

Plant Rhabdoviruses

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Abstract This chapter provides an overview of plant rhabdovirus structure and taxonomy, genome structure, protein function, and insect and plant infection. It is focused on recent research and unique aspects of rhabdovirus biology. Plant rhabdoviruses are transmitted by aphid, leafhopper or planthopper vectors, and the viruses replicate in both their insect and plant hosts. The two plant rhabdovirus genera, *Nucleorhabdovirus* and *Cytorhabdovirus*, can be distinguished on the basis of their intracellular site of morphogenesis in plant cells. All plant rhabdoviruses carry analogs of the five core genes: the nucleocapsid (N), phosphoprotein (P), matrix (M), glycoprotein (G) and large or polymerase (L). However, compared to vesiculoviruses that are composed of the five core genes, all plant rhabdoviruses encode more than these five genes, at least one of which is inserted between the P and M genes in the rhabdoviral genome. Interestingly, while these extra genes are not similar among plant rhabdoviruses, two encode proteins with similarity to the 30K superfamily of plant virus movement proteins. Analysis of nucleorhabdoviral protein sequences revealed nuclear localization signals for the N, P, M and L proteins, consistent with virus replication and mor-

phogenesis of these viruses in the nucleus. Plant and insect factors that limit virus infection and transmission are discussed.

1

Abbreviations

1.1

Virus acronyms

- BYSMV Barley yellow striate mosaic virus
- LNYV Lettuce necrotic yellows virus
- MFSV Maize fine streak virus
- MIMV Maize Iranian mosaic virus
- MMV Maize mosaic virus
- NCMV Northern cereal mosaic virus
- PYDV Potato yellow dwarf virus
- RTYV Rice transitory yellows virus
- SSMV Sorghum stunt mosaic virus
- SYNVSonchus yellow net virus
- SYVV Sowthistle yellow vein virus
- VSV Vesiculostomatitis virus
- WASMV Wheat American striate mosaic virus

1.2

Other Abbreviations

- ER Endoplasmic reticulum
- G Glycoprotein
- GFP Green fluorescent protein
- L Large polymerase protein
- LR Leaf rub inoculation
- M Matrix
- ORF Open reading frames
- NLS Nuclear localization signal
- N Nucleocapsid
- P Phosphoprotein
- SE Size exclusion limit
- TEM Transmission electron microscopy
- VIGS Virus induced gene silencing
- VPI Vascular puncture inoculation

2 Introduction

In this chapter, we will present an overview of the current information on plant rhabdovirus structure and taxonomy, genome structure, protein function, and insect and plant infection. Rather than being comprehensive, we will focus on research that is either recent or that emphasizes unique aspects of rhabdovirus biology. The majority of the rhabdovirus species have two natural hosts: either insects and plants, or insects and vertebrates (Hogenhout et al. 2003). Plant-infecting rhabdoviruses include a number of economically important pathogens and are transmitted to their plant hosts by insect vectors including aphids, planthoppers and leafhoppers (Jackson 1987). The family *Rhabdoviridae* also contains several human and animal pathogens of which the economically important livestock-infecting vesiculoviruses and ephemeroviruses are also insect transmitted. In fact, the widespread ability of rhabdoviruses to infect arthropods has led to speculation that the family evolved from an ancestral insect virus (Nault 1997) and that the rhabdovirus host range is largely determined by the insect host (Hogenhout et al. 2003).

Most plant viruses are composed simply of nucleic acid encapsidated by a protein coat. However, the composition of rhabdovirus particles is more complex. Like other members of the *Rhabdoviridae*, plant rhabdoviruses are bacilliform and are composed of RNA encapsidated by a protein coat surrounded by a lipid bilayer derived from the plant or insect host by budding from the cell's nuclear, endoplasmic reticulum (ER) or cell surface membranes. The rhabdovirus genomic RNA has negative polarity. Thus, the plant rhabdoviruses must carry proteins in the mature virion that enable synthesis of viral mRNAs after introduction into host cells. At 13–14 kb and encoding six to nine proteins, the plant rhabdovirus genome is a large relative to those of most other plant viruses, which range from about 3 to 20 kb and encode from 1 to 12 proteins (Hull 2002).

The ICTV database (Brunt et al. 1996) lists eight definitive cytorhabdoviruses and 13 nucleorhabdoviruses (Table 1). Plant rhabdovirus species are generally distinguished by serological relationships combined with the virus' plant host range and vector species. These viruses are distributed worldwide. The spread of rhabdovirus diseases in crops is restricted by several factors. Rhabdoviruses may have limited plant host range, and some crop germplasm in otherwise susceptible species can be resistant to systemic rhabdovirus infection. Further, plant rhabdoviruses are usually not transmitted vertically to seed or insect progeny, and subsequently are dependent on acquisition and inoculation by their arthropod host. The efficiency of rhabdovirus transmission by insects can be as low as 5% (Ammar 1994; Sylvester and

Table 1. Plant-infecting rhabdoviruses

Virus species name	Acronym	LRI ^a	Vector family	Vectors
Barley yellow striate mosaic cytorhabdovirus	BYSMV	N	Delphacidae	<i>Loadelphax striatellus</i>
Broad bean yellow vein cytorhabdovirus	BBYVV	N	–	–
Broccoli necrotic yellows cytorhabdovirus	BNYV	Y	Aphididae	<i>Breviconyne brassicae</i>
Cereal northern mosaic cytorhabdovirus	NCMV	N	Delphacidae	–
Festuca leaf streak cytorhabdovirus	FLSV	N	–	–
Lettuce necrotic yellows cytorhabdovirus	LNVV	Y	Aphididae	<i>Hyperomyzus lactucae</i>
<i>Sonchus</i> cytorhabdovirus	SonV	Y	–	–
Strawberry crinkle cytorhabdovirus	SCV	Y	Aphididae	<i>Chaetosiphon</i> sp.
<i>Gynodon</i> chlorotic streak nucleorhabdovirus	CCSV	N	Delphacidae	<i>Toya propinqua</i>
Coriander feathery red vein nucleorhabdovirus	CFRW	Y	Aphididae	<i>Hydaphis foeniculi</i> , <i>Myzus persicae</i>
Carrot latent nucleorhabdovirus	CLV	N	Aphididae	<i>Brachycaudus heraclei</i>
<i>Datura</i> yellow vein nucleorhabdovirus	DYVV	N	Not Aphididae	–
Eggplant mottled dwarf nucleorhabdovirus	EMDV	Y	–	–
Maize fine streak nucleorhabdovirus	MFSV	N	Cicadellidae	<i>Graminella nigrifrons</i>
Maize mosaic nucleorhabdovirus	MMV	N	Delphacidae	<i>Peregrinus maidis</i>
Pittosporum vein yellowing nucleorhabdovirus	PVYV	Y	Not Aphididae	–
Potato yellow dwarf nucleorhabdovirus	PYDV	Y	Cicadellidae	<i>Agallia constricta</i> , <i>A. quadripunctata</i> , <i>Aceratagallia sanguinolenta</i>
Rice transitory yellows virus	RTV	N	Delphacidae	<i>Nephotettix</i> sp.
<i>Sonchus</i> yellow net nucleorhabdovirus	SYNV	Y	Aphididae	<i>Aphis coreopsidis</i>
Sorghum stunt mosaic virus	SSMV	N	Cicadellidae	<i>Graminella sonora</i>
Sowthistle yellow vein nucleorhabdovirus	SYVV	N	Aphididae	<i>Hyperomyzus lactucae</i>
Tomato vein clearing nucleorhabdovirus	TVCV	Y	–	–
Wheat Am. striate mosaic nucleorhabdovirus	WASMV	N	Cicadellidae	<i>Elymana virescens</i> , <i>Endria inimica</i>

^aThe ability of the virus to be transmitted (Y) or not (N) by leaf rub inoculation (LRI) under laboratory conditions. SCV and PYDV cannot be transmitted to all hosts by LRI.

Richarson 1992), and rhabdovirus plant hosts appear to be mostly annuals, whereas perennial plant hosts provide a long-term and continuous reservoir of virus inoculum. Thus, most rhabdovirus species have limited geographic ranges, largely because of the limited distribution of insect vectors and plant hosts. Nonetheless, losses from rhabdovirus diseases in sweet corn can reach 100% (Brewbaker 1981).

The distinctive morphology of rhabdoviruses allows for easy identification of the virions by transmission electron microscopy (TEM) of negatively stained plant extracts. However, further characterization of plant rhabdoviruses is more difficult, because these viruses are not easy to purify from infected plants. In addition, most plant rhabdoviruses are recalcitrant to mechanical transmission and appear to be obligately transmitted by insect vectors, particularly those transmitted by leafhoppers (*Cicadellidae*) and planthoppers (*Delphacidae*) (Hogenhout et al. 2003). Hence, there are a number of plant-infecting rhabdoviruses for which only preliminary descriptions are available (Jackson et al. 1999). Of the 60 or so potential plant-infecting rhabdoviruses, a few have been characterized at the biochemical and molecular levels. Viruses for which sequence information is available include the nucleorhabdoviruses *Rice transitory yellows nucleorhabdovirus* (RTYV), *Sonchus yellow net nucleorhabdovirus* (SYNV), and maize fine streak virus (MFSV), and the cytorhabdoviruses *Northern cereal mosaic cytorhabdovirus* (NCMV) and *Lettuce necrotic yellow cytorhabdovirus* (LNYV).

3 Morphology and Taxonomy

The basic structure and morphology of plant rhabdoviruses in electron micrographs is similar to that of vertebrate rhabdoviruses. The virions are bacilliform with a spiked surface and a striated capsid core. Virion sizes vary depending on the virus species and fixation methods with lengths of 130–350 nm and widths of 45–100 nm (Jackson et al. 1999). Rhabdovirus virions are composed of a negative-strand RNA genome encapsidated by the nucleocapsid (N) protein surrounded by a phospholipid membrane. The lipid-embedded glycoprotein (G) and matrix (M) proteins are also structural components of the virion. The N, G and M proteins are generally detectable after SDS-PAGE of purified virions. The phosphoprotein (P) and large polymerase (L) proteins, together with the N protein are required for mRNA synthesis and genome replication. These two proteins are present in the virion, but are usually detectable only by serological analyses (Jackson 1987; Wagner et al. 1996).

The plant-infecting rhabdoviruses are separated into the two genera, *Nucleorhabdovirus* and *Cytorhabdovirus*, depending on the intracellular site of virus maturation in plant cells (Walker et al. 2000). Nucleorhabdoviruses accumulate within the nucleus and perinuclear space of the plant and some insect cells (Ammar and Nault 1985; McDaniel et al. 1985). Cytorhabdoviruses appear to replicate solely within the cytoplasm, undergoing morphogenesis in the ER in masses of thread-like structures that are often referred to as viroplasms. However, the distinction between cytorhabdoviruses and nucleorhabdoviruses is not always clear, as budding from nuclear membranes has been observed for some cytorhabdoviruses such as LNYV (Dietzgen et al. 1994). The two plant rhabdovirus genera cannot be distinguished on the basis of plant host or insect vector range (Table 1). For example, both genera contain species that infect maize and sowthistle (*Sonchus arvensis*). In addition, examples of aphid-, leafhopper- and planthopper-transmitted viruses are found in both virus genera.

In insects, the site of rhabdovirus morphogenesis appears to be cell-type-specific. The nucleorhabdovirus *Maize mosaic virus* (MMV) buds primarily from inner nuclear membranes of most tissues in both its planthopper vector (*Peregrinus maidis*) and in maize cells (Ammar and Nault 1985; McDaniel et al. 1985). However, in salivary gland secretory cells, MMV particles commonly bud from the plasma membranes, and accumulate in the intercellular spaces. These spaces ultimately connect to the salivary ducts, where the virus can move to a new plant host via the saliva.

4 Plant Rhabdovirus Genome Organization

At 13–14 kb, plant rhabdovirus genomes are somewhat larger than most rhabdoviral genomes (Walker et al. 2000). Each plant virus encodes homologs of the five structural rhabdoviral genes found in the prototypical *Vesicular stomatitis virus* (VSV) genome (Fig. 1). The five structural genes occur in the same order as in VSV, and are thought to serve analogous roles in plant rhabdoviruses (Jackson et al. 1999). In addition to these five genes, nucleorhabdovirus and cytorhabdovirus genomes carry one to four additional open reading frames (ORF).

The intergenic regions of rhabdoviruses are highly conserved both among genes of a virus species and between members of the virus family (Table 2). Similarly to VSV intragenic sequences, plant rhabdovirus intergenic regions are comprised of an AT-rich region (region I), thought to be the intracellular polyadenylation signal or stutter sequence, followed by a short, variable, non-

SYNV (N)	N P	<i>sc4</i>		M G	L
RTYV (N)	N P	3		M G 6	L
NCMV (C)	N P	3 4 5 6		M G	L
LNYV (C)	N P	4b		M G	L
VSV	N P			M G	L

Fig. 1 Organization of plant rhabdovirus genomes. The genes and detected open reading frames (ORF) are indicated for the sequenced nucleorhabdoviruses (N), cytorhabdoviruses (C) and vesiculostomatitis virus (VSV). The five core genes found in VSV encode the nucleocapsid (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the large or polymerase (L) protein. The location of the *sc4* and 4b genes and other ORF (shown as *numbers*) found in plant rhabdoviruses are indicated. The plant rhabdovirus sequences used are as indicated in Table 2

transcribed region (region II) and a highly conserved transcription start site (region III). The MFSV intergenic sequence shows a high degree of similarity to those of SYNV and RTYV (Tsai et al., unpublished results). Although there is information available for only a few plant rhabdovirus species, it appears that the region I sequences are more conserved among the nucleorhabdoviruses and that region II is longer in the two cytorhabdoviruses. These patterns suggest conservation of intragenic regions within a genus.

As expected, the L genes are the most conserved of the five core rhabdovirus genes. There is 40%–60% sequence identity among the plant rhabdoviruses, and 37%–39% identity between plant rhabdoviruses and VSV, as determined by ClustalX alignment (Thompson et al. 1997). The other core rhabdovirus genes are somewhat less conserved, with 30%–48% identity among plant rhabdoviruses, and 30%–40% identity between the plant rhabdoviruses and VSV. Phylogenetic analyses of aligned sequences using a neighbor-joining dis-

Table 2. Conservation of plant rhabdovirus intergenic region sequences

Virus ^a	I ^b	II	III
SYNV	<u>AUAUAAGAAAA</u>	<u>CC</u>	<u>AAC</u>
RTYV	<u>AUAUAAUAAAA</u>	<u>CCC</u>	<u>AAC</u>
NCMV	<u>AAUUAAAGAAAA</u>	<u>CUGAGAU</u>	AAC
LNYV	<u>GAUUUAAGAAAA</u>	<u>CNACTGA</u>	<u>GAA</u>
VSV	<u>NUAUGAAAAAA</u>	<u>CU</u>	<u>AAC</u>

^aSYNV, *Sonchus yellow net virus* (Genbank L32603); RTYV, *Rice transitory yellows virus* (AB011257); NCMV, *Northern cereal mosaic virus* (AB030227); LNYV, *Lettuce necrotic yellows virus* (Wetzel et al. 1994); VSV, *Vesiculostomatitis virus* (NC001560).

^bThe consensus sequences for the putative polyadenylation signal (I), the untranscribed intergenic sequence (II) and the known (SYNV, LNYV) or putative transcription start site (III) indicated are from the + strand. Nucleotides conserved for all genes in a viral genome are underlined.

tance algorithm (Swofford 2002) indicate that the three nucleorhabdoviruses (SNYV, MFSV and RTYV) form a group separate from the cytorhabdoviruses (NCMV and LYNV) for the L and N genes (Redinbaugh et al., unpublished results). These data suggest that the genus distinction between nucleorhabdoviruses and cytorhabdoviruses is reflected in the phylogeny of the species. However, similar phylogenetic distinctions between nucleorhabdoviral and cytorhabdoviral P, M and G genes were not found.

Relative to VSV, each of the plant rhabdoviruses carries at least one additional ORF. Several factors indicate that these are expressed genes. Each of the ORFs is downstream of a conserved intergenic region, and transcripts corresponding to these ORFs can be detected by Northern blot analysis of infected plants or insects (Tanno et al. 2000; Scholthof et al. 1994; Huang et al. 2003; Wetzel et al. 1994; Tsai et al., unpublished results). In addition, the ORF-encoded proteins corresponding to the SYN *sc4* (Scholthof et al. 1994) and the RTYV ORF 6 (Huang et al. 2003) have been detected in virions, plants and insects.

In the plant rhabdoviruses, at least one of the additional ORFs is located between the P and M genes (Fig. 1). The fact that each virus has at least one gene inserted between P and M in the genome suggests a conserved role for these additional genes. In spite of the conserved configuration, no significant sequence similarity can be detected among these genes. The nucleorhabdovirus SYN and the cytorhabdovirus LYNV each encode six genes, with the *sc4* (Scholthof et al. 1994) and 4b (Wetzel et al. 1994) genes being inserted into the genome between the P and M genes. The nucleorhabdoviruses RTYV and MFSV both encode seven genes. For MFSV, ORF3 and ORF4 are both located between the P and M genes in the viral genome (Tsai et al., unpublished results), while the RTYV ORF6 is located between the G and L genes in a similar location to the NV gene of novirhabdoviruses (Walker et al. 2000). Finally, the cytorhabdovirus NCMV encodes nine ORFs, with ORFs 3–6 being inserted between the putative P and M genes (Tanno et al. 2000). These four ORFs are quite small, encoding proteins of 12.8–18.6 kDa. Because each ORF is preceded by a conserved intergenic region, and transcripts hybridizing to each ORF accumulated in insects, they appear to be genes (Tanno et al. 2000).

4.1 Plant Rhabdovirus Proteins

Direct experimental evidence for plant rhabdovirus protein function and localization is limited to studies of SYN (Jackson et al. 1987; Scholthof et al. 1994; Choi et al. 1992; Martins et al. 1998; Goodin et al. 2001, 2002). For

the other plant rhabdoviruses, most studies have involved analysis of the genome-encoded open reading frames and comparison of these proteins with those of other more studied viruses.

Rhabdovirus L proteins show the highest conservation with ~~about 20%~~ amino acid sequence identity among the plant rhabdoviruses. The L proteins are all large (220–240 kDa) proteins that carry conserved motifs characteristic of RNA-dependent RNA polymerases. The SYN V L protein has been associated with an RNA polymerase activity in the nuclei of infected plants (Choi 1992; Wagner et al. 1996; Wagner et al. 1997). This L protein forms a complex with the viral N and P proteins that is important for virus replication and protein synthesis (Wagner et al. 1997). Most plant rhabdovirus L proteins are positively charged at neutral pH. The predicted L protein sequences for the three nucleorhabdoviruses each have consensus nuclear localization signals (NLS) and are predicted to be localized in the nucleus based on PSORTII analysis (Table 3). Nuclear localization signals are also found in BEFV and the novirhabdovirus L proteins.

Although the predicted plant rhabdovirus N proteins show only about 20% sequence identity, all are basic (pI 8–10) relative to VSV (pI 6.3). Immunocytochemical analyses and the distribution of GFP:N fusion proteins both indicate that the SYN V N protein is targeted to the nucleus (Martins et al. 1998; Goodin et al. 2001). This protein carries a bipartite NLS between amino acids 441 and 461 in the carboxyterminus that is required for nuclear localization (Goodin et al. 2001). PSORTII analyses of the N proteins of the nucleorhabdoviruses RTYV and MFSV also indicate the presence of strong NLS and predict a nuclear localization. Further, ~~preliminary evidence suggests that~~ the MFSV N protein is also directed to the nucleus (~~Goodin et al., unpublished results~~). Neither cytorhabdovirus N protein has a predicted NLS, and none were identified among other rhabdoviruses.

The plant rhabdovirus P proteins have little homology to one another or to other rhabdovirus P proteins. The SYN V P (or M2) protein is found in the virus nucleocapsid core and is associated with the nuclear replicase complex (Wagner et al. 1997). Although immunohistochemical analyses indicate a nuclear localization for the protein (Martins et al. 1998), a GFP:P fusion protein expressed in tobacco cells is distributed between the cytoplasm and nucleus (Goodin et al. 2001). The SYN V P protein does not contain a canonical arginine-lysine-rich NLS, but the protein does have a karyophilic region between amino acids 40 and 124, suggesting that P utilizes an alternative nuclear import pathway (Goodin et al. 2001). Another facet of the complex distribution of P in plant cells is the fact that a GFP:P fusion protein with a carboxyterminal deletion from amino acids 247 and 346 had more pronounced nuclear localization than the wild-type P protein fused to GFP (Goodin et

Table 3. Plant rhabdovirus protein nuclear localization signals predicted with PSORTII^a

Pro.	SYNV2 ^b	RTYV	MFSV	NCMV	LNYY
N	446 RKR ² 457 KP ⁴ 0.6 nuc.	404 KK LGPPRANAHS RRKEP 0.96 nuc.	435 KR SSDGTGNVSK KKS ² 0.82 nuc.	None	None
P	none	264 RK DSHHYRTVVS RIEKK 0.76 nuc.	None	None	None
M	229 RKRK 266 RKHR 0.6 nuc.	None	206 KRKR 194 KK EDKAEKATTE KRKRQ 0.91 nuc.	None	None
G	590 KKKR 616 RKKK	None	None	None	None
L	1647 K ² RP 2054 K ² PRR 0.80 nuc.	1240 HRRK	None	351 RK FKEIFYMEYF KKNRK 0.7 nuc.	–

^aNuclear localization signals were identified using PROSITEII (Horton and Nakai 1999) (<http://psort.nibb.ac.jp/>).

^bVirus acronyms are as indicated in Table 2.

^cThe starting amino acid and sequence of the predicted NLS (top line) and the certainty score from PSORTII (bottom line). Only nuclear localization certainties more than 0.5 are listed.

al. 2001). Taken together, the results suggest that P has the ability to shuttle between the nucleus and cytoplasm. Interestingly, the RTYV P protein, but not the MFSV P protein, carries a strong bipartite NLS (Table 3). ~~However, preliminary evidence suggests that~~ the MFSV P protein is also directed to the nucleus (~~Goodin et al., unpublished results~~). No P proteins from the other rhabdovirus genera (including cytorhabdoviruses) carry NLS.

Several specific interactions among SYNIV proteins are likely to have roles in virus replication and morphogenesis. The N, P and L proteins are thought to interact to form the replicase complex in the plant cell nucleus (Wagner et al. 1997). All three proteins have karyophilic sequences and are localized in plant nuclei. Interactions between N and P occur in yeast two hybrid systems and in vivo (Goodin et al. 2001). Interestingly, co-expression of N and P drastically affected their localization patterns and resulted in a shift of both proteins to a subnuclear region. Because the karyophilic domain in P is located within the N-binding domain, it is likely that nuclear transport for the two proteins occurs independently and N-P interactions occur after nuclear import (Goodin et al. 2001).

Similarly with the P protein, the plant rhabdovirus M proteins do not share significant sequence homology. As in other rhabdoviruses, these basic proteins are thought to play a role in nucleocapsid coiling and interaction with the G protein (Jackson et al. 1999). ~~Fusion proteins constructed from the SYNIV M protein and GFP~~ localized to the nucleus (Goodin et al. 2001), and ~~this protein contains~~ NLS and is predicted by PSORTII to be localized to the nucleus (Table 3). The MFSV M protein, but not that of RTYV, also appears to carry strong NLS. The M proteins from cytorhabdoviruses do not carry NLS, whereas novirhabdovirus (*Snakehead rattle virus* and *Viral hemorrhagic septicemia virus*) M proteins do.

Plant rhabdovirus G proteins are glycosylated and form the virion spikes (Jackson et al. 1999). In SYNIV-infected tobacco cells, inhibiting glycosylation with tunicamycin prevented virion morphogenesis and resulted in accumulation of nucleocapsid cores at the periphery of the nucleus (van Beek et al. 1985). The plant G proteins do not share significant homology with each other, or with other rhabdovirus G proteins. However, all five plant G proteins are predicted to have one (SYNIV, MFSV and NCMV) or two (RTYV and LYNV) transmembrane domains, consistent with their role as viral membrane glycoproteins. The SYNIV G protein has a NLS, while the other rhabdoviruses do not (Table 3). In addition, immunofluorescence localization with SYNIV virion antibodies suggests that most of the virion G protein is localized in the periphery of the nucleus (Martins et al. 1998).

Specific roles for the additional genes in plant rhabdoviruses remain to be investigated. A role in vector transmission is less likely, because VSV

is capable of replicating in animals with just the five core genes. Further, VSV can be transmitted by sandflies and blackflies, although it is not yet known whether the virus spreads throughout these insects similarly to the spread of plant rhabdoviruses in their aphid, leafhopper and planthopper vectors. A more likely role for the additional genes is in facilitating cell-to-cell and/or systemic movement in plants. Movement proteins involved in cell-to-cell virus transport have been described for many plant viruses, including the NS_M protein of the thrips-transmitted plant pathogenic tospoviruses that belong to the *Bunyaviridae* (Storms et al. 2002). Interestingly, the human and animal infecting members of the *Bunyaviridae* do not have the NS_M protein.

In contrast to animal systems in which viruses enter cells by receptor-mediated endocytosis, movement of plant viruses between plant cells occurs through the symplast, the continuous protoplasm of plants that interconnects cells via plasmodesmata. Many plant viruses encode proteins that function to increase the size exclusion limit (SEL) of plasmodesmata, allowing relatively large molecules to move between cells (Oparka and Roberts 2000). Since rhabdoviruses are more than ten times larger than the normal 3-nm diameter of plasmodesmata and the viral genome is not itself infectious; it is highly likely that a plant rhabdovirus protein(s) must function to increase the plasmodesmata SEL so that unenveloped nucleocapsid cores may move from cell to cell. The *sc4* protein is proposed to have a role in cell-to-cell movement. The protein is expressed in plants and binds loosely to the virion (Scholthof et al. 1994). When expressed as a GFP: *sc4* fusion protein, it is partially localized at the plant cell periphery (Goodin et al. 2002). In addition, the protein is related to the 30K superfamily of plant virus movement proteins (Melcher 2000). Interestingly, the predicted LNYV 4b protein has similarity to the capillovirus and trichovirus movement proteins, which are in a different group of movement proteins in the 30K superfamily from *sc4* (U. Melcher, personal communication).

Another possible function of the additional genes in plant rhabdoviruses is to manipulate or suppress plant defense responses such as virus-induced gene silencing (VIGS) (Melcher 2000; Voinnet 2001). VIGS involves sequence-specific degradation of viral RNA transcripts or genomes, and spreads systemically throughout the plant in response to localized virus infection (Vionnet 2001). Many plant viruses have evolved counter-defensive proteins against VIGS known as suppressors of gene silencing. These include the helper component-protease of potyviruses and the *Cucumber mosaic virus 2b* protein (Vionnet et al. 1999). Many of the identified viral suppressors of gene silencing were previously described as having phenotypes involved with systemic or long-distance virus movement in the plant. Although most of the

viral suppressors of gene silencing have been identified from positive-strand RNA viruses, negative-strand RNA virus genomes also encode suppressors. The tospovirus and tenuivirus NS₅ proteins serve this function (Bucher et al. 2003). Although no suppressor of gene silencing has been identified in plant rhabdoviruses, RTYV is capable of inducing VIGS in rice as virus resistance is seen in transgenic rice expressing RTYV N gene sequences (Fang et al. 1996).

Recently, it was discovered that that the *Drosophila*-infecting *Flock house virus* both induces gene silencing and encodes a suppressor of silencing that is active in the insect host (Li et al. 2002). This raises the possibility that insect-transmitted plant rhabdoviruses manipulate the gene silencing system of their insect hosts in order to be transmitted. It is unclear whether and how gene silencing plays a role in organisms that have an adaptive immune response; however, it seems likely that most, if not all, plant-infecting viruses, including rhabdoviruses, have suppressors of gene silencing.

Other than *sc4*, the only additional plant rhabdovirus gene that was shown to be expressed is the RTYV ORF 6. This gene encodes a small (10.5-kDa) phosphorylated, acidic protein with limited sequence similarity to the novirhabdovirus non-virion proteins (Huang et al. 2003). The protein can be detected in the virion and leafhopper vector, but not in infected rice, suggesting a role in insect transmission for this protein.

5 Virus Infection of Plant Hosts

Insects are thought to inoculate plants with rhabdoviruses in nature by injecting the virus into the plant cell through wounds produced by their stylets. In the laboratory, some rhabdoviruses can be transmitted using mechanical inoculation techniques such as leaf rub inoculation (LRI) or vascular puncture inoculation (VPI) that may mimic the inoculations by vectors as they use their stylets to probe leaf epidermal cells prior to feeding (Hull 2002). For LRI, a virus-containing solution containing an abrasive (e.g., Celite) is rubbed on the leaf surface, making wounds in the epidermal cells and allowing for virus entry. LRI works well for many of the aphid-transmitted rhabdoviruses including SYNIV (Table 1). Most of the rhabdoviruses transmitted by planthoppers and leafhoppers are recalcitrant to LRI, indicating the virus must be delivered to specific, nonepidermal cells for infection to begin (Nault and Ammar 1989). This is consistent with the feeding pattern of leafhoppers and planthoppers, as they generally move their stylets between cells until they find the sugar-rich phloem cells. However, leafhopper- and planthopper-transmitted maize rhabdoviruses can be mechanically transmitted using VPI, which

uses a jeweler's engraving tool to drive minuten pins through virus inoculum and into the scutellum of germinating maize kernels (Louie 1995). It is thought that this technique produces wounds in those specific cells that are susceptible to infection by the viruses (Hogenhout et al. 2003).

Inoculation efficiency of plant rhabdoviruses is strongly affected by the level of resistance the plant has to the virus. Plants, like vertebrates and arthropods, have an innate immune response that is triggered by microbial infection, and there is evidence that this system is important in the plant response to virus infection (Nurnberger and Scheel 2001). For example, the N gene of tobacco confers resistance to tobacco mosaic virus in a classic gene-for-gene manner, by inducing a hypersensitive response and rapid cell death at the site of infection when plants carrying the N gene are inoculated with virus (Whitham et al. 1996). While plant genes conferring resistance to rhabdoviruses have yet to be isolated, there is genetic evidence for rhabdovirus resistance in maize (Ming et al. 1997; Redinbaugh et al. 2001).

Although rhabdoviruses can be transmitted mechanically, the insect is likely to be more than an injection needle, because insect saliva may play a significant role in the establishment of rhabdovirus infection in vertebrate and plant hosts. Insects are much more efficient transmitters of rhabdoviruses, requiring significantly lower concentrations of virus to establish infections than are needed for mechanical transmission. This may be due, at least in part, to factors such as P-450-monoxygenases, glutathione S transferases and proteases in insect saliva that suppress plant defense responses (Foissac et al. 2002; Feyereisen 1999; Gatehouse 2002). In addition, insects produce and excrete proteases that are insensitive to the protease inhibitors secreted by plants in the defense response (Gatehouse 2002). This modulation of the host defense response might facilitate the initial replication of the virus and thus enhance infectivity.

6

Virus Infection of Insect Hosts

Plant rhabdoviruses are transmitted by cicadellid leafhoppers, delphacid planthoppers and aphids in a persistent propagative manner. For transmission to occur, the insect must acquire virus from a plant host over a period of hours to days, then be retained in the insect for a period of days to weeks prior to transmitting the virus to plants (Ammar and Nault 2002). The insect can transmit the virus for the remainder of its lifetime. The virus replicates within the insect vector, as demonstrated by serial dilution transmission assays, injection experiments and quantitative serology.

To be transmitted by the insect, viruses must cross the cells of the gut, move from the gut to the salivary gland, cross the salivary gland cell layers, move into the saliva and be introduced into the plant or animal host by the insect with the saliva. This path suggests that receptor proteins in the brush border or apical membrane of the insect digestive tract are the first insect cellular molecules with which plant rhabdovirus particles interact (Adam and Hsu 1984; Jackson et al. 1987). It is likely that the virions interact with virus receptors on these membranes and enter cells by receptor-mediated endocytosis in a manner similar that described for VSV and rabies virus (Lewis and Lentz 1998; Superti et al. 1987). From the gut cells, viruses move to other insect organs and tissues including muscle, nervous tissues and into the hemolymph (Ammar and Nault 1985). It is generally thought that salivary gland cells are infected from the insect hemolymph; however, it is also possible that the salivary gland is infected via other tissues such as muscle cells or neurons. The latter possibility is more in agreement with the spread of lyssaviruses and vesiculoviruses through host nervous tissue prior to infection of the salivary glands (Tyler and McPhee 1987). Nonetheless, virus entry into muscle, neuron, salivary gland and other cells is also likely to occur by receptor-mediated endocytosis. After replication in salivary gland cells, viruses are secreted into the insect saliva and introduced into animal or plant hosts.

The assembly and accumulation sites of maize mosaic virus (MMV) in its planthopper host *P. maidis* were determined by transmission electron microscopy (TEM) (Ammar and Nault 1985). MMV particles were found in most insect organs. In the insect head, virions were observed in the brain, epidermis, fat and connective tissue, retinula cells, muscle and trachea. Virions also were observed in the principal and accessory salivary glands, nerve ganglia, muscle, foregut, midgut, male and female reproductive systems, but not in hindgut cells or Malpighian tubules. As mentioned above, the intracellular accumulation and assembly sites of MMV varied among vector tissues, with MMV budding from inner nuclear membranes of most cell types. In addition to budding from the ER of the secretory cells of the salivary gland, the virus accumulated in large vesicles in the cytoplasm in some nerve cells.

7

Factors Affecting Movement Between Insect and Plant Hosts

There is a difference between an insect being a virus host and a virus vector. To be transmitted to a new plant host by an insect the rhabdovirus must traverse a number of barriers in the insect host (Ammar 1994; Jackson et

al. 1999; Hogenhout et al. 2003). These barriers include being acquired from the plant host, entry into and infection of midgut cells, release from midgut cells, movement through the insect hemolymph or other organs, infection of the salivary gland, release of the rhabdovirus into the saliva, inoculation into a new susceptible plant host, and establishment of systemic infection in the new plant host. Blockage at any step may prevent transmission of viruses to plants.

There are at least five serologically distinct rhabdoviruses that infect maize, and each of these viruses has a different planthopper or leafhopper vector. Two strains of *Potato yellow dwarf virus* (PYDV) are differentially transmitted by *Aceratagallia* and *Agallia* species (Adams and Hsu 1984), and two serologically related maize rhabdoviruses, *Sorghum stunt mosaic virus* (SSMV) and MFSV, are differentially transmitted by *Graminella* species (Creamer et al. 1997; Redinbaugh et al. 2001). These results indicate there are viral factors that affect insect host range and vectoring capability that are unrelated to the plant host.

Plant rhabdovirus transmission can be blocked in the insect gut, because injection of virus into the hemolymph can increase transmission efficiency and may allow for transmission of viruses by non-vectors. For example, *Maize Iranian mosaic virus* (MIMV), which is serologically related to some MMV isolates, is naturally transmitted by the planthopper *Ribautodelphax notabilis*, but is transmitted at very low efficiency (0.4%–1.6%) by the MMV vector *P. maidis* (Izadpanah 1989). In contrast, *P. maidis* transmits MIMV at 64% efficiency after acquisition by hemolymph injection (Ammar 1994). Similar results were reported for several aphid-transmitted rhabdoviruses (Sylvester and Richardson 1992).

There is also evidence for non-gut barriers to virus transmission in insects. For example, the aphid *Macrosiphum euphorbiae* remains an inefficient vector of *Sowthistle yellow vein virus* (SYVV) when the virus is acquired by injection into the hemolymph (Behncken 1973), indicating that barrier to virus transmission could not be overcome by bypassing the insect gut. In this case, the virus was detected by TEM in various insect tissues, but was not found in the salivary glands. At the molecular level, organ barriers could result from a failure to enter or replicate in cells, a triggering of host defense responses (immune response and apoptosis), failure to move between cells, or failure to exit cells.

8 Conclusions and Future Perspectives

Analysis of genome sequence information suggests that the differential morphogenesis of cytorhabdoviruses and nucleorhabdoviruses is reflected in the phylogeny of the viral N and L genes. However, the differences in genome structure and sequence among the nucleorhabdoviruses are greater than those identified among species of other rhabdovirus genera, and the situation is similar for cytorhabdoviruses. Further phenotypic and genotypic characterization of plant rhabdoviruses will lead to a better understanding of the distinctions among members of these diverse genera. The sequenced nucleorhabdoviruses have relatively divergent plant hosts and include one aphid-, one leafhopper- and one planthopper-transmitted species. To aid in identifying genomic components important for insect vector and plant host specificity, sequence information from plant rhabdovirus species with overlapping plant host ranges and vector specificity would be useful (e.g., MMV/*P. maidis*, MIMV/*R. notabilis*, MFSV/*G. nigrifrons*, WASMV/*Endria inimica*, SSMV/*G. sonora*, NCMV/*Laodelphax striatellus* and BYSMV/*L. striatellus*).

Development of infectious virus clones would greatly facilitate association of functions with specific rhabdovirus proteins in both plants and insects. Expression of infectious clones will require production of a system expressing the rhabdoviral P and L proteins, as has been used for VSV and rabies virus (Pattniak et al. 1992; Mebatsian et al. 1996). More immediately, systems for transient expression of viral proteins in plants such as those developed by Goodin et al. (2001, 2002) will be useful for localizing viral proteins in plants and for elucidating some virus gene functions, especially identification of viral suppressors of gene silencing.

Insect vectors play a critical role in determining the host range of vertebrate and plant rhabdoviruses, and multiple barriers to rhabdovirus transmission have been identified in insects. Characterization of these barriers at the molecular level will aid in elucidation of the pathway the virus takes through the insect from the gut to the salivary gland before being transmitted to a new plant host. A major question is whether movement of virus through the insect nervous system is required for vector activity. Further, identification of rhabdovirus receptors in the vector midgut and salivary gland may provide information on vector specificity, as would some information on the nature of the insect immune response to rhabdovirus infection. While rhabdoviruses do not generally cause disease in their insect vectors, it will be interesting to determine whether virus infection produces changes in behavior that affect virus transmission to plant hosts.

In agricultural systems, plant resistance to pathogens is usually the most economically feasible and environmentally sustainable method for controlling disease. With the exception of maize, naturally occurring rhabdovirus resistance has not been identified in plant hosts. Therefore, further characterization of the genetic resistance in other crops affected by rhabdovirus diseases is warranted. In addition, characterization of the rhabdovirus resistance response in maize will aid in identification and development of virus-resistant germplasm for a variety of plants.

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