parent virus. Consistent with its critical role in driving RNA encapsidation during genome replication, moving the N gene from its promoter proximal position diminished viral replication. These experiments suggest that the conserved gene order may reflect an ancestral gene order that has remained frozen because of the lack of a mechanism for homologous recombination in the *Mononegavirales*.

Geographic and Seasonal Distribution

VSVs are typically restricted to the Americas. Within this region, VSIV and VSNJV are the two most commonly isolated serotypes and have the broadest geographic distribution ranging from Peru to Canada. Vesicular stomatitis Alagoas virus (VSAV) is the major serotype that is isolated in Brazil. Both VSIV and VSNJV are seen in the United States although most outbreaks are associated with VSNJV. In the US, the last outbreak was from 2004 to 2006. In this outbreak, viral infection was reported in Texas, New Mexico, and Colorado in 2004, and the outbreak spread to include Arizona, Utah, Wyoming, Nebraska, Montana, and Idaho in 2005, with a small number of cases reported in Wyoming in 2006. Overall some 1000 animals were affected, representing the most significant outbreak

of disease in the US for almost 10 years. Vesicular stomatitis is endemic in many Latin American countries. In 2002, VSNJV and VSIV were reported in Panama, Costa Rica, Nicaragua, El Salvador, and Mexico, and VSNJV was reported in Honduras, Guatemala, and Belize. In 2004, VSNJV and VSIV were isolated in Columbia, Venezuela, Ecuador, and Peru, and VSIV in Brazil and Bolivia. Although infection is reported throughout the Americas, the highest incidence usually is in tropical regions with more sporadic outbreaks in temperate regions. In temperate regions, outbreaks typically peak in late summer and end by the first frost. In tropical regions, peak incidence typically coincides with the end of the rainy season. These peaks correlate with high insect population levels and, consistent with the role of insect vectors in disease spread, outbreaks tend to spread along waterways. Although, on occasion, VSV has been reported in parts of Europe and Africa, such reports are rare and are probably linked to importation of infected animals.

Pathogenesis and Pathology

Following exposure of animals to VSV, the incubation period is usually 2–4 days. Prior to the development of lesions, animals can show signs of depression, lameness, a fever of up to 41.5 °C, and excessive salivation. In infected cattle, swine and horses, pink to white papules appear in the mouth, lips, gums, nose, teats, and feet. In these regions, the epithelium separates from the basal layer forming a vesicle that fills with clear yellowish fluid that contains very high titers of infectious virus. These vesicles combine and readily rupture. In some cases, lesions appear at a secondary site relative to the initial point of inoculation. The development of secondary lesions is suggestive of a viremia, but virus has not been isolated from the blood of experimentally inoculated natural hosts. Infection results in significant losses for the livestock industry as weight loss can be significant (up to 135 kg in beef cattle), and dairy cattle usually cease milk production. In the absence of secondary infections, the vesicular lesions typically heal in 1–2 weeks and the animals start to regain weight. Viral RNA has been isolated from animals several months after infection although infectious virus has not been recovered.

Immune Response

Our understanding of the immune response to VSV in natural hosts is limited. However, in experimental animals, notably mice, the immune response to VSV has been characterized. Interferon plays an important role in the resistance of older mice to VSV infection and, consistent with this, adult mice that are unable to produce

interferon succumb to infection. Different strains of VSV induce different levels of interferon, but there is no clear correlation between levels of interferon *in vitro* and pathogenesis in animals. Neutralizing antibody has also been shown to play a role in the defense against VSV infection in experimentally infected swine and mice. The target of these neutralizing antibodies is the viral attachment protein G, and adoptive transfer experiments have shown that they are sufficient to protect young mice against infection. In endemic areas, susceptible hosts often have detectable neutralizing antibody responses to VSV. However, these responses are not protective, perhaps reflecting the fact that viral replication is largely confined to the epithelium. Experimental infection of mice with VSV also induces a strong cytotoxic T-lymphocyte (CTL) response. The N protein is the predominant antigen recognized and the resulting CTLs are not serotype specific. However, the functional significance of these CTLs in protection against infection is uncertain as mice incapable of mounting such a response survive VSV infection.

Diagnosis, Prevention, and Control

The similarity of symptoms between VSV and FMDV make rapid diagnosis important, especially in countries that are free of FMDV. Diagnosis is achieved typically by analysis of samples of fluid from vesicular lesions using reverse transcription-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), or growth of virus in cell culture. VSV is listed as a notifiable disease by the World Organisation for Animal Health (OIE) and a positive diagnosis results in quarantine of the affected area. To control spread of disease, farm equipment is disinfected using a 2% bleach solution to interrupt animal-to-animal spread, and insect control measures can be introduced. Vaccines against VSIV and VSNJV have been used to control outbreaks in Central and South America. Although vaccination is not routinely practiced throughout the Americas, experiments demonstrate a substantive decrease in clinical infections in vaccinated animals.

Future Perspectives

VSVs will continue to serve as an important prototype of the nonsegmented negative-strand RNA viruses. The advantages of VSV as a model for these viruses are: (1) its relative safety, in that the virus is not a significant human pathogen; (2) abundant viral replication in a broad range of cultured cells yielding up to 10 000 pfu cell⁻¹ 8–12 h post inoculation of mammalian cells; (3) a robust reverse genetic system which permits the generation of helper-dependent viruses; (4) crystal structures of the N-RNA template, a portion of the P and M proteins,

and the pre- and postfusion forms of the G protein; and (5) an *in vitro* system for viral mRNA synthesis using purified recombinant polymerase. Capitalizing on these advantages will continue to provide new mechanistic insights into the molecular details of viral entry, gene expression, and assembly. Among the key questions to address are understanding: (1) how and where the viral RNP core enters a cell; (2) how and where the N-RNA serves as template for gene expression; (3) how the large multifunctional polymerase serves to initiate, cap, methylate, polyadenylate, and terminate mRNA synthesis in response to specific sequence elements; (4) how the activities of the polymerase are controlled between mRNA synthesis and genome replication; and (5) how the nucleocapsid templates are selected for assembly and budding from the cell. In addition to the fundamental questions regarding the molecular biology of these viruses, the future holds promise for unraveling the significance of the different host species and vectors in the biology of infection. Genomic approaches will likely yield clues as to how VSV interacts with host cells of insect and mammalian origin, and how these cells respond to infection. Many questions remain to be answered regarding the ecology of the virus including the maintenance of the virus, its transmission, and its relative genetic stability in nature. Such studies are not only warranted in understanding VSV as a model virus, but also for the prospects of using VSV as an oncolytic virus and a live-attenuated vaccine vector for human disease. These potential applications should only add to the urgency with which studies on this prototypic virus are pursued.

See also: Chandipura Virus; Rabies Virus; Quasispecies.

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Viral Killer Toxins

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Glossary

ER-associated degradation (ERAD) A cellular quality control mechanism ensuring efficient removal of misfolded and/or unassembled proteins from the endoplasmic reticulum (ER) lumen and their subsequent elimination by the cytoplasmic ubiquitin–proteasome system.

Heterokaryon Coexistence of two or more genetically different nuclei in a common cytoplasm.

Importins A family of proteins that transport macromolecules into the eukaryotic nucleus.

Killer virus system A killer virus system consists of a helper totivirus and a satellite double-stranded (ds)

RNA encoding the unprocessed precursor of a secreted protein toxin that also gives functional immunity.

Preprotoxin Toxin precursor in the cytoplasm of a killer yeast which is post-translationally imported into the secretory pathway for toxin processing, maturation, and secretion.

Satellite dsRNA An encapsidated dsRNA that is dependent on a helper virus for encapsidation and replication.

Spheroplasts A yeast cell whose cell wall has been enzymatically removed.