Plant Rhabdoviruses

A O Jackson, University of California, Berkeley, CA, USA
R G Dietzgen, The University of Queensland, St. Lucia, QLD, Australia
R-X.Fang, Chinese Academy of Sciences, Beijing, People's Republic of China
M M Goodin, University of Kentucky, Lexington, KY, USA
S A Hogenhout, The John Innes Centre, Norwich, UK
M Deng, University of California, Berkeley, CA, USA
J N Bragg, University of California, Berkeley, CA, USA

Introduction

The plant rhabdoviruses have distinctive enveloped bacilliform or bullet-shaped particles and can be distinguished based on whether they replicate and undergo morphogenesis in the cytoplasm or in the nucleus. Consequently, they have been separated into two genera, *Cytorhabdovirus* or *Nucleorhabdovirus*. More than 90 putative plant rhabdoviruses have been described although, in many cases, molecular characterizations necessary for unambiguous classification are incomplete or lacking. Recent analyses indicate that the eight sequenced plant rhabdoviruses have the same general genome organization as other members of the *Rhabdoviridae*, but that each encodes at least six open reading frames (ORFs), one of which probably facilitates cell-to-cell movement of the virus. Thus, plant rhabdoviruses have a number of similarities to members of other rhabdovirus genera, but they differ in several respects from rhabdoviruses infecting vertebrates.

Rhabdoviruses infect plants from a large number of different families, including numerous weed hosts and several major crops. Symptoms of infection vary substantially and range from stunting, vein clearing, mosaic and mottling of leaf tissue, to tissue necrosis. The most serious pathogens include maize mosaic virus (MMV), lettuce necrotic yellows virus (LNYV), rice yellow stunt virus (RYSV), also known as rice transitory yellowing virus (RTYV), eggplant mottled dwarf virus (EMDV), strawberry crinkle virus (SCV), potato yellow dwarf virus (PYDV), and barley yellow striate mosaic virus (BYSMV), which is synonymous with maize sterile stunt virus (MSSV), and wheat chlorotic streak virus (WCSV). A number of other rhabdoviruses also have disease potential that can be affected by agronomic practices, incorporation of genes for disease resistance, and control of insect vectors.

The spread of most plant rhabdoviruses is dependent on specific transmission by phytophagous insects that support replication of the virus, so their prevalence and distribution is influenced to a large extent by the ecology and host preferences of their vectors. Although some rhabdoviruses can be transmitted mechanically by abrasion of leaves, this mode of transmission does not contribute significantly to their natural spread due to the labile nature of the virion. Moreover, seed or pollen transmission of plant rhabdoviruses has not been described; thus, aside from vegetative propagation, direct plant-to-plant transmission is unlikely to be a major factor in the ecology or epidemiology of these pathogens.

This article focuses on recent findings concerning the taxonomy, structure, replication, and vector relationships of plant rhabdoviruses. More extensive aspects of plant rhabdovirus biology, specifically ecology, disease development and control, can be found in earlier reviews.

Taxonomy and Classification

The International Committee on Taxonomy of Viruses (ICTV) has used subcellular distribution patterns to assign plant rhabdoviruses to the genera *Cytorhabdovirus* and *Nucleorhabdovirus* (**Table 1**). Currently the ICTV has assigned eight virus species (BYSMV, *Broccoli necrotic yellows virus* (BNYV), *Festuca leaf streak virus* (FLSV), LNYV, *Northern cereal mosaic virus* (NCMV), *Sonchus virus* (SV), SCV, and *Wheat American striate mosaic virus* (WASMV)) to the genus *Cytorhabdovirus* and seven viruses (*Datura yellow vein virus* (DYVV), *Eggplant mottled dwarf virus* (EMDV), MMV, PYDV, RYSV, *Sonchus yellow net virus* (SYNV), and *Sowthistle yellow vein virus* (SYVV)) to the genus *Nucleorhabdovirus*. However, sufficient new information has been documented to justify provisional inclusion of the recently described maize fine streak virus (MFSV) and taro vein chlorosis virus (TaVCV) in the genus *Nucleorhabdovirus* (**Table 1**). Cereal chlorotic mottle virus (CCMoV) has also been provisionally included in the genus *Nucleorhabdovirus* based on its intracellular distribution and serology. The complete genomic sequences have been determined for three cytorhabdoviruses, LNYV, NCMV, and SCV and for five nucleorhabdoviruses, MFSV, MMV, RYSV, SYNV, and TaVCV. Phylogenetic analyses of these rhabdoviruses have confirmed their taxonomic classification. Most other plant rhabdoviruses have not been investigated in much detail beyond cursory infectivity studies, crude physicochemical analyses of virus particles, and electron microscopic observations of morphogenesis. Consequently, more than 75 putative rhabdoviruses await assignment to a genus (**Table 1**).

Table 1 List of plant rhabdoviruses and their host and vector specificity

Virus	*Host*	*Vector*
Cytorhabdovirus		
Barley yellow striate mosaic virus (BYSMV)	M	P
[Maize sterile stunt virus]		
[Wheat chlorotic streak virus]		
Broccoli necrotic yellows virus (BNYV)	D*	A
Festuca leaf streak virus (FLSV)	M	
Lettuce necrotic yellows virus (LNYV)	D*	A
Northern cereal mosaic virus (NCMV)	M	P
Sonchus virus (SonV)	D*	
Strawberry crinkle virus (SCV)	D*	A
Wheat American striate virus (WASMV)	M	L
[Oat striate mosaic virus]		
Nucleorhabdovirus		
Cereal chlorotic mottle virus (CCMoV)	M	L
Datura yellow vein virus (DYVV)	D	
Eggplant mottled dwarf virus (EMDV)	D*	L
[Pittosporum vein yellowing virus]		
[Tomato vein yellowing virus]		
[Pelargonium vein clearing virus]		
Maize fine streak virus (MFSV)	M	L
Maize mosaic virus (MMV)	M	P
Potato yellow dwarf virus (PYDV)	D*	L
Rice yellow stunt virus (RYSV)	M	L
[Rice transitory yellowing virus]		
Sonchus yellow net virus (SYNV)	D*	A
Sowthistle yellow net virus (SYVV)	D	A
Taro vein chlorosis virus (TaVCV)	M	
Unassigned Plant Rhabdoviruses		
Asclepias virus	D	
Atropa belladonna virus	D	
Beet leaf curl virus	D	LB
Black current virus	D	
Broad bean yellow vein virus	D	
Butterbur virus	D*	
Callistephus chinensis chlorosis virus	D	
Caper vein yellowing virus	D	
Carnation bacilliform virus	D	
Carrot latent virus	D	A
Cassava symptomless virus	D	
Celery virus	D	
Chondrilla juncea stunting virus	D	
Chrysanthemum vein chlorosis virus	D	
Clover enation (mosaic) virus	D	
Colocasia bobone disease virus	D	P
Coriander feathery red vein virus	D*	A
Cow parsnip mosaic virus	D*	
Croton vein yellowing virus	D*	
Cucumber toad skin virus	D	
Cynara virus	D*	
Cynodon chlorotic streak virus	M	P
Daphne mezereum virus	D	
Digitaria striate virus	M	P
Euonymus fasciation virus	D	
Euonymus virus	D	
Finger millet mosaic virus	M	P
Gerbera symptomless virus	D	
Gloriosa fleck virus	D	
Gomphrena virus	D*	
Gynura virus	D	
Holcus lanatus yellowing virus	M	
Iris germanica leaf stripe virus	M	
Ivy vein clearing virus	D*	
Kenaf vein-clearing virus	D	
Laburnum yellow vein virus	D	
Launea arborescens stunt virus	D	
Lemon scented thyme leaf chlorosis virus	D	
Lolium ryegrass virus	M	
Lotus stem necrosis	D	
Lotus streak virus	D	A
Lucerne enation virus	D	A
Lupin yellow vein virus	D	
Maize Iranian mosaic virus	M	P
Maize streak dwarf virus	M	P
Malva sylvestris virus	D	
Meliotus (sweet clover) latent virus	D	
Melon variegation virus	D	
Mentha piperita virus	D	
Nasturtium vein banding virus	D	
Papaya apical necrosis virus	D	
Parsely virus	D*	
Passionfruit virus	D	
Patchouli mottle virus	D	
Peanut veinal chlorosis virus	D	
Pigeon pea proliferation virus	D	L
Pinapple chlorotic leaf streak virus	M	
Pisum virus	D*	
Plantain mottle virus	M	
Poplar vein yellowing virus	D	
Ranunculus repens symptomless virus	D	
Raphanus virus	D*	
Raspberry vein chlorosis virus	D	A
Red clover mosaic virus	D	
Sainpaulia leaf necrosis virus	D	
Sambucus vein clearing virus	D	
Sarracenia purpurea virus	D	
Sorghum stunt mosaic virus	M	L
Soursop yellow blotch virus	D	
Soybean virus	D	
Triticum aestivum chlorotic spot virus	M	
Vigna sinensis mosaic virus	D	
Viola chlorosis virus	D	
Wheat rosette stunt virus	M	P
Winter wheat Russian mosaic virus	M	P

Names in brackets are synonymous to those immediately above. Host: D, dicot; M, monocot. (*) indicates ability to be mechanically transmitted. Vectors: A, aphid, L, leafhopper; LB, lacebug; P, planthopper. Blank spaces indicate that no insect vector has been identified.

Particle Morphology and Composition

Plant rhabdoviruses are normally bacilliform after careful fixation (Figure 1(a)) and estimates of their sizes range from 45 to 100 nm in width and 130 to 350 nm in length. The outer layer consists of 5–10 nm surface projections that appear to be composed of G protein trimers that penetrate a host-derived membrane (Figure 1(b)). The nucleocapsid core is composed of the genomic RNA, the nucleocapsid protein (N), the phosphoprotein (P), and the L polymerase protein (Figures 1(a) and 1(b)). Rhabdovirus virions also contain a matrix protein (M) that interacts with the G protein to stabilize the particle. A sixth protein (sc4) is associated with the membrane fractions of SYNV particles but the presence of an sc4 derivative has not been found in virions of other plant rhabdoviruses.

The overall chemical composition (∼70 % protein, 2 % RNA, 20–25% lipid, and a small amount of carbohydrate associated with the G protein) of the plant and animal rhabdoviruses is similar. The minus-sense RNA genomes of plant rhabdoviruses, which range in size from ∼11 to 14 kb based on sedimentation, gel electrophoretic analyses, and genome sequencing, are slightly larger than those of most described animal rhabdoviruses. The lipids of plant and animal rhabdoviruses consist of fatty acids and sterols that are derived from sites of morphogenesis. The four sterols predominating in SYNV closely approximate sterols in the nuclear envelope, whereas those of NCMV, a cytorhabdovirus, are more typical of cytoplasmic membranes.

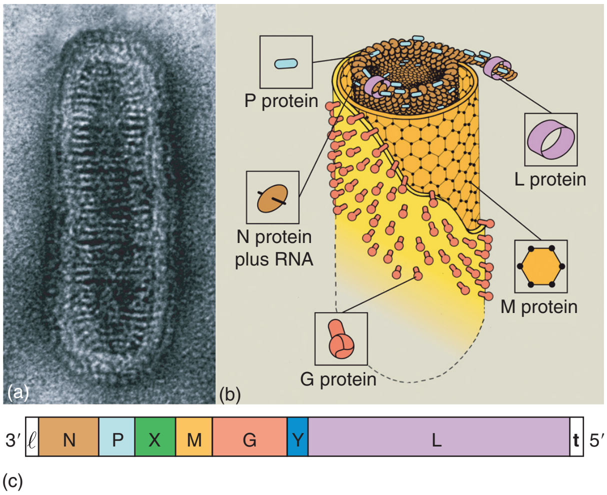

Figure 1 Electron micrograph, diagram, and genome organization of plant rhabdoviruses. (a) Transmission electron micrograph of a negative-stained virus showing the striated inner core, envelope, and glycoprotein spikes. (b) Architecture of the virus particle. The nucleocapsid core is composed of the minus-sense genomic RNA, the nucleocapsid protein (N), the phosphoprotein (P), and the polymerase protein (L). The matrix protein (M) is involved in coiling the nucleocapsid, attachment of the nucleocapsid to the envelope, and associations with the transmembrane glycoprotein (G). (c) Schematic representation of the negative-sense arrangement of genes encoded in the genomes of plant rhabdoviruses. The order of the genes is 3′-ℓ-N-P-X-M-G-Y-L-**t**-5′, where ℓ represents the leader RNA, **t** represents the trailer sequence, X denotes putative movement and undefined plant rhabdovirus genes, and Y shows the location of open reading frames of unknown function in the genomes of several plant and animal rhabdoviruses. Reprinted, with permission, from the Annual Review of Phytopathology, Volume 43, © 2005 by Annual Reviews.

Genomic Structure and Organization

The consensus plant rhabdovirus genome deduced from the eight sequenced viruses is 3-ℓ-N-P-X-M-G-Y-L- **t**-5′ (Figure 1(c)). The N, P, M, G, and L genes appear in the same order as in other rhabdoviruses and their encoded proteins are thought to be functionally similar to the five proteins of vesicular stomatitis virus (VSV). A variable number of genes at the X site have been found in each of the sequenced viruses and some of these appear to be involved in movement. The Y sites between the G and L genes encode short ORFs of unknown functions that are present in the nucleorhabdovirus, RYSV, and the cytorhabdoviruses, NCMV and SCV.

Rhabdovirus ORFs are separated by intergenic or 'gene-junction sequences' that provide vital regulatory functions during transcription and replication (Figure 2(a)). The gene-junction sequences can be grouped into three elements consisting of (1) a poly (U) tract at the 3′ end of each gene, (2) a variable intergenic element that is not transcribed in the mRNAs, and (3) a short element complementary to the first 5 nt at the 5 start site of each mRNA. In general, the gene-junction sequences of each virus are highly conserved, and those of the plant rhabdoviruses share substantial relatedness and differ mostly at element II. Slightly more limited divergence is noted when comparing the genomes of other families within the order *Mononegavirales*, suggesting that these regulatory sequences have been stringently conserved.

The coding regions are flanked by 3 leader (ℓ) and 5 trailer (**t**) noncoding sequences that represent recognition signals required for nucleocapsid assembly and regulation of genomic and antigenomic RNA replication. These sequences have short complementary termini and small amounts of common sequence relatedness (Figure 2(b)). However the plant rhabdovirus ℓ RNAs differ in sequence from the ℓ and **t** sequences of vertebrate rhabdoviruses and are considerably longer than those of VSV. The transcribed ℓ RNA of SYNV is polyadenylated and differs in this respect from the ℓ RNA of VSV and other known rhabdoviruses.

Properties of the Encoded Proteins

The most comprehensive biochemical analyses of the encoded proteins have been carried out with SYNV, LNYV, and RYSV. Overall, the plant rhabdovirus proteins have very little sequence relatedness to analogous proteins of animal rhabdoviruses, with the exception of

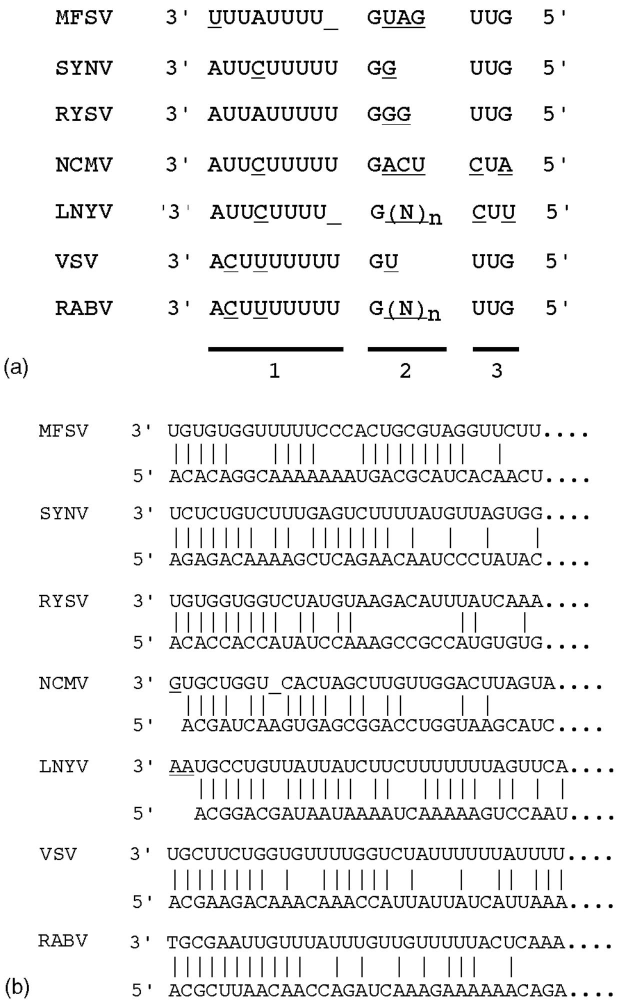

Figure 2 Comparisons of intergenic and terminal noncoding regions of rhabdovirus genomes. (a) Intergenic sequences separating the genes. (b) Complementary sequences at the 3′ and 5′ termini of the genomic RNAs. MFSV (maize fine streak virus), SYNV (sonchus yellow net virus), RYSV (rice yellow stunt virus), NCMV (northern cereal mosaic virus), LNYV (lettuce necrotic yellows virus), VSV (vesicular stomatitis virus), RABV (rabies virus). Modified from figures 2 and 3 of Tsai C-W, Redinbaugh MG, Willie KJ, Reed S, Goodin M, and Hogenhout SA (2005) Complete genome sequence and *in planta* subcellular localization of maize fine streak virus proteins. *Journal of Virology* 79: 5304–5314, with permission from American Society for Microbiology.

the L protein, which has conserved polymerase motifs common to those of most rhabdoviruses. A description of these proteins and their probable functions is outlined below.

The nucleocapsid protein (N)

The N protein functions to encapsidate the viral genomic RNA and is a component of the viroplasms and of the polymerase complex (Figure 1). The N genes of the nucleorhabdoviruses, SYNV, MMV, MFSV, TaVCV, and RYSV, and the cytorhabdoviruses, LNYV, SCV, and NCMV have been sequenced. The SYNV, MFSV, MMV, and RYSV nucleorhabdovirus N proteins exhibit short stretches of sequence similarity, suggesting that these four viruses are closely related. These regions of the nucleorhabdovirus N proteins are not significantly related to those of the cytorhabdoviruses and have no extensive relatedness to vertebrate rhabdovirus N proteins.

Experiments conducted in plant and yeast cells have shown that SYNV N protein contains a bipartite nuclear localization signal (NLS) near the carboxy-terminus that is required for nuclear import, and biochemical studies have shown that the protein interacts *in vitro* with importin α homologs. Related nuclear localization sequences are also present in the MFSV and TaVCV N proteins, but this signal is lacking in the N protein of MMV and RYSV. During transient expression in plant cells, the SYNV N protein forms subnuclear foci that resemble the viroplasms found in infected plants, and coexpression of the N and P proteins results in colocalization of both proteins to subnuclear foci (Figure 3). These foci require homologous interactions of the N protein that are mediated by a helix-loop-helix motif near the amino-terminus. Interestingly, the SYNV subnuclear foci are distinct from those of nucleolar marker proteins, whereas foci formed during interactions of the MFSV N and P proteins appear to colocalize to the nucleolus.

The phosphoprotein (P)

Direct experiments showing phosphorylation of the P protein are available only for SYNV. The amino-terminal half of the SYNV P protein is negatively charged, as is the case for the other rhabdoviruses. In SYNV, the P protein is phosphorylated *in vivo* at threonine residues and hence differs from the VSV P protein, which is phosphorylated at serine residues. No discernable sequence relatedness is evident between the P protein of SYNV and those of other rhabdoviruses. However, the plant rhabdovirus P proteins have similar hydrophilic cores and the cytorhabdovirus P proteins overall are hydrophilic with similar isoelectric points. In addition, there is nearly 50% sequence identity between the P proteins of MMV and TaVCV. Although these results suggest that the provisional assignments of the P proteins of other plant rhabdoviruses are probably correct, additional data need to be accumulated to obtain a consensus of the functional activities and the biochemical interactions of the putative plant rhabdovirus P proteins.

The P protein is a component of the viral nucleocapsid core and the polymerase complex. The SYNV P protein forms complexes *in vivo* with the N and L proteins that are analogous to N:P and P:L complexes found in VSV-infected cells, and hence the P protein probably functions in SYNV polymerase recycling. Biochemical experiments have shown that the solubility of the SYNV N protein is

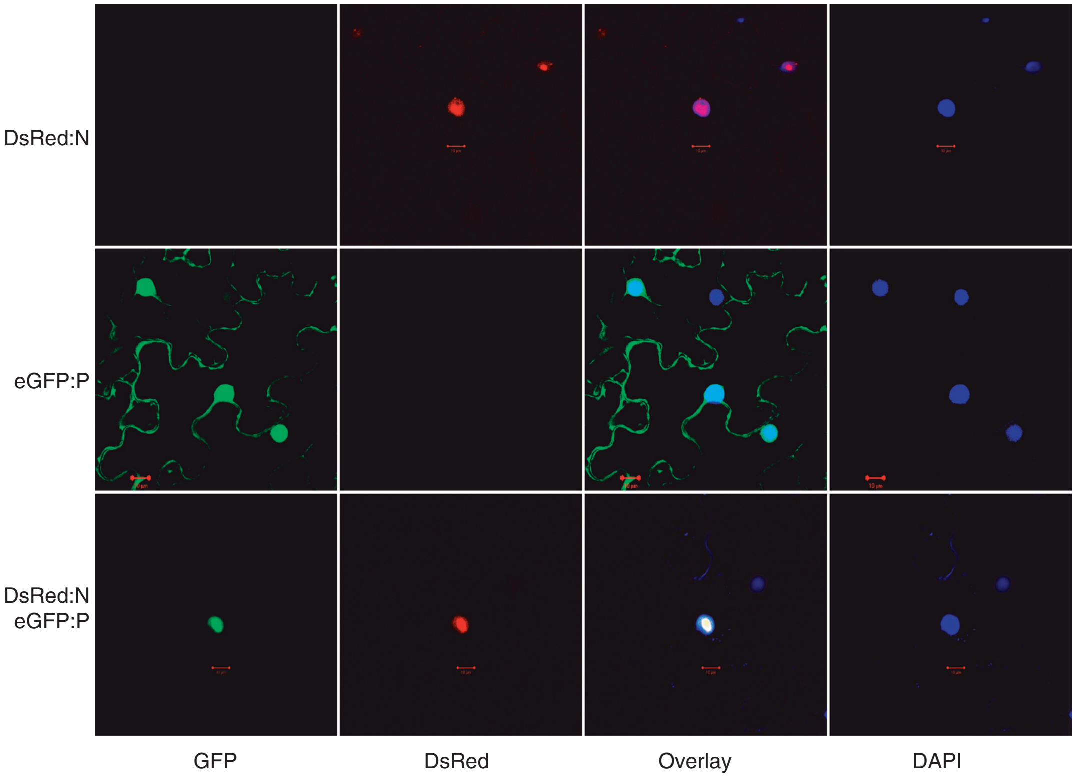

Figure 3 Subcellular localization of the N and P proteins of SYNV. The DsRed:N and eGFP:P fusion proteins were transiently expressed in *Nicotiana benthamiana* leaf tissue via infiltration with *Agrobacterium tumefacians* containing pGD vectors. The confocal micrographs show the subcellular localization of proteins at 3 days after infiltration. The top row depicts the individual expression of the DsRed:N fusion protein. The middle row shows fluroescence in cells expressing eGFP:P alone. The bottom row shows cells coexpressing the DsRed:N and eGFP:P proteins. Nuclei are identified by staining with DAPI (4′-6-diamino-2-phenylindole dihydrochloride). From Deng *et al*., unpublished.

increased during P protein interactions, so the P protein appears to have chaperone activity. Heterologous N:P protein complexes form by interactions of an internal region of the P protein with the amino-terminal helix-loop-helix region of N that overlaps the N:N protein binding site. The P protein also engages in homologous interactions that are mediated near the amino-terminus of the P protein. Hence, the SYNV P protein has functions similar to those of the P proteins of other well-characterized vertebrate rhabdoviruses.

Reporter gene fusions show that the SYNV P protein, when expressed alone, accumulates in both the nucleus and the cytoplasm (Figure 3). The central third of the SYNV P protein is required for nuclear import, but other regions of the protein affect the import efficiency. Recent experiments indicate that the SYNV P protein binds directly to human importin β derivatives *in vitro* and, since the N protein has an NLS site and interacts with importin α, the N and P proteins have different mechanisms for nuclear import. Interestingly, sequence analyses show that the SYNV P protein does not have a bipartite NLS, whereas the P proteins of RYSV and MMV have a bipartite NLS, and both P proteins have a pronounced nuclear localization pattern. Hence, due to these differences between the SYNV and the RYSV and MMV proteins, it is likely that the viruses have diverged in their strategies of nuclear localization. In SYNV and MFSV, coexpression of the P protein with the N protein of the same virus results in colocalization of the complexes to subnuclear foci characteristic of viroplasms. In SYNV, formation of these foci requires interactions of P with the amino-terminus of the N protein. However, the N and P interactions appear to be virus-specific because heterologous combinations of the SYNV and MFSV N and P proteins fail to form subnuclear foci, and the P protein continues to be expressed in both the nucleus and the cytoplasm.

The SYNV P protein also has a leucine-rich nuclear export signal located within the first third of the protein. Reporter proteins fused to the P protein are retained in the nucleus following treatment with Leptomycin B, an inhibitor of nuclear export. Interaction with the host nuclear export receptor XpoI provides further evidence for P protein nuclear export functions. These results and P protein mutagenesis experiments provide strong evidence suggesting that the SYNV P protein is involved in nuclear shuttling activities.

In addition to its role as a structural protein, the SYNV P protein shares many of the hallmarks of

RNA-silencing suppressor proteins. These characteristics include suppression of reporter gene silencing in transgenic plants and the ability to bind small interfering (siRNAs) and single-stranded RNAs *in vitro*. Together, these activities clearly point to key roles of the P protein in nucleocapsid structure, replication, countering innate host defenses, and possibly intercellular movement.

Position X proteins

Like other plant viruses, plant rhabdoviruses must encode proteins to assist in cell-to-cell movement of virus derivatives through the plasmodesmata and their systemic transport through the vascular system. Considerable evidence for a movement function has been accumulated for proteins encoded at position X between the P and M genes (**Figure 1(c)**). The predicted secondary structures of several plant rhabdovirus proteins, including SYNV sc4, LNYV 4b, RYSV P3, MMV P3, and MFSV P4, have a distant relatedness to the TMV 30 K superfamily structural motifs. Additional evidence for a role of the position X-encoded proteins in cell-to-cell movement is their association with host and viral membranes during transient expression. Unpublished evidence indicates that the sc4 protein is phosphorylated, as is also the case with the TMV 30 K movement protein. The movement hypothesis for genes occupying the X position has been reinforced by experiments carried out with RYSV. With this nucleorhabdovirus, the P3 protein is able to *trans*-complement cell-to-cell movement of a movement-defective potato virus X in *Nicotiana benthamiana* leaves. The P3 protein also interacts with the N protein, the major component of nucleocapsids; hence, it could possibly facilitate movement of nucleocapsids through these interactions.

Despite the persuasive evidence for a movement function of genes encoded at position X, considerable diversity appears in the X ORF(s) of several plant rhabdoviruses. For example, although the TaVCV gene X codes for a protein of a size similar to the other X protein genes, the protein has no obvious sequence similarity to proteins in the 30 K superfamily. MFSV encodes two proteins between the putative P and M proteins, and both have different localization patterns from sc4 of SYNV. In addition, four small ORFs reside between the P and M genes of NCMV. Thus, additional studies need to be undertaken to clarify the functional activities of the 'unusual' X proteins.

The matrix protein (M)

The M proteins of plant rhabdoviruses are basic and are thought to function in nucleocapsid binding and coiling, and interactions with the G protein. Sequence alignments of the M proteins of several plant rhabdoviruses have not revealed extensively conserved motifs. Unpublished studies suggest that the SYNV M protein is phosphorylated *in vivo* at both threonine and serine residues. When expressed ectopically, the M proteins of SYNV and MFSV localize in the nucleus. A central hydrophobic region of the M protein is thought to mediate membrane–lipid interactions with the G protein during morphogenesis. In addition to their roles in viral morphogenesis, preliminary experiments indicate that rhabdovirus M proteins have important roles in host–virus interactions because they appear to be able to inhibit host gene expression.

Position Y ORFs

NCMV, RYSV, and SCV contain a short ORF at position Y that separates the genes encoding the G and L proteins (**Figure 1(c)**). Small, nonvirion ORFs preceding the L gene are also found in the genomes of some animal viruses, but the products of these ORFs are either nonstructural or have not been detected in infected cells. The three predicted plant rhabdovirus Y proteins are small (<100 amino acids) and do not share obvious sequence identity with each other or with the nonvirion genes of the animal rhabdoviruses. However, short stretches of the Y ORFs have limited relatedness to other negative-strand RNA virus proteins, suggesting that these regions of the genome may have originated by gene duplication or recombination.

The RYSV P6 protein at position Y is predicted to contain an aspartic protease motif (DTG) and has five potential phosphorylation sites (S/T-X-X-D/E). *In vitro* phosphorylation assays using a GST:P6 fusion protein have shown that P6 is phosphorylated at both serine and threonine residues. Although P6 could not be detected in total protein extracts from infected leaf tissue, immunoblots of purified virus and protein extracts from viruliferous leafhoppers suggest that P6 is associated with virions, so it may have a structural role in infection.

The glycoprotein (G)

The G protein forms the glycoprotein spikes of rhabdovirus virions (**Figure 1**). The plant rhabdovirus G proteins do not have extensive similarity, but they are more closely related to each other than to the G proteins of several vertebrate rhabdoviruses. The plant rhabdovirus G proteins share putative N-terminal signal sequences, a transmembrane anchor domain, and several possible glycosylation sites. In addition, the SYNV G protein contains a putative NLS near the carboxy-terminus that could be involved in transit to the inner nuclear membrane prior to morphogenesis. Several glycosylation inhibitors interfere with N-glycosylation of the SYNV G protein, and tunicamycin treatment blocks SYNV morphogenesis, leading to accumulation of striking arrays of condensed nucleocapsid cores that fail to bud through the inner nuclear membrane. Thus, the G protein has a prominent role in morphogenesis, and the available evidence suggests that glycosylation is required for interactions of the protein with coiled nucleocapsids.

The polymerase protein (L)

The L proteins of plant rhabdoviruses are present in low abundance within nucleocapsids and in infected cells. The L proteins are the most closely related of the rhabdovirus-encoded proteins and are positively charged with conserved polymerase domains and RNA-binding motifs. The L protein of SYNV is required for polymerase activity, because antibodies directed against the GDNQ (polymerase) motif inhibit transcription. Alignment of the L protein sequence with polymerases of several other nonsegmented negative-strand RNA viruses reveals conservation within 12 motifs. Phylogenetic trees derived from L protein alignments indicate that the nucleorhabdoviruses and cytorhabdoviruses cluster together in two clades separated from the vertebrate rhabdoviruses. This suggests that the plant rhabdoviruses have diverged less from each other than from the vertebrate rhabdoviruses.

Polymerase Activity

A viral RNA-dependent RNA polymerase is activated after treatment of LNYV and BNYV cytorhabdovirus virions with mild nonionic detergents, and this activity cosediments with loosely coiled nucleocapsid filaments that are released from virions. The transcribed products are complementary to the genome, as expected of mRNAs. Thus, the described polymerases of these plant cytorhabdoviruses appear to be similar to the extensively studied polymerases of the vesiculoviruses.

In contrast, no appreciable polymerase activity is evident in dissociated preparations of SYNV or other nucleorhabdovirus virions that have been analyzed. However, an active polymerase can be recovered from the nuclei of plants infected with SYNV. Polymerase activity is associated with a nucleoprotein derivative, consisting of the N, P, and L proteins, that cosediments with SYNV nucleocapsid cores. The polymerase complex can be precipitated with P protein antibodies, but the activity of the complex is not inhibited by these antibodies. However, antibody inhibition experiments demonstrate that the L protein is required for polymerase activity. Kinetic analysis of transcription products also reveals that the complex is capable of sequentially transcribing a polyadenylated plus-sense leader RNA and polyadenylated mRNAs corresponding to each of the six SYNV-encoded proteins. Potential replication intermediates consisting of short incomplete minus-strand products homologous to the genomic RNA are also transcribed. These results thus provide a model whereby nucleorhabdovirus particles require polymerase activation by host components early in infection. In contrast, the polymerases of the cytorhabdoviruses appear to be present in an active form in virions and the released cores are capable of initiating primary transcription immediately upon uncoating *in vitro*.

Cytopathology and Replication

The plant rhabdoviruses vary profoundly in their sites of replication and morphogenesis, and those that replicate in the nucleus differ substantially from vertebrate rhabdoviruses that replicate and assemble in the cytoplasm. In plants, nucleorhabdoviruses replicate in the nucleus, bud in association with the inner nuclear membrane, and accumulate in enlarged perinuclear spaces formed between the inner and outer nuclear envelopes. Similar patterns normally occur in the majority of insect tissues, but MMV also buds in the outer membranes of the salivary glands and nerve cells of its leafhopper vector. Clearly, studies on insect cells need to be emphasized in future work to clarify aspects of insect transmission.

The limited evidence available indicates that the cytorhabdoviruses replicate in the cytoplasm, bud in association with the endoplasmic reticulum (ER), and accumulate in ER-derived vesicles (**Figure 4(a)**). Two slightly different variations in replication of LNYV and BYSMV have been proposed, based on extensive electron microscopic observations of infected cells. Indirect evidence suggests that a nuclear phase may be involved in LNYV replication because the outer nuclear membrane blisters and develops small vesicles that contain some virus particles. However, later in the life cycle, masses of thread-like viroplasms appear in the cytoplasm and these are located close to dense networks of the ER that appear during infection. These proliferated membranes form vesicles that may serve as sites for morphogenesis of the accumulating nucleocapsids. A similar scenario lacking a nuclear phase has been outlined for BYSMV. In this case, membrane-bound viroplasms appear in the cytoplasm and virus particles are found exclusively in association with cytoplasmic membranes that proliferate in close proximity to the viroplasms. Unfortunately, both of the cytorhabdovirus models have been derived solely from ultrastructural observations, and none of these studies has utilized specific antibodies to identify individual virus proteins, viral-specific probes for *in situ* hybridization, or modern techniques of cell biology to probe replication.

No direct information is yet available about the early entry and uncoating events, but a model for nucleorhabdovirus infection predicts that, after entry into the cell during vector feeding or mechanical transmission, rhabdovirus particles associate with the ER to release the nucleocapsid cores into the cytoplasm (**Figure 4(b)**). Released cores may then utilize the host nucleocytoplasmic transport

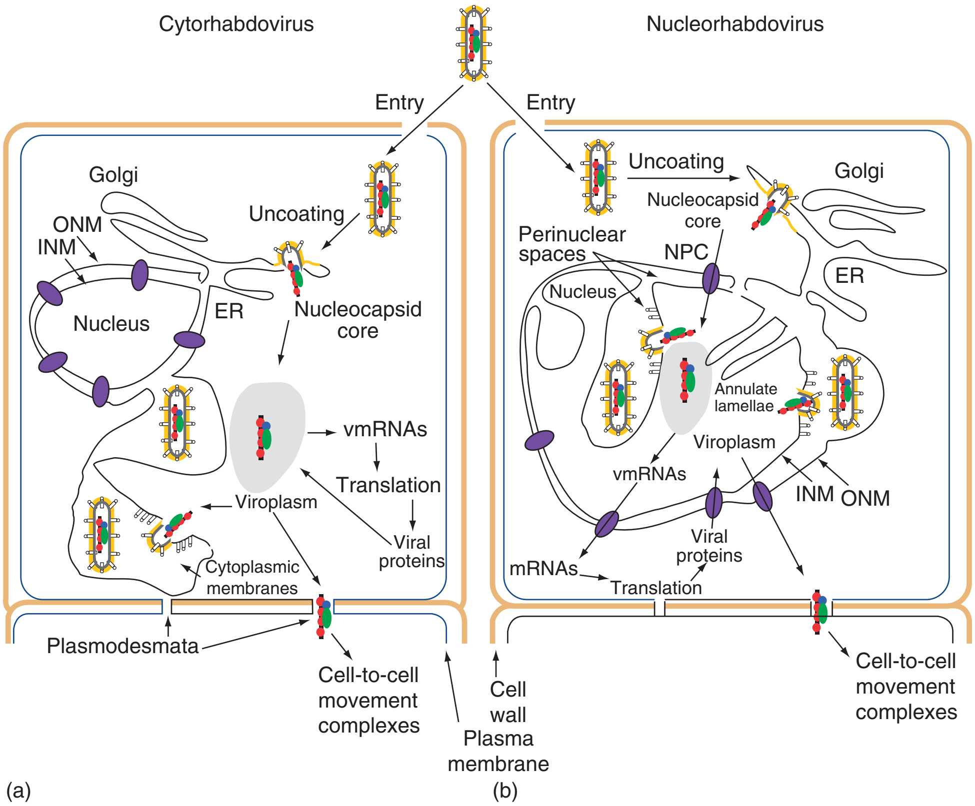

Figure 4 Models for cytorhabdovirus and nucleorhabdovirus replication in plant cells. Most rhabdoviruses are believed to enter plant cells during insect vector feeding and the nucleocapsid is thought to fuse with the endoplasmic reticulum (ER) and be liberated into the cytoplasm. Panels (a) and (b) provide contrasts between the cytorhabdovirus and the nucleorhabdovirus replication strategies, respectively. (a) Cytorhabdovirus replication model. The available information about cytorhabdovirus replication relies almost entirely on ultrastructural observations, and molecular or modern cytological information has not been obtained to extend these observations. After nucleocapsid release, primary and secondary rounds of transcription are followed by nucleocapsid accumulation in viroplasms to form dense masses that are associated with proliferated membrane vesicles. Morphogenesis occurs by budding of nucleocapsids into the ER. During the later stages of replication, large aggregates of bacilliform virions accumulate in pronounced vesicles that are thought to have originated from the ER. (b) Nucleorhabdovirus replication model. The nucleocapsid is thought to be transported into the nucleus through the nuclear pore complex and host components are thought to activate the nucleocapsids to initiate primary rounds of transcription to produce polyadenylated leader RNA and mRNAs for each of the viral proteins. The mRNAs are transported to the cytoplasm and translated, and the N, P and L proteins are transported through the nuclear pore complex into the nucleus. As the N, P, and L proteins increase in abundance, a switch occurs from primary transcription of mRNAs to a mode consisting of intermittent rounds of replication to produce antigenomic and genomic nucleocapsids, followed by secondary rounds of transcription to increase the pool of mRNAs. This phase of replication is regulated by a feedback mechanism that relies on the abundance of the core proteins for encapsidation of nascent leader RNAs. As replication progresses, the nuclei become greatly enlarged, and subnuclear viroplasms appear that consist of large masses of granular material that contain viral RNA and the N, P, and L proteins. Early during replication, some of the newly synthesized nucleocapsids are transported to the cytoplasm where they associate with movement proteins and are transported to other cells through the plasmodesmata. Late in replication, the M protein reaches sufficient concentration to coil the genomic nucleocapsids and mediate interactions with G protein patches at the inner nuclear membrane. During this process, virions undergo morphogenesis by budding through the inner nuclear membrane and accumulate in the perinuclear spaces. Reprinted, with permission, from the Annual Review of Phytopathology, Volume 43, © 2005 by Annual Reviews.

machinery to recognize karyophylic signals present on the N and P proteins to facilitate nucleocapsid entry into the nucleus. During the early stages of infection, the virion-associated polymerase is probably activated by host components to produce an active transcriptase that copies the genomic RNA into capped and polyadenylated mRNAs that are transported to the cytoplasm and translated. The translated N and P proteins are imported into the nucleus by separate mechanisms using host importin α and β proteins, respectively.

After entry into the nucleus, the N, P, and L proteins probably participate in multiple rounds of mRNA transcription, and antigenomic and genomic RNA replication. As replication proceeds, the viroplasms form discrete foci that appear near the periphery of dramatically enlarged nuclei. During the early stages of infection, small amounts of the nucleocapsids are postulated to be exported to the cytoplasm by interactions of the nuclear export signals on the P protein with host nuclear export receptor proteins. These exported nucleocapsids then interact with movement protein homologs to mediate transport through the plasmodesmata to adjacent cells. As infection progresses, the M protein accumulates in the nucleus and reaches concentrations sufficient to downregulate transcription and participate in coiling of minus-sense RNA nucleocapsid cores. The coiled cores then associate with G protein at sites on the inner nuclear envelope that are located in close proximity to the viroplasms. During budding, numerous enveloped virions accumulate in perinuclear spaces between the inner and outer nuclear envelope where they may be ingested during vector feeding.

A recent discovery that may shed new light on the processes of nucleorhabdovirus replication and maturation has been noted during infection of transgenic *N. benthamiana* plants that express green fluorescent protein (GFP) targeted to the ER. During infections with either SYNV or PYDV, a substantial proportion of the GFP appears to be redistributed to form spherules within the nuclei. In the case of SYNV, the spherules colocalized with foci formed by the N protein. A model to explain this phenomenon is that the spherules contacting the viroplasms in SYNV-infected plants are derived from the ER and become redistributed to the inner nuclear membrane to serve as sites for replication and virion maturation.

Vector Relationships, Distribution, and Evolution

Plant pathogenic rhabdoviruses are highly dependent on arthropod vectors for their distribution between plants. Although some plant rhabdoviruses have no known vector, most well-characterized members are transmitted by insects in which they also multiply, so it is possible that the plant rhabdoviruses radiated from a primitive arthropod. Plant rhabdoviruses are most commonly transmitted by aphids (Aphididae), leafhoppers (Cicadellidae), or planthoppers (Delphaccidae) (Table 1). An incompletely characterized putative rhabdovirus, beet leaf curl virus (BLCV), reportedly has a heteropteran beet leaf bug (*Piesma quadratum*) vector, but more extensive molecular and cytological analyses of this virus and other poorly characterized plant rhabdoviruses need to be carried out so their properties and vector relations can be clarified.

Vector–host relationships have profoundly affected plant rhabdovirus distribution and host range. For example, leafhoppers, planthoppers, and aphids are prevalent on both monocots and dicots, but the rhabdoviruses causing diseases of the Gramineae are all transmitted by leafhoppers or planthoppers. Except for PYDV and EMDV, which have leafhopper and planthopper vectors, respectively, dicot-infecting rhabdoviruses whose transmission has been investigated are transmitted by aphids. In all cases of insect transmission that have been carefully examined, rhabdoviruses are persistently transmitted in a propagative fashion, and in many cases, can be transmitted to vector progeny. Long latent periods are required before transmission occurs; insects often remain viruliferous throughout their lives, and transovarial passage has been observed through eggs and nymphs. In addition, strain-specific infection of tissue culture lines and explants combined with serological detection in vector cells provides unequivocal proof that rhabdoviruses replicate with high specificity in leafhopper and aphid vectors.

Several classical studies with PYDV in leafhoppers and SYVV in aphids, as well as recent studies with MMV in its planthopper vector *Peregrinus maidis*, have provided models for tissue-specific events in insect infection (Figure 5). After virus acquisition from plants, MMV initially accumulates in epithelial cells of the anterior part of the insect midgut and subsequently in nerve cells. Then, the virus appears in tracheal cells, hemocytes, muscles, and the salivary glands, and finally, in the fat cells, mycetocytes, and epidermal tissues. MMV infection is most extensive in the anterior portion of the gut, nerve cells, tracheal cells, and salivary glands of the planthopper. Based on this order of events, it is postulated that epithelial cells of the midgut are the first virus-entry sites,

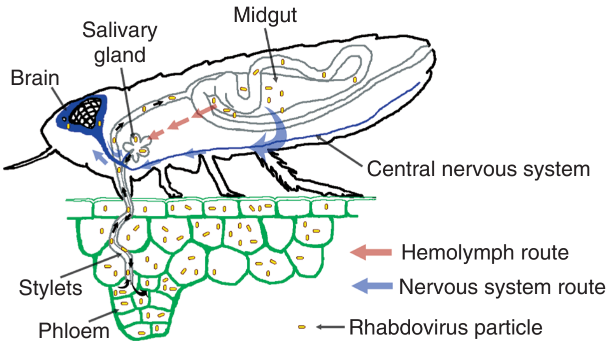

Figure 5 Events occurring during the infection cycle of rhabdoviruses in leafhopper vectors. Viruses are acquired during feeding on plant cells and move from the stylet to the midgut lumen of the digestive tract where they are hypothesized to invade epithelial cells by receptor-mediated endocytosis. From the epithelial cell layers, the virus moves into the nervous system, trachea, and hemolymph and spreads throughout the insect into the salivary glands and reproductive tissues. The salivary glands accumulate high levels of virus particles that are released by exocytosis, transported through the salivary canal, and transmitted to new host plants during subsequent feeding. Reproduced from figure 2a, Hogenhout SA, Redinbaugh MG, and Ammar E (2002) Plant and animal rhabdovirus host range: A bug's eye view. *Trends in Microbiology* 11: 264–271, with permission from Elsevier.

and that the virus quickly moves to the nervous system, trachea, and the hemolymph. From these tissues, MMV can be transmitted systemically to other tissues, including the salivary glands, which support high levels of virus accumulation.

MMV buds through the inner nuclear membrane in plant cells and has a similar pattern of morphogenesis in most cells of *P. maidis* tissues. However, MMV has also been observed to bud frequently from outer membranes in cells of nervous tissue and salivary glands of the planthopper, and similar observations have been made with other virus–vector combinations. Thus, the cellular budding site of MMV, and probably other rhabdoviruses, in insect hosts may be dependent on the cell type. Budding from outer cell membranes in salivary glands may be important because the process could allow release of virions into the saliva and permit introduction into plant cells during feeding.

Genetic experiments with PYDV have shown that highly efficient and inefficient leafhopper vectors can be selected. Continuous passage of PYDV by serial injection of insects can result in isolates that are unable to infect plants. Additional studies have shown that strains that have lost their capacity to be insect-transmitted can be recovered after protracted passage in plants. This phenomenon could provide a mechanism for evolution of vectorless rhabdoviruses, particularly in cases where infections were established in vegetatively propagated hosts.

Rhabdoviruses normally have the capacity to infect a greater range of plant hosts than the narrow range of species colonized by their insect vectors, because experimental host ranges usually can be extended considerably through mechanical transmission. In addition, plant rhabdoviruses have a wider insect host range than their natural insect vector hosts would indicate. For example, the majority of maize-feeding leafhoppers and planthoppers can acquire MFSV, but only one leafhopper, *Graminella nigrifrons*, can transmit this virus to maize. In addition, studies have shown that surrogate nonvectors of SCV injected with infected plant extracts can support replication and transmit virus to dicot hosts that do not support feeding by the native vector. Finally, cowpea protoplast infectivity experiments with the grass rhabdovirus FLSV and with SYNV show that both viruses are able to infect the legume protoplasts, but neither virus is able to infect cowpea plants. Together, these observations indicate that some plant rhabdoviruses have the ability to infect cells of several distantly related hosts, but that the natural host specificity is determined by (1) the insect vector feeding range; (2) the ability of the virus to move through the insect vector into the salivary glands and into the plant; and (3) systemic movement in the infected plant.

During evolution, plant rhabdoviruses faced two major challenges of a fundamentally different nature brought about by the necessity to alternately infect plants and insects. In each host, the virus must utilize different entry methods and accommodate distinct cellular and defense mechanisms. Rhabdovirus acquisition by the vector probably necessitates attachment to specific receptors at the surface of cells in the digestive system, followed by active invasion of the reproductive organs, fat bodies, and salivary glands. Very different barriers must be circumvented to establish systemic infections of plants. In order to establish a primary infection focus, the cell wall must first be breached by mouthparts of the insect, the virus must be introduced into the plant cell, where it uncoats and initiates the replication cycle. To establish systemic infections in plants, the virus must move from cell to cell through very small plasmodesmatal connections, enter the vascular system and spread throughout the plant. Therefore, plant rhabdoviruses are anticipated to have evolved a number of sophisticated mechanisms to circumvent the barriers to infection of their insect and plant hosts.

See also: Fish Rhabdoviruses.

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