Annu. Rev. Phytopathol. 2005. 43:623–60
doi: 10.1146/annurev.phyto.43.011205.141136

First published online as a Review in Advance on May 19, 2005

BIOLOGY OF PLANT RHABDOVIRUSES*

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Key Words rhabdoviruses, plant viruses, negative strand viruses, nucleorhabdoviruses, cytorhabdoviruses

■ **Abstract** The *Rhabdoviridae*, whose members collectively infect invertebrates, animals, and plants, form a large family that has important consequences for human health, agriculture, and wildlife ecology. Plant rhabdoviruses can be separated into the genera Cytorhabdovirus and Nucleorhabdovirus, based on their sites of replication and morphogenesis. This review presents a general overview of classical and contemporary findings about rhabdovirus ecology, pathology, vector relations, and taxonomy. The genome organization and structure of several recently sequenced nucleorhabdoviruses and cytorhabdoviruses is integrated with new cell biology findings to provide a model for the replication of the two genera. A prospectus outlines the exciting opportunities for future research that will contribute to a more detailed understanding of the biology, biochemistry, replication and host interactions of the plant rhabdoviruses.

INTRODUCTION

The rhabdoviruses form a large family whose collective host ranges include vertebrates, invertebrates, and plants. Plant rhabdoviruses have traditionally been recognized based on their distinctive enveloped bacilliform or bullet-shaped particles. These large complex particles can be easily distinguished from the constituents present in uninfected tissue by electron microscopy of extracts or thin sections of infected tissue. Consequently, putative plant rhabdoviruses have been described in a large number of plant species based solely on electron microscopic observations, without the necessary accompanying molecular characterizations

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needed for unambiguous identification. As a result, about 100 distinctive members have been described, but most of these need to be purified and analyzed in considerable detail to verify their tentative inclusion in the *Rhabdoviridae* family. Nevertheless, microscopy of infected cells reveals that many of the assigned and putative plant rhabdoviruses can be distinguished depending on whether the viruses elicit inclusions in the nucleus, bud from the inner nuclear envelope, and accumulate in the perinuclear spaces, or whether they develop cytoplasmic viroplasms, undergo morphogenesis from cytoplasmic membranes, and accumulate in the cytoplasm. Thus, the major cytopathic changes accompanying infection by plant rhabdoviruses provide evidence for at least two different sites of replication and form the basis for separating these viruses into the *Cytorhabdovirus* and *Nucleorhabdovirus* genera (10, 89).

Rhabdoviruses infect a large number of monocot and dicot species. Assigned rhabdoviruses, with acronyms, appear in Table 1; Table 2 presents putative rhabdoviruses. Most plant rhabdoviruses are dependent on transmission by phytophagous insects, so their prevalence and distribution is influenced to a large extent by the ecology and host preferences of their vectors. Virus-vector interactions are highly specific, and in all cases where known vectors have been carefully examined, they have been shown to support the replication of the plant rhabdoviruses they transmit (7, 71, 87). Although some rhabdoviruses can be transmitted mechanically by abrasion of leaves, this mode of transmission does not contribute significantly to spread in nature. Moreover, seed or pollen transmission of plant rhabdoviruses has not been described. Thus, aside from vegetative propagation, which is a major factor in maintenance of SCV, PYDV, and TaVCV, direct plant-to-plant transmission is not normally a factor in the ecology or epidemiology of these pathogens.

The last comprehensive reviews describing the biological and physical properties of plant rhabdoviruses appeared over 15 years ago (25, 26, 52). Several concise reviews for specialty audiences have subsequently provided information on the biology, biochemistry, and replication of the better-studied plant rhabdoviruses (19, 36, 44, 53, 74, 87). During the past five years, a number of advances have been made in understanding the molecular and cell biology of the infection processes of the nucleorhabdoviruses, and the complete genomic sequences have been determined for three cytorhabdoviruses, LNYV, NCMV, and SCV, and five nucleorhabdoviruses, SYNV, MFSV, MMV, RYSV, and TaVCV (Table 3). For this review, we have summarized the older literature on the biology, pathology, and ultrastructural aspects of the plant rhabdoviruses, and we refer those interested in more detailed information about this area to previous reviews. We have also addressed more recent findings about the molecular biology of selected rhabdoviruses and cellular biology of their infection processes, and where appropriate, we have cited the animal rhabdovirus literature for comparison. Finally, we have concluded with a prospectus for future research with the intent of stimulating others to initiate studies on this intriguing group of viruses.

TABLE 1 List of recognized plant rhabdoviruses, their hosts, and vectors

Virus	Acronym	Natural host range	Vector
Genus Cytorhabdovirus			
*Barley yellow striate mosaic virus**	BYSMV	Graminae	Delphacid planthopper
(Maize sterile stunt virus)[a]	MSSV		Delphacid planthopper
(Wheat chlorotic steak virus)	WCSV		Delphacid planthopper
*Broccoli necrotic yellows virus**	BNYV	Brassicae	Aphid
*Festuca leaf streak virus**	FLSV	*Festuca gigantea*	
*Lettuce necrotic yellows virus**	LNYV	Chenopodiaceae, Compositae, Leguminosae, Liliaceae, Solanaceae	Aphid
*Northern cereal mosaic virus**	NCMV	Graminae	Delphacid planthopper
Sonchus virus	SonV	*Sonchus oleraceus*	
*Strawberry crinkle virus**	SCV	*Fragaria* sp.	Aphid
*Wheat American striate mosaic virus**	WASMV	Graminae	Cicadellid leafhopper
(Oat striate mosaic virus)	OSMV		Cicadellid leafhopper
Genus Nucleorhabdovirus			
Cereal chlorotic mottle virus	CCMoV	Graminae	Cicadellid leafhopper
*Datura yellow vein virus**	DYVV	*Datura stramonium*, *Thunbergia alata*	
*Eggplant mottled dwarf virus**	EMDV	Solanaceae	Cicadellid leafhopper
(Pittosporum vein yellowing virus)	PVYV		
(Tomato vein yellowing virus)	TVYV		
(Pelargonium vein clearing virus)	PVCV		
Maize fine streak virus	MFSV	Graminae	Cicadellid leafhopper
*Maize mosaic virus**	MMV	Graminae	Delphacid planthopper
*Potato yellow dwarf virus**	PYDV	*Nicotiana* sp., *Trifolium* sp., Potato	Cicadellid leafhopper
*Rice yellow stunt virus**	RYSV	Rice	Cicadellid leafhopper
(Rice transitory yellowing virus)	RTYV		
*Sonchus yellow net virus**	SYNV	Compositae	Aphid
*Sowthistle yellow vein virus**	SYVV	Compositae	Aphid
Taro vein chlorosis virus	TaVCV	*Colacasia esculenta*	

[a]Synonyms are given in brackets.

*Species recognized by ICTV (Tordo et al. 2005).

TABLE 2 List of unassigned plant rhabdoviruses and their hosts, symptoms, and vectors

Virus	Plant species	Disease symptoms	Transmission
Asclepias virus	*Asclepias currassavicia*	Diffuse leaf chlorosis, mosaic	
Atropa belladonna virus	*Atropa belladonna*	Symptoms unknown, virus complex	
Beet leaf curl virus	*Beta vulgaris*	Vein deformation, leaf curl	Lacewing
Black current virus	*Ribes nigrum*	Reversion disease complex	
Broad bean yellow vein virus (C)[a]	*Vicia faba*	Vein chlorosis	
Butterbur virus (N)	*Petasites officinalis*	Symptomless	Mechanical
Callistephus chinensis chlorosis virus	*Callistephus chinensis*	Leaf chlorosis	
Caper vein yellowing virus (N)	*Capparis spinosa*	Vein yellowing	
Carnation bacilliform virus	*Dianthus* sp.	Not specified	
Carrot latent virus (N)	*Daucus carota*	Symptomless	Aphid
Cassava symptomless virus	*Manihot* spp.	Symptomless	
Celery virus (C)	*Apium graveolens*	Symptoms unknown, occurs with celery mosaic potyvirus	Grafting
Chondrilla juncea stunting virus (N)	*Chondrilla juncea*	Leaf chlorosis and distortion, stunting	
Chrysanthemum vein chlorosis virus	*Chrysanthemum* spp.	Chlorotic vein banding	
Citrus leprosis virus	*Citrus sp.*	Chlorotic blotches on fruits, leaves, twigs	Mite, grafting
Clover enation (mosaic) virus (N)	*Trifolium repens*	Enations on main leaf veins	Grafting
Coffee ringspot virus	*Coffea arabica*	Chlorotic lesions	Mite, mechanical
Colacasia bobone disease virus	*Colocasia esculenta*	Stunting, distortion, and leaf mosaic	Planthopper
Coriander feathery red vein virus (N)	*Coriandrum sativum*	Red vein banding	Aphid, mechanical
Cow parsnip mosaic virus (N)	*Heracleum spondyllum*	Mild chlorosis	

(*Continued*)

TABLE 2 (*Continued*)

Virus	Plant species	Disease symptoms	Transmission
Croton vein yellowing virus (N)	*Codiaeum variegatum*	Dwarfing and yellow/pink vein banding	Grafts, mechanical
Cucumber toad-skin virus	*Cucumis sativus*	Vein clearing, severe leaf crinkling, and stunting	
Cynara virus	*Cynara* sp.	Symptoms unknown, virus complex	Mechanical
Cynodon chlorotic streak virus (N)	*Cynodon dactylon*, *Zea mays*	Chlorotic streaking	Planthopper
Daphne mezereum virus	*Daphne mezereum*	Symptomless	
Digitaria striate virus	*Digitaria* sp.	Chlorotic spots and stripes	Planthopper
Euonymus fasciation virus	*Euonymus japonica*	Fascinated stems	
Euonymus virus	*Euonymus japonica*	Vein yellowing, chlorotic spotting	
Finger millet mosaic virus (N)	*Eleusine coracana*	Mosaic, streaking, mottling, chlorosis	Planthopper
Gerbera symptomless virus	*Gerbera* sp.	Symptomless	
Gloriosa fleck virus (N)	*Gloriosa rothchildiana*	Leaf flecking	
Gomphrena virus	*Gomphrena globosa*	Local lesions	
Gynura virus	*Gynura aurantiaca*	Vein clearing	
Holcus lanatus yellowing virus	*Holcus lanatus*	Leaf chlorosis	
Iris germanica leaf stripe virus	*Iris germanica*	Leaf stripe	
Ivy vein clearing virus (C)	*Hedera helix*	Vein chlorosis	Mechanical
Kenaf vein-clearing virus	*Hibiscus cannabinus*	Chlorotic vein banding	
Laburnum yellow vein virus (N)	*Laburnum* sp.	Chlorotic vein banding	
Launea arborescens stunt virus	*Launea arborescens*	Stunting	

(*Continued*)

TABLE 2 (*Continued*)

Virus	Plant species	Disease symptoms	Transmission
Lemon scented thyme leaf chlorosis virus (N)	*Thymus x citriodorus*	Leaf chlorosis	
Lolium ryegrass virus (N)	*Lolium* sp.	Symptoms unknown	
Lotus stem necrosis virus	*Lotus* sp.	Stem necrosis	
Lotus streak virus (N)	*Nelumbo nucifera*	Chlorotic (ring)spots, root streaking	Aphid
Lucerne enation virus (N)	*Medicago sativa*	Enations on leaf veins	Aphid, grafting
Lupin yellow vein virus	*Lupinus polyphyllus x L.arboreus*	Chlorotic vein banding	
Maize Iranian mosaic virus (N)	*Zea mays*	Fine chlorotic vein striping	Planthopper
Maize streak dwarf virus (N)	*Zea mays*	Chlorotic streaking of leaf & sheaths	Planthopper
Malva sylvestris virus	*Malva* sp.	Mottle, vein clearing	
Melilotus (sweet clover) latent virus (N)	*Melilotus* sp.	Symptomless	Grafting
Melon variegation virus (C)	*Cucumis melo*	Stunting, chlorosis of leaves & fruits	
Mentha piperita virus	*Mentha piperita*	Not specified	
Nasturtium vein banding virus	*Tropaeum major*	Vein banding	
Papaya apical necrosis virus	*Carica papaya*	Apical necrosis	
Parsley virus	*Petroselinum crispum*	Symptoms unknown, virus complex	
Passionfruit virus (N)	*Passiflora edulis*	Symptoms unknown, virus complex	Grafting
Patchouli mottle virus	*Pogostemon patchouli*	Leaf mottle	
Peanut veinal chlorosis virus	*Arachis hypogaea*	Stunting and vein chlorosis	
Pelargonium vein clearing virus	*Pelargonium* sp.	Mild vein clearing	
Pigeonpea proliferation virus	*Cajanus cajan*	Tissue proliferation	Leafhopper

(*Continued*)

TABLE 2 (*Continued*)

Virus	Plant species	Disease symptoms	Transmission
Pineapple chlorotic leaf streak virus (N)	*Ananas comosus*	Chlorotic leaf streaks	
Pisum virus (C)	*Pisum sativum*	Symptomless	Mechanical
Plantain mottle virus	*Plantago lanceolata*	Leaf mottle, necrotic spotting	
Poplar vein yellowing virus (N)	*Populus balsamifera*	Clearing and yellowing of leaf veins	
Ranunculus repens symptomless virus	*Ranunculus repens*	Symptomless	
Raphanus virus (C)	*Raphanus* sp.		
Raspberry vein chlorosis virus (N)	*Rubus idaeus*	Vein chlorosis	Aphid
Red clover mosaic virus (N)	*Trifolium incarnatum*	Mosaic	
Saintpaulia leaf necrosis virus	*Saintpaulia ionantha*	Leaf necrosis	
Sambucus vein clearing virus	*Sambucus* sp.	Vein clearing	
Sarracenia purpurea virus	*Sarracenia purpurea*	Not specified	
Sorghum stunt mosaic virus (N)	*Sorghum vulgare*	Mottling & streaking, severe stunting	Planthopper
Soursop yellow blotch virus (C)	*Annona muricata*	Vein-clearing, yellow blotch & leaf distortion, stunting	Mechanical, grafting
Soybean virus	*Glycine max*	Mosaic	
Triticum aestivum chlorotic spot virus	*Triticum aestivum*	Chlorotic spots	
Vigna sinensis mosaic virus	*Vigna sinensis*	Mosaic	
Viola chlorosis virus	*Viola* spp.	Leaf chlorosis	
Wheat rosette stunt virus	*Triticum aestivum*	Stunting	Planthopper
Winter wheat Russian mosaic virus (C)	*Triticum aestivum*	Leaf mosaic	Planthopper

[a]Electron microscopic studies have indicated particles associated with the nucleus/perinuclear space (N) or the cytoplasm (C).

TABLE 3 List of fully sequenced plant rhabdoviruses

Virus	Genome size (nt)	Genbank accession number
Cytorhabdovirus		
LNYV	12,807	AJ867584
NCMV	13,222	AB030277
SCV	14,547	–
Nucleorhabdovirus		
MFSV	13,782	AY618417
MMV*	>12,133	AY618418
RYSV	14,042	AB011257
SYNV	13,720	L32603
TaVCV	12,020	AY674964

*3′ and 5′ ends not completed.

BIOLOGY, PATHOLOGY, AND VECTOR RELATIONSHIPS

Rhabdoviruses infect numerous weed hosts and several major crop plant families in temperate, subtropical, and tropical climates. The individual viruses induce a wide spectrum of disease symptoms in their respective host plants, and these vary from a lack of discernible effects to death. However, symptoms of infection most often include stunting, clearing, and yellowing of leaf veins, mottling or mosaic of systemically infected plants, and tissue necrosis (Figure 1; Tables 1, 2). Plant growth conditions, particularly temperature and light intensities, and age of plants when inoculated can also have marked effects on symptom expression, disease development, and accumulation of virus particles. Several rhabdoviruses cause crop diseases, and serious economic losses have been reported in plants infected by BYSMV, EMDV, LNYV, SCV, MMV, RYSV, RTYV, and PYDV. SYNV in lettuce (22) is an example of a rhabdovirus-host combination that has previously resulted in significant crop loss. Such losses are no longer observed, probably because of changes in cropping practices and biological variables. A summary of factors affecting the diseases caused by LNYV, MMV, RTYV, and PYDV can be found in Reference 52. An extensive review of the disease epidemiology and vector relationships of LYNV is also available (75), and the diseases caused by a number of rhabdoviruses infecting the *Gramineae* have been discussed (54). The disease potential of SCV has also recently been described (74).

Although resistance has not been emphasized for control of most rhabdovirus diseases, control of MMV, which can cause 100% yield losses in susceptible maize during severe epiphytotics, relies on the deployment of resistance genes (8). The *Mv1* gene for MMV resistance has been mapped and incorporated into several maize lines by conventional breeding and shown to provide effective and durable resistance (69). Some maize lines also carry a dominant resistance gene for MSSV, and resistance to CCMoV and MFSV has been reported in other maize

lines and hybrids (37, 38, 76). Resistance genes against *Raspberry vein chlorosis virus* (RVCV) have also been used to protect raspberries (56). These successes underscore the importance of continuing efforts to identify resistance genes that can be incorporated into susceptible cultivars.

Genetic engineering also is potentially useful for creating resistant plants in situations where disease-resistance genes have not been identified in genetically compatible germplasm. In preliminary experiments, transgenic Japonica rice varieties expressing translatable or frame-shifted RYSV nucleocapsid protein gene sequences under the control of the *Act*1 promoter showed high levels of resistance to RYSV in insect transmission trials. However, the resistance phenotype was not stable in subsequent generations (23; R.X. Fang, personal communication). Nevertheless, recent understanding of the basis for eliciting transgenic protection has provided promising new avenues for transforming plants with cloned derivatives that produce double-stranded RNAs that elicit effective gene silencing of targeted viral RNAs (101). Application of this approach to degrade rhabdovirus mRNAs is already being tested to protect crisphead lettuce against LNYV (13), and should reinvigorate attempts to generate stable synthetic resistance that can be employed against a range of rhabdovirus diseases.

Most rhabdoviruses infecting cereals are resistant to experimental transmission by rub-inoculation of leaves, but vascular puncture inoculation has been successful for some of these viruses (44, 71). Although, a number of rhabdoviruses infecting dicots can be transmitted mechanically, efficient transfer usually requires specific buffers tailored for individual viruses. In this regard, SYNV can be efficiently transmitted from *Nicotiana* species only in the presence of strong reducing agents, but the transmission of SYVV, which appears to have a number of properties in common with SYNV, is not facilitated by these buffers (A.O. Jackson, unpublished). The host also affects the efficiency of transmission of a number of rhabdoviruses. As an example, SCV must be transferred from strawberry to more amenable hosts for successful mechanical transmission (87). Seed transmission of rhabdoviruses in plants has not been reported, but foci for aphid transmission of SCV are maintained through propagation of infected stolons, and EMDV and PYDV can be transmitted through infected potato tubers. Rhabdovirus-like particles have also been detected in parasitic dodder (79), but efficient host-to-host transmission by this vehicle has not been documented.

Some viruses such as MMV, BNYV, and SCV occur throughout the world, but the geographical distribution of most plant rhabdoviruses is restricted and appears to be closely linked to insect vector distribution (Tables 1, 2). Although some plant rhabdoviruses have no known vector, most are transmitted in a persistent and propagative manner by aphids (*Aphidae*), leafhoppers (*Cicadellidae*), or planthoppers (*Delphacidae*). Comparisons of virus-vector relationships have revealed puzzling patterns of transmission among the monocots and dicots. For example, leafhoppers, planthoppers, and aphids are prevalent on and transmit a wide range of viruses to both monocots and dicots. However, all known rhabdoviruses causing diseases of the *Gramineae* are transmitted by leafhoppers or planthoppers. Moreover, except

for the nucleorhabdoviruses PYDV and EMDV (2), which have solanaceous hosts and closely related leafhopper vectors, all other rhabdoviruses infecting dicots are transmitted by aphids.

Indirect evidence for rhabdovirus replication in vector insects is that long latent periods are required before transmission occurs, that virus is often retained throughout the life of the insect, and that transovarial passage can be observed through eggs and nymphs (71, 75, 87). More direct evidence has been obtained by continued transmission of virus after repeated serial dilution passages from insect to insect. The most comprehensive of these serial transmission trials were conducted by injecting PYDV into leafhoppers (6), and SYVV into aphids (87). These studies, as well as less extensive findings with several other leafhopper- and aphid-transmitted rhabdoviruses, are all consistent with replication in the vector (71, 75, 87). In addition, electron microscopy, serological detection of virus, and strain-specific infection of insect tissue culture lines and cultured explants from organs provide proof that rhabdoviruses replicate in their vectors (7, 45, 87).

A high degree of vector specificity has been observed for the rhabdoviruses. For example, among the viruses that infect maize, MMV is transmitted by *Peregrinus maidis*, MFSV by *Graminella nigrifrons*, WASMV by *Endria inimical* and *G. nigrifrons*, *Maize Iranian mosaic virus* (MIMV) by *Ribautodelphax notabilis* and *Sorghum stunt mosaic virus* (SSMV) by *Graminella sonora* (76). Genetic experiments with PYDV have shown that highly efficient and inefficient leafhopper vectors can be selected (4). Continuous passage of PYDV by serial injection of insects can also result in isolates that are unable to infect plants (5). Additional studies have shown that strains that have lost their capacity to be insect transmitted can be recovered after protracted passage in plants (7). This phenomenon could provide a mechanism for evolution of vectorless rhabdoviruses, in cases where infections become established in vegetatively propagated hosts.

The natural host range of rhabdoviruses is primarily determined by vector specificity and feeding preferences, so vector dynamics have major effects on the distribution of these viruses. A striking illustration of this dependence has occurred in Berkeley, California, where a precipitous decline in the incidence of SYVV has been attributed to the displacement of the aphid vector (*Hyperomyzus lactucae*) with an invader aphid (*Uroleucon sonchi*) that is not a vector for SYVV (87). Although plant-to-plant transmission does not have a major role in ecology, rhabdoviruses that can be mechanically transmitted normally have the capacity to infect a greater range of experimental plant hosts than the narrow range of species colonized or used as food sources by their vectors. A specific example is transmission of SCV, which is restricted in nature to cultivated and native strawberry due to feeding preferences of its aphid vectors (*Chaetosiphon* sp.). SCV is very difficult to transmit mechanically from strawberry. However, alternate solanaceous hosts can be infected by surrogate nonvector aphid species that have been injected with extracts from the strawberry aphid, and the virus can then be mechanically transmitted from these plants (87). These results indicate that some rhabdoviruses have the ability to infect plant hosts that are quite distantly related to their native hosts,

and suggest that changes in vector feeding preferences could result in emergence of these viruses beyond their natural host ranges.

For successful vector transmission, rhabdoviruses must be acquired during feeding, establish infection foci in the insect, move from these foci to the salivary glands, and be regurgitated into the saliva. The insect gut appears to be one of the major barriers that must be circumvented for transmission to occur, because bypassing the gut by injection of virus into the hemolymph can increase transmission efficiency or allow transmission by nonvectors (44, 87). After ingestion, rhabdoviruses probably enter epithelial cells of the midgut by receptor-mediated endocytosis. The glycoprotein spikes protruding from the virion surface appear to be involved in the recognition of insect cell surface receptors because blocking of the G protein by antibodies or its enzymatic removal from PYDV drastically reduces infectivity for insect vector cells (31). Transmission from the infected gut cells into the hemolymph followed by infection of the salivary glands and release of virus into the saliva also provides additional potential barriers to rhabdovirus transmission. Such organ barriers could be based on failure to enter, replicate in, move between, or exit from insect cells and organelles, and each of these features could be interdicted by innate or virus-induced defense responses (44). We currently have only rudimentary knowledge about the scenario of infection events within vector tissues; therefore, a major focus on vector interactions is essential to move towards a mechanistic understanding of the transmission specificities of the plant rhabdoviruses.

CLASSIFICATION AND NOMENCLATURE

The International Committee on Taxonomy of Viruses (ICTV) has classified the Rhabdoviruses in the Order Mononegavirales, whose members consist of large enveloped viruses with linear, nonsegmented, single-stranded (ss) RNA genomes that are organized in a negative-sense orientation (89). The order contains four families (*Bornaviridae*, *Filoviridae*, *Paramyxoviridae*, and *Rhabdoviridae*) that are classified based on particle morphology, genome organization, and mechanisms of gene expression. Members of each of these families collectively cause an enormous number of serious diseases that impact public health and wildlife, and pose substantial threats to the agriculture and fisheries industries.

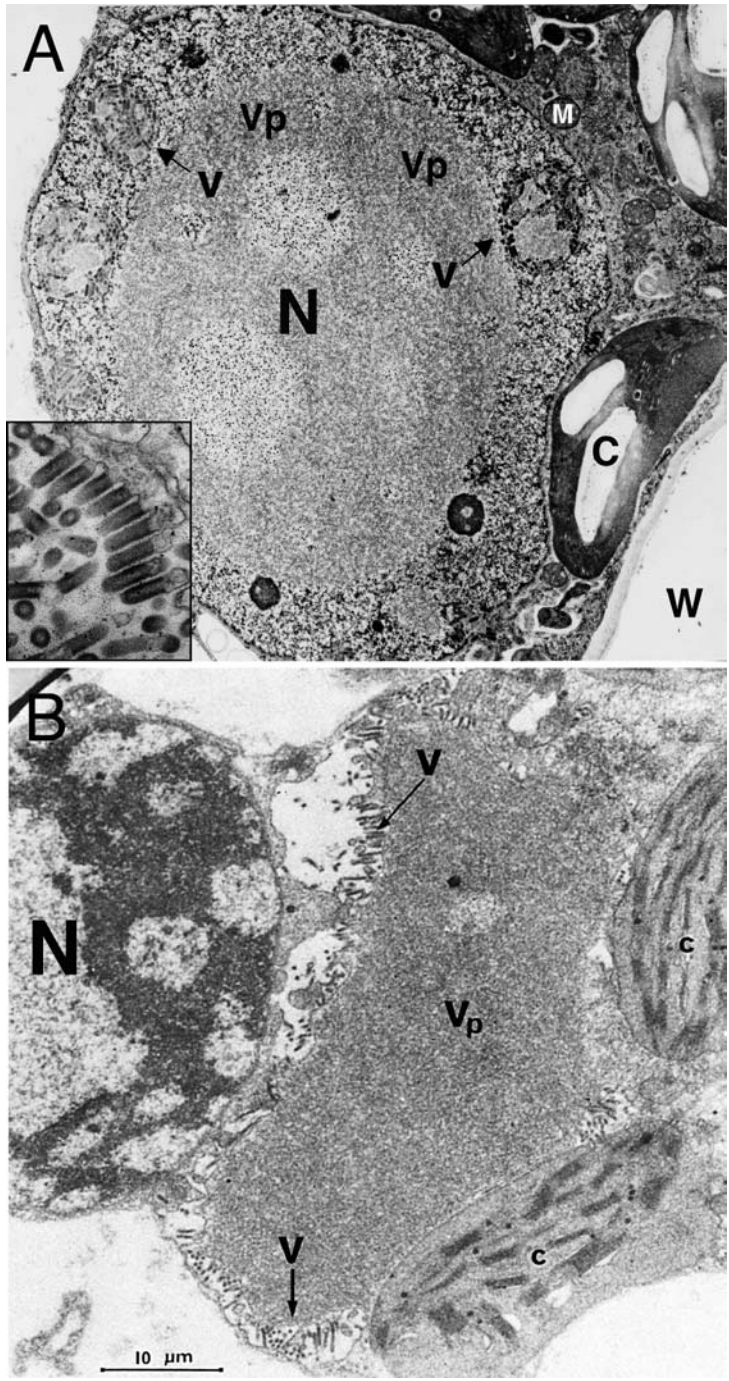
Members of the *Rhabdoviridae* share a number of common properties that account for their inclusion as a distinct family within the Mononegavirales. The major defining characteristics of the family are enveloped virions with a bacilliform or bullet-shaped particle morphology. Although rhabdovirus particles vary substantially in length and width, their virions all consist of a tightly coiled nucleocapsid core that is enveloped by a lipoprotein membrane (Figure 2). The core contains at least three structural proteins surrounding a monopartite, negative-sense, ss genomic RNA, and the envelope consists of host-derived lipids and a single type of viral glycoprotein that spans the membrane and is attached to the nucleocapsid

by matrix protein associations. Six genera have currently been designated within the family by the ICTV to accommodate variations in the number of encoded proteins and biological properties (89). Members of four of the genera (*Vesiculovirus*, *Lyssavirus*, *Ephemerovirus*, and *Novirhabdovirus*) infect vertebrates, and two genera (*Nucleorhabdovirus* and *Cytorhabdovirus*) infect plants. Many members of the vesiculoviruses, cytorhabdoviruses, and nucleorhabdoviruses also infect arthropod vectors, so collectively the members of the family have adapted to an enormous variety of host requirements.

The cytorhabdoviruses and nucleorhabdoviruses are primarily distinguished based on their sites of maturation in the cytoplasm or the nucleus (Figure 3). The significance of this property is unknown, but the current classification scheme has so far been supported by the available genome sequence data and the cell biology evidence. However, more than 75 putative rhabdoviruses have not been assigned to a genus because their replication sites have yet to be clearly determined and/or their molecular properties have not been adequately described (Table 2). Moreover, the identification of many of these viruses relies on unsubstantiated ultrastructural observations and general biological properties, so some of them could be members of other virus families. A particularly notable example is *Orchid fleck virus* (OFV), which was initially classified as an unassigned rhabdovirus, but the virus subsequently has been shown to contain a bipartite genome (60). *Coffee ringspot* and *Citrus leprosis viruses*, which closely resemble OFV in particle morphology, cytopathic effects, and mite transmissibility, may also belong to this new group of viruses. Therefore, one of the pressing needs for definitive classification is to provide more complete molecular and cytological analyses of these viruses.

The type species of the *Cytorhabdovirus* genus is LNYV, which has undergone the most detailed characterization. Other assigned cytorhabdoviruses are BYSMV, BNYV, FLSV, NCMV, SonV, SCV, and WASMV. In addition, BYSMV, MSSV, and WCSV have identical vectors, similar particle morphologies, and such strong serological cross-reactivity that they are considered to be synonymous. Although WASMV and its likely synonym, OSMV, are currently assigned to the *Cytorhabdovirus* genus, we think that their taxonomic status and relationships should be

Figure 3 Electron micrograph of nucleorhabdovirus- and cytorhabdovirus-infected cells. (*A*) Cytopathology of cells infected with the nucleorhabdovirus SYNV. Note the greatly enlarged nucleus and virus budding (V) at several locations (see *arrows*) near the periphery of the nucleus. Electron-dense granular areas throughout the nucleus are thought to represent viroplasms (Vp). The nucleus (N), chloroplast (C), mitochondria (M), and the cell wall (W) are identified. The inset shows virus particles budding from the inner nuclear envelope and accumulating in the perinuclear space. (Photograph from A.O. Jackson archive.) (*B*) Cell infected with the cytorhabdovirus BYSMV. The cells contain cytoplasmic viroplasms (Vp) from which virus particles (V) appear to be budding (see *arrow*). Nucleus (N), chloroplast (C). (Modified from Figure 18 in Reference 28).



confirmed by molecular data because their virions have been found in both the cytoplasm and in the nuclei of infected cells.

Seven viruses are currently assigned to the *Nucleorhabdovirus* genus by the ICTV based on their ability to establish nuclear viroplasms and on the available physico-chemical and/or molecular information (89). Recently, sufficient new information has been documented to justify provisional inclusion of MFSV and TaVCV in the genus (Table 1). CCMoV has also been provisionally included based on accumulation of its virions in perinuclear spaces and serological differentiation from other cereal rhabdoviruses (39). PYDV is the type species of the Nucleorhabdovirus genus, but SYNV and RYSV have been subjected to the most detailed molecular analyses. Other members of the *Nucleorhabdovirus* genus are DYVV, EMDV (note that EMDV has three synonyms, PVYV, PVCV, or TVYV), MMV, and SYVV.

Species in both plant rhabdovirus genera are primarily differentiated by host range and vector specificity. Nucleic acid hybridization using cloned probes, serology to verify common species that infect different hosts, and more recently, genomic sequence data have been used to underpin species demarcation. Decoration of nucleocapsids of some planthopper-transmitted rhabdoviruses of cereals with specific antisera first indicated that the cytorhabdoviruses BYSMV and NCMV are related but distinct viruses, that MSSV and WCSV are strains of BYSMV, and that *Wheat rosette stunt virus* (WRSV) may be a strain of NCMV (Tables 1, 2) (68). *Digitaria striate virus* resembles BYSMV in structure, vector species, and cytopathology, but the two viruses are only distantly related serologically (39). Analysis of maize-infecting rhabdoviruses by ELISA and western blots has revealed a serological relationship between MFSV and SSMV and confirmed that MFSV, MMV, WASMV, and MIMV, which is a putative nucleorhabdovirus, are distinct (76). Complete nucleotide sequences are now available for three species in the *Cytorhabdovirus* genus, LNYV, NCMV, and SCV, and five species in the *Nucleorhabdovirus* genus, MFSV, MMV, RYSV, SYNV, and TaVCV (Table 3). Analyses of sequence homologies between the analogous nucleocapsid (N), glycoprotein (G), and L (polymerase) genes of these viruses and their molecular phylogenies are compatible with the current taxonomic classification of plant rhabdoviruses within the two genera (59, 78).

Partial nucleotide sequences of a Chinese isolate of WRSV have recently been reported (105 and references therein). Although there is serological evidence to suggest that WRSV is a strain of NCMV (62, 68), recent nucleotide sequence comparisons of the N, phosphoprotein (P), and matrix protein (M) genes of WRSV with the analogous genes of NCMV revealed only 25% or less sequence identity (R.G. Dietzgen, unpublished). Therefore, WRSV should remain in the list of unassigned rhabdoviruses until this discrepancy has been resolved.

Definitive information about the genetic and biological diversity within species has been slow to appear due to difficulties in conducting comparative analyses. However, we expect this situation to change rapidly as sequence comparisons begin to supplement biological observations about strain specificity among

rhabdovirus members. Early studies first revealed the presence of two distinct strains of PYDV based on differences in leafhopper vectors and serology. The biological properties of these two strains, *Sanguinolenta yellow dwarf virus* and *Constricta yellow dwarf virus*, have been investigated in some detail (6, 7), but more definitive molecular data are needed to clarify their relationships. In addition, serologically related strains of EMDV appear to have distinct host ranges and symptoms (18, 21). In other examples, isolates of MMV and SCV differ in symptom severity on specific indicator hosts (59, 70). Field isolates of LNYV can also be distinguished by the severity of symptoms on the indicator host *Nicotiana glutinosa*, but no serological differences between isolates have been reported (30). Recently, two subgroups of LNYV (11a) and SCV (59) have been distinguished based on nucleotide sequence phylogeny among field isolates, but it is unknown whether these sequence differences result in phenotypic effects sufficient to separate these isolates into distinct biological strains. Hence, a considerable amount of additional biological, molecular, and diagnostic experimentation needs to be conducted to more clearly define the relationships and properties of these and other plant rhabdoviruses.

RHABDOVIRUS PARTICLE STRUCTURE AND GENOME ORGANIZATION

Virion Morphology and Composition

Rhabdovirus particles are quite easily distorted and a variety of shapes ranging from pleomorphic to bullet-shaped can be observed by electron microscopy of unfixed particles (26). However, after careful fixation, plant rhabdoviruses often have a bacilliform morphology (Figure 2) and particle size estimates range from 45 to 100 nm in width and 130 to 350 nm in length. This suggests that different rhabdoviruses vary considerably in size, but swelling of virions, shrinking of the nucleocapsid core, and other preparation artifacts undoubtedly contribute to the variation in these estimates. Three layers of varying electron density can be discerned in most virus particles. The outer layer contains spike-like surface projections composed of the glycoprotein (G) that protrude 5 to 10 nm above the surface of the particle. The spikes appear to be arranged as surface hexamers and the G protein subunits are thought to associate as trimers. The middle layer of the particle consists of a host-derived lipid membrane penetrated by the G protein. The membrane surrounds a striated inner core, with a periodicity of 4 to 5 nm that is composed of a helical ribonucleoprotein containing the genomic RNA. This core forms a coiled structure when liberated from the virus by mild detergents (51) and is thought to consist of the N protein, the P protein, and a large polymerase protein (L) associated with the genomic RNA (Figure 2). The matrix protein (M) probably participates in coiling of the nucleocapsid and interacts with the G protein to stabilize the particle. In addition to the five proteins forming the particles of

all rhabdoviruses, a sixth protein thought to be required for cell-to-cell movement is encoded by all plant rhabdoviruses. In SYNV, this gene, which is designated sc4, encodes a protein that appears to form a minor component of the envelope of purified SYNV particles (83), but the virion association of analogous proteins encoded by other plant rhabdoviruses has not been investigated.

A detailed analysis of plant rhabdovirus particles depends on the ability to purify individual viruses from host contaminants. A number of factors complicate the purification of most rhabdoviruses from their hosts and a considerable amount of attention has previously been focused on purification and reviewed previously (52, 55). Nevertheless, there is general agreement that the chemical composition of rhabdoviruses varies from 65% to 75% protein, 1% to 2% RNA, 15% to 25% lipid, and ~3% G protein carbohydrate (89). The minus-sense RNA genomes of the eight sequenced plant rhabdoviruses range in size from ~12 to ~14.5 kb (Table 3), and consist of six to nine open reading frames (ORFs) that are separated by short spacer sequences between their genes designated "gene junction regions" (Figure 4). The coding regions are flanked by leader and trailer sequences whose 3′ and 5′ termini contain sequences that exhibit varying degrees of potential base pairing (16, 106). Rhabdovirus lipids have differences in fatty acid and sterol composition that are derived from their hosts during morphogenesis. Two nucleorhabdoviruses, SYNV (84) and PYDV (1), have been examined in the most detail, and the results indicate that a variety of fatty acids and free and esterified sterols are present in purified virus particles. Four sterols predominating in SYNV closely approximate the sterols of the nuclear envelope, whereas those of NCMV (90), a cytorhabdovirus, are more typical of cytoplasmic membranes. These results support the hypothesis that lipid composition is dependent on the host and specific membranes used for morphogenesis, although it is plausible that additional selection of lipids could occur at localized sites of virus assembly.

Genomic Structure and Organization

The eight sequenced rhabdoviruses are thought to contain five consensus genes flanked by leader (*ℓ*) and trailer (*t*) regions (3′-*ℓ*- N, P, M, G, L –*t*-5′) that appear in the same order as the genes of the vertebrate rhabdovirus prototype, *Vesicular stomatitis virus* (VSV) (89). All sequenced plant rhabdoviruses also contain one to four additional genes (or more correctly, ORFs) residing at position X between the P and M genes. In addition, SCV and RYSV and some animal rhabdoviruses contain genes at position Y between the G and L genes (Figure 4). The gene order for SYNV is 3′-*ℓ* -N-P-sc4-M-G-L-*t* -5′, and the order for LNYV is 3′-*ℓ*-N-4a-4b-M-G-L-*t*-5′ (where 4a is thought to represent a phosphoprotein and 4b probably corresponds to SYNV sc4). Similarly, MMV and TaVCV contain one additional gene, provisionally named gene 3, and MFSV contains two genes, designated 3 and 4, at position X. RYSV and SCV (C. Schoen, personal communication) contain a gene 3 located at position X, and a gene 6 at position Y. The NCMV genome is the most complex and consists of four small genes located between the P and M genes.

Since LNYV and SYNV, and SCV and RYSV, have the same gene order, respectively, it appears that the number and location of these additional genes transcends the *Nucleorhabdovirus* and *Cytorhabdovirus* genera. The coding regions of these viruses are all flanked at the 3′ end of the minus-sense genomes by noncoding leader sequences ranging from 84 to 206 nucleotides, and by noncoding trailer sequences ranging from 145 to 389 nucleotides at the 5′ end of the genomes. The leader and trailer sequences are considerably longer than those of VSV and they have little sequence in common with each other or with other rhabdoviruses. However, they do have short regions of terminal complementary, as is the case with other minus-strand RNA viruses. A notable difference among the animal rhabdoviruses is that the transcribed leader RNAs are not polyadenylated (see section on polymerase protein L), whereas the SYNV leader RNA is polyadenylated (96). Our hypothesis is that this feature represents an adaptation to facilitate the nuclear mode of replication of the nucleorhabdoviruses, but verification of this hypothesis requires additional comparisons.

The intergenic "gene junction" regions can be grouped into three components constituting a poly (U) tract at the 3′ end of each gene on the genomic template (element I), a short nontranscribed element that separates each gene (element II), and a conserved element located at the beginning of each subsequent gene (element III). Overall, the gene junction sequences are highly conserved between the genes of each virus and between plant rhabdovirus genomes (Table 4). The SYNV element I is identical to that of the nucleorhabdoviruses MMV and TaVCV and that of the cytorhabdovirus NCMV, and differs by a single nucleotide from that of RYSV, whereas elements I of LNYV, SCV, and MFSV are most similar to each other. Although element II is conserved between the nucleorhabdoviruses MMV, RYSV, and TaVCV, it is most divergent among the eight rhabdovirus genomes. LNYV and NCMV have similar element III sequences (CUU versus CUA, respectively), but differ from those of other plant rhabdoviruses, whose sequences are UUG. Element I (AUUCUUUUU) of SYNV (16) is also nearly identical to the analogous region (AUUCUUUUUU) of Ebola virus (48), suggesting that some conservation of regulatory features may extend to the *Filoviridae*. Sequence similarities in this region are also found in Paramyxoviruses and borna disease virus. Thus, intergenic regions of the genome that play an important role in regulating mRNA transcription and replication appear to have been conserved. However, the leader and trailer sequences and the genes encoding the proteins appear to have undergone extensive evolution to accommodate diverse host requirements.

Properties of the Encoded Proteins

The structural properties of plant rhabdovirus proteins deduced from nucleotide sequence analyses are described below in the order of their appearance from the 3′ end of the genome (Figure 4). Only rudimentary biochemical analyses have been conducted on the proteins, and these analyses are mostly confined to SYNV, LNYV, and RYSV. Overall, the plant rhabdovirus proteins appear to have very

TABLE 4 Comparison of rhabdovirus gene junction regions

Virus		Element I	Element II	Element III	
Consensus	3′	AUUNUUUUU	GNN	UUG	5′
Plant nucleorhabdovirus					
SYNV	3′	AUUCUUUUU	GG	UUG	5′
MFSV	3′	UUUAUUUU	GUAG	UUG	5′
MMV	3′	AUUCUUUUU	GGG	UUGW[a]	5′
RYSV	3′	AUUAUUUUU	GGG	UUG	5′
TaVSV	3′	AUUCUUUUU	GGG	UUGW[a]	5′
Plant cytorhabdovirus					
LNYV	3′	AUUCUUUU	G(N)$_n$[b]	CUU	5′
NCMV	3′	AUUCUUUUU	GACU	CUA	5′
SCV	3′	AUUAUUUU	GAU	N/A[d]	5′
Animal virus					
VSV	3′	ACUUUUUUU	GU	UUG	5′
RABV	3′	ACUUUUUUU	G(N)$_n$[b]	UUG	5′
Ebola virus[c]	3′	AUUCUUUUUU	(N)$_n$[b]	CUN[e]	5′

Nucleotides that differ from the consensus are underlined.

[a]W represents A or U nucleotide.

[b](N)$_n$ represents variable number of nucleotides.

[c]This virus belongs to *Filoviridae*.

[d]N/A, not available.

[e]N represents G, C, A, or U.

little sequence relatedness to analogous proteins of animal rhabdoviruses, with the exception of 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 genomic RNA and it is a component of the viroplasms and of the polymerase complex that can be isolated from nuclei of infected plants (96). The N protein genes of the nucleorhabdoviruses SYNV, MMV, MFSV, TaVCV, and RYSV, and the cytorhabdoviruses LNYV, SCV, and NCMV have been sequenced. The deduced amino acid sequences of the N proteins of these viruses have no extensive sequence relatedness to animal rhabdovirus N proteins, although their hydropathy patterns have some similarity. However, the SYNV and RYSV N proteins have limited regions of weak homology that are more closely related to each other than to analogous regions of the N protein of LNYV. The SYNV, RYSV, MFSV, and MMV N proteins show sequence similarities in database searches, while the TaVCV N protein is most similar to that of MMV. The 475-amino acid SYNV N protein contains a nucleoplasmin-like nuclear localization signal (NLS) close to the carboxy (C) terminus that is required to facilitate the nuclear localization of the N protein (33). Putative NLSs

in a similar location of the N protein have also been identified in the nucleorhabdoviruses RYSV, MFSV, and TaVCV, but not in any of the cytorhabdoviruses. Other short regions of sequence homology are also present in the N proteins of the cytorhabdoviruses LNYV, NCMV, and SCV. The SYNV, LNYV, RYSV, and NCMV N proteins also contain regions located approximately two thirds of the way towards the C termini that appear to be weakly conserved with that of VSV.

The SYNV N protein is localized to the nucleus and the C terminus has a classic bipartite NLS that mediates association with the nuclear import protein, Importin α(33). In the absence of the P protein, N is generally distributed throughout the nucleus, but in the presence of the P protein, heterologous interactions occur that lead to the subnuclear accumulation of the complexes (Figure 5). The N and P proteins interact in yeast two-hybrid assays and these interactions appear to be mediated near the amino (N) terminus of the N protein. Nuclear localization of its N protein has also recently been demonstrated for MFSV. Here the interaction between N and P proteins leads to subnuclear colocalization to the nucleolus (92), and hence is different from SYNV, which exhibits a distinct pattern from the *Arabidopsis* Fib 1 nucleolar marker (34). This distinction between the two viruses is further accentuated because heterologous expression of the SYNV and MFSV N and P proteins does not lead to associations that result in subnuclear relocalization (92).

THE PHOSPHOPROTEIN (P) Biochemical data supporting the identity of the P protein of plant rhabdoviruses are available only for SYNV, which encodes a 308-amino acid protein with no direct amino acid sequence relatedness to the P proteins of other rhabdoviruses. Putative P proteins from the other plant rhabdoviruses have been assigned based on their genome location next to the N protein gene. Although this provisional assignment probably is correct, sequence analyses have failed to reveal discernible relatedness among these proteins, except for the deduced P protein sequence of TaVCV, which is 46% identical to that of MMV (78). Nevertheless, P proteins tend to share a hydrophilic core and the provisional P proteins of cytorhabdoviruses are overall hydrophilic with isoelectric points ranging from 4.3 to 5.1.

Irrespective of the lack of appreciable relatedness, the SYNV P protein (and probably that of other plant rhabdoviruses) appears to have functions similar to those of VSV because it is a component of the viral nucleocapsid core and the nuclear-associated polymerase complex (66, 97). The SYNV P protein is capable of forming complexes in vivo with the N and L proteins that are analogous to N:P and P:L complexes found in VSV-infected cells that may function in transcription and replicase recycling. The N-terminal half of the SYNV P protein is negatively charged, as is the case with the other rhabdoviruses, but little charge similarity or sequence resemblance is present at the C terminus. The SYNV P protein is phosphorylated in vivo at threonine residues (J. Wagner, M. Fujita, M.M. Goodin & A.O. Jackson, unpublished) and hence differs from the VSV P protein, which is phosphorylated at serine residues (14). Based on studies with fluorescent protein fusions, the SYNV P protein accumulates throughout the nucleus (but is sometimes

found near the outer edge of the nucleus) and in the cytoplasm. Deletion analyses have revealed that several regions of the P protein appear to contribute to its karyophilic properties (M. Deng & A.O. Jackson, unpublished). Yeast two-hybrid analyses also indicate that the P protein interacts with the N protein near regions that facilitate nuclear entry. In the presence of the N protein, fluorescence of the GFP:P protein fusion is restricted to the nucleus and forms compact subnuclear foci (Figure 5). The P protein also appears to have nuclear export signals and a portion of the protein interacts with the yeast nuclear export receptor Xpo1 (A.O. Jackson & M.M. Goodin, unpublished; 85). The significance of this activity is discussed in more detail below in the section on cell biology.

THE MATRIX PROTEIN (M) The M protein is basic and is thought to function by condensing the nuceocapsid and associating with the G protein during morphogenesis. Sequence data indicate that the M proteins vary in size from 19.7 kD for NCMV to 32 kD for SYNV (41). Sequence alignments of the SYNV, NCMV, RYSV, and LNYV M proteins have failed to reveal conserved consensus motifs, but short stretches of amino acids display some similarities in composition to the M proteins of other rhabdoviruses, and the SYNV and RYSV M proteins are more closely related to each other than to other rhabdovirus proteins (63). The M proteins of TaVCV and MMV have some sequence homology (78). A putative NLS has been identified in the MFSV M protein and the nuclear localization of GFP:M fusion protein has been demonstrated (92). The SYNV M protein has a predicted NLS residing between nucleotides 226 to 236, but the function of this motif has not been demonstrated experimentally. A hydrophobic region of 67 amino acids extending almost to the middle of the M protein could be involved in membrane-lipid interactions with the G protein. Recent studies suggest that the SYNV M protein is phosphorylated in vivo at both threonine and serine residues (M. Fujita, M.M. Goodin & A.O. Jackson, unpublished). In addition to its functions in nucleocapsid coiling, the SYNV GFP:M protein fusions are localized to the nucleus and the M protein appears to interfere with accumulation of nonviral reporter proteins (M.M.Goodin & S. Mathews, unpublished). The significance of these findings is discussed in the section on cell biology.

THE GLYCOPROTEIN (G) The G protein forms the glycoprotein spikes of the rhabdovirus virions. The calculated sizes from sequence information range from 54 kD for NCMV to 75 kD for RYSV. The plant rhabdovirus G proteins have no direct relatedness to G proteins of other rhabdoviruses, albeit the overall identity of the G proteins of nucleorhabdoviruses SYNV, MFSV, MMV, and RYSV is higher than that between the G proteins of several animal rhabdoviruses. In addition, the RYSV and SYNV G proteins contain conserved peptide sequences that have not been reported for other rhabdoviruses (64). The sequenced plant rhabdovirus G proteins also contain putative N-terminal signal peptides comprised of 17 to 32 amino acids, a transmembrane anchor domain, and 3 (LNYV) (12) to 10 (RYSV) potential glycosylation signals (Asn-X-Ser/Thr). The G protein of LNYV purified

from *N. glutinosa* contains a network of N-acetylchitobiose N-linked to asparagine residues (20), but the type of glycosylation appears to be influenced by the host (104). In addition, the SYNV G protein contains a putative nuclear targeting signal near the C terminus, which could be involved in transit to the inner nuclear membrane prior to morphogenesis (32). Glycosylation inhibitors interfere with G protein N-glycosylation (58) and the protein is stable in tunicamycin-treated cells (93). Interestingly, the latter treatment blocks SYNV morphogenesis and results in striking arrays of condensed nucleocapsid cores that accumulate in the nucleus and fail to undergo morphogenesis.

THE POLYMERASE PROTEIN (L) The positively charged rhabdovirus L (polymerase) protein contains polymerase and RNA binding domains, and is present in low abundance with the N and P proteins within nucleocapsids. The calculated sizes of the L proteins from sequence information range from 223 kD for RYSV and MFSV to 241 kD for SYNV. Alignment of the L protein sequences with polymerases of several other nonsegmented negative-strand RNA viruses reveals conservation within 12 motifs that appear sequentially in the protein (15).

Recent rhabdovirus phylogenies based on the L gene suggest a monophyletic origin (44, 89). Phylogenetic trees of the conserved polymerase module place the plant rhabdoviruses into two distinct clades, which correspond to the cyto- and nucleorhabdovirus genera, respectively (Figure 6). The members of the nucleorhabdoviruses fall into two sister clades; TaVCV and MMV appear to be

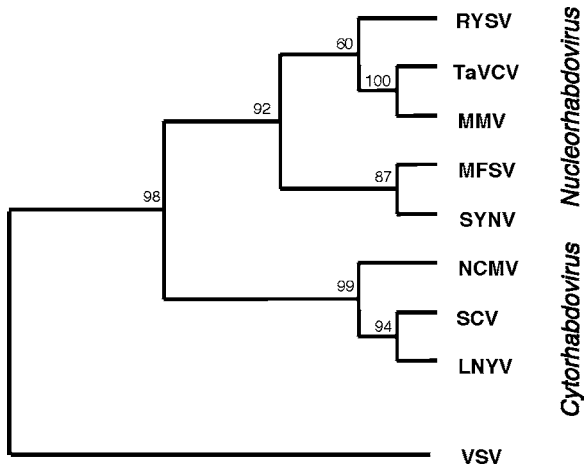


Figure 6 Phylogenetic relationships between plant rhabdoviruses based on amino acid sequence alignment of the conserved polymerase module (pre-A to E domains) of the L gene, using the analogous sequence of *Vesicular stomatitis virus* (VSV) as an outgroup. The tree was generated using the neighbor-joining method. Bootstrap values of 100 tree replicas are shown above each branch node (courtesy of B. Callaghan).

evolutionarily closely related whereas SYNV and MFSV form a separate subclade. The association of RYSV with either sister clade varies with the L gene sequence fragment analyzed, and may change depending on whether neighbor-joining or maximum parsimony methods are used to generate the trees (11, 76, 78). Interestingly, the L protein of *Lettuce big-vein virus* (LBVV), a nonenveloped, bipartite negative-sense ssRNA virus, is evolutionarily closely related to that of plant rhabdoviruses. Furthermore, conserved gene junctions that resemble those of cytorhabdoviruses suggest that LBVV may use a similar transcription termination and initiation strategy (80, 81).

A viral RNA-dependent RNA polymerase is activated after treatment of LNYV and BNYV cytorhabdovirus virions with mild nonionic detergents (27, 28). This activity cosediments with the 40 to 45S loosely coiled nucleocapsid filaments that are released from virions by detergent treatment (91). The transcribed products are complementary to the genome, as expected of mRNAs. In the LNYV in vitro transcription system, two major discrete RNAs of ~1.9 kb and ~1.4 kb and a minor RNA of ~0.85 kb, which comigrated with mRNAs for the N, P, and M genes, respectively, have been identified (G. Kurath, personal communication). Thus, the described polymerases of this cytorhabdovirus appear to be similar to the extensively studied polymerase of the vertebrate rhabdovirus prototype, VSV.

In contrast to these cytorhabdoviruses, no appreciable polymerase activity is evident in dissociated preparations of SYNV and other nucleorhabdovirus members. In this regard, the negligible levels of activity are similar to those obtained from rabies virus preparations. However, an active polymerase can be recovered from the nuclei of plants infected with SYNV (96, 97). The polymerase activity is associated with a nucleoprotein derivative consisting of the N, P, and L proteins, and it cosediments with nucleocapsid cores released from purified virions. The polymerase complex can be precipitated in an active form by P protein antibodies. L protein antibodies inhibit transcription activities of the complex, showing that the L protein is a functional component of the polymerase. 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 support the hypothesis that the polymerases of cytorhabdoviruses are present in an active form in virions and that released cores are capable of initiating primary transcription immediately upon uncoating. In contrast, the Nucleorhabdovirus particles appear to differ by containing an inactive polymerase that requires activation by host components early in infection.

ANCILLARY PROTEINS In addition to the five consensus proteins thought to be encoded by all rhabdoviruses, some rhabdoviruses encode additional proteins at positions provisionally designated X and Y (Figure 4). Genes residing at position X are not found in vertebrate-infecting rhabdoviruses, but ORFs at this position

encode the sc4 and 4b proteins of SYNV (83) and LNYV (102), respectively. Recent sequence information also shows that P3 of RYSV, MMV, and SCV, P3 and P4 of MFSV, and P3, P4, P5, and P6 of NCMV are located at position X (Figure 4). Refined secondary structure predictions have shown that the SYNV sc4 and LNYV 4b proteins have limited regions of structure similar to those of the 30 K superfamily of plant virus movement proteins (67). BlastP database searches (R.G. Dietzgen, B. Callaghan, T. Wetzel, J.L. Dale, unpublished) and secondary structure prediction of the LNYV 4b protein have also revealed close similarities with the movement proteins of capillo- and trichoviruses (family *Flexiviridae*) (http://opbs.okstate.edu/Virevol/web/Rhabdo.html), which induce movement-associated tubules in infected cells. The membrane and cell wall associations of SYNV sc4 protein also suggest a role in cell-to-cell movement (34, 83). Recent detailed secondary structure predictions of P3 of RYSV also reveal a relationship with the 30 K protein superfamily (46). These analyses indicate that the central region of P3 consists of six β-elements separated by four α-helical regions that form three core structures. This central region is very similar to the central region of the 30 K superfamily consensus structure and has a higher degree of similarity than the SYNV sc4 and LNYV 4b proteins. In addition, the MMV P3 and MFSV P4 have conserved core structures similar to those of the MPs of the 30 K superfamily and amino acid sequence alignments of all these putative MPs reveal a conserved $IXDX_{46-71}G$ motif that is similar to a motif in the 30 K-like proteins (92).

Direct experimental evidence now strongly suggests that RYSV P3 functions as a viral cell-to-cell MP (46). The results show that P3 is able to *trans*-complement cell-to-cell movement of a movement-defective potexvirus in *Nicotiania benthamiana* leaves. P3 also exhibits nonspecific ssRNA binding in vitro, as is common for the MPs of numerous viruses (40, 98). Moreover, GST pull-down assays show that P3 specifically binds to the N protein, providing support for involvement of the nucleoprotein in movement (46). Interestingly, the available evidence also shows some differences in the putative MP of SYNV and MFSV because the SYNV sc4 protein is primarily localized in the cytosol, whereas MFSV P4 is localized in the nucleus. Therefore, future studies are needed to elucidate the molecular and biochemical properties of these putative MP, and the mechanisms whereby movement is mediated.

Three plant rhabdoviruses, NCMV, SCV, and RYSV, contain a short ORF at position Y, separating the genes that encode the G and L proteins (47, 88; C. Schoen, personal communication). Small, nonvirion (NV) 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 unidentified (47). In contrast to the NV ORFs of novirhabdoviruses that encode putative proteins of approximately 120 amino acids, the NCMV P9, SCV P6, and RYSV P6 proteins are predicted to be 52, 68, and 93 amino acids in length, respectively (88). These three proteins do not share identifiable sequence similarity. However, limited amounts of similarity can be identified with other viral proteins. The RYSV P6 shares 24% similarity to the N-terminal 110 amino acids of the SYNV L protein. In addition, RYSV

P6 has some similarity to the NV proteins of *Infectious hematopoietic necrosis virus* (IHNV) (22.6%) and *Viral hemorrhagic septicemia virus* (VHSV) (25.4%) and noncoding regions preceding the L gene in Hendra virus (38.3%) and rabies virus (36.6%). The pIs of SCV P6 and NCMV P9 are both approximately 8.9, but RYSV P6 is highly acidic (pI 3.49). The large negative charge of this protein is reminiscent of the acidic N-terminal domain of the LNYV L protein, although RYSV P6 and LNYV L lack sequence similarity.

Of the three position Y proteins, only RYSV P6 has been experimentally characterized. This protein is predicted to contain an aspartic protease motif (DTG) and five potential phosphorylation sites (S/T-X-X-D/E). In vitro phosphorylation assays using a GST:P6 fusion protein demonstrate that P6 is phosphorylated on both serine and threonine residues. Western blots of purified virus and protein extracts from viruliferous aphids suggest that P6 is associated with virions and that it may have some structural role, but P6 was not detected in total protein extracts from infected leaf tissue, indicating that the protein is present in low amounts during infection. The function of the position Y proteins is yet unknown. However, similarities of RYSV P6 with the SYNV and RYSV L proteins and the observation that the IHNV NV protein improves virus growth (47) suggest that the proteins located at position Y might have a role in rhabdovirus replication.

SEQUENCE VARIATION AMONG RHABDOVIRUS FIELD ISOLATES

Comparative analyses of nucleotide and deduced amino acid sequences within the N and L genes of three plant rhabdoviruses have indicated that considerable genetic variability exists between field isolates. Phylogenetic analysis of the complete N gene of eight LNYV isolates has revealed two distinct subgroups. Nucleotide sequences within each subgroup were more than 96% identical, but differed by about 20% between subgroups. However, amino acid sequence difference between subgroups was less than 4%, indicating a strong conservation for N protein function. Isolates belonging to either subgroup naturally infect lettuce and appear to have coexisted in time and space across Australia (11a). Evidence for two subgroups of SCV has been provided by phylogenetic analysis of a region enclosing the GDN motif in the L gene in eight European isolates (59). Nucleotide sequences within each subgroup were 98% identical, but ~11% different between the subgroups. The grouping did not appear to be linked to symptom severity on strawberry indicator plants or geographic origin of the isolates. In another study, high levels of nucleotide sequence diversity within ~1 kb RT-PCR products of the L and N genes were identified between TaVCV isolates across six Pacific Island countries (78). Nucleotide sequences of the L gene differed by up to 27.4% among 20 isolates, while maximum variability within the N gene was 19.3% among 15 isolates. Deduced amino acid sequences for the L and N gene fragments differed by up to 11.3% and 6.3%, respectively, indicating evolutionary constraints on variability

to maintain the functions of both proteins. Phylogenetic analysis of the L gene nucleotide sequences indicated that TaVCV isolates generally grouped according to geographic origin, but with numerous exceptions (78).

CYTOPATHOLOGY AND INFECTION PROCESSES

All ultrastructural investigations have shown that plant rhabdoviruses accumulate within membrane-bound vesicles (25, 26, 29, 65, 66, 76). It is evident however that the cytorhabdoviruses and nucleorhabdoviruses differ considerably in the events that lead to the formation of cytoplasmic and nuclear viroplasms, respectively (Figure 3). Although very little progress has been made in understanding the early phases in the infection processes of the cytorhabdoviruses over the past 15 years (52), electron microscopy evidence has suggested that some cytorhabdoviruses may elicit changes in the host nucleus in the early stages of infection and that the structure of the viroplasms of the Cytorhabdoviruses may differ. A nuclear phase has been suggested during the initial phases of LNYV infection, because blistering of the outer nuclear envelope has been observed, and a few virus particles appear in these membranes (19, 25, 29). These nuclear events were not detected in BYSMV infections and the viroplasms of the two viruses also appear to differ. Late in LNYV infection, masses of thread-like particles appear in the cytoplasm and large numbers of virus particles bud from the endoplasmic reticulum and accumulate in the cytoplasm. However, in BYSMV-infected cells, large granular viroplasms accumulate in the cytoplasm in association with extensive proliferation of membranes into which budding occurs (3). Unfortunately, these differences rely only on ultrastructural observations taken at different times and on different plants, so the cell biology of cytorhabdovirus infections needs to be evaluated more thoroughly with modern cell biology techniques to verify details of the replication model described in Figure 7. The recent cloning and sequencing of LNYV, NCMV, and SCV provide the materials necessary to initiate such studies with these viruses.

The defining characteristic of nucleorhabdovirus infections is the formation of large inclusions or "viroplasms" in the nuclei of infected cells. In the case of SYNV and other nucleorhabdoviruses (29, 52), electron microscopy reveals the presence of viruses budding from viroplasm-like structures in the nucleus and accumulating in the perinuclear spaces surrounding the nucleus (Figure 3). Immunolocalization also shows that the N and P proteins accumulate in the nucleus and in situ hybridization demonstrates that the viral genomic RNAs accumulate in the nucleus (Figure 8). Viral polymerase activity containing the three nucleocapsid proteins can also be recovered from purified nuclei and this activity can be inhibited by L protein antibodies (96, 97). Therefore, these results provide persuasive evidence that SYNV replication occurs in the nuclei of infected cells and that the viroplasms contain polymerase activity mediated by the N, P, and L proteins.

Proliferation of membranes around the nucleus also often accompanies nucleorhabdovirus infections, and in the case of SYVV, membranes appear to be redistributed to form "annulate lamellae" in the phloem cells of infected *Sonchus oleraceous* plants (86). Annulate lamellae are structurally similar to both the endoplasmic reticulum and the nuclear envelope, because they contain pore complexes similar to those of the nuclear envelope. These structures are usually found either singly or in parallel stacks in the perinuclear region, but sometimes are located elsewhere in the cytoplasm or within the nucleus itself (86). It is possible that these structures represent the invagination of the nuclear membranes into the nucleoplasm and that viral cores bud into the reorganized membranes during morphogenesis.

Recent findings with SYNV may provide some explanation for the SYVV observations (35). This study has shown that the sizes of the nuclei of cells infected with SYNV and PYDV increase in cross-sectional areas by more than twofold over nuclei of mock-infected cells. On the other hand, nuclei of plants infected with *Tobacco rattle virus*, *Impatients necrotic spot virus*, and *Tobacco etch virus* (TEV), each of which replicate in the cytoplasm, failed to show significant increases in area (Figure 9). Interestingly, when *N. benthamiana* plants expressing an endomembrane-associated GFP (9) were inoculated with SYNV and PYDV, GFP fluorescence shifted dramatically away from the cytoplasm to the nucleus (Figure 10). In live cells, colocalization experiments with an endomembrane-specific dye, staining of the nuclei with DAPI, GFP fluorescence, and SYNV immunolocalization also revealed that the membranes containing the GFP were relocalized near the subnuclear sites of SYNV replication. The regions that stained most intensely with antibodies to GFP also had the most intense N protein antibody reactions (Figure 10). Taken together, these data strongly suggest that infection of *N. benthamiana* with SYNV results in the redistribution of host cytoplasmic membranes to the nuclear sites of viral accumulation (35). These experiments thus provide the first study to use live cell imaging to characterize rhabdovirus-induced alterations of host cell membrane flow, and the use of fluorescence probes to track these processes. The relocalization of GFP also provides a very convenient and distinctive marker to follow movement of SYNV and PYDV in live tissue because the infection process elicits dramatic shifts to nuclear fluorescence. In the future, this powerful method should facilitate studies of rhabdovirus cell-to-cell movement and may enable more detailed kinetic analyses of the cellular anomalies and membrane reorientation occurring along the viral infection front and in tissues behind the infection front.

Based mainly on studies of SYNV, recent work with PYDV, and long-standing studies with VSV (reviewed in 42), we are proposing a model for plant rhabdovirus replication (Figure 7). According to this model, upon entry into the cell via insect vectors or wounding, the virus associates with the endoplasmic reticulum to release the nucleocapsid core into the cytoplasm. Following liberation, the nucleocapsids of the cytorhabdoviruses establish viroplasms at sites within the cytoplasm, whereas the nucleorhabdoviruses associate with the nuclear transport

apparatus of the host to facilitate translocation to the nucleus. At some stage in this process, the cores are modified to activate transcription and, upon localization in subnuclear sites, they begin to transcribe viral mRNAs that are exported to the cytoplasm and translated. The N, P, and L proteins are subsequently transported to the nucleus. The accumulation levels of the N protein, and probably the P protein, are thought to regulate mRNA synthesis and an alternating series of antigenomic and genomic nucleocapsid amplification. As N and P increase in abundance sufficient to encapsidate newly synthesized leader RNAs, antigenomic RNA replication is initiated and the levels of free N and P protein are depleted because of their use in formation of the nascent antigenomic nucleocapsids. This transient decrease in the concentration of N and P triggers new rounds of mRNA transcription to replenish the N and P concentration, which then functions to encapsidate nascent genomic RNA transcribed from the N-, P-, and L-containing antigenomic nucleocapsids. Thus, the availability of the N and P proteins provides an ingenious feedback mechanism to regulate mRNA transcription and nucleocapsid replication. As interspersed rounds of replication occur, increasing numbers of nucleocapsids accumulate, discrete granular viroplasms appear in the nuclei, and the nuclei increase dramatically in volume. Several lines of evidence show that SYNV viroplasms contain the N, P, and L proteins, and therefore they appear to be the source of the polymerase activity that can be recovered from nuclei (66, 96, 97). Recent results (35) suggest that at some stage in the replication process, the G protein associated with the endoplasmic reticulum becomes glycosylated and the flow of these membranes is redirected to the nucleus where membranes form close associations with the growing viroplasms (Figure 10). As replication proceeds into the late stages of infection, the M protein associates with the genomic nucleocapsids and condenses these nucleocapsids to form tightly coiled core structures that are unable to function in mRNA transcription. These cores then bud through the redirected membranes at sites containing the G protein. Blocking G protein glycosylation with tunicamycin prevents budding, possibly by interfering with cytoplasmic membrane redirection, and results in the accumulation of large numbers of coiled core particles around the periphery of the nuclei (93). However, in untreated cells, the coiled genomic nucleocapsids undergo rapid morphogenesis at G protein-containing membranes to produce enveloped virions that subsequently accumulate in perinuclear spaces. These virus particles probably then function to infect insect vectors that feed on the infected plant.

Our model also suggests mechanisms whereby plant rhabdoviruses establish systemic invasions in plants. In order to move from the initially infected cells, the virus must move from cell to cell through plasmodesmatal channels into the phloem cells of the vascular system and throughout the plant (Figure 7). Because the plasmodesmata normally restrict movement of macromolecules, viruses generally move to adjacent cells via mechanisms that increase the permeability/gating capacity of the plasmodesmata. Many plus-strand RNA viruses encode specific nucleic acid binding proteins that function to enlarge plasmodesmata, bind RNA, and shuttle the genome through the plasmodesmata to neighboring cells (40, 98).

Plant rhabdoviruses face a special challenge at this stage of infection because the approximately 3-nm diameter plasmodesmata are at least an order of magnitude smaller than virus particles. Therefore, transit of intact viruses would require enormous alterations to plasmodesmata structure that ought to be easily visible by electron microscopy. However, a large number of ultrastructural studies have been carried out with many different rhabdoviruses and enlarged plasmodesmata have not been described in these studies. Because nucleocapsids are the minimal infectious units of negative strand RNA viruses, a reasonable hypothesis is that some form of genomic nucleocapsids are involved in cell-to-cell spread and in vascular movement. It is also likely that dedicated movement proteins (MP) encoded by the virus facilitate nucleocapsid translocation through the plasmodesmata.

We propose that shortly after the genomic nucleocapsids begin to increase, a small number of them associate with viral MP. According to our model, these associations mediate transit of nucleocapsids to the plasmodesmata. The MP causes enlargement of the desmotubules sufficiently to permit nucleocapsid translocation into adjacent cells where they are redirected into the nucleus (for nucleorhabdoviruses) to engage in a new series of replication events. Clearly, this general model raises a number of major questions that need to be addressed in future studies, but we are rapidly developing the resources to carry out the cellular and biochemical studies needed to clarify some of these issues.

EFFECTS OF RHABDOVIRUS-ENCODED PROTEINS ON HOST GENE EXPRESSION

In addition to their contributions to virion structure and replicative processes, rhabdoviral proteins are undoubtedly multifunctional and have additional roles in interacting with their hosts. Although little direct information is yet available, studies of these interactions may explain long-standing observations of viral infection processes. For example, two phases of infection can be distinguished during the infections of the nucleorhabdovirus SYNV in *N. edwardsonii*. During the acute phase, SYNV rapidly spreads systemically to leaves and roots, and accumulates to high levels in the nucleus and perinuclear spaces. After about 10 days, the plants enter a chronic or recovery phase characterized by a rapid decrease of viral RNA and structural proteins and the dislocation of virions and nucleocapsids to the cytoplasm (49). This recovery has been attributed to the accumulation of defective-interfering particles in chronically infected plants (50), but innate host defense responses such as transcriptional and posttranscriptional gene silencing and translational repression are probably major contributors to recovery.

Evidence for gene silencing in animal rhabdovirus infections has implicated the M proteins, which are known to be involved directly in shutting off host gene expression (73, 95). Conservation of a similar activity in plant rhabdoviruses has recently been suggested by experiments conducted in one of our labs (Goodin) with SYNV-encoded proteins (Figure 11). The results show that the SYNV M

protein has the ability to shut off gene expression, and it appears that this activity can be regulated. In these experiments, agroinfiltration (34) was used to express a cyan fluorescent reporter protein (CFP) in plant cells in the presence or absence of SYNV-M (Figure 11*A*). When the SYNV M protein was coexpressed with CFP, fluorescence disappeared, indicating that the M protein possesses the ability to shut off reporter gene expression. However, when the M protein and CFP were coexpressed in the presence of the SYNV P protein, high levels of CFP expression were observed, showing that the P protein can interfere with the reporter gene shut-off activities of the M protein.

Additional experiments indicated that the ability of the P protein to suppress GFP shut off by the M protein was abrogated when the P and N proteins were coexpressed (Figure 11*A*). This result suggests that the interference of the P protein with M protein host interactions may occur in the cytoplasm, and that this interference is relieved as the result of relocalization of P protein to subnuclear sites during associations with the N protein. The interference of the P protein on M protein down-regulation of GFP could also be replicated by known viral suppressors of RNA silencing. Three strong suppressors, the p19 protein of *Tomato bushy stunt virus*, HC-Pro of *TEV*, and the 2b protein of *Tomato aspermy virus*, were all effective in interfering with the M protein activity. Since p19, HC-Pro, and 2b target host defenses that result in degradation of the RNAs of invading viruses, these results imply that the P protein itself may have a role in the suppression of host antiviral defenses. Independent support for such activity has been determined in transient agroinfiltration assay designed to identify gene-silencing proteins (57). In these experiments, bacteria capable of transient expression of GFP were infiltrated in various combinations with mixtures of bacteria capable of expressing dsRNA-containing GFP sequences and bacteria expressing the P protein. When bacteria expressing GFP were infiltrated alone, high levels of GFP expression resulted in the infiltrated areas and, as expected, infiltration with bacteria expressing dsRNAs complementary to GFP resulted in activation of host gene silencing activities directed against GFP. In contrast, tissue infiltrated with bacteria expressing GFP, dsRNA and P protein expressed high levels of GFP, suggesting that the P protein is able to suppress the host gene-silencing responses (Figure 11*B*).

Taken together, these data suggest a model whereby the ability of the SYNV M protein to shut off host gene expression is prevented by the P protein and is regulated by the concentrations of free P protein (Figure 11*D*). These findings are significant, given that the coordinated interactions of the SYNV M, N, and P proteins that appear to regulate RNA silencing are without precedent in the animal rhabdovirus literature. There is no evidence that the animal rhabdovirus M protein interference with gene expression can be reversed by the cognate P protein or by other viral proteins. Likewise, no evidence exists for control of the activity of viral-encoded suppressor proteins by interactions with other virus proteins. Thus, the interactions of the N, P, and M proteins may provide a novel regulatory model to account for the rapid burst of SYNV replication, followed by the chronic phase in which down-regulation of viral expression occurs. This model accommodates

changes in the relative levels of the SYNV M, N, and P proteins during the course of infection that may exert temporal effects on expression of host genes and on virus replication and movement.

FUTURE DIRECTIONS

Plant rhabdoviruses provide excellent material for future research, and a variety of experimental approaches can be emphasized in their study. The complete and partial sequences obtained for several rhabdoviruses over the past five years should provide a major focus for the analysis of the remaining members of this group of viruses. The availability of sequence information will clearly permit a more detailed understanding of the relationships among current and provisional members of the genera, and may provide a more definitive analytical basis for taxonomy than the current criteria based on electron microscopy studies. Our understanding of the cellular events occurring during replication of the nucleorhabdoviruses has increased dramatically over the past few years, and we anticipate similar advances in our understanding of the cytorhabdoviruses as recombinant probes based on cloned sequences become available. Thus, many opportunities exist to extend our knowledge about rhabdovirus biology, and there are numerous challenges that need to be addressed to conduct sophisticated analyses of gene function and host-vector interactions. Meeting these challenges will provide fundamental information about the infection processes of plant rhabdoviruses as well as the basic defenses of host plants and insect vectors that must be exploited during the virus life cycle.

The conservation of the gene junction regions of the sequenced rhabdovirus genomes indicates that representative primers for these sequences can be utilized for polymerase chain reaction amplification of genomic and antigenomic RNAs present in infected cells. This will circumvent the necessity for purification of virus particles and should greatly accelerate studies of those viruses that have previously proven difficult to investigate by classical means. Furthermore, the availability of sequence information encompassing the assigned and provisional rhabdoviruses will not only permit a comprehensive molecular approach for taxonomy to refine analysis of the relationships among these viruses, but will also enable application of a variety of molecular strategies to address important questions about infection processes.

Continued efforts to understand the replication of model nucleorhabdoviruses and cytorhabdoviruses should soon provide a wealth of new information. This should result in development of more refined models for host and viral interactions that lead to the establishment of the sites of replication, development of viroplasms, mechanisms involved in rhabdovirus movement, and the sequence of events involved in morphogenesis. The availability of cloned genes will also permit biochemical experiments to evaluate function and interactions of host and viral proteins. The use of recombinant materials applied to infected protoplasts should provide a medium for studies of synchronous replication (58, 93, 94), while in

plants, considerable information can be gained from agrobacterium-mediated ectopic expression of proteins (34). Yeast expression systems and two-hybrid assays also have enormous potential for probing biochemical interactions, cell biology, and the function of rhabdovirus-encoded proteins (33). Studies of the interactions of plant rhabdoviruses with insect vectors have languished over the past 20 years, but application of recombinant probes and microarray analysis of expressed genes could provide a wealth of new information about the mechanisms of infection of whole insects, how these events differ from those observed in plants, the details of transovarial passage to progeny, and the steps required for transmission to plants. Early work summarized by Black (7) provided a foundation for the use of insect tissue cultures for studies of PYDV and other viruses, and his studies provide a strong argument that development of additional cell lines suitable for infectivity studies could be profitable for comparisons with the emerging cell biological information being accumulated in plant cells.

Another area that is ripe for exploitation is viral ecology and vector interactions. The use of diagnostic molecular probes from cloned genomes and antibodies derived from recombinant proteins now provide powerful tools to determine virus distribution in hosts and vectors. The use of such probes can be combined with more traditional infectivity assays to determine whether the serological titer in a vector provides a good correlation with transmission ability. If so, ecological analyses to evaluate viruliferous vectors and the conditions required for transmission from weeds to crops can be used to develop predictive models for disease development and may result in simple changes in agronomic practices for disease control. Hence, a better understanding of epidemiology, host range, and vector relations will have practical benefits in devising rational and durable disease control measures.

The development of reverse genetics strategies that can be applied to studies of plant rhabdoviruses is probably the most pressing area. Recovery of positive-strand RNA viruses from recombinant DNAs was implemented relatively easily owing to the infectious nature of the viral RNAs, and the first studies with infectious full-length clones of viruses appeared about two decades ago. However, successful reverse genetic strategies with the negative-strand viruses required much more complex approaches because the nucleocapsid core consisting of the polymerase proteins and the genomic RNA is the minimal infectious unit. Consequently, formation of a biologically active ribonucleoprotein complex is essential for recovery of recombinant rhabdoviruses. Approximately ten years ago, a variety of approaches using various delivery systems for the N, P, and L proteins and minigenome derivatives culminated in the in vivo generation of recombinant rabies virus (82) and VSV (61, 103). Since those pioneering experiments, similar reverse genetics strategies have been applied to members of most families of negative-strand viruses infecting animals (17, 43, 72). In most cases, the key elements leading to reconstitution of these viruses revolve around the coexpression of polymerase proteins in cells coupled with transcription of authentic antigenomic (positive-sense) RNAs that become encapsidated to form recombinant nucleocapsids. The subsequent

application of reverse genetics has lead to an explosion of research with animal rhabdoviruses and to enormous advances in our understanding of replication and biology. Pilot studies have also indicated that recombinant viruses can be exploited for a wide range of biotechnology and biomedical applications, including attenuated virus derivatives for protection of livestock (99), viruses with rearranged genes to produce live attenuated vaccines (24), and replication incompetent viruses with protective efficacy (100). In addition, proteins encoded by other viruses can be engineered into recombinant negative-strand virus vectors, and upon infection, the expressed proteins provide protection against the source virus (see 77). These developments thus provide great optimism that the ability to recover recombinant plant rhabdoviruses that can be genetically manipulated will affect almost all areas of plant rhabdovirus research.

Unfortunately, the application of reverse genetics to the plant negative-strand viruses has been hampered by two major problems not encountered with their animal virus counterparts. The most serious difficulty revolves around an inability to express the complex of proteins needed for assembly of infectious nucleocapsids in cultured plant cells or in whole plants. Lack of a plaque assay in plant or insect cell lines creates a second hurdle in visualizing and recovering biologically active virus from the primary infection foci. The walls of cultured plant cells are highly resistant to the introduction of plasmids needed for infectivity studies, so alternative approaches must be used. Although protoplasts can be infected with some purified rhabdoviruses and used to study synchronous replication, their routine use is tedious. It is quite difficult to introduce multiple plasmids into cells, and the cells often fail to regenerate walls and begin to deteriorate during the time required for maximal accumulation of progeny virus (58). Other possible approaches, such as use of insect cell lines for selection of recombinant plant rhabdoviruses, seem remote at the present time because of the limited availability of suitable vector lines, and because discernible cytopathologies are not obvious in rhabdovirus-infected cell lines (7). Nevertheless, a reinvigorated approach to insect vector studies will, it is hoped, result over the next few years in lines that can be useful for transformation. If so, plaque detection might be visualized by use of reporter genes. *Drosophila*, lepidopteran, and possibly mammalian cell lines might also be usefully applied to some rhabdoviruses, and given their broad host ranges, it is possible that yeast might support the replication of some plant rhabdoviruses. Thus, our feeling is that recovery of recombinant negative-strand plant viruses will require substantially different approaches from those used with animal viruses.

One attractive approach being investigated in our labs to circumvent these difficulties is the use of *Agrobacterium*-mediated transient expression to produce proteins and various viral genomic transcripts in cells of infiltrated leaf tissue. Agroinfiltration permits several proteins to be expressed to very high levels in most of the cells in infiltrated regions of a leaf (34). Thus, the N, P, and L proteins are being expressed and tested for their ability to encapsidate antigenomic and/or genomic RNAs. We believe that this approach provides a solid basis for

reproducible delivery and high level of expression of SYNV genome derivatives that might be suitable for reconstitution of fully recombinant virus that can move from the infiltrated regions of the leaves and spread systemically throughout plants.

In summary, we anticipate that the next few years will lead to an acceleration in our understanding of several areas of plant rhabdovirus biology. In particular, application of molecular and biochemical tools will provide a better understanding of viral taxonomy, replication, vector relations, and ecology. Over the next decade, these tools, in combination with application of reverse genetics, should result in reinvigorated fundamental and applied plant rhabdovirus research that will be attractive to a broad range of biologists.

ACKNOWLEDGMENTS

We thank Mr. Ben Callaghan for providing Figure 6 and for unpublished information on the LNYV genome sequence prior to thesis submission, and Drs. Cor Schoen, Saskia Hogenhout, Peter Revill, and Rong-Xiang Fang for manuscripts prior to publication. Dr. Peg Redinbaugh is also acknowledged for the photograph of MFSV shown in Figure 1, Ms Ann Boughton (Thumbnail Graphics) for Figure 2, and Debashish Ghosh and Sharon Yelton for Figure 9. Drs. Steve Ruzin and Denise Schichnes and the Center for Biological Imaging at UC Berkeley provided invaluable training, support, and advice during the cell biology phases of our research. Ralf Dietzgen was supported by Horticulture Australia Ltd and the Department of Primary Industries and Fisheries, Queensland, Michael Goodin by Grant 5-41,184 from the Kentucky Tobacco Research and Development Center, and Andy Jackson by National Science Foundation Grant Number MCB-03,16907 and National Institutes of Health Award 1 RO3 AI059178-01.

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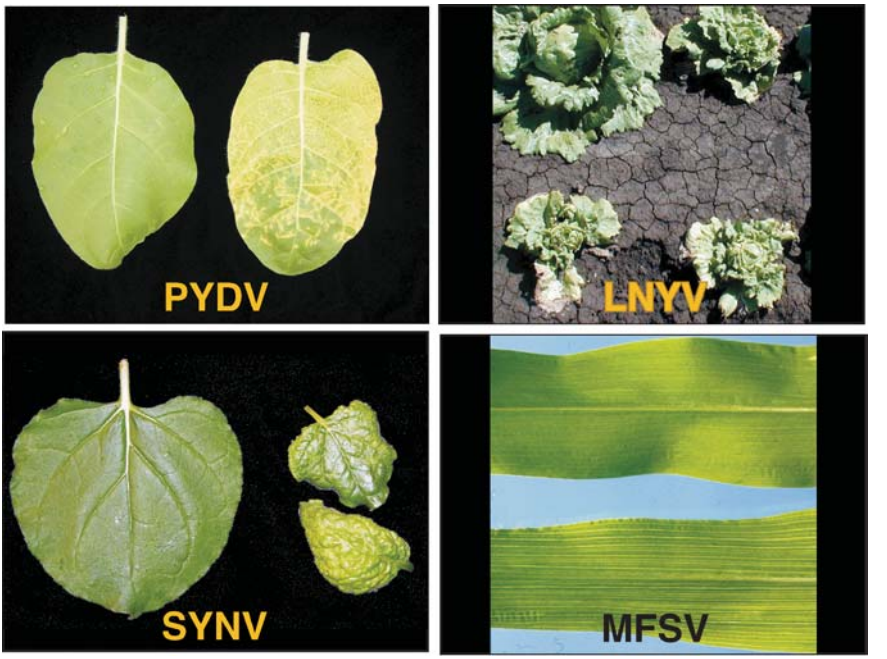

Figure 1 Symptoms of rhabdovirus-infected plants. Shown clockwise from top left are PYDV in *N. rustica*, LNYV in *Lactuca sativa*, MFSV in *Zea mays*, and SYNV in *N. benthamiana*. Typical symptoms include veinal chlorosis mosaic and stunting. Photographs were provided by R. Dietzgen (LNYV), M. Goodin (PYDV and SYNV), and P. Redinbaugh (MFSV).

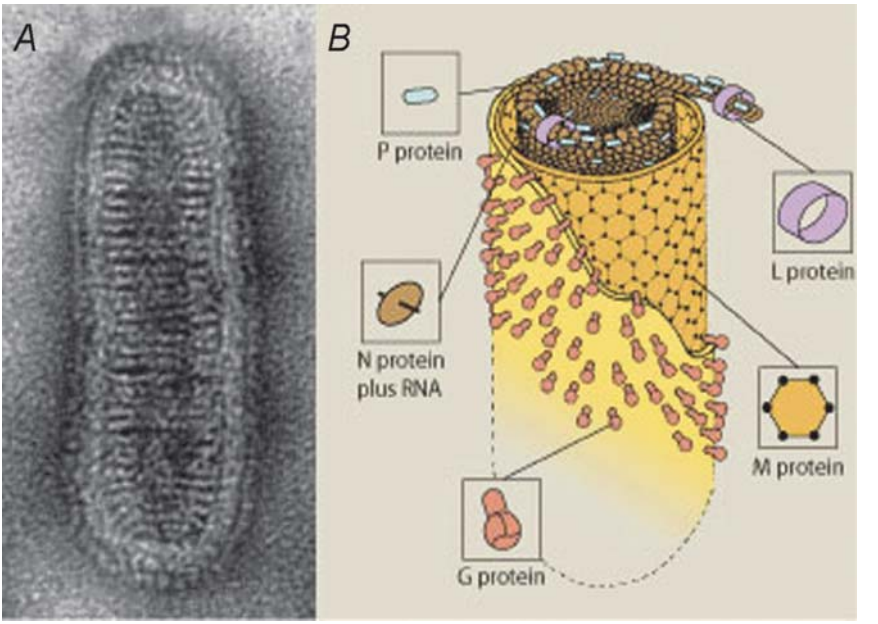

Figure 2 Electron micrograph and diagram illustrating SYNV morphology. (*A*) Transmission electron micrograph of a negative stained virion showing the striated inner core, envelope and glycoprotein spikes. (*B*) Depiction of the architecture of the virus particle. The nucleocapsid core is composed of 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 core to the envelope, and associations with the G protein. The membrane lipids are host-derived and are interspersed with trimeric glycoprotein (G) spikes arranged as surface hexamers. The sc4 protein (not depicted) is believed to form a minor component of the envelope of purified SYNV particles. Modified from Reference 29 by Ann Boughton (Thumbnail Graphics).

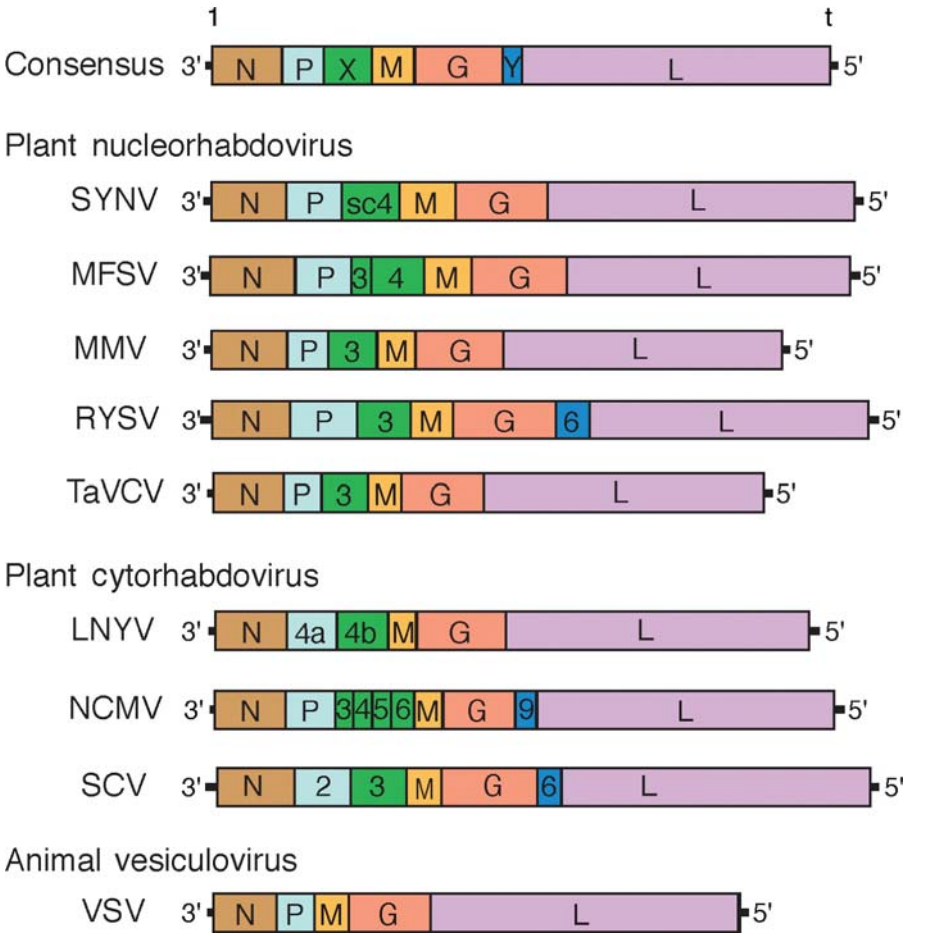

Figure 4 Schematic representation of the 3′ to 5′ negative-sense arrangement of genes in the genome of plant and animal rhabdoviruses. ℓ: 3′ leader sequence; N: nucleocapsid protein gene; P: phosphoprotein gene; X: site containing the putative movement genes and unknown genes of plant rhabdoviruses; M: matrix protein gene; G: glycoprotein gene; Y: location of unknown genes of several plant and animal rhabdoviruses; L: polymerase gene; t: 5′ trailer sequence. Other sequenced genes whose functions have not been clearly defined are numbered according to their overall location on the genome starting at the 3′ end.

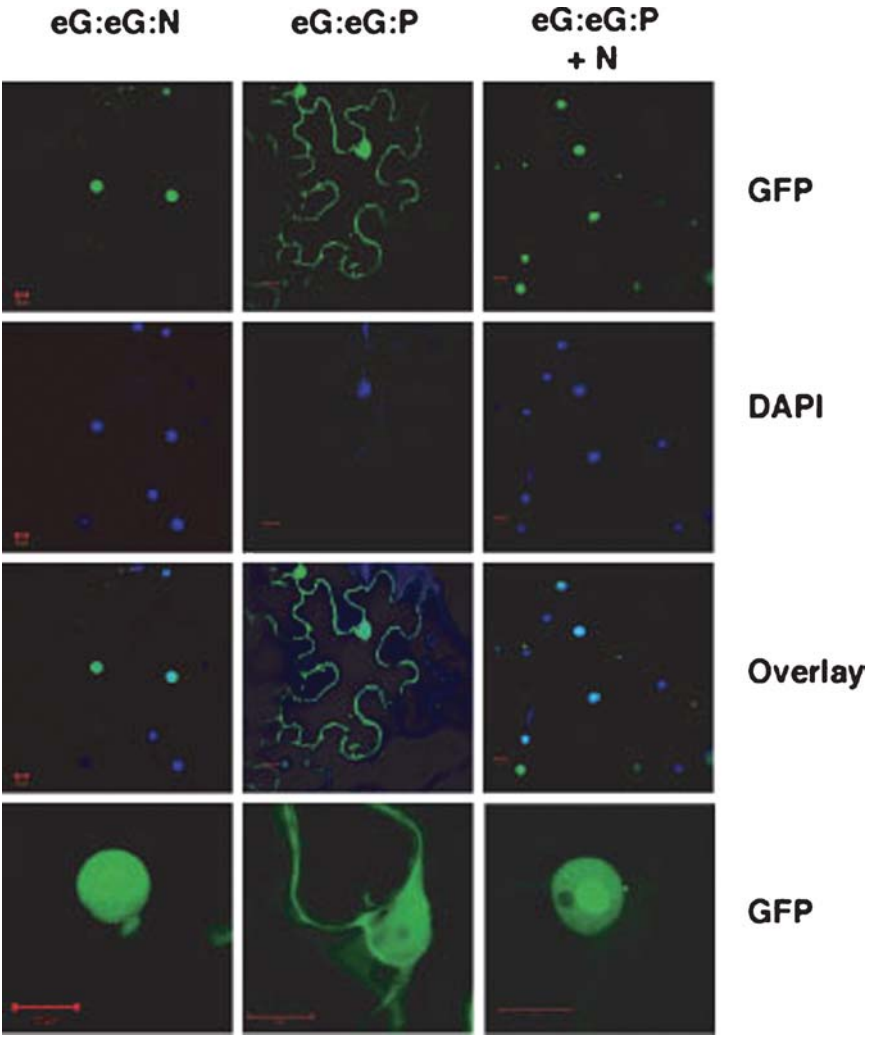

Figure 5 Confocal micrographs showing the subcellular localization of SYNV nucleocapsid protein (N) and phosphoprotein (P) in the epidermal cells of *Nicotiana benthamiana*. Localization of fusion proteins consisting of enhanced GFP (eGFP) N-terminal fusions to either the N (eG:eG:N) or P (eG:eG:P) proteins. The columns show transient expression of eG:eG:N or eG:eG:P individually or coexpression of eG:eG:P with N. In each column, the first row shows GFP fluorescence representing the localization of the fusion proteins when expressed alone or coexpressed; the second row identifies the nuclei with DAPI staining; the third row presents an overlay of the GFP and DAPI images; and the fourth row shows GFP fluorescence around the nucleus at a higher magnification. Bar size = 10 mm.

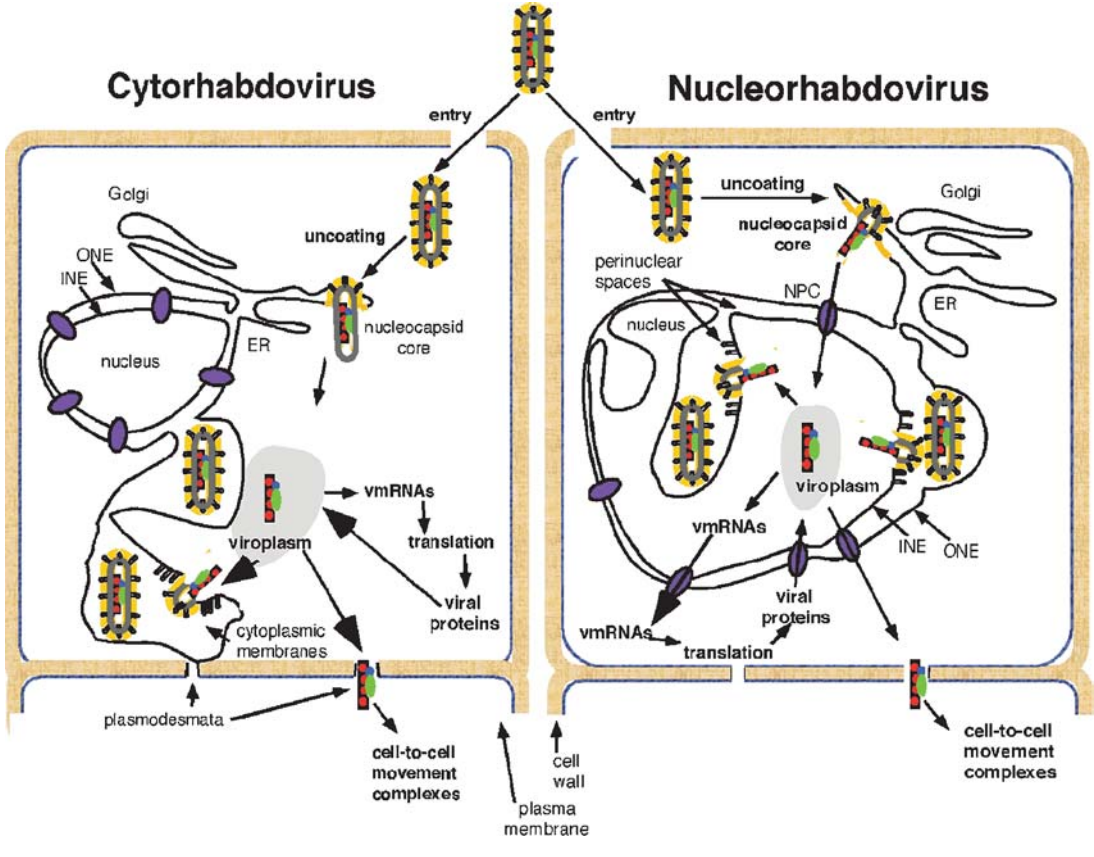

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Figure 7 Contrasts between the replication cycles of cyto- and nucleorhabdoviruses. Most rhabdoviruses gain entry into host cells during insect vector feeding. Uncoating is believed to take place on ER membranes, followed by release of the nucleocapsid core into the cytoplasm. At this point, the replication cycles of the two genera diverge. In the case of the cytorhabdoviruses, the newly released cores become transcriptionally active and associate with the endoplasmic reticulum to establish viroplasms that function in transcription of viral mRNAs (vmRNAs) and replication of genomic and antigenomic viral RNAs. Following translation of the vmRNAs, viral proteins involved in replication accumulate in the viroplasm. Viral glycoproteins (□) are targeted to cytoplasmic membranes or, possibly, the outer nuclear envelope (ONE). Maturation of cytorhabdoviruses takes place via matrix protein-mediated condensation of cores at sites of G protein accumulation in the endoplasmic reticulum. In the case of the nucleorhabdoviruses, released cores are transported into the nucleus through nuclear pore complexes (NPC). Following transcription and export, vmRNAs are translated and viral proteins are imported into the nucleus, where they participate in replication and formation of large viroplasms. During intermediate stages of infection of plant rhabdoviruses, movement of infectious units from cell to cell occurs. Nucleocapsids most likely are the transported form, and these interact with viral-encoded movement proteins that participate in number of activities, including nucleocapsid binding, transport through the NPC to the plasmodesmata, and modifications to the plasmodesmatal size exclusion limits. Morphogenesis occurs near the end of active transcription and replication and involves interactions with the M protein to coil the viral nucleocapsids and form associations with membrane-associated G protein. In the cytorhabdoviruses, electron microscopic observations suggest that budding occurs into proliferated ER associated with the viroplasms. Currently, at least two models can be proposed for morphogenesis of nucleorhabdovirus virions. In recent data outlined in the text, the inner nuclear envelope (INE) proliferates due to redistribution of cytoplasmic membranes and invaginates to form intranuclear spherules, into which viral budding occurs. In the classical model, virus budding occurs through intact INE resulting in an expansion of the outer nuclear envelope. In both models, mature virions accumulate in the perinuclear spaces of infected cells where they may be reacquired during subsequent insect feeding.

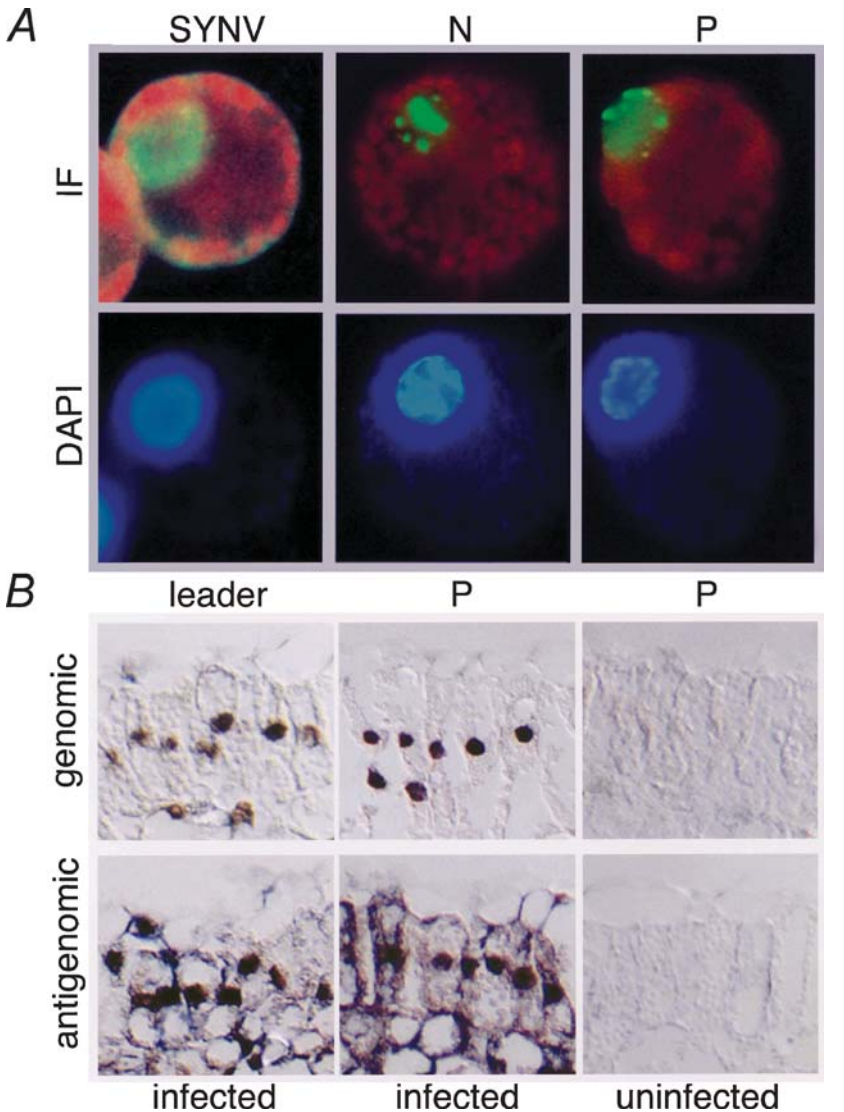

Figure 8 (*A*) Immunofluorescence (IF) localization of SYNV proteins, the N protein, or the P protein in SYNV-infected *N. benthamiana* protoplasts that were isolated from systemically infected leaves. Antibodies raised against disrupted SYNV particles, the N protein, or the P protein were incubated with the protoplasts, and secondary antisera conjugated to FITC were used to visualize the location of the proteins. The protoplasts were also incubated with DAPI before examination. Note that the most intense areas of IF coincide with regions of DAPI exclusion. (*B*) In situ hybridization of SYNV-infected *N. benthamiana* leaf tissue (11 to 14 dpi). Probes specifically recognize specific leader or P regions of the genomic and antigenomic RNAs. (Figure modified from figures 1 and 2 in Reference 66.)

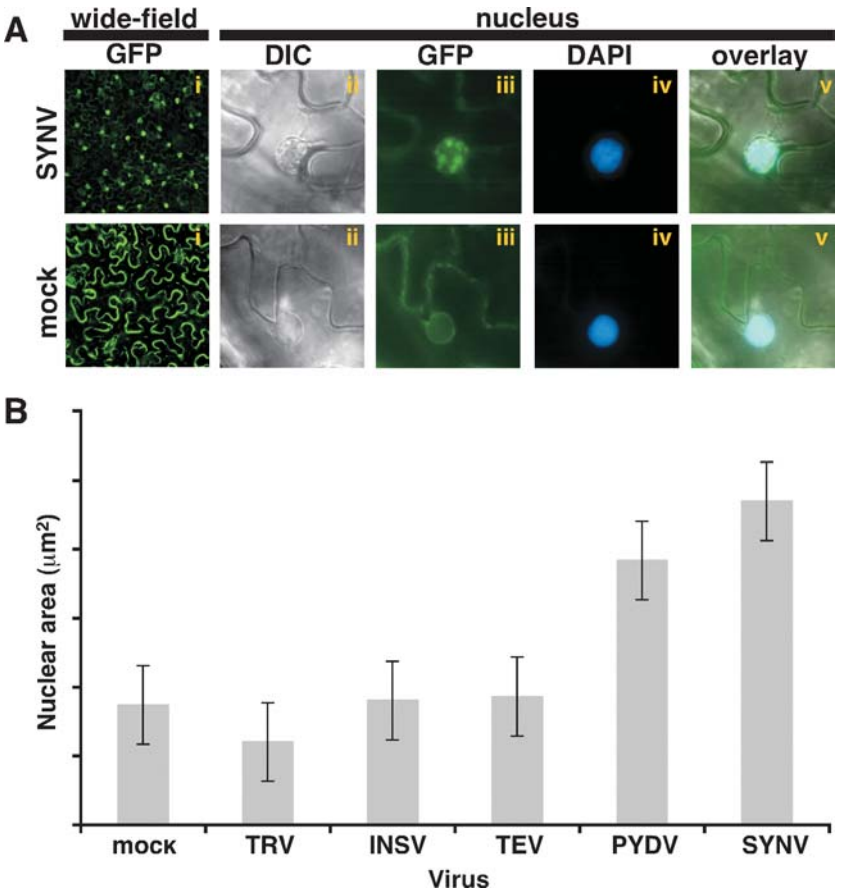

Figure 9 Changes in the nuclei of rhabdovirus-infected cells. *Panel A*: Mock inoculated or *Sonchus yellow net virus*-infected green fluorescent protein (GFP)-expressing *Nicotiana benthamiana* 16c transgenic plants. (*i*) membrane-targeted GFP fluorecence in wide field view of leaf-epidermal cells. (*ii*) Differential interference contrast (DIC) micrograph of a single cell. (*iii*) GFP fluorescence in a single cell. (*iv*) DAPI fluorescence to identify the nucleus. (*v*) Overlay of images of (*ii*), (*iii*), and (*iv*). Note the changes from hyaline poorly refractile nuclei in cells of mock-inoculated plants contrasted with highly refractile nuclei in SYNV-infected plant cells, and the reorientation of GFP fluorescence from the cytoplasm of mock inoculated cells to the nuclei of SYNV infected cells. *Panel B*: Comparisons of the sizes of nuclei in mock- and virus-infected 16c plants. TRV, *Tobacco rattle virus*; INSV, *Impatiens necrotic spot virus*; TEV, *Tobacco etch virus*; SYNV, *Sonchus yellow net virus*; PYDV, *Potato yellow dwarf virus*. TRV, INSV, and TEV replicate in the cytoplasm and SYNV and PYDV replicate in the nuclei. The area bounded by the greatest area of fluorescence was measured in mock-inoculated or virus-infected leaves of 16c plants. The mean and 95% confidence intervals are shown for measurements of 100 nuclei in each treatment.

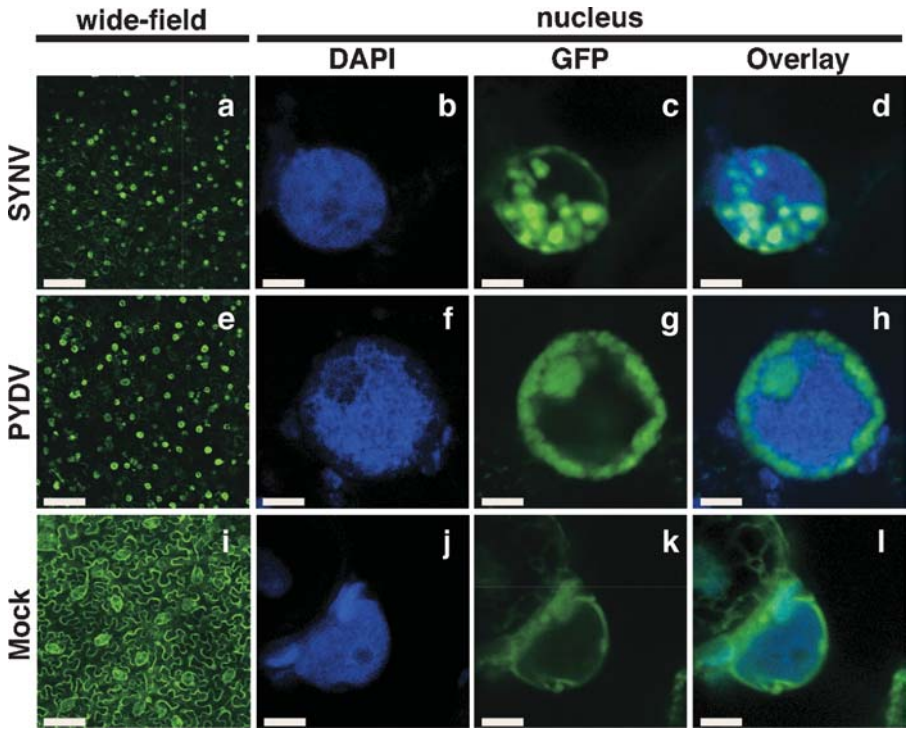

Figure 10 Confocal micrographs of rhabdovirus-induced nuclear inclusions in line 16c *N. benthamiana* plants expressing an endoplasmic reticulum targeted green fluorescent protein. (*a–d*) Leaves of 16c plants systemically infected with SYNV, (*e–h*) PYDV, or (*i–l*) mock-inoculated. (*a*), (*e*), and (*i*) GFP fluorescence in wide-field micrographs of the abaxial leaf surface. Contrast the predominant cytoplasmic fluorescence of membrane-targeted GFP in mock-inoculated tissue with the punctate nuclear fluorescence occurring after SYNV and PYDV infection. Scale-bar, 200 μm. (*b–d*), (*f–h*), and (*j–l*): DAPI and GFP fluorescence, and the overlay of these images in nuclei of epidermal cells of SYNV-, PYDV- and mock-inoculated leaves, respectively. Scale bar, 5 μm. (*b–d*) The punctate areas of GFP fluorescence in the SYNV-infected nuclei represent inclusions that are thought to result from membrane reorientation from the cytoplasm. (*f–h*) The punctate areas of GFP fluorescence in the PYDV-infected nuclei also represent membrane reorientation and accumulate primarily around the area of DAPI fluorescence and within a large internal region that excludes DAPI. (*j–l*) Note that the GFP fluorescence in mock-inoculated tissue occurs at the nuclear periphery and surrounding membranes. Discrete GFP inclusions were not detected within these nuclei. (Image courtesy of MPMI and the authors.)

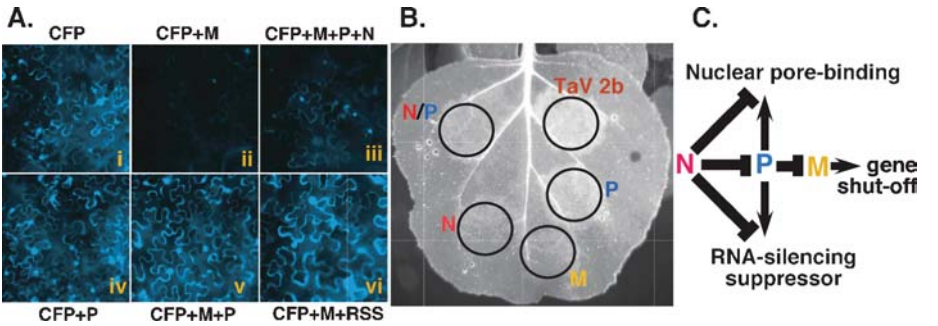

Figure 11 (*A*) Shut off of transient reporter gene expression by the SYNV M protein can be prevented by coexpression of SYNV P or an RNA silencing suppressor protein. (*i*) A cyan fluorescent protein (CFP) under the control of the single 35S promoter is easily detected when transiently expressed in epidermal cells of wild-type *N. benthamiana*, (*ii*) but fluorescence is not evident when CFP and SYNV M are coexpressed. (*iii*) CFP fluorescence is greatly reduced in cells coexpressing CFP, SYNV-M, SYNV P, and SYNV N. (*iv*) High levels of fluorescence occur in cells coexpressing CFP and SYNV P or (*v*) CFP, SYNV M, and SYNV P. (*vi*) Fluorescence is also easily detected in cells coexpressing CFP, SYNV M and several RNA silencing suppression proteins tested. (*B*) SYNV-P is a putative RNA-silencing suppressor (RSS). Transient expression of a known RSS (*Tomato aspermy virus* 2b protein) or SYNV P results in enhanced GFP fluorescence (*white areas*) at the sites of agroinfiltration in 16c *N. benthamiana* leaves. Expression of SYNV M, N, or coexpression of N and P does not lead to enhanced GFP fluorescence compared to expression of the P protein. (*C*) A model to integrate data presented in panels (*A*) and (*B*). The SYNV M protein is able to shut off gene expression, but SYNV P interferes with this M protein activity, and is a putative RSS protein. Interaction with SYNV N results in loss of the ability of SYNV P to interfere with SYNV M. Thus, interactions of all three proteins are involved in regulation of gene expression. (*Arrows* indicate interactions/activities and *blunt-ended lines* indicate inhibition.)

Annual Review of Phytopathology
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